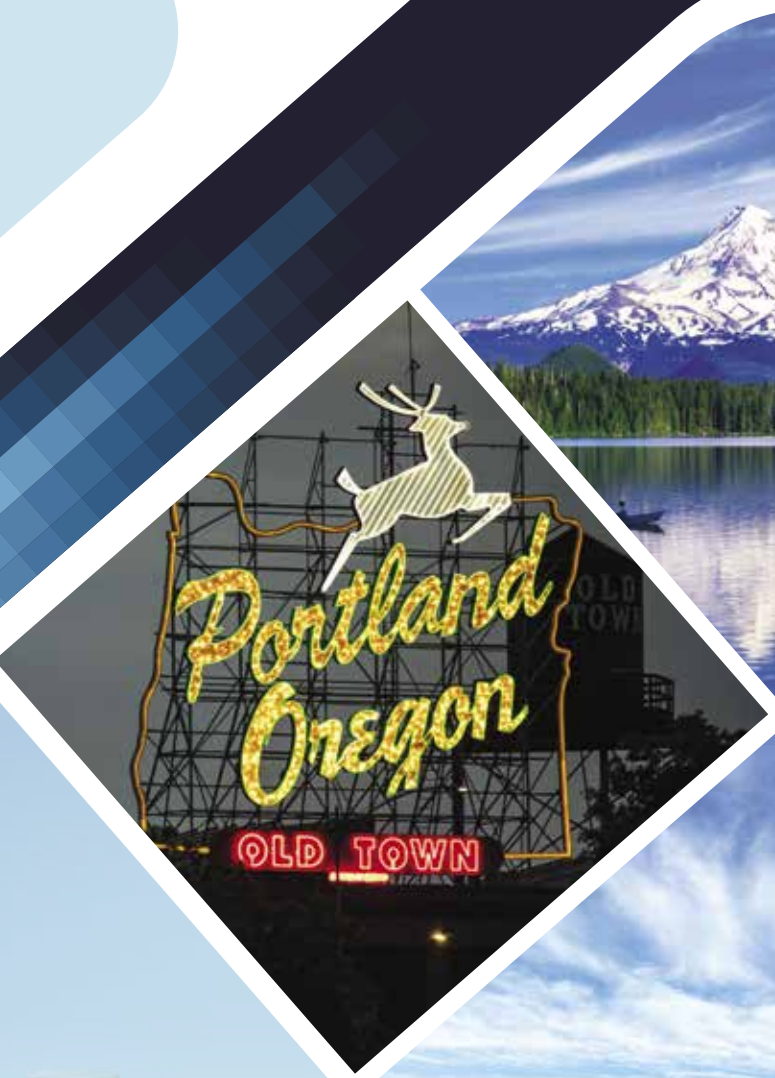
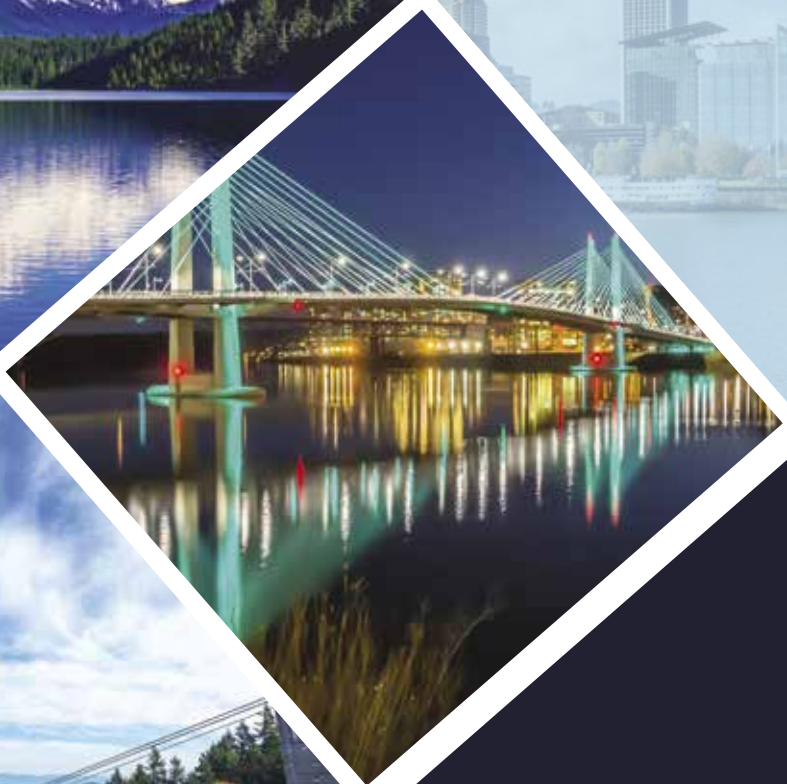
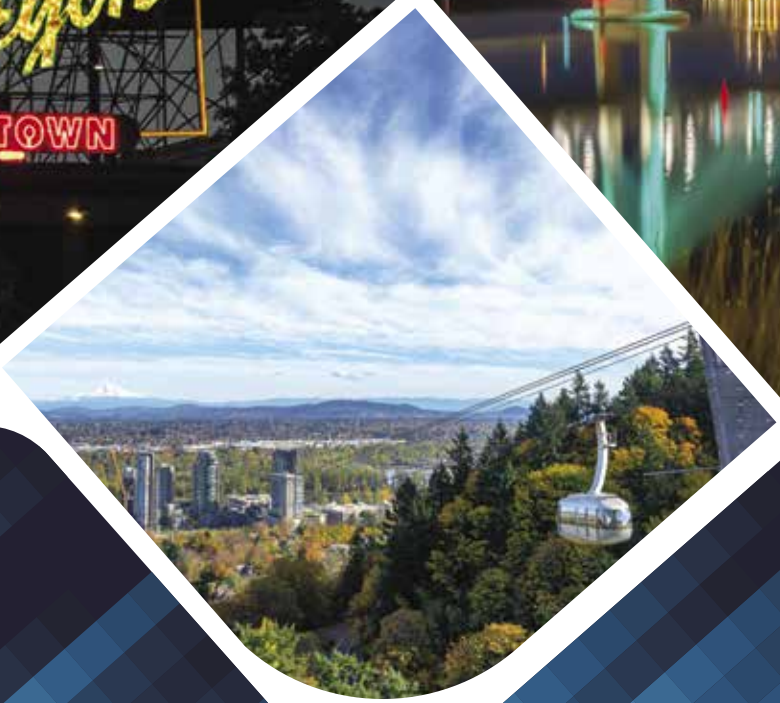
SID ANNUAL MEETING

PORTLAND 22

MAY 18-21, 2022







ABSTRACT BOOKLET

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Late-Breaking ABSTRACTS

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001

Inhibition of tissue resident memory-T cells as a therapy for contact hypersensitivity

T. Khosravi-Hafshejani, M. Ghoreishi, J. P. Dutz
Dermatology and Skin Science, The University of British Columbia Faculty of Medicine, Vancouver, British Columbia, Canada

Background: Systemic therapy for eczema targets the immune system during active inflammation. Disease inevitably returns to previously inflamed skin due to the persistence of tissue resident memory T (Trm) cells responding to autoantigens/allergens. The survival of Trm cells is regulated by IL-15. We present a mouse model of recurrent contact hypersensitivity (CHS) and suggest a novel approach to the treatment of inflammatory skin diseases through inhibition of Trm cells. Methods: C57BL/6 Mice were sensitized on the abdomen with the allergen 2,4-dinitrofluorobenzene (DNFB) (day -5), and then challenged on the ear on day 0. Mice then received peritoneal injections of IL-15-receptor neutralizing antibodies, twice a week for four weeks, and then re-challenged with DNFB on day 30 and 60. The control group received no antibodies. Ear swelling was measured every 12-hours for 96-hours post challenge. Ear skin was harvested 2-days (inflamed skin) and 15-days (healed skin) after each DNFB ear re-challenge. Results: In the control group, serial DNFB challenges produced stronger peak CHS responses, with a significant increase at 2- months ($p<0.05$). The number of Trm cells and expression of IL-15-receptor significantly increased in healed skin compared to inflamed skin ($p<0.05$). There were significant increases in Trm cell number and IL-15-receptor expression in healed skin at 2-months compared to 1-month DNFB re-challenge ($p<0.05$). In our inhibitor group, IL-15-receptor inhibition resulted in significantly less ear swelling ($p<0.05$) and significantly reduced number of Trm cells expressing IL-15 receptors in inflamed and healed skin ($p<0.05$) compared to control. Conclusions: Trm cells expressing IL-15-receptors accumulate in healed skin following inflammation. Inhibition of IL-15-receptors during disease quiescence prevents skin inflammation following allergen re-challenge, and correlates with a reduction of Trm cells expressing IL-15-receptors. This may be a novel strategy to prevent dermatitis recurrence and maintain long-term remission.

002

Shiitake dermatitis: Not mush-room for shrooms

K. Mai[1], D. Adams[2]
[1]*College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, Pomona, California, United States*, [2]*Dermatology, Lassen Medical, Red Bluff, California, United States*

In rare occasions, ingestion of raw or undercooked shiitake mushrooms (Lentinus edodes) can result in a unique dermatologic manifestation known as shiitake flagellate dermatitis (SFD). The diagnosis of SFD is often overlooked due to the rarity of the condition and the commonality of shiitake mushroom consumption. As the consumption of shiitake mushrooms increases globally, a rise in cases may follow suit. Therefore, awareness of the presentation and clinical background of SFD is critical. Here, we present a case of SFD in a middle-aged female. A middle-aged female presented with an abrupt onset of pruritic skin lesions. She denied any other symptoms or previous episodes of a similar rash. Past medical history was noncontributory. However, the patient recalled eating shiitake mushrooms just prior to the outbreak. Physical examination was significant for erythematous linear papules that formed flagellate/whip-like patterns across her body. The lesions extended from her neck to her abdomen and bilateral thighs. A 2x3mm biopsy showed superficial perivascular, lymphocytic infiltrate with a few eosinophils. Fungal staining with appropriate controls were positive for spores in the follicular ostia. Her clinical presentation and history conformed with SFD. While the mechanism of SFD is still unclear, theories suggest it stems from a hypersensitivity reaction to lentinan. Lentinan is a polysaccharide found in the cell wall of shiitake mushrooms. The heat-labile nature of lentinan may explain why cases originate following the consumption of undercooked or raw shiitake. Biopsies commonly demonstrates dermal edema, and lymphocytic perivascular infiltrates with eosinophils and neutrophils. Our case further demonstrates that fungal staining may be beneficial when the clinical presentation is insufficient for diagnosing SFD. SFD often self-resolves, however, oral histamines and topical steroids have shown to alleviate symptoms. A few cases have also demonstrated SFD triggered or exacerbated by sun exposure suggesting a possible photosensitivity component to the condition.

003

Atypical bullous pemphigoid due to radiation therapy

A. Belzer[1], C. J. Ko[2], J. S. Leventhal[2]
[1]*Yale School of Medicine, New Haven, Connecticut, United States,* [2]*Dermatology, Yale School of Medicine, New Haven, Connecticut, United States*

Introduction: Both breast cancer and radiation therapy (RT) have been associated with an increased risk of bullous pemphigoid (BP). In the setting of RT, the majority of patients develop BP following completion of treatment, with a mean time to onset of 15.8 months1. Case Report: A 68-year-old Black female with a history of ductal carcinoma of the right breast status post partial mastectomy with sentinel lymph node biopsy and RT was referred to Oncodermatology over two years after completing oncologic treatment for painful erosive blisters that developed in the right inframammary fold with extension to the left breast. Physical exam showed clustered vesicles and erosions overlying annular pink patches isolated to the inframammary folds. In addition, beefy red gingival erythema was observed. Herpes simplex virus testing and superficial wound culture were performed, as well as two punch biopsies for routine staining and direct immunofluorescence testing. The differential diagnosis included delayed radiation-induced bullous pemphigoid, irritant or allergic contact dermatitis, and superficial infection such as bullous impetigo or herpes simplex virus. Treatment with triamcinolone 0.1% cream and mupirocin ointment was initiated. Biopsy findings included focal subepidermal clefting and immunofluorescence studies showed linear IgG and weak, patchy C3 at the dermoepidermal junction. BP180 was normal, BP230 was elevated to 39, and IgE was elevated to 278. The patient was diagnosed with atypical bullous pemphigoid isolated to the breasts, with RT as the most likely trigger given localization to irradiated breast and adjacent skin. The patient improved with topical corticosteroids and systemic treatment was therefore deferred. Discussion: Atypical presentations of localized BP triggered by RT may present years after completion of treatment and should be on the differential of inflammatory dermatoses localized to previously irradiated skin. References: 1Nguyen T, Kwan JM, Ahmed AR. Relationship between Radiation Therapy and Bullous Pemphigoid. J. Dermatol. 2014;229:88–96.

004

Calcitonin gene-related peptide (CGRP) acts on endothelial cells (ECs) at a site not in skin to favor th17-type immunity

Y. Kim, W. Ding, L. L. Stohl, R. D. Granstein
Weill Cornell Medicine, New York, New York, United States

The neuropeptide CGRP can influence the outcome of in vitro antigen presentation through actions on ECs acting as bystanders. To examine this pathway *in vivo*, we used an inducible, conditional knockout (KO) mouse with RAMP1 (part of the CGRP receptor) deleted on ECs. Upon skin immunization with dinitrofluorobenzene (DNFB), stimulated CD4+ T cells from draining lymph nodes (LNs) had significantly reduced IL-17A expression with significantly increased IFNγ and IL-22 expression at the protein and mRNA levels compared to control RAMP1 floxed, Cre- mice (Cre-). To determine the locus of relevant ECs targeted by CGRP, skin from wild-type (WT) mice was grafted onto KO mice and onto Cre- mice. Mice were immunized to DNFB 7 wk later on the grafts. Draining LNs were harvested 3 d later, CD4+ cells isolated and stimulated with anti-CD3 and anti-CD28 monoclonal Abs. Supernatants were harvested 48-hr later and cytokine content determined by ELISA. With cells from KO mice grafted with WT skin, there was a trend to reduced IL-17A production with increased IFNγ and IL-22 production compared to Cre- mice grafted with WT skin, with statistical significance only for IL-22. When KO skin was similarly grafted onto Cre- mice and compared to Cre- mice given Cre- skin grafts, no significant differences were seen. However, genetic analyses of grafts found both donor and host cells, perhaps explaining the uncertain results. So, a second type of experiment was performed. Mice were grafted, as above, with grafts painted with DNFB 1-hr before being put on recipients. With this procedure, vascular and lymphatic channels are not yet present, but host cell content of grafts is likely greatly reduced. When WT skin was grafted onto Cre- mice and KO mice, significantly less IL-17A, significantly more IL-22 and a trend toward more IFNγ production by LN CD4+ cells from KO mice was seen compared to cells from Cre- mice. These data suggest that relevant ECs are not in the skin. In this regard, it may be expected that antigen presentation is taking place in the regional lymph nodes.

005

Novel cutaneous squamous cell carcinoma cell lines constrained by T cell-mediated immune responses

A. C. Adams[1], E. S. Borden[1], A. M. Macy[1], K. H. Buetow[2, 3], M. A. Wilson[2, 3], D. J. Roe[4, 5], K. T. Hastings[1, 4]

[1]College of Medicine - Phoenix, University of Arizona, Phoenix, Arizona, United States, [2]Center for Evolution and Medicine, Arizona State University, Tempe, Arizona, United States, [3]School of Life Sciences, Arizona State University, Tempe, Arizona, United States, [4]Cancer Center, University of Arizona, Tucson, Arizona, United States, [5]Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, Arizona, United States

T cell-mediated immune responses can control *in vivo* cutaneous squamous cell carcinoma (cSCC) growth, but the determinants of anti-tumor T cell responses are not fully understood. To address this need, we developed a murine model of cSCC induced by the same etiologic factor as human disease which allows for evaluation of tumor-specific T cell responses. Solar simulated light induced cSCC tumors from BALB/c mice were used to create a panel of six clonal cSCC cell lines. Athymic mice, lacking mature T cells, and immunocompetent wild-type mice were injected with 1e6 cells of each cSCC cell line. Each cSCC cell line generated tumors in all athymic mice, demonstrating that, in the absence of T cells, the cSCC cell lines reliably form tumors. However, in wild-type mice all cSCC cell lines formed small tumors and then regressed, demonstrating that T cells constrain cSCC tumor growth. In four of the cSCC cell lines, injection of a higher cell number (5e6) resulted in immune evasion and tumor formation in a portion of mice, while no mice injected with the other two cSCC cell lines developed tumors. The cSCC cell lines express BALB/c-specific MHC class I alleles (H2Kd, H2Dd, H2Ld) in vitro. Whole exome and RNA sequencing was performed on the cSCC cell lines. In each cSCC cell line, there were >4000 missense mutations and >400 predicted MHC class I neoantigens, with 2-5 predicted to be immunogenic. Immunogenicity was predicted based on high affinity of the peptide:MHC class I interaction and high mRNA expression in the tumor cell. This cSCC model can be used to identify determinants of tumor-specific T cell responses.

006

IL-6 enhances IL-6 expression by epidermal langerhans cells (LCs)

W. Ding, L. L. Stohl, V. Isak, J. A. Wagner, Z. Bulmer, R. D. Granstein

Weill Cornell Medicine, New York, New York, United States

Exposure of endothelial cells (ECs) to the neuropeptide calcitonin gene-related peptide (CGRP) endows them with the ability, as bystanders, to bias the outcome of LC antigen (Ag) presentation to T cells toward the Th17 pole and our previous work indicates that EC IL-6 production mediates most of this effect. Preliminary experiments suggested that IL-6 could enhance IL-6 expression by LCs. We have further examined this effect as IL-6 released by CGRP-exposed ECs may stimulate LCs to produce additional IL-6, potentially involved in regulating the outcome of Ag presentation. To examine the impact of IL-6 on gene expression, purified BALB/c LCs were exposed to complete medium (CM) alone, 10 ng/ml of IL-6 or 100 ng/ml of IL-6 for 3-hrs or 6-hrs. Then, total RNA was extracted for analysis by RNAseq. IL-6 exposure induced significant increases in IL-6 mRNA: 4.63-fold for 6-hrs and 4.16-fold for 3-hrs with 100 ng/ml; 1.80-fold for 6-hrs and 2.15-fold for 3 hrs with 10 ng/ml. To determine if IL-6 exposure enhanced LC IL-6 protein expression, LCs were treated with 10 or 100 ng/ml of IL-6 or CM alone. After 3-hrs of culture, cells were washed 4 times and placed in CM containing 10 ng/ml GM-CSF (known to enhance LC viability) or CM alone. Supernatants were harvested 24-hrs later and IL-6 concentration assessed by ELISA. Exposure of LCs to GM-CSF or 10 ng/ml of IL-6 alone failed to increase IL-6 release into the medium. However, exposure to IL-6 at 100 ng/ml led to a significant increase in release of IL-6 [118.5+/-6.8 (SEM) pg/ml vs. 27.1+/-6.6 for CM]. IL-6 at 10 or 100 ng/ml combined with exposure to GM-CSF substantially and significantly increased IL-6 release further [173.7+/-12.9 pg/ml and 311.8+/-17.2 pg/ml, respectively, vs. 6.8+/-0.8 pg/ml for GM-CSF alone]. Thus, IL-6 released by ECs in Ag presenting cultures containing LCs may greatly enhance IL-6 release by LCs. This IL-6, combined with IL-6 released by ECs, may be trans-presented by LCs to responding T cells with consequent biasing of immunity toward the Th17 pole. T cell products, such as GM-CSF, may enhance this effect of IL-6.

007

Immunohistochemical expression of immune regulatory proteins in interface dermatoses

S. G. McAlpine, D. A. Culton, M. Duplisea, Z. Liu, P. Googe

University of North Carolina System, Chapel Hill, North Carolina, United States

Immunological therapies such as anti-programmed cell death protein 1 (PD-L1) are proving useful in the treatment of many cancers. As they inhibit self-tolerance, immunotherapies are associated with immune-related adverse events (irAEs) including rashes that histologically resemble a variety of lichenoid interface dermatoses. These cutaneous irAEs suggest PD-1/PD-L1 pathway or other immune checkpoint factors may be involved in the pathobiology of lichenoid interface dermatitis in naïve patients. We studied the staining pattern, level and prevalence of PD-L1, STING, IL-36, PD-1, CD8 and LAG-3 in skin biopsies from treatment naïve patients with five types of interface dermatitis, oral lichen planus (OLP) (n=10), cutaneous lichen planus (CLP) (n=10), chronic cutaneous lupus erythematosus (CLE) (n=11), erythema multiforme (EM) (n=11), toxic epidermal necrolysis (TEN) (n=13) and normal human skin (NHS) (n=5), by immunohistochemistry (IHC) analysis. Expression was evaluated semi-quantitively, according to the percentage of keratinocytes stained (0, 1-50, or >50%) and density of infiltrating lymphocytes (absent, non-brisk, or brisk). The expression of PD-L1, STING, IL-36, PD-1, CD8 and LAG-3 in lesional skin was normalized to NHS. All interface dermatitis had keratinocyte surface staining for PD-L1 and cytoplasmic staining for STING that was not present in NHS. The portion of lymphocytes that express LAG-3 was increased in all pathologies. Nuclear and cytoplasmic expression of IL-36 in the basal layer keratinocytes of OLP, CLP and CLE was more intense. The lymphocytic infiltration expressed CD8 and PD-1 more significantly in OLP, CLP and CLE than the resident pericapillary lymphocytes of NHS. CD8 lymphocyte population was increased in TEN but not in EM. The current thinking is that interface dermatitis is the result of a cell-mediated immune reaction that triggers cytotoxic CD8 T cell-mediated apoptosis of keratinocytes. The findings in this study suggest while cell-mediated immunity may contribute to the pathobiology, adaptive immune mechanisms are participating as well.

008

Smad7 dampened IL22 signaling-induced inflammation through IL22RA2 upregulation

Y. Ke[1], B. Li1, D. Wang[1, 2], S. Wang[1, 2], C. Young[1, 2], X. Wang[1, 2]

[1]University of Colorado - Anschutz Medical Campus, Aurora, Colorado, United States, [2]Allander Biotechnologies, LLC, Aurora, Colorado, United States

IL22 signaling plays a critical role in the pathogenesis of skin inflammatory diseases such as psoriasis and atopic dermatitis. Smad7 is a transforming growth factor β (TGFβ) inhibitor consisting of an N-terminal domain, a C-terminal domain, and a linker region harboring a PY motif. Smad7 transgene expression in Keratin 5 (K5)+ keratinocytes attenuated K5.TGFβ1 transgene-induced psoriatic inflammation. The downstream mechanistic players and functional domains of Smad7 that mediates its anti-inflammatory properties and whether the anti-inflammatory function of Smad7 applies to other skin conditions remain to be determined. Here, we showed that mice expressing K5.Smad7, but not the N-terminal domain of Smad7, caused resistance to imiquimod (IMQ)-induced dermatitis compared to their wildtype littermates. Further, we generated a truncated Smad7 protein with C-terminal Smad7 and PY motif (PYC-Smad7) fused with the cell-penetrating Tat peptide (Tat-PYC-Smad7). Topical application of Tat-PYC-Smad7 significantly attenuated IMQ-induced psoriatic inflammation, 2,4-dinitrofluorobenzene-induced atopic dermatitis, and tape-stripping-induced skin inflammation. RNA sequencing analysis and spatial phenotyping with multiplex immunofluorescence identified that Tat-PYC-Smad7 not only mitigated TGFβ and NFκβ signaling but alleviated inflammation-induced epidermal hyperproliferation, neutrophil, macrophage and T-cell infiltration, angiogenesis, and STAT3 activation. These anti-inflammatory effects of Tat-PYC-Smad7 were mediated by Tat-PYC-Smad7 upregulation of IL22RA2, a negative regulator of IL22/STAT3 signaling. Upregulation of IL22RA2 by Tat-PYC-Smad7 was dependent upon transcriptional factor C/EBP-β, which binds to IL22RA2 promotor in cells expressing PYC-Smad7. Thus, PYC-Smad7 contains the functional domain of Smad7 to dampen multiple IL-22-dependent pro-inflammatory signaling pathways and Smad7-based Tat protein is a potential therapeutic agent for treating skin inflammatory diseases.

009

Adiponectin-derived pentapeptide ameliorates psoriasiform skin inflammation by suppressing IL-17 production in γδT cells

J. Suh[1, 2, 3], Y. Lee[1, 2, 3], J. Ohn[1, 2], E. Kim[2], T. Kim[4], J. Chung[1, 2, 3]
[1]Dermatology, Seoul National University College of Medicine, Seoul, Korea (the Republic of), [2]Dermatology, Seoul National University Hospital, Jongno-gu, Seoul, Korea (the Republic of), [3]Seoul National University Graduate School Department of Biomedical Science, Seoul, Korea (the Republic of), [4]Dermatology, Yonsei University College of Medicine, Seodaemun-gu, Seoul, Korea (the Republic of)

Adiponectin, a hormone secreted from adipocytes, is closely associated with psoriasis and suppresses psoriasiform inflammation. Recently, a small-sized transdermally deliverable 5-mer peptide (GLYYF; P5) was discovered as a potential adiponectin receptor 1 agonist. This study aims at confirming the reduction of adiponectin protein level in the human psoriasis skin and investigating whether functional adiponectin replenishment by topical P5 application improves psoriasiform skin inflammation. We examined the adiponectin protein expression in the skin of individuals with psoriasis and normal skin. Next, we adopted imiquimod-induced psoriasis-like dermatitis mouse model in wild-type (WT) and adiponectin-deficient (Adipoq−/−) mice. As a result, adiponectin protein expression was downregulated both in the epidermis and dermis of psoriatic lesions as compared to that in the normal skin. Topically applied P5 attenuated the severity of imiquimod-induced psoriatic dermatitis in both WT and Adipoq−/− mice by decreasing the expression of psoriasis-related cytokines (Il17a, Il1b, Il6, and Tnfα). P5 application significantly reduced the proportion of interleukin-17A–producing γδT cells in flow cytometric analysis. In conclusion, transdermally deliverable adiponectin receptor 1 agonist, P5, can be a potential peptide drug to manage psoriasis by mediating the anti-psoriatic effect of adiponectin. This study proposes P5 as a topical peptide drug that replenishes the beneficial effects of adiponectin in a non-invasive and practically feasible manner to treat psoriasis.

010

Both IL-17RA and IL-17RC receptor complexes are required for IL-17A- and IL-17F-driven inflammation

A. Maroof, A. Manghera, S. Shaw
UCB Pharma, Slough, United Kingdom

Interleukin (IL)-17A is a key driver of inflammation and the principal target of anti-IL-17 therapeutic monoclonal antibodies. IL-17A and its structurally similar family member IL-17F have been shown to be functionally dysregulated in certain human immune-mediated inflammatory diseases such as psoriasis, psoriatic arthritis, and axial spondylarthritis. The IL-17 receptor C (IL-17RC) is thought to act in concert with IL-17RA to transduce IL-17A-, IL-17F-, and IL-17AF-mediated signals. A recent report resolving the crystal structure of the extracellular domain of human IL-17RC in complex with IL-17F has shown that IL-17RC forms a symmetrical 2:1 complex with IL-17F, suggesting the possibility of IL-17RA-independent IL-17 signaling pathways. To investigate whether IL-17F signals independently of IL-17RA to drive proinflammatory responses, we used CRISPR technology to knock-out IL-17RA and/or IL-17RC in synoviocytes or normal human dermal fibroblasts (NHDFs). NHDFs or synoviocytes stimulated with either IL-17A, IL-17F, or IL-17AF promoted expression of key proinflammatory mediators, such as IL-6, IL-8, and CXCL1. In contrast, in the absence of either IL-17RA or IL-17RC, complete abrogation of IL-17A- and IL-17F-mediated activation was observed. In contrast to recent publications, our data suggest that, in humans, both IL-17A and IL-17F require expression of IL-17RA and IL-17RC receptor complexes to mediate IL-17-driven inflammation, reinforcing the overlapping biology observed with these two IL-17 isoforms.

011

A new immunocompetent skin model recapitulates molecular features of psoriasis

C. Mainzer, M. Laclaverie, M. Mangier, S. Bordes, E. Aymard, B. Closs
R&D Department, SILAB, Brive, France

Psoriasis is a chronic inflammatory skin disease involving interactions between keratinocytes and both the innate and adaptive immune systems. The IL-17/IL-23 axis is described to drive the release of inflammatory and immune factors in keratinocytes (cytokines, antimicrobial peptides, chimiokines), which sit at the heart of an inflammation amplification loop. To this date, there are very few immunocompetent models recapitulating as close as possible psoriatic skin. In this context, the aim of this work was to develop a 3D model of psoriatic lesional skin summarizing biological features of the pathology. As the dialogue between Th17 lymphocytes (LTh17) and keratinocytes plays a central role in psoriasis pathogenesis, a model taking into account these two biological actors was developed. A reconstructed epidermis (RE) was co-cultured with differentiated LTh17. RE morphology as well as proliferation/differentiation imbalance and inflammatory immune response were explored. As it is reported in psoriatic lesions, results showed that IL-17 family cytokines were increased. Histological analysis demonstrated that the psoriatic mimic epidermis exhibited morphological changes. Moreover, mechanisms of proliferation and differentiation appeared profoundly altered while a high keratinocyte inflammatory response characteristic of psoriasis was observed. In conclusion, we have succeeded in designing a 3D in vitro model reproducing molecular alterations of psoriatic skin. The co-culture of the RE with differentiated LTh17 led to a typical inflammatory response in keratinocytes, thus proving the successful communication of the two biological compartments. This physiological relevant model is suitable for the assessment of natural therapeutic molecules dedicated to psoriasis treatment.

012

Tissue-specific manipulation of regulatory T cells reveals the skin to be a site of immune tolerance

J. Cohen[1], J. Moreau[2], V. Gouirand[2], C. Macon[2], I. Boothby[2], I. Gratz[3], A. Stoecklinger[3], C. Weaver[4], A. Sharpe[5], R. Ricardo-Gonzalez[2], M. Rosenblum[2]
[1]Dermatopathology, University of California San Francisco, San Francisco, California, United States, [2]Dermatology, University of California San Francisco, San Francisco, California, United States, [3]Universitat Salzburg Naturwissenschaftliche Fakultat, Salzburg, Salzburg, Austria, [4]The University of Alabama at Birmingham School of Medicine, Birmingham, Alabama, United States, [5]Harvard Medical School, Boston, Massachusetts, United States

It is commonly accepted that immune tolerance to self-antigens occurs in the thymus and secondary lymphoid organs. Despite the presence of rich immune microenvironments in non-lymphoid organs, such as skin and gut, it is currently unknown if self-tolerance is actively maintained in these tissues. We developed a novel technique to induce genetic recombination only in regulatory T cells (Tregs) that reside in skin. Cell lineage-tracing revealed that skin Tregs undergo significant attrition and replenishment during their natural life cycle. Deletion of these cells only in skin resulted in autoimmune attack of hair follicles, by both CD4+ effector T cells and CD8+ T cells. Suppressive capacity partially relied on high affinity interleukin-2 receptor (CD25) expression by skin Tregs, functioning exclusively in a cell extrinsic manner. Interestingly, CTLA-4 expression was completely dispensable for Treg function in steady-state skin. In a novel model of hair follicle stem cell-associated autoimmunity, we reveal that skin Tregs play a critical role in immunologically protecting this stem cell niche. Collectively, these results delineate the function of Tregs in a peripheral tissue relative to the systemic cellular pool and elucidate a mechanism of self-tolerance active exclusively in a non-lymphoid organ.

013

TRM create their own pro-survival niche in skin via IL-32

Y. Watanabe[1], A. Klosowicz[1], K. Yu[1], J. Moore[2], A. Gehad[1], J. Teague[1], N. Smith[3], A. Villani[3], S. Essien[1], Q. Zhan[1], R. A. Clark[1]
[1]Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States, [3]Massachusetts General Hospital, Boston, Massachusetts, United States

Skin resident memory T cells (TRM) appear to have the unique capacity to persist long-term in the absence of antigen while remaining poised to rapidly respond. The signaling pathways that support TRM survival in skin are unknown. We immunostained healthy human skin and found that TRM were located in close approximation to CD141+CD11c+ dendritic cells (cDC1) expressing TNF family costimulatory molecules including OX40L and CD40L. Imaging flow cytometry of collagenase digested skin confirmed that DC and TRM were biologically interacting with each other, as shown by actin localization to cell-cell contacts. Single cell RNAseq also identified cell clusters composed of TRM and cDC1 expressing multiple TNF family costimulatory ligand receptor pairs. T cells in clusters also expressed high levels of IL-32, a uniquely human cytokine known to induce APC maturation and activation. IL-32 added to human PBMC in vitro generated CD11c+ DC expressing OX40L that supported autologous T cell and B cell survival for over three weeks, suggesting that IL-32 may generate costimulatory molecule expressing cDC1 that form a niche to support T cell survival in skin. Neonatal human skin lacks TRM and we found markedly reduced IL-32 expression and a lack of TNF family costimulatory receptors on DC. In adult skin, IL-32 expression linearly correlated with T cell presence (CD3z) and CD40L and OX40L expression, suggesting that TRM may induce their own niche via IL-32. To test this hypothesis, we grafted neonatal skin to NSG mice, infused allogeneic T cells, and observed TRM generation over 6 weeks. IL-32 levels and DC expression of OX40L and CD40L increased as T cells migrated into skin and differentiated into TRM. In summary, our results suggest that T cells entering skin create their own pro-survival niche by producing IL-32 which induces cDC1 differentiation and upregulation of costimulatory molecules capable of supporting long term T cell survival.

014

Differential gene expression in lesional skin may signify immune-mediated lung parenchymal damage in dermatomyositis patients

K. Shaw, N. Doudican, N. Frazzette, A. Caplan, A. Femia, J. Carucci
Dermatology, NYU Langone Health, New York, New York, United States

Dermatomyositis (DM) is an autoimmune connective tissue disease that most commonly affects skin and muscles. Clinical manifestations can be protean, with some patients experiencing skin-limited disease while others develop serious systemic complications including interstitial lung disease (ILD). While both T cell-mediated and humoral immune dysregulation have been purported to play a role in manifesting said end-organ damage, our understanding of immunophenotypic prognostic factors associated with the development of ILD in DM patients remains limited. Thus, we sought to identify potential biomarkers that might be differentially expressed in lesional skin obtained from DM patients with versus without ILD. Utilizing NanoString technology, we assessed differential mRNA expression of 800 immune-related genes in formalin-fixed, paraffin-embedded lesional skin samples obtained from five DM patients with ILD compared to five DM patients without ILD. Among 42 highly regulated genes, 10 were significantly ($p<0.05$) upregulated in DM patients with ILD: CD44, NFKBIZ, CD24, EGR1, DEFB103B, CCL18, CCL22, CXCL2, S100A8 and S100A9. Notably, serum levels of S100A8/A9 heterodimer (an endogenous ligand of Toll-like receptor 4) are known to correlate with clinical severity in patients with DM-associated ILD, thereby supporting NanoString's utility in identifying salient genes associated with end-organ damage in DM. We also identified several novel genes downregulated in patients with DM-associated ILD that play a role in autophagy and adaptive immune activation: CLEC7, LEF1, KLRC2, BTLA, MUC1, and TCF7. Taken together, our results begin to characterize an immune-related gene expression signature in lesional DM skin that may be associated with comorbid ILD. While validation experiments are ongoing, the identification of biomarkers of systemic disease in lesional DM skin not only has the potential for risk stratifying DM patients at the time of tissue diagnosis but may provide novel targets for early intervention against DM-associated ILD.

015

Optimal methods for human skin T-cell analysis

T. Sato, Y. Ogawa, S. Shimada, T. Kawamura
Department of Dermatology, Yamanashi Daigaku Igakubu Daigakuin Sogo Kenkyubu Igakuiki, Chuo, Yamanashi, Japan

Background:Tissue-resident memory T (TRM) cells reside in peripheral tissues, including the skin. In humans, TRM cells exist in both the epidermis and dermis, but their composition, phenotype, and function are not fully elucidated. To analyze T cells in both the epidermis and dermis, the skin needs to be incubated with Dispase II to separate the two layers. The next step varies among researchers; the subsequent enzymatic digestion of epidermis and dermis is popular, whereas the spontaneous migration method can also be done. Objective: This study aimed to determine the strengths and limitations of the enzymatic digestion method and spontaneous migration method. Methods: Healthy, non-inflamed human skin was incubated with Dispase II to separate the epidermal and dermal sheets, which were subject to either subsequent enzymatic digestion or floated on a medium to obtain emigrants. Results: An enzyme combination of Dispase II and collagenases, but not collagenase alone, cleaved CD4, CD8, and CD69 on skin T cells. The spontaneous migration method lost ~20% of T cells in the floating sheets. However, there was no significant bias with regard to CD103 expression between emigrants and remaining T cells in the sheets. Through the spontaneous migration method, it was determined that there were 104 and 105 CD3+ T cells per 1 cm2 of epidermis and dermis, respectively. Conclusions: The spontaneous migration method might be useful for skin T-cell analysis.

016

Phenotypic effects of genetic loss of function in tyrosine kinase 2 (TYK2) using large-scale biobanks

N. Raghupathy, E. Kvikstad, E. Holzinger, I. M. Catlett, J. Maranville
Bristol Myers Squibb Co, Princeton, New Jersey, United States

We used a naturally occurring germ-line genetic variant in tyrosine kinase 2 (TYK2 [P1104A, rs34536443]), which has been shown to cause partial loss of function and protect against autoimmune disease, to survey the phenotypic consequences of reduced TYK2 function. We performed phenome-wide association studies (PheWAS) in 2 large biobanks (FinnGen and UK Biobank) and across public case-control genetic studies using Open Targets Genetics. This study found additional support for a protective effect (odds ratio [OR] <0.8, P<1x10-4) of TYK2 partial loss of function in multiple autoimmune diseases, including rheumatoid arthritis, psoriasis, psoriatic arthritis, systemic lupus erythematosus, sarcoidosis, type 1 diabetes, inflammatory bowel disease, and hypothyroidism. We did not observe any novel phenotypic associations that could highlight safety concerns for TYK2 inhibition. Additionally, we used well-powered and focused analyses to demonstrate that TYK2 partial loss of function is not associated with nonselective Janus kinase inhibitor safety concerns in any of the genetic studies. Our meta-analysis across the included studies showed no association with increased risk of cardiovascular disease (OR=0.97; P=0.02; 258,279 cases / 549,387 controls), venous thromboembolism (OR=0.97; P=0.52; 11,966 cases / 260,704 controls), and lymphoma (OR=1.06; P=0.47; 2687 cases / 220,721 controls). TYK2 P1104A, a partial loss-of-function polymorphism, enables assessment of TYK2 involvement in immune-mediated disease and other pathologies. Loss of function in TYK2 reduces risk of immune-mediated disease but does not significantly increase risk of cardiovascular disease, thromboembolism, or lymphoma.

017

Immunogenic catagen initiates alopecia areata

J. Kim[1], A. M. Christiano[1, 2]
[1]Department of Dermatology, Columbia University, New York, New York, United States, [2]Department of Genetics and Development, Columbia University, New York, New York, United States

In autoimmunity, emerging evidence suggests that intrinsic dysregulation in the target organ itself may contribute to the onset of disease. Alopecia areata (AA) is autoimmune disease in which cytotoxic CD8+ T cells attack anagen hair follicles (HFs). The mechanism of disease initiation is largely unknown, since patients develop AA after the preceding immunogenic events have already occurred. Using C3H/HeJ inbred mice, we discovered an immunogenic cascade that occurred during the catagen stage of hair cycle prior to the onset of spontaneous AA. In C3H/HeJ catagen, regressing HF epithelium underwent necroptosis, a highly immunogenic form of cell death, rather than the immune-silent apoptosis that occurs in normal C57BL/6 catagen. Necroptotic epithelial debris was taken up by classical dendritic cells (DCs), instead of the normal clearance mechanism of catagen debris via epithelial phagocytosis by neighboring HF epithelial cells. Classical DCs migrated to distal lymphoid tissues, including lymph nodes and spleen, and triggered a systemic immune response. Using K14-CreER; Rac1flox/flox mice, we found that genetically blocking the engulfment of catagen debris by neighboring HF epithelial cells was sufficient to induce necroptosis and initiate the immunogenic cascade. Introduction of an ectopic catagen accelerated the onset of spontaneous AA when skin grafts from C3H/HeJ mice (in catagen) were transferred onto C3H/HeJ recipients (in telogen), which was dependent on classical DCs. To determine the translational relevance of these findings, we systemically treated C3H/HeJ mice with the necroptosis inhibitor necrosulfonamide, which prevented disease onset and attenuated disease progression. Our findings established that the disease initiating mechanism in AA is immunogenic death within the HF epithelium during the catagen of hair cycle prior to the disease onset, and uncovered inhibition of necroptosis as a novel therapeutic axis in AA.

018

Dysregulation of natural killer cell activity in dermatomyositis

G. EL-Banna, D. F. Fiorentino, K. Y. Sarin
Dermatology, Stanford University School of Medicine, Stanford, California, United States

Natural killer (NK) cell number and cytotoxicity are decreased in the blood of patients with a wide range of auto-immune conditions. However, the role of NK cells in dermatomyositis (DM) has not been explored. To quantify NK activity, we utilized a signature score, summing the standardized z-scores of a previously validated NK-specific gene set: Nkp46, CD160, KLRC3, CD244, KLRF1, KLRC1, GNLY, PRF1, XCL2, and IL18RAP. Upregulation and downregulation of NK signature were defined as NK scores above 2sd and below -2sd, respectively. The NK score in the blood of DM patients (n= 201) was on average -6.44sd below the mean of healthy controls (p=0.0045, t-test) whereas the NK score in the skin of DM patients (n=181) was on average 40.24sd above the mean of healthy controls (p= 2.75E-18, t-test). The NK signature in blood was inversely associated with skin disease activity as measured by CDASI and age of disease onset (p= 0.02 and 0.002, respectively, multivariate linear regression). In addition, longitudinal blood samples from 73 DM patients showed a significant inverse correlation between temporal changes in CDASI and blood NK signature (p=0.038, linear regression).136 skin biopsies (n=181, 75.14%) had elevated NK signature, and lesional biopsies had on average twice as much NK signature expression as non-lesional biopsies (p= 4.37E-06, t-test). There was no significant correlation between NK signatures in blood and skin samples from the same patients. Elevated NK signature in skin was associated with increased dyskeratotic keratinocytes, basal vacuolization, and vascular dilatation on histopathology ($\chi 2$= 9.72, p= 0.021; $\chi 2$= 12.01, p=0.017; $\chi 2$=10.19, p=0.017, respectively). The NK signature in blood and skin tissue did not significantly differ among DM autoantibody groups. In summary, our study shows consistent downregulation of NK signature in the blood and upregulation of NK signature in the skin of DM patients. This NK signature is associated with skin disease activity and keratinocyte death, highlighting a potential role for NK cells in DM skin disease.

019

Investigating OX40L and its role in mediating cutaneous and systemic autoimmune disease

E. Xing, A. Billi, R. Wasikowski, O. Plazyo, M. Gharaee-Kermani, X. Xing, J. M. Kahlenberg, L. C. Tsoi, J. E. Gudjonsson
University of Michigan, Ann Arbor, Michigan, United States

Systemic lupus erythematosus (SLE) is a devastating systemic inflammatory disease, with prominent female bias. Recent literature suggests the OX40/OX40L costimulatory pathway may play an important role in development of SLE. GWAS have identified TNFSF4 (OX40L) as an SLE susceptibility locus. OX40L stimulation of T cells through the OX40 receptor has been shown to promote a T follicular helper (Tfh) phenotype, and Ox40l knockout has been demonstrated to ameliorate the SLE phenotype in transgenic and induced lupus mouse models. We recently reported a mouse model (K5-Vgll3) where epidermal overexpression of the female-biased transcription cofactor Vgll3 results in upregulation of Ox40l in epidermis as well as development of SLE. To address the role of OX40L in this model, we demonstrate that Ox40l KO results in decreased disease severity, consistent with this pathway being important for propagating VGLL3-mediated inflammation. To investigate the link between VGLL3 and OX40L expression in SLE skin, we created an N/TERT VGLL3-Myc-DDK overexpression cell system. IP-mass spectrometry of VGLL3 complexes identified transcription factor TEAD1, previously reported to bind upstream of OX40L, as a top VGLL3 interactor. Assessing OX40L and OX40 expression via immunofluorescence and single-cell RNA-seq (scRNA-seq), we found increased expression of OX40L on KCs and OX40 on CD4+ T cells in both K5-Vgll3 and SLE skin compared to controls. ScRNA-seq also showed that OX40+ T cells in K5-Vgll3 and lupus skin were skewed towards a Tfh/T peripheral helper (Tph) phenotype. Similarly, spatial-seq identified a population of Tfh/Tph-like population in SLE skin not present in healthy controls. Overall, our data indicates cutaneous VGLL3 controls transcriptional regulation of OX40L through TEAD1, and OX40L expression is required to mediate downstream SLE-like inflammation, possibly through promoting Tfh/Tph-like cells in SLE compared to healthy skin.

020

Topical ruxolitinib for the treatment of lupus-like skin disease in MRL/lpr mice

T. Curran[1], J. Yon[3], J. Anolik[2], C. T. Richardson[1, 2]
[1]Dermatology, University of Rochester Medical Center, Rochester, New York, United States, [2]Medicine, Allergy/Immunology and Rheumatology, University of Rochester Medical Center, Rochester, New York, United States, [3]University of Rochester, Rochester, New York, United States

Cutaneous lupus erythematosus (CLE) is a difficult to treat autoimmune skin disease in need of new therapies. Interferons (IFN) are key inflammatory cytokines in CLE pathogenesis and signal through the JAK-STAT pathway. Oral ruxolitinib, a Janus kinase inhibitor, has been shown to be effective in preventing skin disease in the MRL/lpr mouse model of lupus. Topical ruxolitinib has not been studied in the MRL/lpr model or in human CLE, but has recently been approved for atopic dermatitis. In this study, we sought to determine the efficacy of ruxolitinib 1.5% cream for the prevention of MRL/lpr skin disease. 4-week old MRL/lpr mice were exposed to ultraviolet B (UVB) radiation for 6 weeks to induce skin lesions. Ruxolitinib cream or vehicle cream was applied starting at the end of UVB exposure for 4 additional weeks. The primary endpoint of this study was the change in skin severity score at 14 weeks. At 14 weeks, fewer ruxolitinib-treated mice had developed skin disease, with a significantly decreased average skin severity score ($p < 0.05$). Quantitative RT-PCR analysis of treated skin demonstrated significantly decreased MX1, IFNG, CD8, and CD4 expression in ruxolitinib-treated mice as compared to controls, while no difference was observed in the spleen. Consistent with a lack of systemic effect, splenomegaly and lymphadenopathy (by weight) were not significantly different between groups. There was also no difference in anti-dsDNA antibody titers. In summary, these results suggest a local effect of ruxolitinib cream with the potential to prevent lupus-like skin disease in MRL/lpr mice.

021

CXCL13-producing peripheral T helper cells as potential mediators of photosensitivity in dermatomyositis

K. Afshari[1], N. Haddadi[1], Y. Wang[1], J. M. Richmond[1], R. Vleugels[2], M. Garber[1], M. Rashighi[1]
[1]*University of Massachusetts Chan Medical School, Worcester, Massachusetts, United States,* [2]*Harvard Medical School, Boston, Massachusetts, United States*

Dermatomyositis (DM) is a rare autoimmune disease that causes photosensitivity, disfiguring rash, and pruritus. Type I interferons (IFN-Is) are expressed in lesional (L) skin of DM, suggesting their role in disease pathogenesis. Using suction blistering on both L and non-lesional (NL) skin of treatment-naïve patients with DM followed by single-cell RNA sequencing (scRNA-seq) and flow cytometry, we found a distinct population of T cells expressing high levels of CXCL13, only in L DM. Their immunophenotype (CD4+/PD-1hi/CXCR5-) was characteristic of T peripheral helper (Tph) cells, a new subset of T cells recently described to drive autoimmunity in rheumatoid arthritis via CXCL13 mediated recruitment of B cells to synovium. However, consistent with previous reports, we did not find any B cells infiltrating L DM skin. Intriguingly, we observed high expression of CXCR5 on a subset of CD8+ T cells in both DM blood and L skin, suggesting CXCL13 promotes recruitment of cytotoxic CD8+ T cells to the inflamed skin. ELISA on the interstitial skin fluid confirmed higher levels of CXCL13 in L DM than NL and healthy ($p<0.001$), and immunohistochemistry highlighted CXCL13 staining colocalizing within perivascular CD4+ T cells. Using gene set enrichment analysis in our scRNA-seq data, we identified a robust IFN-I signature in Tph cells in DM skin. Further, we found that CD4+ T cells from DM blood displayed a significantly higher CXCL13 expression in response to IFNA than those from healthy subjects in vitro ($p<0.01$). Moreover, we observed that keratinocytes (KCs) isolated from L DM exhibit an enhanced IFNA expression in response to UVB than healthy in vitro, indicating an exaggerated IFN-I response to UVB in DM KCs induces differentiation of CXCL13-producing Tph cells in the skin, leading to the recruitment of circulating cytotoxic CD8+ T cells. These findings suggest a novel role for the IFN-I/CXCL13 axis in DM pathogenesis and its potential contribution to photosensitivity.

022

The role of ISG15-USP18 axis in oxidative stress-induced vitiligo

E. Lee[1], J. Kim[1], Y. Bae[1], S. Park[1], J. Lee[2], S. Oh[1]
[1]*Yonsei University College of Medicine, Seodaemun-gu, Seoul, Korea (the Republic of),* [2]*Yonsei University College of Pharmacy, Incheon, Incheon, Korea (the Republic of)*

Vitiligo is a relatively common, acquired depigmenting disease caused by the loss of epidermal melanocytes. Oxidative stress is known to participate in the initiation of vitiligo by triggering autoimmunity. In this study, we tried to identify factors serving as a bridge between oxidative stress and autoimmunity in vitiligo. According to RNA sequencing analysis on H2O2-treated melanocytes, interferon-stimulated gene 15 (ISG15) among interferon signaling-related genes were particularly upregulated. Vitiligo melanocytes showed significantly higher expression of ISG15 compared to normal melanocytes. Also, ubiquitin-specific peptidase 18 (USP18), which deconjugates ISG15 was significantly downregulated in vitiligo melanocytes. USP18 knockout melanocytes displayed senescence changes and upregulation of ISG15 similar to vitiligo melanocytes. Additionally, hypermethylation of the USP18 promoter region was observed in H2O2-treated melanocytes, vitiligo melanocytes, and skin tissues of vitiligo. Skin tissues and blood of vitiligo patients exhibited elevated expressions of ISG15. Finally, extracellular ISG15 was found to induce interferon-γ production from peripheral blood mononuclear cells via LFA-1 receptor. In conclusion, oxidative stress increases ISG15 expression in melanocytes through USP18 hypermethylation, which leads to interferon-γ production from immune cells, a main pathogenic cytokine in vitiligo. The ISG15-USP18 pathway might be important in oxidative stress-induced autoimmunity of vitiligo pathogenesis.

023

Lenabasum, a cannabinoid type 2 receptor agonist, exerts anti-inflammatory effects in dermatomyositis in Th1 cells

D. Diaz[1, 2], T. Vazquez[1, 2], N. Kodali[1, 2], M. Grinnell[1, 2], E. Keyes[1, 2], J. Dan[1, 2], G. Sprow[1, 2], Y. Li[1, 2], M. Bashir[1, 2], M. Sharma[1, 2], M. Momohara[1, 2], V. Werth[1, 2]
[1]*Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [2]*VA Medical Center, Philadelphia, Pennsylvania, United States*

Dermatomyositis (DM) is a chronic, systemic autoimmune disease affecting the skin, muscle, and lungs. Lenabasum, a non-psychoactive cannabinoid type 2 receptor (CB2R) agonist, is currently being investigated as a non-immunosuppressive treatment option for DM. The activation of CB2R has been shown to reduce several key pro-inflammatory cytokines implicated in DM. Our lab has previously demonstrated via immunohistochemistry (IHC) that Lenabasum decreases CD4, IFNβ, IFNγ and IL31 expression in DM skin at 12 weeks ($p<0.05$), with no differences in IL4 compared to placebo ($p>0.05$). In this study, we then utilized multiplexed flow cytometry of leukocytes eluted from DM skin to further analyze the expression of CB2R on 12 cell lineages. When evaluating cell lineages, CD4 T helper (Th) subsets were gated on CD4+ IFNγ+ Th1 and CD4+IL4+ Th2. There was a significantly higher frequency of parent (FOP) percentage ($p<0.05$) of CB2R in Th1 (61.05%, n=6) versus Th2 cells (25.5%, n=6). Among myeloid cell lineages, there was greater FOP of CB2R in M2 macrophages (CD68+CD163+), CD14++CD16++ macrophages, and monocyte derived dendritic cells (moDCs; CD11c+CD14+). With 15mmol of Lenabasum, there was a trend towards a decrease of: TNFα in M2 macrophages; IFNγ and IFNβ in CD4+ Tcells; IFNβ in CD16+ cells; and IL31 in Th1 cells, CD14++CD16+ macrophages, and M2 macrophages. There was also a significant decrease in moDCs secreting IL31 with Lenabasum ($p<0.01$). Imaging mass cytometry of untreated DM skin demonstrated the highest IL31 MPI in moDCs. IL31 was also elevated to a lesser extent in other myeloid cell lineages. These data suggest Lenabasum may exert specific anti-inflammatory effects in DM, particularly on Th1 cells and Th1-derived IL31.

024

Asymmetric cell division for fate induction of chimeric antigen receptor (CAR) T cells.

C. Ellebrecht, C. Lee, R. O'Connor, A. Payne
University of Pennsylvania, Philadelphia, Pennsylvania, United States

Early expansion and long-term persistence predict efficacy of genetically-engineered T cells. While this is thought to reflect induction of effector and memory T cell populations to provide both short-term clearance and long-lasting remission, the cellular mechanisms of fate induction after T cell activation through synthetic receptors are unknown. A better understanding of such processes could improve therapeutic outcome. Here we show that human T cells engineered to express chimeric antigen receptors (CARs) undergo asymmetric cell division (ACD) with distinct proximal and distal daughter cells that adopt effector and memory phenotypes, respectively. Using molecular proximity labeling to distinguish first division proximal and distal daughter cells, we demonstrate that target-engaged CAR molecules remain on the proximal first division daughter cell and establish cellular asymmetry between daughter cells in proliferative pace, cytolytic function and metabolic program. The single cell transcriptional program of proximal first division daughter cells is driven by c-myc, mTORC1 and JAK-STAT3 activation (each with adjusted $p<0.001$) resulting in predominance of glycolytic metabolism, features consistent with effector T cell differentiation. Conversely, distal daughter cells utilize BACH-2, ETS-2 and KLF2 (each with adjusted $p<0.001$) to shape their transcriptome and rely on oxidative phosphorylation, indicating a memory precursor phenotype. *In vivo* characterization of first division daughter cells in two xenograft leukemia mouse models to evaluate cytolytic function and memory induction confirms superior leukemia elimination (n=14, p=0.0004) and long-term persistence (n=12, p=0.0051) by distal daughter cells, establishing these cells as memory precursors responsible for long-term efficacy of human CAR T cells. Collectively, these studies uncover ACD as a novel framework for understanding mechanisms of CAR T cell differentiation and influencing therapeutic outcomes.

025

Transcriptional profiling of cutaneous immunotoxicity from melanoma immunotherapy

D. Y. Chang[1], H. Claussen[1], B. Pollack[1], H. Yeung[1], S. C. Chen[2], R. Feldman[1], E. Cole[1]

[1]Emory University, Atlanta, Georgia, United States, [2]Duke University, Durham, North Carolina, United States

Immunotherapy has drastically improved the survival prospect for advanced melanoma. Unfortunately, these treatments often come with cutaneous side effects which may cause the patient to discontinue or delay therapy. To understand the mechanism of cutaneous immunotoxicity in relation to clinical presentation, we characterized cutaneous symptom clusters by clinical and histologic morphology, severity rating, and transcriptional analyses in patients before, during, and after immunotherapy. As of 2020, 30 subjects were recruited, and 12 developed rashes (83% eczematous, 17% psoriasiform) who were treated only with topical steroids. Histologically, 75% demonstrated spongiotic dermatitis; 42% demonstrated a purely lymphocytic infiltrate while only 8% were primarily eosinophilic. Quality of life (QoL) was impacted by the rash, as demonstrated by a score increase on the Skindex, ItchyQoL and ItchyQuant. We next extracted RNA from FFPE punch biopsies to assess the transcriptional differences between baseline prior to therapy, and corresponding normal with rash tissues during immunotherapy. Transcriptional differences between normal tissues at baseline and during therapy, and between different histologic subtypes were not significant, as defined by 2-fold change and FDR<0.05. However, in the rash lesions compared to baseline or corresponding normal tissues, 3140 or 1287 genes, mainly immune-related such as T cell receptor signaling molecules, co-stimulatory receptors, cytotoxic effector molecules, and chemokine receptors, were upregulated, while 1522 or 934 genes corresponding mostly of tight junctional adhesion molecules and lipid syntheses were downregulated. GSEA revealed that rash samples reflected signatures similar to effector and memory CD8 T cells found after vaccination, PD-1 signaling, allograft rejection, and various cytokine signaling pathways. In conclusion, cutaneous immunotoxicity is mediated by pro-inflammatory processes and downregulation of tight junctions and lipid syntheses, which clinically manifests in impact on patient QoL.

026

Bioactivity of alpha-linolenic acid on T cell adhesion in a psoriatic skin model produced by tissue-engineering

S. Morin[1, 2], M. Simard[1, 2], G. Rioux[1, 2], R. Pouliot[1, 2]

[1]Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, Axe Médecine Régénératrice, Centre de recherche du CHU de Québec-Université Laval, Quebec, Quebec, Canada, [2]Faculté de Pharmacie, Université Laval, Quebec, Quebec, Canada

Psoriasis is a long-lasting skin disease identified by excessive proliferation of epidermal keratinocytes. Psoriasis triggers an inflammatory reaction, which involuntary activates the immune system, leading to leukocyte infiltration into the skin. However, the supplementation of the diet with diverse supplements, including n-3 polyunsaturated fatty acids (n-3 PUFAs), namely alpha-linolenic acid (ALA), is beneficial for psoriatic patients. However, the exact mechanisms of action of ALA on psoriatic immunity are still unknown. Therefore, this study was aimed to evaluate the bioaction of ALA on the lymphocyte component of psoriasis, using a psoriatic skin model enriched in T cells. To do so, healthy and psoriatic skin substitutes were produced according to the self-assembly technique, using unsupplemented culture media or culture media supplemented with 10 uM of ALA. T cells were isolated from whole blood of healthy donors. The supplementation of the medium with ALA regulated the hyperproliferation and the abnormal cell differentiation of psoriatic keratinocytes. Moreover, skin substitutes produced with T cells secreted high quantities of cytokines and chemokines, such as CXCL1 and IL-6, while the addition of exogenous ALA normalized the production of those cytokines. Finally, ALA slowed down the infiltration of T cells into the epidermis of the skin substitutes, remarkable by a diminished expression of CD45 and ICAM-1 in the epidermal compartment. Ultimately, our results show that in this model of psoriatic skin substitutes, ALA exerts its anti-inflammatory action by decreasing the production of biological mediators produced by T cells.

027

Profilin-1 prevents psoriasis pathogenesis through IκBζ regulation

B. Mok[1, 2], A. Kim[1, 2], S. Baek[2], J. Shin[2], D. Kim[2]

[1]Biomedical Science, CHA University - Bundang Campus, Seongnam, Gyeonggi-do, Korea (the Republic of), [2]Dermatology, CHA Bundang Medical Center, Seongnam, Gyeonggi-do, Korea (the Republic of)

Profilin-1 (PFN-1) is an actin-binding protein that regulates actin polymerization, cell proliferation, apoptosis, angiogenesis, and carcinogenesis. Its dysregulation has been reported in diverse pathologic diseases; however, the role of PFN-1 in psoriasis has not yet been elucidated. In this study, we demonstrate that PFN-1 expression is increased in both skin and serum of patients with psoriasis. PFN-1 was markedly expressed in the epidermis of psoriatic lesions and its expression positively correlated with psoriasis severity. The expression of PFN-1 in human keratinocytes was induced by IL-17A and TNF-α stimulation, but only in the presence of TNF-α stimulation, PFN-1 was secreted out of the cell. In addition, knockdown of PFN-1 with shRNA resulted in an altered expression of psoriasis-associated inflammatory markers, HBD-2, S100A7, S100A9, and Ki67, and recombinant PFN-1 suppressed the IL-17A-induced inflammatory response in keratinocytes. Interestingly, recombinant PFN-1 also suppressed IL-17A-induced IκBζ, an important player in immune response in psoriasis. Considering these facts, it is suggested that the balance of PFN-1 intracellularly or extracellularly is important for IκBζ regulation in the pathogenesis of psoriasis. Our results showed that PFN-1 acts as a negative regulator of psoriatic inflammation through the suppression of IκBζ. Furthermore, epidermal hyperplasia observed in IL-17A induced psoriasis-like reconstructed skin equivalents were reduced by PFN-1, which indicates that PFN-1 not only regulates the pathogenesis of psoriasis but also regulates aberrant epidermal development due to psoriatic inflammatory response. In conclusion, our findings demonstrate that PFN-1 can be used as a biomarker for disease severity, and allude that it can be considered as a possible target for suppressing and preventing the onset of psoriasis and alleviating symptoms.

028

Patient genetics shape the autoimmune response in pemphigus vulgaris

J. D. Baker, K. Seiffert, A. Sinha

Dermatology, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, Buffalo, New York, United States

Pemphigus Vulgaris (PV) is a rare autoimmune blistering disease with a multifactorial origin. Key Human Leukocyte Antigen (HLA) alleles, including DRB1*0402 and DQB1*0503, have been linked to the development of PV. However, it is not known how HLA types vary with ethnicity and how they shape autoimmunity in PV. We assessed correlations between factors including HLA genotype, ethnicity, auto-antibody levels, and lesion distribution in a cohort of 293 patients. Eighty-one percent of patients typed as either DRB1*0402 or DQB1*0503 with a high prevalence of DRB1*0402 in patients of Ashkenazi Jewish or Caucasian (non-Jewish) descent and DQB1*0503 in patients of Southeast Asian descent. Patients typing as HLA DRB1*0402 had higher levels of anti-desmoglein (Dsg)3 antibodies than patients without DRB1*0402 and had mucosal only lesions more often than cutaneous only or mucocutaneous lesions. Patients typing as DQB1*0503 had high levels of anti-Dsg1 antibodies compared to other groups and higher rates of mucocutaneous disease than other lesion types. We also report an unexpected HLA association with DRB1*0804 in PV patients of African descent, of which 64% carried the allele. DRB1*0804 was rarely seen in other ethnicities. DRB1*0804 positive patients presented with highly elevated levels of anti-Dsg3. However, neither African heritage nor DRB1*0804 correlated with a predilection to any specific lesion morphology. A group of patients that carried neither DRB1*0402, nor DQB1*0503 or DRB1*0804 had the lowest levels of anti-Dsg3 and the highest rate of solely cutaneous disease compared to carriers of these alleles. These data show the impact of genetic factors on the autoimmune response. It is important to note though that these correlations are not strict; any patient can present with any lesion morphology or antibody profile. Nevertheless, our study advances the goal of elucidating mechanisms that determine disease activity, knowledge necessary to identify individuals at risk, predict disease prognosis, and eventually achieve individually tailored medical therapies.

029

DRB1*0804 is associated with PV in patients of African descent

J. D. Baker, K. Seiffert, A. Sinha
Dermatology, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, Buffalo, New York, United States

Pemphigus Vulgaris (PV) has one of the strongest known HLA disease associations. The majority of PV patients studied carry either DRB1*0402 or DQB1*0503. However, studies on HLA associations in PV have largely focused on populations with specific ethnic backgrounds, often Ashkenazi Jewish (AJ) and non-Jewish Caucasians and there are limited studies on patients of African descent. We investigated the HLA status of 11 African American (AA) PV patients in comparison to 282 patients of non-African descent to identify HLA-associations and potential disease relevance in this understudied population. We find that 64% of our AA patients carry the HLA class II allele DRB1*0804. These patients had the highest levels of anti-desmoglein (Dsg) 3 antibodies compared with patients with other PV HLA alleles, but unlike other PV associated HLAs, no skewing towards mucosal only, muco-cutaneous or cutaneous only lesions. The DRB1*0804 allele is believed to be of African origin, and is most frequent in Northwestern Africa near modern Burkina Faso. This allele has previously been reported in association with PV in studies from Egypt and Brazil, however, a disease relevance has not been established. Given the similar disease phenotype we see in the DRB1*0804-carrying population (AA) and the main DRB1*0402 carrying population (AJ) we compared DRB1*0804 to DRB1*0402 at the DNA and protein level. For the DRB1*0402 allele, it has been shown that the P4 pocket plays a critical role in MHC-linked susceptibility to PV. Interestingly, 92.9% of DRB1*0804 residues overlap with those of DRB1*0402, and the crucial residues of the P4 pocket, DRB 70 and 71, are identical. For comparison, disease non-relevant alleles, such as DRB1*01:01, -10:01, and -07:01 had slightly lower protein similarities and less sequence homology in the residues surrounding the P4 pocket. Our study indicates that different HLA alleles in different ethnic populations may predispose PV patients to presentation and recognition of similar Dsg3-associated peptides leading to disease initiation and similar phenotypic expression.

030

Immune checkpoint inhibitor-induced bullous pemphigoid skin has elevated interleukin-4 and interleukin-13 expression and responds to IL-4R inhibition

W. D. Shipman[1], K. Singh[1], J. M. Cohen[1], J. S. Leventhal[1], W. Damsky[1, 2], M. M. Tomayko[1, 2]
[1]Department of Dermatology, Yale School of Medicine, New Haven, Connecticut, United States, [2]Department of Pathology, Yale School of Medicine, New Haven, Connecticut, United States

Immune check point inhibitors (ICIs) have revolutionized the treatment of advanced malignancies but can induce skin toxicities including ICI-induced bullous pemphigoid (BP). The pathogenesis of ICI-induced BP is unclear and selection of effective treatment options that do not inhibit the anti-tumor response is a challenge. If cytokine expression in ICI-induced BP and conventional BP skin is similar, emerging therapies for conventional BP may be effective for ICI-induced BP as well. RNA-in situ hybridization was utilized to examine expression of IL4Rα ligands, IL4 and IL13, in formalin-fixed paraffin-embedded skin biopsy specimens from patients with conventional BP, ICI-induced BP, atopic dermatitis and normal, control skin. IL4 and IL13 expression was similarly elevated and localized in conventional and ICI-induced BP when compared to healthy controls. IL4 expression was largely limited to the dermis, while IL13 was expressed in the dermoepidermal junction and within the dermis. In a retrospective case series of four patients with laboratory-confirmed ICI-induced BP, treatment with the IL-4Rα inhibitor, dupilumab, resulted in satisfactory responses in two patients and complete clearance in two others. Together, these findings indicate that conventional autoimmune BP and ICI-induced BP share key underlying pathomechanisms and suggest that blockade of IL-4 and/or IL-13 signaling may be an effective treatment for ICI-induced BP. In addition, IL4 and IL13 expression are potential biomarkers for ICI-induced BP development and patient response to IL-4Rα blockade.

031

Tape strips better capture epidermal barrier abnormalities of patients with atopic dermatitis compared to skin biopsies

A. D. Pagan[1], J. Cheng[1], E. Del Duca[1], A. B. Pavel[1, 2], J. G. Krueger[3], R. Bissonnette[4], E. Guttman-Yassky[1]
[1]Department of Dermatology and Laboratory of Inflammatory Skin Diseases, Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Department of Biomedical Engineering, University of Mississippi, University Park, Mississippi, United States, [3]Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York, United States, [4]Innovaderm Research, Montreal, Quebec, Canada

Our knowledge of atopic dermatitis/AD is largely derived from whole skin biopsies, which may cause scarring and infection. Tape strips are a minimally invasive technique to collect skin samples. A study directly differentiating the two approaches is unavailable. We thus compared the transcriptomic profile of lesional AD skin as captured by tape strips and skin biopsies taken from 20 moderate-to-severe AD patients and 20 healthy controls. We performed RNA-sequencing, using criteria of fold-change/FCH ≥2.0 and false discovery rate/FDR ≤0.05 to identify differentially expressed genes/DEGs. 4107 DEGs (2552 up; 1555 down) and 1001 DEGs (534 up; 467 down) were respectively detected in tape strips and biopsies. Both techniques were able to capture upregulation of key Th2- (IL13, IL4R, CCL17, CCR4, OX40/TNSFR4) and Th22-related (IL22) genes as well as genes linked with epidermal barrier abnormalities (FLG2, FA2H, CLDN8). Tape strips better or uniquely captured itch-related genes (IL31, TRPV2), as well as primarily-epidermal-based immune markers (FCER1A, CCL24, IL23A/IL23p19, CXCL2/3, STAT3), as well as genes related to lipid metabolism or epidermal differentiation (DGAT2, PSORS1C2). Biopsies better captured genes linked to hyperplasia (KRT16, COL6A5), and Th1 or Th17 (IFNg, IL12B, LCN2, DEFB4) pathways. Our results suggest that while key features of the AD signature are commonly depicted by both techniques, tape strips better capture epidermal barrier and lipid abnormalities, while biopsies better represent the hyperplasia and dermal dysregulation of AD.

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IL-36 axis is a sex-biased immune amplification circuit localized to the supraspinous epidermal compartment

M. K. Sarkar[1], F. Ma[2], A. Kidder[1], B. E. Perez White[3], R. Uppala[1], N. L. Ward[4], C. Dobry[1], A. Coon[1], L. C. Tsoi[1], J. M. Kahlenberg[2], J. E. Gudjonsson[1]
[1]Department of Dermatology, University of Michigan Michigan Medicine, Ann Arbor, Michigan, United States, [2]Division of Rheumatology, Department of Internal Medicine, University of Michigan Michigan Medicine, Ann Arbor, Michigan, United States, [3]Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [4]Department of Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States

Both psoriasis and pustular psoriasis are characterized by prominent involvement of IL-36 family of cytokines, which consists of three pro-inflammatory cytokines: IL-36A, IL-36B and IL-36G, and the IL-36 receptor antagonist (IL-36RA/IL36RN). IL-36 cytokines are significantly increased in psoriatic skin, and on average about 5-10-fold elevated in pustular forms of psoriasis. Using single-cell and spatial-seq approaches we have demonstrated that IL-36 responses are primarily localized to the supraspinous compartment of the epidermis, and strongly correlate with, and act downstream of, both IL-17A and TNF responses. Deletion of each of the IL-36 family members using CRISPR/Cas9 targeted KO in keratinocytes, demonstrate that both IL36G and IL36R KOs, but not IL36A KO, markedly suppress both IL-17A and TNF responses ($p<0.001$), compared to WT keratinocytes. Notably, the suppressive role of IL36G KO occurred in the absence of neutrophil proteases, which have been considered primary activators of the IL-36 axis in skin. Furthermore, bulk RNA-seq data from keratinocytes (n=47) showed marked sex bias in IL-36G response, with female keratinocytes having a more robust response to IL-36G ($p<0.001$). These data provide novel insights into IL-36 biology, demonstrate its role in amplifying IL-17 and TNF responses within the supraspinous compartment of the epidermis, and suggest that the sex-bias of IL-36 response underlie the marked female-bias of pustular forms of psoriasis.

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Bullous pemphigoid autoantibodies induce keratinocyte PAI-1 expression resulting in decreased plasmin activation

C. Cole, K. Amber, J. Li, L. Bao
Dermatology, Rush University Medical Center, Chicago, Illinois, United States

Bullous pemphigoid (BP) has been associated with an increase in venous thromboembolism due to impaired fibrinolysis. As plasma levels of plasminogen activator inhibitor type 1 (PAI-1) are elevated in BP patients, this represents one possible mechanism. We sought to better understand the role of BP autoantibodies in inducing a prothrombotic state. We first reviewed our previous RNA-seq database of BP-IgG treated keratinocytes relative to controls, confirming overexpression of SERPINE1 (Log2fold (1.01 P-adjusted < 0.0001). To confirm these results at the protein level, we next treated primary human keratinocytes overnight with IgG from BP patients and normal controls and performed a Luminex assay on supernatants revealing a 28-fold increase in supernatant PAI-1 levels in BP-IgG treated keratinocytes relative control-IgG (P < 0.0001). To confirm whether keratinocyte supernatants would carry a net anti-fibrinolytic effect, we performed a plasmin activation assay. In this assay, activation of plasminogen to plasmin results in cleavage of a fluorogenic substrate. Recombinant human plasminogen was treated with supernatants from BP-IgG or control-IgG treated keratinocytes, and fluorometric activity was measured at 30 minutes. Relative fluorescence units (RFU) were significantly increased in control-IgG versus BP-IgG (P = 0.02), indicating a relative inhibition of plasmin activation in BP-IgG treated keratinocytes. These findings reveal a direct mechanism by which BP autoantibodies induce a prothrombotic state through increased expression of PAI-1 in keratinocytes. Further research is needed to determine whether additional mechanisms of blister formation in BP contribute towards impaired fibrinolysis.

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Tissue specificity of dendritic cells supersedes subset identity

Q. Huang[1], A. S. Doane[2], O. Elemento[2], N. Anandasabapathy[1]
[1]*Department of Dermatology, Weill Cornell Medicine, New York, New York, United States,* [2]*Department of Physiology and Biophysics, Weill Cornell Medicine, New York, New York, United States*

To prevent acute life-threatening auto-inflammation/autoimmune of the tissues, our immune system must mount protective immunity, and rapidly resolve inflammation. Dendritic cells (DCs) are specialized immune sentinels that maintain immune homeostasis by efficiently regulating the balance between protective immunity and tolerance to self-antigens. It is critical to understand how tissue DCs subsets and their tissue specificity are governed to mediate protective or tolerant immunity. By assaying chromatin accessibility of LN resident (cDC1 vs cDC2) and migratory DCs (migDC1 vs migDC2) genome-wide through ATACseq analysis, together with our prior lab work using RNAseq analysis, we found that tissue myeloid maturation and migration programing supersedes subset diversification, and is enforced at the level of the chromatin. No major differences in chromatin accessibility were detected on DC subsets from CD11cCre-/IFNgR1flfl (WT) versus CD11cCre+/IFNgR1flfl (IFNgR1ΔDC) mice however, indicating IFNg/IFNgR1 signaling may function as a potential local, instead of global instructive cue. IFNgR1 promotor chromatin accessibility analysis detected major differences between cDCs vs migDCs, confirms a site-specific regulation of IFNgR1 signaling in DCs leading to differences in IFNgR1 level at the protein level. Further studies will explore how IFNg/IFNgR1 signaling regulates DCs in a site-specific manner and identify tissue-specific transcription factors (TFs) controlling IFNgR1 promoter accessibility in homeostasis.

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Blocking IL-7, but not TLR7, signaling prevents the development of lupus-like autoimmunity in mice

O. Plazyo[1], A. Billi[1], M. Gharaee-Kermani[2], J. M. Kahlenberg[1,2], J. E. Gudjonsson[1]
[1]*Dermatology, University of Michigan, Ann Arbor, Michigan, United States,* [2]*Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, United States*

Systemic lupus erythematosus (SLE) is a female-biased multisystem inflammatory disease with substantial morbidity and mortality and limited treatment options. Our lab previously found that VGLL3, a putative transcription co-factor with increased activity in female cells, can drive lupus-like systemic inflammation when overexpressed in mouse epidermis under the K5 promoter. IL-7 signaling was found to be elevated in the skin of lupus patients and K5-Vgll3+ mice alike. Our current results demonstrate that deleting peripheral Il7r in these mice prevents the development of disease phenotype, with amelioration of dermatitis, splenomegaly, lymphadenopathy, and kidney damage. Cell and molecular analyses indicate down-regulation of inflammatory signaling and dampened lymphocyte activation that accompany the ameliorated phenotype in the absence of Il7r. On the other hand, deleting Tlr7, an X-linked pro-inflammatory gene that has been hypothesized to promote lupus in women, not only fails to rescue the autoimmune phenotype in K5-Vgll3+ mice, but further exacerbates the severity of their disease. Therefore, VGLL3-driven systemic autoimmunity is dependent on IL-7 signaling and independent of TLR7. Further research is underway to determine whether neutralizing anti-IL-7 antibodies can halt or slow down the progression of end-organ damage in K5-Vgll3+ mice to better assess the therapeutic potential of targeting IL-7 signaling for treatment of SLE.

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Unique proteins appear at different times during the course of a delayed-type hypersensitivity reaction in human skin

J. Han[1], J. Correa Da Rosa[1], S. Owji[1], Y. Estrada[1], J. Ungar[1], J. G. Krueger[2], N. Gulati[1]
[1]*Dermatology, Icahn School of Medicine at Mount Sinai, New York, New York, United States,* [2]*Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York, United States*

Diphencyprone (DPCP) is a hapten that induces delayed-type hypersensitivity (DTH) reactions and is used therapeutically for various conditions including alopecia areata and melanoma metastases. Prior work has shown gene expression markers of major T cell subsets (Th1, Th2, and Th17) are significantly increased at 3 days after a single application of DPCP in healthy volunteers. We evaluated the cutaneous proteome of DPCP reactions in the skin of eight healthy volunteers at days 3, 14, and 120 after application. We assessed 96 proteins using the Olink immuno-oncology panel. Compared to placebo-treated sites, DPCP-challenged skin at 3 days (peak clinical inflammation) had 68 proteins significantly deregulated (P<0.05). At 14 days (during resolution of inflammation), 63 proteins were significantly deregulated (P<0.05). At 120 days (when inflammation had completely resolved), no proteins were significantly deregulated. 15 proteins were significantly deregulated at day 3 but not day 14, 10 at day 14 but not day 3, and 53 at both days 3 and 14 (P<0.05). Pathways that were significantly upregulated at both days 3 and 14, but progressively decreased with time, included: promoting tumor immunity, apoptosis/cell killing, inhibitory checkpoints, suppressing tumor immunity, chemotaxis, vascular tissue remodeling, Th1, Th2, and Th17 (P<0.05). IL12 was significantly upregulated only at day 14, thus potentially directing Th1 intensification over time with repeated DPCP application as is done therapeutically. As many but not all proteins were differentially expressed at the distinct time points studied, the proteins uniquely deregulated during the course of a DTH reaction may function to polarize T cell subsets or resolve skin inflammation. The marked proteomic changes at day 14 suggest that the duration of a human T cell recall response to a defined antigen may be more prolonged than suggested by many current model systems.

037

A systems immunology approach to classify melanoma tumor infiltrating lymphocytes (TILs) informs and models overall survival.

A. Jaiswal[1], A. Verma[1], R. Dannenfelser[2], M. Melssen[3], I. Tirosh[4], B. Izar[5], T. Kim[6], C. Nirschl[7], S. Devi[1], W. Olson[3], C. Slingluff[3], V. Engelhard[3], L. Garraway[8], A. Regev[9], C. Yoon[7], O. Troyanskaya[2], O. Elemento[1], M. Suarez-Farinas[10], N. Anandasabapathy[1]

[1]Weill Cornell Medicine, New York, New York, United States, [2]Princeton University, Princeton, New Jersey, United States, [3]University of Virginia, Charlottesville, Virginia, United States, [4]Weizmann Institute of Science, Rehovot, Israel, [5]Columbia University, New York, New York, United States, [6]Yonsei University, Seodaemun-gu, Seoul, Korea (the Republic of), [7]Harvard University, Cambridge, Massachusetts, United States, [8]Dana-Farber Cancer Institute, Boston, Massachusetts, United States, [9]Broad Institute, Cambridge, Massachusetts, United States, [10]Icahn School of Medicine at Mount Sinai, New York, New York, United States

Faithful modeling of human tumor infiltrating leukocytes (TILs) is necessary to advance therapeutic strategies and inform patient outcomes. However, TILs have traditionally been assigned to a state using a small number of markers, often redundant to several cell states, such as PD-1. We utilize robust classification methods to interrogate TILs status. First, we generated viral T cell activation, memory, resident memory, and exhaustion signatures and scored bulk and single cell TILs spanning a variety of tumor types including melanoma. We also queried single cell TILs clusters associated with response/nonresponse along multiple kinetic T cell differentiation trajectories in response to several infections, tumor exhaustion models, and human vaccines. In addition, we compared TILs programs to T cell programs from > 25 pre-clinical mouse models to see which model transcriptionally resembled TILs the most. Finally, we score metastatic melanoma tumors from patients both naïve to and receiving immune checkpoint blockade to test if high, medium, or low expression of T cell state signatures can predict survival outcomes. We believe these methods are less susceptible to bias and more accurately characterize the differentiation of TILs. Additionally, usage of these methods to characterize TILs associated with survival/improved response to immune checkpoint blockade may suggest novel combination therapies that are desirable adjuncts to immune checkpoint blockade.

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Single-cell sequencing of freshly isolated cells from lesional and peri-lesional skin to explore cellular origins of IL-17 isoforms in psoriasis

A. Skelton[1], K. Pappelbaum[2], X. Li2, V. Oji[3], A. Tsianakas[4], M. Page[1], M. Bertolini[2], S. Shaw[1], A. Maroof[1]

[1]UCB Pharma, Slough, United Kingdom, [2]Monasterium Laboratory, Münster, Germany, [3]Hautarztpraxis am Buddenturm, Munster, Germany, [4]Fachklinik Bad Bentheim, Bad Bentheim, Germany

Psoriasis is the most common chronic inflammatory skin disease in adults, characterized by immune cell infiltration, expansion of disease-associated lymphocytes, and epidermal hyperplasia. Increasing evidence highlights the central role of interleukin (IL)-17A and IL-17F in its pathobiology. IL-17A and IL-17F, known to be produced by activated T helper 17 cells, can form homodimers or heterodimers to drive aberrant keratinocyte biology. We applied single-cell RNA sequencing to characterize the immune infiltrate of psoriatic skin and further understand cellular sources of IL-17A and IL-17F. Biopsies of psoriatic lesions and peri-lesional skin were collected from six patients with moderate to severe plaque psoriasis. Transcriptomic profiles of 15,690 isolated CD45+ cells were determined from fresh tissue rapidly dissociated by mechanical and short enzymatic dissociation, and lymphocyte sub-clusters manually curated based on canonical cell-type markers. IL-17+ cells were markedly expanded in psoriatic lesions compared with peri-lesional samples, consistent with a central pathobiological role. Cells that separately express IL-17A or IL-17F exist in psoriatic lesions and were significantly more abundant than co-expressing cells. IL-17-producing cells were predominantly CD4+ and CD8+ T cells expressing skin-resident or -homing markers, but were not limited to these cell types. IL-17F+ cells were the most abundant subset and had similar transcriptional signatures to IL-17A+ and co-expressing cells, supporting IL-17F in addition to IL-17A as a key proinflammatory cytokine in plaque psoriasis. Our data suggest that, to fully ameliorate IL-17 signaling, both IL-17 isoforms must be neutralized, consistent with clinical findings from the phase 3b BE RADIANT study in plaque psoriasis patients, in which bimekizumab was superior to secukinumab.

039

Single-cell RNA-sequencing captures the cellular diversity within lesional and non-lesional skin of patients with dermatomyositis

G. Hile[3], F. Ma[3], A. Victory[3], B. Xu[3], E. A. Pedersen[3], R. Wasikowski[3, 2], C. C. Berthier[3], N. Nechiporchik[3], V. Ognenovski[3], E. Schiopu[3], A. Billi[3], J. E. Gudjonsson[3], J. M. Kahlenberg[3, 1]

[1]Internal Medicine, Division of Rheumatology, University of Michigan, Ann Arbor, Michigan, United States, [2]Computational Medicine & Bioinformatics, University of Michigan, Ann Arbor, Michigan, United States, [3]University of Michigan, Ann Arbor, Michigan, United States

Dermatomyositis (DM) is a devastating and poorly understood autoimmune disease characterized by prominent muscle weakness and relapsing refractory skin rashes. To investigate the cellular composition and comprehensive transcriptional effects of DM, we analyzed lesional and sun-protected, non-lesional skin from 8 patients with active cutaneous DM using scRNA-seq. Samples were analyzed in parallel with skin from 8 sex-matched healthy controls. Clustering and annotation revealed 12 major cell types, with prominent disease-associated shifts observed in both stromal and immune cell populations in DM skin. In particular, keratinocytes (KCs) from both lesional and nonlesional DM skin exhibited an exaggerated interferon (IFN) gene response that was most evident in spinous, lesional KCs. Top canonical pathways enriched in DM inflammatory KC related to oxidative phosphorylation mitochondrial function, indicative of cellular stress (p=10-40), response to type I interferon (p=10-15), and interleukin 1 signaling (p=10-13). Sub-clustering of immune cell subsets revealed major shifts in myeloid cells, including an increase in the proportion of Langerhans cells (LCs) in non-lesional DM skin relative to lesional and healthy control, and increased proportion of classical type 2 dendritic cells (cDC2A) and perivascular macrophages (PVM) in lesional DM skin compared to non-lesional and healthy control. Collectively, our data outline the cellular composition of dermatomyositis skin lesions at unprecedented resolution. We demonstrate a skewed transcriptional IFN-rich profile in both non-lesional and lesional DM skin and identify KC abnormalities that may contribute to its pathogenesis.

040

The C3H/HeJ mouse model of alopecia areata as a resource for training graduate and undergraduate students in clinical laboratory research techniques.

C. S. Potter

Biology, Central Connecticut State University, New Britain, Connecticut, United States

Alopecia areata (AA) is a cell-mediated autoimmune disease that targets actively growing hair follicles in both humans and mice, resulting in hair loss [1]. Alopecia Areata occurs naturally in about 20% of C3H/HeJ mice by 12 months of age [2]. Transplantation of skin or cells from the lymph nodes of affected animals into younger, histocompatible C3H/HeJ mice will reliably produce AA symptoms within 10-20 weeks of transplant [2, 3] providing both a useful tool for investigating the underlying mechanisms of AA and a preclinical model for drug testing. In addition, we have found this model to be a valuable tool for training undergraduate and graduate students with an interest in clinical research. Here we review the establishment of a C3H/HeJ colony at Central Connecticut State University as a resource for in house AA research, for use in small scale drug studies by outside entities, and as a mechanism for the instruction of students, in both individual and classroom settings, in areas including basic experimental design, laboratory animal management, necropsy, histology, immunohistochemistry, genetic analysis, etc. The Bureau of Labor Statistics projected a nationwide need for a 13% average increase in the number of medical laboratory technologists and technicians between 2016 and 2026, which is nearly double the underlying average increase in all occupations. Similarly, the US Department of Health and Human Services, Human Resources and Service Administration (HRSA), projects a substantial increase in demand/growth for medical and clinical laboratory technologists and technicians between 2012 and 2025 of 22%.4 [4] Given these statistics, the C3H/HeJ transplant model provides not only a useful research model but also a valuable tool in training students in technologies needed for careers in clinical laboratory sciences.

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Specific extracellular vesicles correlate with skin activity in dermatomyositis

M. Ogawa-Momohara[2, 1], Y. Li[2, 1], N. Kodali[2, 1], J. Dan[2, 1], T. Vazquez[2, 1], D. Diaz[2, 1], G. Sprow[1], V. Werth[2, 1]
[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Dermatology, Philadelphia VAMC, Philadelphia, Pennsylvania, United States

Background: Extracellular vesicles (EVs) are small, lipid-bilayer membrane structures that are actively released by normal, diseased, and transformed cells in vitro and *in vivo*. EVs carry nucleic acids, lipids, and proteins to mediate cell-cell communication. Many of the molecules carried by EVs act as damage-associated molecular patterns (DAMPs) that activate intrinsic immunity. EVs also can transport molecules from donor to recipient cells. We previously reported that proinflammatory EVs are elevated in the plasma of dermatomyositis (DM) patients and the EV particle number was correlated with Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI) scores. In the current study, we determined cells of origin for EVs relative to CDASI scores. Methods: We collected EVs from fresh plasma of 8 healthy controls (HC) and 15 DM patients using ultracentrifugation and size-exclusion chromatography. Signal intensities of 37 surface markers on EVs were measured by bead-based flow cytometry. Results: Tetraspanin markers of exosomes showed a positive correlation with the CDASI score [CD81 ($p<0.05$), CD63 ($p<0.05$), CD9 ($p<0.05$)]. Several cell-type specific markers on EVs also correlated with CDASI scores [CD69 ($p<0.01$), CD25 ($p<0.001$), CD31 ($p<0.0001$), CD40 ($p<0.01$), and MHC Class I ($p<0.0001$)]. Numbers of CD31-, CD40-, and CD25-positive EVs were highly increased in skin-active DM patients compared with HC ($p<0.05$, $p<0.0001$, $p<0.01$ respectively). Conclusions: EVs that carry specific biologically informative molecules are prevalent in the plasma of DM patients and correlate with DM skin activity.

042

Epidermal loss of RORα accelerates skin inflammation in a mouse model of atopic dermatitis

X. Hua, H. Dorsey, R. Hsung, J. Dai
School of Pharmacy, University of Wisconsin-Madison, Madison, Wisconsin, United States

One of the most common skin diseases is atopic dermatitis (AD), a chronic inflammation featuring skin barrier dysfunction and immune dysregulation. Previously, we reported that the nuclear orphan nuclear receptor RORα was highly expressed in the epidermis of human skin and positively regulated the expression of skin barrier-related genes, including filaggrin, in human keratinocytes. In contrast, RORα was downregulated in AD lesions. We then aimed to evaluate the *in vivo* function of RORα in regulating AD pathogenesis, using the newly generated mice with epidermis-specific Rora knockout (RoraΔepi) and an AD mouse model induced by MC903. We observed that the MC903-triggered ear thickening was greatly accelerated and enhanced in RoraΔepi mice compared to their wild-type littermates. On day 11, RoraΔepi mice displayed severe AD symptoms, including scaling, excoriation, and the histological features of epidermal hyperplasia and heavy dermal infiltrations (of eosinophils, neutrophils, and macrophages). In addition, we found the earlier AD onset in RoraΔepi mice to be associated with a marked reduction of keratin 10 and filaggrin expression in the epidermis and not a higher production of TSLP, the key cytokine for AD initiation in this model. These results substantiate the importance of epidermal RORα in suppressing AD development and that it functions at least in part through regulating keratinocyte differentiation and skin barrier function. Further mechanistic studies should help uncover the potential of RORα as a novel therapeutic target for AD and other diseases related to skin barrier dysfunction.

043

Deep immunoprofiling in pemphigus reveals significant shifts in dendritic-, natural killer- and T cell compartments at the single-cell level

E. Christie, I. Kozik, K. Seiffert, A. Sinha
Dermatology, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, Buffalo, New York, United States

Pemphigus Vulgaris (PV) is a rare, potentially life-threatening blistering disorder of the skin and mucous membranes characterized by IgG autoantibodies directed against specific components of epidermal adhesion structures. Although previous studies have attempted to address changes in immune cell phenotype and frequency in PV largely by flow cytometry, these studies have been limited by the number of parameters that can be simultaneously analyzed, thus restricting its utility. Consequently, the subpopulations of autoreactive and regulatory T and B lymphocytes, and other relevant immune cells that contribute to disease pathogenesis remain topics of intense investigation. In this study, we utilized mass cytometry by time of flight (CyTof), a powerful method with single-cell resolution, to interrogate the phenotype and frequency of a comprehensive range of immune cellular subsets assessed using 30 cellular markers simultaneously in the blood of 12 pemphigus patients and 15 healthy controls. Within the lymphocyte compartment, we observe a marked shift in the CD4/CD8 ratio with a significant increase of CD4 cells and decreased numbers of both CD8 and MAIT/NKT cells in PV patients vs. healthy controls. Across both CD4 and CD8 populations there is a shift from naïve to memory compartments. Among CD4+ cells there is an additional increase in Th2 and Th17 cells, accompanied by a significant decrease in regulatory T cells in PV. In NK cells we observe a shift from early to late cells, while there is no apparent change in overall B cell numbers or subtypes. However, as expected, treatment with the CD20 inhibitor rituximab strongly impacts B cell numbers and distribution. Finally, among dendritic cells, a redistribution from plasmacytoid to myeloid phenotypes is apparent. In summary, our study illuminates previously unattainable details regarding the composition, frequency and phenotype of circulating immune cell subsets in PV to advance our understanding of autoimmune mechanisms.

044

IL-2C treatment expands regulatory T cells *in vivo* and arrests development of murine alopecia areata

M. Lensing[1, 2], S. J. Connell[1, 2], P. Christy[2], A. Jabbari[1, 2, 3]
[1]Interdisciplinary Graduate Program in Immunology, The University of Iowa, Iowa City, Iowa, United States, [2]Department of Dermatology, The University of Iowa, Iowa City, Iowa, United States, [3]Iowa City VA Medical Center, Iowa City, Iowa, United States

Alopecia Areata (AA) is a common autoimmune disease characterized by the loss of immune privilege of the hair follicle. CD8+ T cells have been identified as the main effectors in AA, however, little is known about the role of regulatory T cells (Tregs) and why they fail to control disease. The imbalance of Tregs: effectors is thought to influence the development of many autoimmune diseases. Enrichment of the regulatory compartment has been gaining momentum in the field and currently there are many ongoing clinical trials involving Treg therapy as a treatment for autoimmune diseases. We were interested in exploring the effect of enhancing Treg numbers on the onset and development of AA in a murine C3H skin graft-induction model. *In vivo* treatment with recombinant IL-2 complexed to anti-IL-2 monoclonal antibody (IL-2C) resulted in selective expansion of CD4+ CD25+ FoxP3+ Tregs and enhanced expression of functional Treg markers compared to IgG control. Both continuous treatment and transient treatment with IL-2C resulted in the complete prevention of AA. Continuous IL-2C treatment resulted in an increased frequency of Tregs in the skin (2,652 vs. 594 Tregs/g skin), suggesting their protective role in hair follicle tolerance. Treg levels remained elevated in skin at 3 months after stopping treatment, indicating how IL-2C treatment may deliver long-lasting tolerogenic benefits. Ongoing studies may elucidate the potential for IL-2C to inhibit hair loss during active disease. Findings from these studies may provide translational insight into the use of *in vivo* Treg enrichment as a clinical therapeutic option for AA patients.

045

An EGFR ligand maintains scleroderma skin and lung fibrosis

I. Odell[1], H. Steach[4], S. Gauld[2], T. Carr[2], J. Wetter[2], L. Phillips[2], M. Hinchcliff[3], R. Flavell[4, 5]

[1]Dermatology, Yale University, New Haven, Connecticut, United States, [2]AbbVie Inc, North Chicago, Illinois, United States, [3]Internal Medicine, Yale School of Medicine, New Haven, Connecticut, United States, [4]Immunobiology, Yale School of Medicine, New Haven, Connecticut, United States, 5Howard Hughes Medical Institute, Chevy Chase, Maryland, United States

Systemic sclerosis (SSc/scleroderma) is an autoimmune disease that causes skin and internal organ fibrosis. Molecular signals that regulate the persistence of fibrosis are poorly understood. In SSc skin, Epidermal Growth Factor Receptor (EGFR) ligand expression correlates with skin fibrosis severity. However, EGFR inhibition has shown inconsistent results in fibrosis mouse models. We hypothesized that an immune-mesenchymal signaling circuit underlies SSc-related skin and lung fibrosis, and that targeting a specific EGFR ligand may prevent receptor activation on pathologic fibroblasts. Through scRNA-Seq of skin and lung tissues from patients with diffuse cutaneous SSc, we identified EGFR activation as a marker of pathogenic fibroblasts in both organs. Examination of ligand-receptor enrichment identified a unique dendritic cell-derived EGFR ligand as a driver of fibroblast EGFR activation. In mice, this ligand was essential for the persistence of skin and lung fibrosis, and its inhibition with a neutralizing antibody resulted in complete normalization of dermal skin thickness, 50% reduction in collagen content, and 190% reduction in Col1a1 expression (to below control levels). In the lungs, EGFR ligand inhibition led to a 2-point reduction in modified Ashcroft score and 38% reduction in collagen content. We further validated the antibody on lung explants from a patient with idiopathic pulmonary fibrosis, which reduced collagen secretion and expression by 56% and 70%, respectively. Mechanistically, this EGFR ligand signals downstream of type I interferon to drive a multicellular circuit, in which EGFR induces NOTCH activation and excess extracellular matrix production by fibroblasts. Since antibody patent is pending, target withheld until meeting. Our findings reveal an EGFR ligand as a crucial signal that maintains skin and lung fibrosis in SSc and other fibrotic diseases.

046

Construction of a detailed historical longitudinal personal medical journey identifies critical non-genetic determinants of disease in a patient with pemphigus vulgaris.

J. Baroukhian, K. Seiffert, A. Sinha

Dermatology, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, Buffalo, New York, United States

While predisposing genetic factors have been implicated across a variety of autoimmune diseases, the inherited risk they carry has failed to completely account for the development of disease in any given individual. In fact, the etiology of autoimmunity is complex and multifactorial, involving both genetic and non-genetic factors. The lack of full concordance as revealed by monozygotic twins studies across autoimmune conditions support the importance of non-genetic factors and the social determinants of health (SDH) in both the initiation and clinical course of disease. Pemphigus vulgaris (PV) has long been studied as a prototypical, humorally mediated, organ-specific, autoimmune disease. Presented here are findings from a complete life course history taken from a 54 year old White female who is a living embodiment of the interplay between genetic susceptibility (HLA DRB1*0402+) and a complex cast of psychological (adverse childhood events, psychosocial stress), physical (sustained exposure to agricultural and darkroom/photographic developing chemicals), autoimmune (Hashimoto's thyroiditis), and dietary factors that demonstrate not only the unique constellation of these well documented risk factors in a single patient who went on to develop PV, and the power of altering selected environmental factors, namely diet in this case, in both ameliorating disease severity and significantly improving patient quality of life. Our data highlight the essential role of key environmental factors in the development and course of PV, and the importance of longitudinal history taking and monitoring to uncover relevant determinants of autoimmune activity in individuals. The use of such longitudinal data collection methods to obtain real world data further the push towards personalized medicine by revealing unique risks underpinning disease development and, perhaps most importantly, provide potential avenues for prevention where environmental factors and SDH are implicated.

047

Pro-dysfunction and weak anti-cancer tumor infiltrating lymphocytes associated with more aggressive BCC subtype

N. Frazzette, N. Doudican, J. Carucci

Dermatology, NYU Langone Health, New York, New York, United States

Background: Immune response is key in defense against basal cell carcinoma (BCC). We aim to better define the immune tumor microenvironment in this cancer. Methods: CD8+ tumor infiltrating lymphocytes (TILs) obtained from fresh BCC tumor specimens from nodular subtype ("nBCC", n=6) versus infiltrative subtype ("iBCC", n=6) were subject to single-cell RNA profiling. Data were analyzed using iCellR. Results: CD8+ TILs were clustered based on gene expression as follows: cytotoxic (GZMA, GZMB, IFN-γ, PRF1); naïve (CCR7, LEF1, TCF7, IL7R, OX40); exhausted (BTLA, CTLA4, PDCD1, TIM3, LAG3, STAT3, 4-1BB); and regulatory (FOXP3, TIM3, OX40); additional subtypes were observed for naïve, exhausted, and regulatory T cells. A significantly greater percentage of cytotoxic TILs and lesser percentage of naïve TILs were observed in iBCC compared to nBCC. Further analysis revealed a unique, pro-tumor-controlling naïve subtype in nBCC and a unique, highly suppressive regulatory subtype in iBCC; moreover, regulatory TILs in nBCC exclusively clustered in a low suppressive activity subtype. Additionally, percentage of all exhausted TILs was not significantly different across tumor subtype; however, there was a significantly greater proportion of pro-tumor-controlling progenitor exhausted and terminally exhausted TILs in nBCC. Conclusion: Two significantly greater proportions of pro-tumor controlling exhausted subtypes and one significantly lower proportion of highly suppressive regulatory subtype were identified in nBCC compared to iBCC, possibly contributing to observed relative aggressiveness of these tumors. Additionally, more exhausted TILs from nBCC tended to cluster in a progenitor exhausted phenotype that may be more responsive to immune checkpoint blockade therapy.

048

CD8 TIL compartment in immunocompetent SCC associated with greater suppressive potential and smaller proportion of exhausted cells than in BCC or immunocompromised SCC

N. Frazzette, N. Doudican, J. Carucci

Dermatology, NYU Langone Health, New York, New York, United States

Background: The immune tumor microenvironment (TME) is key to controlling disease progression in skin cancer. We aim to compare the TME in these cancers to better inform prognosis and therapeutic decision making. Methods: CD8+ tumor infiltrating lymphocytes (TILs) obtained from fresh immunocompetent SCC (n=5) and BCC (n=12) tumors and solid-organ transplant recipient SCC ("TSCC", n=6) tumors were subject to single-cell RNA profiling. Data were analyzed using iCellR. Results: CD8+ TILs were clustered based on gene expression: cytotoxic (GZMA, GZMB, IFN-γ, PRF1); naïve (CCR7, LEF1, TCF7, IL7R, OX40); exhausted (BTLA, CTLA4, PDCD1, TIM3, LAG3, STAT3, 4-1BB); and regulatory (FOXP3, TIM3, OX40); additional subtypes were observed for naïve, exhausted, and regulatory T cells. Similar percentages of cytotoxic TILs were observed in SCC and BCC, significantly greater than in TSCC. Conversely, TSCC had the highest percentage of exhausted TILs. TSCC exhausted cells also tended to be more terminally exhausted, while those in SCC and BCC tended to be more progenitor exhausted. While the overall percentage of the regulatory CD8 compartment was similar across all three groups, SCC had a significantly greater proportion of a more suppressive subtype. Conclusion: Many significant differences in the CD8 TME were observed between SCC, TSCC, and BCC. TSCC was associated with a proportionally larger and extensively terminally exhausted CD8 compartment. BCC was also associated with a significantly greater exhausted compartment than SCC, possibly correlating to the relatively slow-growing clinical course of BCC. Additionally, SCC was associated with a significantly greater proportion of more suppressive regulatory CD8s, possibly correlating to the greater clinical aggressiveness of SCC.

049

Distinct transcriptomic profiles of sensory neurons in mouse model of atopic dermatitis and psoriasis: Insight into the mechanism of chronic itch in atopic dermatitis and psoriasis

S. Kim, I. Jeong, S. Lee
Dermatology, Yonsei University College of Medicine, Seodaemun-gu, Seoul, Korea (the Republic of)

Atopic dermatitis (AD) and psoriasis are common inflammatory skin diseases. Both diseases show distinct clinical features and are driven by different immune signatures, but share some immune and barrier abnormalities. Chronic itch is a central feature of AD, but it is also the common reported symptom in psoriasis patients. Itch signal is transduced by specialized sensory neurons. However, little is known about the neuronal changes in AD and psoriasis. To address this issue, we first profiled the transcriptional changes in sensory neurons in mouse model of AD and psoriasis. Trigeminal ganglion (TGs) obtained in the MC903 AD model and IMQ-induced psoriasis model (n=10 each), were subjected to RNA sequencing. Biomarkers were validated by qRT-PCR. Among the 1,982 differentially expressed genes by using a FCH of ≥2 and an adjusted P ≤ .05, 371 and 731 genes differentially expressed in AD and psoriasis mouse TGs versus TGs of their vehicle controls, respectively. TGs from AD and psoriasis model shared increases in levels of excitability of sensory neurons. The only TRP channels upregulated in MC903-treated mouse was TRPC5. While, TRPA1, TRPC1, and TRPM7 were upregulated only in IMQ-treated mouse TG. Among G-protein-coupled receptors, Mrgprb4 and P2Y were upregulated in both MC903-and IMQ-treated mouse TG, but atopic TGs showed the greater increase in serotonin receptors and glutamate ionotropic receptors. Atopic TGs showed increased tachykinin 2 and vasoactive intestinal polypeptide genes, while psoriatic TGs showed high levels of CGRP genes. TGs from both model showed increased IL1R2, but atopic TGs showed upregulated IL31RA, OSMR, and eosinophil activation marker, eosinophil peroxidase, contrary, psoriatic TGs showed neutrophil chemotaxis and activation marker. Only psoriatic TGs showed increased expression of TLRs and resistin gene. This transcriptomic analysis will lead to an increased understanding of the mechanisms of chronic pruritus and neuroinflammation in AD and psoriasis.

050

Eotaxin-1 and matrix metalloproteinase-9 are critical in anti-BP180 IgE-induced experimental bullous pemphigoid

T. Jordan, J. Chen, N. Li, S. Burette, D. A. Culton, S. Geng, P. Googe, N. Thomas, L. Diaz, Z. Liu
University of North Carolina, Chapel Hill, North Carolina, United States

Bullous pemphigoid (BP) is the most common autoimmune blistering disease characterized by chronic subepidermal blistering, autoantibodies directed against hemidesmosomal components, and a predominant eosinophil infiltrate. BP autoantibodies recognize two hemidesmosomal proteins of basal keratinocytes: BP230 (also termed BPAG1) and BP180 (also termed BPAG2 or collagen XVII) with the NC16A domain of the human BP180 antigen containing the major autoantibody-reactive epitopes. Anti-BP180 autoantibodies belong to IgG and IgE isotypes. We have previously demonstrated the pathogenicity of anti-NC16A IgG and IgE autoantibodies from BP patients using antibody passive transfer animal models. We have shown that anti-NC16A IgE autoantibodies from BP patients recruit eosinophils and induce eosinophil-dependent BP in double humanized NC16A and human IgE receptor (termed NC16A/hFcεRI) mice. In this study, we find that pathogenic anti-NC16A IgE significantly increases levels of key eosinophil chemoattractants, eotaxin 1 and eotaxin 2, and matrix metalloproteinase 9 (MMP-9) in the lesional skin of diseased mice. Treatment with neutralizing antibody against murine eotaxin 1, but not murine eotaxin 2, significantly reduces the pathogenic activity of anti-NC16A IgE. Blockade of the main eotaxin receptor, CCR3, using a small molecule inhibitor also drastically reduces anti-NC16A IgE-induced BP disease severity. Furthermore, NC16A/hFcεRI mice lacking MMP-9 are resistant to anti-NC16A IgE-induced BP. Finally, we find significantly higher levels of eotaxins in blister fluid and sera of BP patients compared to normal controls. These findings identify eotaxin 1 and MMP-9 as key players in experimental BP and potential new therapeutic targets for this disease.

051

Exogenous IL-27 prevents disease induction in murine alopecia areata

P. Christy[1], S. Crotts[1], S. J. Connell[1,2], M. Lensing[1,2], L. Ortolan[1], N. Henderson[1], X. Bai[4], A. Jabbari[1,2,3]
[1]Department of Dermatology, University of Iowa, Iowa City, Iowa, United States, [2]Interdisciplinary Graduate Program in Immunology, University of Iowa, Iowa City, Iowa, United States, [3]Iowa City VA Medical Center, Iowa City, Iowa, United States, [4]Department of Pathology, College of Medicine and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, United States

Alopecia areata (AA) is an autoimmune disease characterized by T cell infiltration of the hair follicle. The breakdown of tolerance to the hair follicle is thought to be the driving force in the nonscarring hair loss seen in patients. There are currently no FDA approved treatments for AA. IL-27 plays a pleotropic role across many other autoimmune diseases; however, whether its immunoregulatory effects can be leveraged for therapeutic applications in AA is unknown. To address this, we used an adeno-associated virus (AAV) that drives overexpression of IL-27 in our C3H/HeJ skin graft-induction model of AA. Briefly, AAV-IL-27 administration was followed by disease induction using skin grafts from an AA-affected donor. Mice were observed weekly for disease progression. We found that exogenous IL-27 protected mice from disease development. Additionally, we saw an increased presence of FoxP3+ CD4 T cells and IL-10-producing CD8 T cells in the skin draining lymph nodes of mice treated with AAV-IL-27, suggesting roles for these immunoregulatory populations in the prevention of autoimmunity. Interestingly, NKG2D-expressing CD8 T cells, previously associated to correlate with the presence of disease, were present in mice treated with AAV-IL-27, raising the possibility that IL-27 may be useful in late stages of disease. Our findings support further studies examining use of IL-27 in the treatment of AA patients.

052

Localized increased in chemokines associated with itch in hidradenitis suppurativa

V. Harbour, J. Kilgour, G. Swaminathan, K. Yekrang, M. Aleshin, K. Y. Sarin
Dermatology, Stanford Medicine, Stanford, California, United States

Hidradenitis Suppurativa (HS) is an autoinflammatory skin disease characterized by painful, pruritic nodules, abscesses and pus-draining tunnels in intertriginous skin. To better elucidate the immune response leading to localized activity in HS skin, we compared cytokines from 15 lesional and perilesional skin biopsies from patients with Hurley Stage II and III HS. Patients additionally filled out a qualitative questionnaire assessing a range of symptoms and environmental factors including pain, itch, sweat levels and diet at the time of biopsy. Cytokine levels were assessed using a bead-based, multi-analyte immunoassay system. The expression of IP-10 (CXCL-10), IL-8, MIP-alpha (CCL-3), MIP-beta (CCL-4), GRO-alpha (CXCL-1), 1L-1ß and eotaxin-1 (CCL-11) were all found to be significantly enhanced in lesional skin compared to clinically normal perilesional skin. Eotaxin-1 displayed 1.8 fold elevation as compared with normal skin (p=0.0234 by paired t-test). Eotaxin-1 is an eosinophil chemoattractant, also found in atopic dermatitis, that plays a critical role in tissue inflammation and may be linked to the pathogenesis of highly pruritic HS flares. Overall, our results indicate that inflammatory markers in HS lesions are those produced by keratinocytes (1L-1ß, IL-8, CCL-3), linked to T-cell or B-cell activation in inflammatory sites (CXCL-10 and CCL-4, respectively) and that regulate neutrophil chemoattraction (CXCL-1) which align with previously reported immune marker expression and known pathogenesis in HS lesions. We are further stratifying these results by patient-reported symptoms, treatment, and environmental factors to develop greater insight into treatment strategies and pathogenic processes underlying HS.

053

TNF-α blockage abolishes M1 macrophage polarization with WNT5A in treating patients with psoriasis vulgaris

S. Lin, C. Lee
Dermatology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan (TWN), Taiwan

Psoriasis vulgaris is a chronic cutaneous inflammatory disease. Our previous study showed that enhanced M1 macrophage infiltration in the psoriatic skin was obliterated by successful clinical treatment. WNT signaling was known to regulate macrophage activation and polarization. We aimed to investigate how WNT signal pathways regulate macrophage polarization in psoriasis, and if so, TNF-α blockage would subvert aberrant macrophage polarization via specific WNT signals. We isolated circulating CD14+ monocytes from 6 patients with psoriasis (average age 48.2 years, M/F=3/3) and make them differentiate to M1 and M2 macrophage by TNF-α/IFN-γ and IL-4, respectively. The transcriptional levels of 19 WNT ligands in monocytes, M1 and M2 macrophage were profiled by PCR. The chemokine and cytokine levels in the supernatants from these cells were measured by multiplex ELISA. The numbers of specific WNT-expressing CD68+ macrophages in psoriatic lesions before and 28 weeks after successful treatment was measured by immunohistochemistry. The results showed a selective and significant WNT5A overexpression in M1 macrophages than that in monocytes or M2 macrophage (3.89 ±0.83, 1.02 ±0.04, and 1.67 ±0.43, respectively, $p<0.05$). The number of CD68+WNT5A+ macrophage was increased in active psoriatic lesions but was reduced by 85% after anti-TNF-α treatment. Among the cytokines and chemokines, CXCL10 level was increased from M1 macrophage than that from CD14+ monocyte (6231± 1236 and 525± 126 pg/ml, respectively, $p<0.01$). We concluded that TNF-α blockage abolishes M1 macrophage polarization, likely through WNT5A, in treating patients with psoriasis vulgaris.

054

IL-18 regulates nerve growth factor and semaphorin 3A in dermatomyositis-related pruritus

L. Wong[1], C. Lee[1], Y. Yen[2, 3]
[1]Dermatology, Chang Gung Memorial Hospital Kaohsiung Branch, Kaohsiung, Taiwan, [2]Fooyin University Hospital, Donggang, Taiwan, [3]Biomedical Sciences, National Sun Yat-sen University, Kaohsiung, Taiwan

Pruritus is common in autoimmune diseases and a prominent symptom especially in patients with dermatomyositis (DM). In contrast, the severity of pruritus in cutaneous lupus erythematosus (CLE) is less severe. Importantly, pruritus is an important symptom to differentiate DM from CLE. It has been found that keratinocytes-derived interleukin (IL)-18 played a pathophysiologic role in DM and IL-18 potentially distinguishes DM from CLE. The present study aimed to investigate the association of IENFD in DM-related pruritus and the possible mechanism. We found IENFD was unchanged in patients with DM compared with healthy control. Increase epidermal expression of nerve growth factor (NGF) and reduction of epidermal expression of semaphorin 3A (Sema3A) in patients with DM. Furthermore, IL-18 induced the production of NGF and inhibited the expression of Sema3A in keratinocytes. Taken together, IL-18 may contribute to the different itch intensity between patients with DM and CLE by influencing the expression of NGF and Sema3A in the skin.

055

CD8 T cell immunity after respiratory viral infection is transient, while CD8 T cell immunity after epidermal vaccination is lung focused and durable

T. Tian, Y. Pan, T. Pan, J. B. Williams, E. Rotrosen, Y. Yan, T. Kupper
Brigham and Women's Hospital, Boston, Massachusetts, United States

Pandemic respiratory viral pathogens like Influenza A and SARS COV2 exhibit continuous and evasive mutations in cell surface molecules, making vaccination with the goal of antibody-mediated protection elusive. CD8 T cells mediate eradication of viral disease, and vaccination to conserved internal viral proteins to elicit CD8 T cell memory is a promising strategy. Using a mouse model, we compared pulmonary infection with H1N1 influenza with skin (epidermal) vaccination using Modified Vaccinia Ankara (MVA) expressing highly conserved NP or another conserved Ags. H1N1 influenza pulmonary infection led to recruitment and lung infiltration with Ag specific CD8 T cells by day 5-10. By day 40, abundant CD8 lung TRM and LN TCM were present. Surprisingly, by day 80, both lung TRM and systemic TCM cells were greatly diminished and were absent at day 120. These mice were protected against lethal challenge at day 40 but not day 80, suggesting built-in obsolescence of CD8 memory. In contrast, epidermal vaccination led to CD8 T cell infiltration of lung at day 5-10, measurable at day 40 and still detectable at day 80 in lung, LN and spleen. In addition, a novel intravascular lung population of CD8 T cells was present at all time points. These mice were completely protected against lethal flu challenge at day 80 and 120. Protection was observed after pulmonary challenge with either H1N1 or H3N2 influenza as well as in B cell depleted mice. We analyzed protective immunity in skin vaccinated mice. At 2 hours after pulmonary challenge, Ag specific CD8 T cells moved from the intravascular space into the lung parenchyma, were abundant at day 3 and persisted for >80 days. Single cell RNA sequencing indicated that these intravascular T cells were transcriptionally distinct from systemic TEM and TCM. We conclude that CD8 T cell immunity after pulmonary infection is powerful but short-lived, while skin vaccine induced CD8 T cell protective immunity is mediated by lung intravascular T cells is protective and durable.

056

IL-15 prolongs hair growth and operates as a guardian of human hair follicle immune privilege

T. Suzuki[1], D. Demetrius[1], A. Rajabi-Estarabadi[1], F. D. Scala[1], J. Gherardini[1, 2], T. Purba[3], J. Rodriguez-Feliz[4], G. Epstein-Kuka[5], C. Nicu[1], M. Harries[3], J. Chéret[1], R. Paus[1, 3, 2]
[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States, [2]Monatserium Laboratory, Münster, Germany, [3]Centre for Dermatology Research, The University of Manchester, Manchester, Manchester, United Kingdom, [4]Skin & Hair, Plastic Surgery Clinic, Coral Gables, Florida, United States, [5]Foundation for Hair Restoration, Miami, Florida, United States

Effective alopecia areata (AA) therapy requires targeting of the underlying key pathomechanisms, namely hair follicle (HF) immune privilege (IP) collapse and premature catagen. Based on mouse data, the multi-functional, anti-apoptotic, autoimmunity-promoting cytokine, interleukin-15 (IL-15), is often postulated to be a key pathogenic cytokine in human AA, even though it is unclear how IL-15 affects HF-IP and catagen. Here, we show that lesional skin of AA patients shows an increased number of perifollicular IL-15+ T and NK cells. Yet, protein expression of both, the private IL-15 receptor (IL-15Ra, which is not stimulated by IL-2), and of the common g chain is significantly decreased in lesional AA HFs. Treating organ-cultured, healthy human anagen scalp HFs with rhIL-15 significantly stimulates hair matrix keratinocyte proliferation and inhibits catagen development *ex vivo*. Most importantly, when rhIL-15 is administered before or after HF IP collapse induction by IFNg, IL-15 provides relative protection from HF IP collapse and can even partially restore HF IP (as assessed by MHC class Ia and a-MSH immunohistomorphometry, $p<0.05$). Instead, IL-15Ra silencing *ex vivo* accelerates catagen development and inhibits hair matrix proliferation. Thus, IL-15 likely exerts highly ambivalent functions in human HF biology and AA: while IL-15 may promote AA-associated T and NK cell infiltration, IL-15Ra-mediated signaling operates as a critical HF IP guardian and hair growth-promoting cytokine. The latter novel IL-15 functions revealed here are blocked by JAK1/3 inhibitors; this may explain why AA relapses rapidly after therapy discontinuation.

057

Targeting keratinocytes to potentiate skin immunization

J. You[1,2], X. Hao[1], L. Falo III[1], R. Hao[1], J. Zhang[1], C. Carey[1], Z. You[1], L. Falo Jr.[1]
[1]*Dermatology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States,* [2]*University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States*

Skin is a unique target for vaccination. Targeting keratinocytes to produce proinflammatory mediators to improve immunity is rational approach for vaccine design. Keratinocytes genetically engineered to overexpress the stress response related transcription factor x-box binding protein 1 (XBP1) increased production of proinflammatory mediators and co-delivered antigen. Keratinocyte-specific overexpression of XBP1 through cutaneous genetic immunization was transient and induced a pro-immunogenic skin microenvironment, including enhanced expression of proinflammatory mediators and co-delivered antigen, and increased skin-infiltration and stimulatory function of dendritic cells. Overexpression of XBP1 in keratinocytes drove non-viral DNA vaccines to enhance induction of antigen-specific humoral and cellular immune responses, including durable antigen-specific skin-resident memory CD8 T cells and effective protective immunity in mouse tumor models. These findings support the strategy of targeting keratinocytes to improve skin immunization, identify XBP1 as a potential molecular 'adjuvant' in for skin immunization, and contribute to the development of efficacious skin-targeted vaccines against cancers and infection diseases.

058

Tissue-resident memory T cells in tumor immunity

J. B. Williams, T. Kupper
Brigham and Women's Hospital, Boston, Massachusetts, United States

Tissue-resident memory T (TRM) cells have emerged as key players and potential immunotherapeutic targets in antitumor immunity. However, the study of TRM cells within the TME has been hampered by a lack of adequate tumor models. Cell lines injected subcutaneously do not contain the same biological cues as tumors arising from the dermis and epidermis, while most other models lack a known tumor-specific antigen to track immune responses. We have addressed these two issues by modifying the Braf/PTEN model of melanoma to express OVA as a tumor antigen (abbreviated as BPO). In this novel model, we can detect a robust endogenous tumor-antigen specific immune response against OVA as measured by IFN-γ ELISPOT and pentamer staining. In addition, after adoptive transfer into tumor bearing hosts, OT-I cells expanded in the TdLN and infiltrated into the tumor. To investigate TRM properties we first looked for expression of CD103 and CD69. We found significantly more CD8 TILs co-expressing CD103 and CD69 in autochthonous BPO tumors compared to subcutaneous tumors from injected BPO-derived cell lines (22.3±2.4 vs 2.5±0.27 SEM). A subset of tumor-antigen specific CD8 T cells also expressed these TRM markers in autochthonous tumors. In contrast, tumor-antigen specific CD8 T cells isolated from tumor-adjacent skin were significantly more enriched for CD69 and CD103.This led us to the hypothesis that the TME interferes with the TRM program of tumor infiltrating CD8 T cells. We performed bulk RNASeq on CD8 T cells expressing CD103 and CD69 from the tumor, adjacent skin, and from the skin of mice that rejected their tumor, respectively. From this analysis we identified several markers and pathways that appear to regulate TRM cells in the tumor. Of particular interest is CD101, which is enriched in CD103+ TILs, and receptors involved the purinergic signaling pathway (P2RX7, CD38, and P2RY6). Together, these data suggest that the TME influences the generation of TRM cells in the tumor and provide potential avenues study this population more deeply.

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Enhanced and suppressed tumor immunity is mediated by IL-1R1 on distinct immune cells

J. B. Williams, T. Tian, T. Kupper
Brigham and Women's Hospital, Boston, Massachusetts, United States

Inflammation is necessary for immune defense against pathogens and a functional antitumor immune response, but chronic inflammation promotes tumor growth and enables tumors to escape from immune-mediated destruction. Recently, we and others demonstrated that deficiency of IL1β strongly favors antitumor immunity, and multiple clinical trials involving antibody mediated IL1β blockade in multiple cancers are underway. The mechanism, however, remains obscure. We demonstrated that intact IL1α signaling was required for IL1β blockade-induced antitumor immunity, leading to the hypothesis that its interaction with the IL1 type I receptor (IL1R1) and subsequent signaling was required for the antitumor immune response. To determine which cell type required the IL1α signal, we studied the antitumor immune response in several mouse lines where the IL1R1 was conditionally deleted in T cells (LckCre), dendritic cells (CD11cCre), or macrophages (LysMCre) and on an IL1β-sufficient or -deficient background. When IL1R1 was absent in the T cell compartment the augmented control of MC38.OVA tumor growth in IL1β-deficient mice was lost. This indicated that IL1α signaling through T cells enabled the observed anti-tumor response. To determine the contribution of other immune cells, we measured the growth of MC38.OVA tumors in mice where the IL1R was absent on dendritic cells or monocytes/macrophages. We did not observe a difference in tumor growth when the IL-1R was absent on dendritic cells. Strikingly, conditional deletion of IL1R in monocytes/macrophages further delayed tumor growth compared to the already significant tumor inhibition in IL1β-deficient mice. Since both IL1α and IL1β only signal through the IL-1R, we propose that IL1α costimulation of T cells provokes antitumor immunity; however, this is strongly antagonized by activation of monocytes/macrophages by IL1 (predominantly IL1β), leading to suppression of tumor immunity by MDSC and tumor macrophages. Understanding this complexity may help optimize IL1β blocking therapy in clinical trials of tumor immunity.

060

Heterogeneity and lineage development of memory CD8+ T cells after viral infection of skin

Y. Yan[1], N. Smith[2], Y. Pan[1], J. Zhao[1], J. B. Williams[1], J. Zhang[1], T. Tian[1], T. Pan[1], K. Wu[1], A. Villani[2], T. Kupper[1]
[1]*Brigham and Women's Hospital, Boston, Massachusetts, United States,* [2]*Massachusetts General Hospital, Boston, Massachusetts, United States*

To better understand CD8 memory T cell development, we analyzed the profile of CD8+ T cells infiltrating skin as well as their circulating counterparts in the dLN after the same VACV skin infection at days 2-60, using the droplet-based microfluidic system Chromium (10X Genomics) single cell RNA sequencing platform. Using the Leiden algorithm, Ag-specific T cells were clustered and mapped through uniform manifold approximation and projection. Early activated T cells clustered together, showing a unique transcriptional profile, and skin and LN effectors began to diverge at day 5. A population destined to be TCM could be identified at day 5-10, and these cells followed a defined trajectory through day 60. When examining T cells from the skin, two populations began to appear at days 10-15 and followed distinct developmental trajectories through day 20-60, resulting in two populations of TRM. TRM1 cells expressed itgae and ifng, and showed high expression of junb and other AP-1 family members. Surprisingly, TRM2 cells showed upregulation genes like tgfb1, cd74, fcer1g and MHCII related genes while maintaining expression of cd3 and cd8. The existence of TRM2s was validated by imaging flow cytometry and further refined after several rounds of sub-clustering and filtering out of possible myeloid contamination. Using force-directed layout embedding, these 2 groups of skin TRMs showed distinct lineage trajectories in parallel with that of TCMs, resulting into 3 distinct terminal states derived from naïve and activated cells. Through Waddington optimal transport analysis, the flow of cells along time in and between trajectories could be observed in detail. Signature genes and regulons were also characterized for development for each lineage. Our study provides a multidimensional profiling of T cells from different anatomic sites after skin immunization in the dimensions of both time and space, providing novel perspectives on memory T cell development.

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Spatial proteomics and transcriptomics with digital spatial profiling reveals overlapping, but distinct inflammatory pathways in discoid lupus and lichen planus

M. D. Vesely[1], P. Divakar[3], Y. Liang[3], L. Chen[1, 2]
[1]*Dermatology, Yale School of Medicine, New Haven, Connecticut, United States,* [2]*Immunobiology, Yale School of Medicine, New Haven, Connecticut, United States,* [3]*NanoString Technologies Inc, Seattle, Washington, United States*

Lichen planus (LP) and discoid lupus erythematosus (DLE) are chronic, inflammatory autoimmune skin diseases with interface dermatitis and significant morbidity whose pathogenesis is incompletely understood. Recently, NanoString Technologies has developed GeoMx® digital spatial profiling (DSP), a novel highly multiplex method for detection of proteins and RNA in fixed tissues. Here, we perform spatial proteomics and transcriptomics using DSP to further characterize immune activation and immunoregulatory function, including immune checkpoints, of these overlapping autoimmune skin diseases. We utilized a 58-marker antibody cocktail and found that DLE and LP express a unique combination of immune inhibitory receptors. For example, programmed death receptor ligand 1 (PD-L1) and lymphocyte-activation gene 3 (LAG3) are detected in greater amounts in DLE than LP, while LP expresses more T-cell immunoglobulin and mucin-domain containing-3 (TIM3). We next used the NanoString Cancer Transcriptome Atlas (>1800 genes) on a separate cohort of patients with DLE and LP. Within CD3 enriched areas, there were 17 differentially expressed genes in DLE and LP. DLE expressed more IRF7, STAT2, TNFSF10 and type I IFN genes (IFITM1, MX1, LY6E). In contrast, LP expressed more type I IFN IFNA17 and NK cell associated KIR2DL1. IFN-g related genes were not significantly different, suggesting that both DLE and LP have strong type II IFN signatures, while DLE has an overall stronger type I IFN signature in addition to TNF and NF-kb signaling. There are a few limitations to our study. Our study and its conclusions are limited by the small discovery cohort of patients we used for spatial proteomics and spatial transcriptomics (n=6). As such, our study should be taken as a preliminary demonstration of how DSP can generate novel insights into immune checkpoint expression and inflammatory pathway activation in LP and DLE.

062

High-throughput quantitative proteomics unveils HIF1α as a driver of IL17A-induced metabolic alterations favoring hyperproliferation in keratinocytes

S. S. Marathe, D. Mukherjee, M. Basu, B. Dhamija, V. V. Sawant, S. Wad, D. Attrish, S. Kumar, R. Purwar
Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, Maharashtra, India

The cytokine IL17A, primarily secreted by Th17 cells, is known to play a crucial role in the pathogenesis of psoriasis where it augments keratinocyte hyperproliferation and inflammation. Although, the signaling cascades downstream of this critical cytokine, and the resultant proteomic and phenotypic alterations remain to be exhaustively explored. The findings of such an exploration could be harnessed to devise safer and more effective therapies. Accordingly, LC-MS/MS-based label-free quantitative proteomic analysis was performed on human primary keratinocytes (HPKs) stimulated with IL17A. Upon functional annotation analysis of high confidence differentially expressed proteins (FC≥ ±1.5) using STRING and PantherDB tools, levels of proteins involved in carbohydrate and lipid metabolism were found to be altered. Key rate-limiting enzymes involved in lipogenesis and cholesterol biosynthesis were found to be consistently upregulated. Moreover, upregulation of enzymes of the glycolytic machinery namely ENO-2, PKM, LDH, SLC16A1 was observed. Upregulation of hypoxia-inducible factor **α** (HIF1**α**), a transcription factor for all four of these proteins, was validated using western blot and intracellular staining ($p<0.05$). Moreover, pharmacological inhibition of HIF1**α** using echinomycin inhibited the glycolytic flux that is indispensable for hyperproliferating cells and reduced neutral lipid content of the HPKs measured by flow-cytometric staining. As a result, echinomycin induced apoptosis and curtailed the IL17A driven hyperproliferative phenotype of HPKs. In conclusion, by the means of a comprehensive proteomic analysis, we delineated several consequential protein dysregulations in HPKs by IL17A stimulation. HIF1**α** has been identified as a key player in IL17A mediated HPK hyperproliferation, thereby presenting this transcription factor and associated pathways as potential druggable targets for psoriasis.

063

CD4 T cells exhibit an effector phenotype and transfer disease in murine alopecia areata

S. J. Connell[1, 2,] S. Crotts[1], P. Christy[1], M. Lensing[1, 2], A. Jabbari[1, 2, 3]
[1]Department of Dermatology, University of Iowa, Iowa City, Iowa, United States, [2]Interdisciplinary Graduate Program in Immunology, University of Iowa, Iowa City, Iowa, United States, [3]Iowa City VA Medical Center, Iowa City, Iowa, United States

Alopecia Areata (AA) is a common autoimmune disease characterized by T cell infiltration of the hair follicle, resulting in nonscarring hair loss. Although CD4 T cells comprise the majority of the infiltrate, little is known about their contribution to disease pathogenesis. Previously we have identified an increased number of IFN-γ producing CD4+ T cells in the skin-draining lymph nodes (SDLN) as compared to unaffected (UA) controls. The objective for this study was to determine the role that CD4 T cells play in the pathogenesis of AA. Utilizing spectral flow cytometry, we found CD4 T cells exhibiting an effector phenotype were more numerous in the SDLN of AA mice as compared to that from UA mice. To address the role CD4 T cells play in AA, we adapted a model of AA whereby in vitro expanded bulk lymph node (LN) cells from previously affected AA mice were adoptively transferred into previously unaffected mice. When CD4 T cells were specifically sorted from SDLNs of AA mice using this protocol, these cells robustly transferred AA, contrasting with minimal induction by CD4 T cells from UA mouse LNs or spleens from AA mice. Additionally, this induction was found to be dose-dependent, with increased numbers of CD4 T cells inducing disease in recipient mice at a higher rate. In addition, we found that the endogenous CD8 T cell population is critically required for the transfer of CD4 T cell-induced disease, and that CD4 T cells-mediated disease relies on IFN-γ signaling for the emergence of AA. Our data suggest that activated CD4 T-helper type 1 effector cells contribute to the activation of CD8 T cells, enabling the attack of the hair follicle. Further studies are needed to elucidate the specific mechanisms by which CD4 T cells and IFN-γ contribute to the development of AA.

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Functional interrogation of lymphocyte subsets in alopecia areata using single-cell RNA sequencing

E. Y. Lee[1, 3], Z. Dai1, E. Wang[1], A. M. Christiano[1, 2]
[1]*Dermatology, Columbia University Irving Medical Center, New York, New York, United States,* [2]*Genetics and Development, Columbia University, New York, New York, United States,* [3]*Medical Scientist Training Program, Columbia University Irving Medical Center, New York, New York, United States*

Alopecia areata (AA) is one of the most prevalent autoimmune diseases, yet the development of innovative therapeutic strategies has been hampered by an incomplete understanding of disease pathogenesis, such as the complex immunological mechanisms that underlie AA pathogenesis. Here, we performed single-cell RNA sequencing (scRNAseq) of skin-infiltrating immune cells from the well-established graft-induced C3H/HeJ mouse model of AA, together with antibody-based depletion experiments to systematically interrogate the role of specific immune cell types in disease pathophysiology *in vivo*. Since AA is a predominantly T cell-mediated disease, we focused on dissecting the function of lymphocyte subsets in AA. Both our scRNAseq and functional studies established that CD8+ T cells, whose depletion was sufficient for both disease prevention and reversal, are the main disease-driving cell type in AA. Focused computational analyses of CD8+ T cells identified five CD8+ T cell subsets, ranging from naïve cells to effector and resident T cell populations. CD8+ T cell heterogeneity in AA was defined by an 'effectorness gradient' in which CD8+ T cells formed a continuum of interrelated transcriptional states that culminated in increased effector function and tissue residency, as opposed to discrete mutually exclusive populations. Our findings also confirmed a role for CD4+ T helper cells in disease initiation and demonstrated that regulatory T cells are protective against AA. Depletion of natural killer cells, B cells, and γδ T cells had no effect on disease prevention or reversal. Guided by our scRNAseq analyses, we performed a highly comprehensive, systematic interrogation of lymphocyte heterogeneity in AA, and uncovered a novel 'effectorness gradient' framework for AA-associated CD8+ T cells with implications for the design of future therapeutics.

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Longitudinal analysis of T cell dynamics in alopecia areata at single-cell resolution

E. Y. Lee[1, 2], A. M. Christiano[1, 3], Z. Dai[1], E. Wang[1]
[1]Dermatology, Columbia University Irving Medical Center, New York, New York, United States, [2]Medical Scientist Training Program, Columbia University Irving Medical Center, New York, New York, United States, [3]Genetics and Development, Columbia University, New York, New York, United States

Alopecia areata (AA) is defined by autoreactive T cells that attack hair follicles and lead to non-scarring hair loss. We previously demonstrated that AA is driven by cytotoxic CD8+ T cells that undergo clonal expansion. However, the relationship between CD8+ T cell pathogenicity and clonality in AA, as well as their temporal changes throughout disease course, remain poorly understood. Here, we conducted a time-course study in which we performed concomitant single-cell RNA and T cell receptor (TCR) sequencing analysis of T cells in both the skin and skin-draining lymph nodes (SDLNs) prior to, during, and after AA onset in the graft-induced C3H/HeJ mouse model. We observed a striking increase in CD8+ T cell clonality in both the skin and SDLNs during AA onset, with little to no clonal expansion in other T cell subsets. Clonality increased throughout AA progression, but later decreased in chronic AA, when the mice exhibited total body hair loss. Although we identified shared TCR sequences between AA skin and SDLNs, the degree of T cell clonality was increased in the skin, suggesting a two-step mechanism in which activated CD8+ T cells undergo an initial round of expansion in the SDLNs, followed by subsequent round(s) upon antigen exposure in the skin. We functionally investigated the causal role of specific TCR αβ pairs by generating retrogenic TCR mice expressing an expanded TCR sequence identified in our single-cell studies. Mice expressing expanded TCRs developed AA-like hair loss, whereas control mice expressing unexpanded TCR sequences did not. Our results demonstrate that individual expanded CD8+ T cell clones are sufficient to cause disease induction. These findings have broad implications for the development of novel therapeutics in AA aimed at the depletion of expanded pathogenic T cells, as well as clinical immunomonitoring in AA patients by following the dynamics of T cell clonality.

066

Plasmacytoid dendritic cells are not major producers of type 1 interferons in cutaneous lupus

T. Vazquez[1, 2], D. Diaz[1, 2], N. Kodali[1, 2], J. Patel[1, 2], E. Keyes[1, 2], G. Sprow[1, 2], M. Sharma[1, 2], M. Ogawa-Momohara[1, 2], M. Grinnell[1, 2], J. Dan[1, 2], V. Werth[1, 2]
[1]VA Medical Center Corporal Michael J Crescenz, Philadelphia, Pennsylvania, United States, [2]University of Pennsylvania, Philadelphia, Pennsylvania, United States

Type 1 interferons (IFN-1) are major drivers of disease activity in systemic (SLE) and cutaneous lupus erythematosus (CLE). Plasmacytoid dendritic cells (pDCs) are the major producers of IFN-1 during viral infection. pDCs are reduced in the circulation in SLE and have recently been shown to have an exhausted phenotype, suggesting that pathogenic pDCs were located in the end organs. We performed imaging mass cytometry of treatment naïve CLE skin (n=48), which revealed only 8.5% (IQR 0.0-22.22) of pDCs were IFNa+. Across all CLE biopsies, pDCs were the second lowest contributor of absolute IFNa+ cells (Median 1; IQR 0-4.5), with B lymphocytes being the smallest contributor. Classic dendritic cells (cDCs) and macrophages (Mf) were the largest relative and absolute contributors of IFNa in CLE. For IFNb, only 16.7% of pDCs were positive, compared to 60.7% of CD14+CD16+ Mf. pDCs were also the second lowest contributors of IFNb+ cells (Median 1; IQR 0-7), with B lymphocytes again being the lowest. We then performed mRNA in situ hybridization for CLEC4C (BDCA2) and IFN-1 (a, b, k) genes to confirm these findings. IFN-1 mRNA was detected in ITGAX (CD11c) positive cDCs but we did not identify colocalization of CLEC4C and IFN-1 mRNA. We then eluted leukocytes from CLE skin for flow cytometry. Flow cytometry showed a median of 5.2% of pDCs were positive for IFNa (IQR 3.8-9.6), compared with other myeloid cell lineages such as CD68+ Mf 35.9% (26.4-40.7), M1 Mf 23.7% (21.6-31.7), and M2 Mf 35.9% (26.4-40.7). We also performed CyTOF on CLE PBMCs with a 41-marker panel, including a pan-IFNa which demonstrated minimal staining of pDCs with IFN-1 compared to other myeloid cell lineages. Taken together, these findings suggest pDCs may not play the central role in CLE as major IFN-1 producers and myeloid cells are larger contributors of IFN-1 in numbers and as a percent. pDCs may have a pathogenic role in CLE through IFN-1-independent mechanisms.

067

Dissecting circulating regulatory T cells in severe Korean psoriasis patients by mass cytometry

B. Lee[1], Y. Bang[2], S. Lim[4], S. Kang[2], C. Park[2], H. Kim[2], T. Kim[3]
[1]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Seoul National University College of Medicine, Seoul, Korea (the Republic of), [3]Dermatology, Yonsei University College of Medicine, Seodaemun-gu, Seoul, Korea (the Republic of), [4]Dermatology, Yonsei University Wonju College of Medicine, Wonju, Korea (the Republic of)

Psoriasis is a chronic inflammatory skin disease with a strong Th17-skewed immune phenotype. It has been generally accepted that regulatory T cells in the lesional psoriasis skin have a functional impairment. However, circulating Cutaneous Leukocyte Associated antigen(CLA)+ regulatory T cell which potentially migrated out from the lesional skin has not yet been explored well so far especially in Asians. Herein, to investigate whether circulating CLA+regulatory T cells show aberrant immuno-phenotype compared to CLA- regulatory T cells, we've collected a total of sixty-four psoriasis patient blood in a single center with age/sex control in Korea. We designed a regulatory T cell panel encompassing 39 surface and intracellular markers with mass cytometry.

068

Lenabasum reduces IFNγ and pIRF3 in dermatomyositis skin: Biomarker results from a double-blind phase 3 international randomized controlled trial

T. Vazquez[2, 1], M. Sharma[2, 1], R. Feng[1], D. Diaz[2, 1], N. Kodali[2, 1], J. Dan[2, 1], M. Grinnell[2, 1], E. Keyes[2, 1], G. Sprow[2, 1], B. White[3], V. Werth[2, 1]
[1]University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]VA Medical Center Corporal Michael J Crescenz, Philadelphia, Pennsylvania, United States, [3]Corbus Pharmaceuticals Holdings Inc, Norwood, Massachusetts, United States

Dermatomyositis is an autoimmune skin disease with limited treatment options, and lenabasum is a cannabinoid type 2 receptor agonist with anti-inflammatory properties. Our previous work showed that IFNg and IL31 are increased in DM skin vs. skin from healthy controls and lenabasum reduces type 1 interferon (IFN-1) and IL-31 production by DM PBMCs in vitro. Lenabasum 20 mg BID treatment improved CDASI activity (CDASI-A) scores vs. placebo at 1 year in an international, double-blind, randomized Phase 3 trial. Imaging mass cytometry was done on 66 total FFPE skin biopsies obtained at Baseline and Week 16 in that trial, testing expression of 12 intracellular biomarkers in 9 cell types and using a zero-based, linear mixed effects model and Spearman's correlation for statistical analyses. Subjects in both treatment groups had similar immune infiltrates in skin at Baseline except plasmacytoid dendritic cells were increased in placebo group ($p<0.05$). At Week 16, subjects treated with lenabasum 20 mg BID vs. placebo had a significant decrease in IFNg ($p<0.05$) and phosphorylated-IRF3 (activated transcription factor for IFN-1) ($p<0.05$) expression. There was a trend towards decrease in IL-31 expression with lenabasum treatment ($p=0.08$). Absolute change in CDASI-A scores was positively correlated with a change in IL-31 staining ($R=0.636$, $p=0.026$). These results suggest clinical benefit of lenabasum on skin disease in DM may be mediated in part through reduction of IFNg and pIRF3 (IFN-1 Type 1) expression.

069

Non-supervised gene expression profiling of circulating regulatory T cells in severe Korean atopic dermatitis patient by scRNA-seq

S. Jin[3], K. Moon[1], K. Lee[2], Y. Bang[3], S. Jung[3], H. Nam[3], B. Cho[3], J. Lee[3], S. Choi[3], J. Lee[3], C. Park[4, 5, 6], D. Lee[3], H. Kim[7], E. Guttman-Yassky[8]

[1]Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut, United States, [2]Department of Biochemistry, Yonsei University, Seodaemun-gu, Seoul, Korea (the Republic of), [3]Seoul National University College of Medicine, Seoul, Korea (the Republic of), [4]Department of Microbiology and Immunology, Seoul National University College of Medicine, Seoul, Korea (the Republic of), [5]Seoul National University Graduate School Department of Biomedical Science, Seoul, Korea (the Republic of), [6]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea (the Republic of), [7]Genomic Medicine Institute, Medical Research Center, Seoul National University College of Medicine, Seoul, Korea (the Republic of), [8]Department of Dermatology and Laboratory for Inflammatory Skin Diseases, Icahn School of Medicine at Mount Sinai, New York, New York, United States

Atopic dermatitis (AD), also known as atopic eczema, is a chronic inflammatory skin disease caused by a combination of factors, such as neuroinflammation, microbiome, barrier dysfunction, and immune dysregulation. Affecting more than 15% of children and 1-3% of adults, the disease has negatively impacted countless patients worldwide, and more research on pathological features is imperative to develop effective patient care. Our group has previously discovered that circulating regulatory T cells in AD patients have diverse immune phenotypes and contribute to their immune dysregulation among European-American patients. However, limited large-scale research has been conducted for Asian patients with AD, who may have distinct pathological mechanisms. In this study, we employed a single-cell RNA-seq analysis on 12 Korean severe AD patients and 8 healthy controls with more than 300,000 cells, and investigated whether Asian patients with severe AD present with varied immunophenotypes in circulating regulatory T cells. Distinct populations in T, Monocytes, and B cells were also found in AD patients and several key regulators were inferred through cell-cell interaction analysis.

070

WITHDRAWN

071

WITHDRAWN

072

The anti-inflammatory cytokine IL-37 inhibits CD4+ T cell activation through the receptor IL-1R8 and supports regulatory T cells

D. G. Osborne[2], J. Domenico[1], C. Dinarello[2], C. Garlanda[3], M. Fujita[1]

[1]Dermatology, University of Colorado Denver School of Medicine, Aurora, Colorado, United States, [2]Medicine, University of Colorado Denver School of Medicine, Aurora, Colorado, United States, [3]Biomedical Sciences, Humanitas University, Milan, Italy

IL-1 family member IL-37 inhibits both innate and adaptive immune responses. Previously, we have shown that intracellular IL-37 expression in monocytes and dendritic cells reduces inflammation and induces immune tolerance. However, the role of extracellular IL-37 on T cell activation remains unknown. Extracellularly, IL-37 has been found to form a tripartite ligand-receptor complex containing IL-1R8 (SIGIRR/TIR8) and IL-18Ra in monocytes. We used human CD4 T cells from healthy donors, transgenic mice expressing human IL-37, and the mice deficient in IL-1R8 to study the role of extracellular IL-37 on T cell activation. Treatment of human CD4+ T cells with recombinant human IL-37 reduced TCR-mediated signaling activation, T-cell proliferation, and pro-inflammatory cytokine production. Because IL-37 was highly expressed in human regulatory T (Treg) cells, we compared the effects of recombinant IL-37 in conventional T (Tconv) cells and Treg cells using human CD4+ T cells, wild-type (WT) mice, IL-37 transgenic mice, and IL-1R8-deficient mice. Recombinant IL-37 directly inhibited the canonical T-cell signaling pathways through IL-1R8 in Tconv cells, indicating a direct role of anti-inflammatory cytokine IL-37 in suppressing CD4+ Tconv cell-mediated immunity. On the other hand, recombinant IL-37 reduced Treg cell activation without affecting Treg-specific gene expression, suggesting that endogenous IL-37 expression helps Treg cells retain high levels of Treg cell gene signatures even in the presence of extracellular IL-37. IL-37's inhibitory definition now includes a direct role in CD4+ T cell-mediated adaptive immunity.

073

D-dopachrome tautomerase overexpression accelerates photocarcinogenesis in mice

T. Shimizu[1], T. Andoh[2], Y. Yoshihisa[1], T. Makino[1]
[1]Dermatology, Toyama Daigaku, Toyama, Toyama, Japan, [2]Pharmacology, Kinjo Gakuin University, Nagoya, Japan

Ultraviolet irradiation (UV) exposure constitutes the most relevant environmental hazard to human skin. UV-mediated cutaneous inflammation and direct DNA damage lead to the promotion of skin carcinogenesis. D-dopachrome tautomerase (D-DT), a functional homolog of macrophage migration inhibitory factor (MIF), has functional similarities to MIF. However, its role, unlike the role of MIF in photocarcinogenesis, is unknown. We therefore explored the role of D-DT in photocarcinogenesis by developing D-DT transgenic (D-DT Tg) mice and provided a research model for future studies targeting D-DT. Chronic UVB exposure accelerated tumor development in D-DT Tg mice compared with wild-type (WT) mice, with a higher incidence of tumors observed in D-DT Tg mice than in WT mice. In irradiated D-DT Tg mouse keratinocytes, the p53, PUMA, and Bax expression were lower than that in WT mice. These results indicate that D-DT Tg overexpression confers prevention against UVB-induced apoptosis in keratinocytes. Taken together, these findings support D-DT is as a functionally important cytokine in photocarcinogenesis and a potential therapeutic target for the prevention of the carcinogenesisphotocarcinogenesis.

074

ALK-positive desmoplastic Spitz naevus in a patient with corresponding ALK-positive anaplastic large cell lymphoma

J. Nguyen[1], A. Huang[1], J. Fleming[2], D. MacGregor[3], D. Wilks[1]
[1]Paediatric Plastic and Reconstructive Surgery, The Royal Children's Hospital Melbourne, Parkville, Victoria, Australia, [2]Children's Cancer Centre, The Royal Children's Hospital Melbourne, Parkville, Victoria, Australia, [3]Department of Anatomical Pathology, The Royal Children's Hospital Melbourne, Melbourne, Victoria, Australia

Aim:Spitz naevus is a benign melanocytic tumour with distinct histopathological features of spindle-shaped melanocytes. An understanding of genetic aberrations can help to characterise the different subsets of Spitzoid neoplasms. Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor that was first described in the pathogenesis of anaplastic large cell lymphoma (ALCL). ALK mutations have been reported in up to 10% of Spitz tumours.This study describes the rare occurrence of an ALK mutation in two different diseases in the same patient – ALCL and Spitz naevus. Method:Presentation, management, and clinical outcomes were retrospectively reviewed for a patient with ALK-positive ALCL and ALK-positive Spitz naevus at a tertiary paediatric hospital. Result:The patient was a 14-year-old female with a 12-month history of a red-brown plaque on the right shoulder.This was on a background stage III ALCL treated with chemotherapy and stem cell transplantation. She underwent surgical excision of the skin lesion. Histopathological examination showed an ALK-positive desmoplastic Spitz naevus. A bone marrow aspirate and trephine (BMAT) of the right axillary lymph node at the time of ALCL diagnosis also revealed an ALK mutation. She is currently in remission 2 years post stem cell transplantation. Conclusion:Our case reports on a rare coincidence of a patient having the same mutation for two distinct disease processes. There has been one similarly reported case in the literature in a patient with ALK-positive cutaneous ALCL and Spitz naevi with the same fusion partner gene (TPM3). Whilst this occurrence is not fully understood, further studies may identify whether this is an independent coincidence in two different cell lineages, or a fragile site similarity which may predispose to the same translocation event.

075

Gene-expression differences in actinic keratosis versus squamous cell carcinoma: A prelude to malignancy

M. Majidian[1, 2], J. M. Anderson[3, 2], J. Rock[4], R. Ai[4], J. Whitaker[4], R. Moy[2]
[1]Tulane University School of Medicine, New Orleans, Louisiana, United States, [2]Moy Fincher Chipps Facial Plastics and Dermatology, Beverly Hills, California, United States, [3]The University of Texas Health Science Center at San Antonio, San Antonio, Texas, United States, [4]DermTech Inc, La Jolla, California, United States

Purpose: Actinic keratosis (AK) is a premalignant lesion that has a 1-10% potential of progression to squamous cell carcinoma (SCC). The purpose of this study was to non-invasively collect skin samples from subjects with lesions clinically suspicious for AK and SCC and identify genes that may indicate the likelihood of AK progressing to SCC. Materials and Methods: Skin samples were collected non-invasively with adhesive patch kits prior to biopsy and RNA was isolated to detect gene expression levels with RNA sequencing. All diagnoses were histopathologically confirmed after non-invasive collection. A threshold fold change >2 and adjusted p value <0.05 was used as a cutoff for determining differentially expressed genes (DEGs). Results: 47 lesions were examined, consisting of 12 AK, 13 in-situ SCC, and 22 invasive SCC. There were 11 differentially expressed genes between AK and invasive SCC ($p<0.05$). After correction for gDNA contamination, there were 219 differentially expressed genes found ($p<0.05$) with 6 overlapping DEGs from before the correction comparing AK with SCC. These genes included KRT1, KRT2, KRT6C, FABP5, IVNS1ABP, and SLC2A3. Conclusions: These finding support the progression model of premalignant lesions evolving to SCC and suggest a non-invasive option may be available to monitor AK as an aid in early detection, treatment, and prevention of disease progression. Additional studies are needed to elucidate the genes that have potential to serve as sentinels to lesion advancement.

076

Can melanoma origin sites be related to metastatic sites?

M. Kaszycki[1], S. Weiss[2], R. Feinn[1], M. Fogarasi[1]
[1]Medical School, Quinnipiac University, North Haven, Connecticut, United States, [2]Medical Oncology, Yale School of Medicine, New Haven, Connecticut, United States

Melanoma is a skin cancer originating from melanocytes that if not diagnosed and treated early can become life threatening as it commonly metastasizes to other body sites such as lymph nodes, lung, liver, bone, and brain. Certain anatomic sites of melanoma indicate poorer prognosis, such as liver and brain metastases, therefore anatomic sites of metastasis should be deliberated when considering treatment with varying side effect profiles. A single-center retrospective chart review was performed, patient data including site of primary melanoma, sites of metastasis, treatment and outcome were collected. Patients with unknown or multiple primary melanoma sites were excluded. A total of 101 patients were included in our data, 50 patients received combination anti-CTLA-4 anti-PD-1 therapy and 51 patients received single agent anti-PD-1 therapy. A median age of diagnosis was 63 years old, and the median number of metastatic sites was 2, with the most common original site being the trunk (39%) followed by head and neck (27%) and the most common metastatic sites being the lymph nodes (65%) followed by the lungs (42%). Cancers that originated in the trunk, compared to other sites, were more likely to spread to soft tissue (46% vs 23%, $p=0.006$) and cancers that originated in the lower extremity were more likely to spread to the bone (31% vs 12%, $p=0.026$). We did not find other associations between other sites of origin and metastatic sites. Additionally, there were no associations between tumor thickness, mitosis, and ulceration of the original melanoma site and site of metastasis. For treatment therapy, combination therapy was more commonly used for brain (24% vs 4%, $p=0.005$) and soft tissue (41% vs 22%, $p=0.038$) metastases, and less commonly for cutaneous (0% vs 12%, $p=0.013$) metastases.

077

Identifying genetic factors that enable basal cell carcinoma to transition from microscopic to macroscopic disease

K. G. Trieu[1], S. Tsai[1], M. Eberl[1], V. Ju[1], N. Ford[1], O. J. Doane[1], J. K. Peterson[1], N. A. Veniaminova[1], M. Grachtchouk[1], P. W. Harms[2], F. J. Swartling[3], A. A. Dlugosz[1], S. Y. Wong[1]

[1]Departments of Dermatology and Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan, United States, [2]Departments of Pathology and Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [3]Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Rudbeck Laboratory, Uppsala Universitet, Uppsala, Sweden

Basal cell carcinoma (BCC) is driven by constitutive activation of the Hedgehog (Hh) signaling pathway, most commonly through loss-of-function mutations in PTCH1. Using animal models, we demonstrate here that microscopic BCC-like tumors initiated by loss of Ptch1 or gain of Smo fail to progress and eventually enter into a dormant state. Because loss-of-function mutations in NOTCH1/2 and TRP53 are commonly detected in human BCC, we sought to determine if these genetic factors modulate tumor progression. Indeed, we found that loss of Ptch1 and concomitant loss of either Notch1 or Trp53 enables rare macroscopic tumors to form. Using whole exome sequencing and IHC, we further found that "successful" macroscopic tumors acquired the ability to hyperactivate downstream Hh signaling by amplifying Gli1/2 and upregulating Mycn. Finally, we demonstrate that concomitant loss of Ptch1 and transgenic overexpression of MYCN promotes tumor progression to macroscopic disease. Overall, we provide functional evidence that BCCs arise through a step-wise process initiated by mutations that activate upstream Hh signaling, followed by the acquisition of secondary mutations that further hyperactivate downstream Hh signaling. Our observations may explain why some BCCs persist during pharmacological inhibition of SMO and highlight downstream MYCN as a potential target for therapy.

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Transcript profiling for TSLP in human squamous cell carcinoma in situ

Y. Suzuki-Horiuchi[1], S. Prouty[1], L. Wushanley[1], V. Lee[1,2], J. T. Seykora[1]

[1]Department of Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Scheie Eye Institute, University of Pennsylvania, Philadelphia, Pennsylvania, United States

SCCIS (squamous cell carcinoma in situ) is a precursor lesion for cSCC which is the second most common cancer in the US. Research on immunoediting and immune evasion has identified potential mechanisms that highly-mutated SCCIS cells use to avoid immunosurveillance. However, little is known about how SCCIS cells acquire immune evasive properties. Our recent work suggests that UV-exposed keratinocytes that form SCCIS attain immune evasive properties during lesion development. First, our whole Exome-seq studies identified UV-signature loss-of-function mutations in NOTCH1-3 in the epidermis and known oncogenic mutations in TP53 in SCCIS. These data indicate that deregulation of the p53/Notch pathway is important for SCCIS development. Our RNA-seq data on the same samples revealed that the largest class of differentially expressed genes between epidermis and SCCIS are immune response genes. One significantly upregulated immunoregulatory gene was TSLP (adjp< 1.2x10-4) known to be associated with decreased p53/Notch signaling. These data raise the hypothesis that transcriptomic changes in TSLP may alter the immune microenvironment associated with SCCIS. To understand how TSLP upregulation promotes SCCIS we identified the cells producing TSLP, its expression level, and transcript specificity using BaseScope duplex assay on human SCCIS samples with adjacent epidermis for the two human transcript variants of TSLP: a long-form (variant 1; lfTSLP) consisting of four exons and the short-form (variant2; sfTSLP) that contains an extended exon 3 and exon 4; the function of the short form transcript remains unclear. Our data show that both the long and short form TSLP transcripts are upregulated in SCCIS lesional cells and in dermal lymphocytes. There was a trend towards increased expression of the short form in dermal immune cells compared with lesional keratinocytes. These data suggest that the elevated TSLP transcript levels may be part of the altered immune environment that permits SCCIS development.

079

Identifying signaling networks in melanoma tumors that promote the uncontrolled growth of BRAF mutant melanocytes

H. Xiao[1], C. Chen[2], J. Shiu[2], R. Ruiz[1], M. G. Caldwell[1], A. D. Lander[1], A. K. Ganesan[2]

[1]Center for Complex Biological Sciences, University of California Irvine, Irvine, California, United States, [2]Department of Dermatology, University of California Irvine, Irvine, California, United States

Melanocytic nevi (AKA moles) are benign proliferations of melanocytes induced by the same BRAF mutation that initiates melanoma. BRAF mutant melanocytes in nevi eventually undergo growth arrest whereas melanomas do not, raising the question that how do melanoma tumors escape growth arrest and continue to progress uncontrolledly? In answering this question, we recently determined that simply crossing the BRAF mutation from black mice into an isogenic albino background was sufficient to induce melanoma formation. BRAF mutant albino tumors did not have any new mutations, indicating that factors that influence tumor formation in these models must be epigenetic. We next sought to define signaling networks present in BRAF mutant albino melanomas that are absent in BRAF mutant black nevi. Single cell RNA sequencing analysis of melanoma tumors identified discrete macrophage and fibroblast populations that were observed in BRAF induced melanomas but not in BRAF induced nevi. Specifically, we identified a distinct population of melanocytes with a Schwann cell-like gene expression signature (termed S cells) that have a similar gene expression pattern to the neural crest stem cells that are thought to regulate BRAF inhibitor resistance. These S cells were observed in both BRAF and BRAF PTEN melanoma tumors as well as human melanomas. Intriguingly, the S cells present in nevi had a different gene expression pattern than the S cells observed adjacent to melanomas. Taken together, these results indicates that discrete cells in the melanocytic lineage promote tumorigenesis by secreting growth promoting signals that allow BRAF mutant melanocytes to overcome growth arrest.

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Current treatment options for patients with unresectable cutaneous melanoma

S. Shimon[1], D. Sharad[2], E. Schlam[3], S. Gonzalez-Escola[2]

[1]Nova Southeastern University College of Allopathic Medicine, Davie, Florida, United States, [2]Westside Regional Medical Center, Plantation, Florida, United States, [3]University of Miami School of Medicine, Miami, Florida, United States

Cutaneous Melanoma (CM) remains the leading cause of skin-related mortality in the U.S. The U.S. Surveillance, Epidemiology and End Results database reports 5.6% of all new cancer cases in 2021 attributed to CM, with incidences rising annually since 2009. Several subtypes of CM are thought to arise from early driver mutations in BRAF, RAS, and NF1 genes resulting in unopposed activation of MAPK and PI3K signaling pathways, unregulated growth and malignant transformation of melanocytes. Due to the metastatic potential of CM, emphasis on systemic treatment has been an area of interest when surgical resection is insufficient for curative prognosis. Our case involves a 65 year old patient who presents for management of a 8.5mm tumor with ulceration and multiple associated subcutaneous satellite lesions on his left scapula. At his most recent follow up, he has experienced a 5kg weight loss, fatigue, shortness of breath, and non-tender axillary lymphadenopathy. Excisional biopsy revealed nodular-variant malignant melanoma, and immunoperoxidase studies were positive for SOX10 and Melan-A, confirming the diagnosis. CT of the chest showed enlarged bilateral axillary lymph nodes with areas of necrosis, as well as punctate bilateral pulmonary nodules, consistent with metastasis. Based on the American Joint Committee of Cancer classification, our patient has T4N2M1b consistent with stage IV melanoma and a 53% one year prognostic survival. Due to his unresectable tumor, systemic treatment using immune checkpoint inhibitors or targeted therapy is indicated. As of 2011, the FDA approved immunomodulator therapy, such as CTLA4 inhibitors and PD1 inhibitors, which promote CD8+ T cell death. Of these, nivolumab, a PD1 inhibitor, has been shown to have increased response rates (43% vs 19%), and 3 year survival (52% vs 37%) than CTLA-4 inhibitors, such as ipilimumab. Due to these benefits and lower side effect profile, we chose nivolumab as the drug of choice for our patient.

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Modulation of the aryl hydrocarbon receptor by adipose tissue: Implications for skin carcinogenesis

S. Shareef[1], J. Veenstra[2], J. J. Bernard[3, 4]
[1]Michigan State University College of Human Medicine, East Lansing, Michigan, United States, [2]Department of Dermatology, Henry Ford Health System, Detroit, Michigan, United States, [3]Department of Dermatology, Department of Medicine, Michigan State University College of Human Medicine, East Lansing, Michigan, United States, [4]Michigan State University Department of Pharmacology and Toxicology, East Lansing, Michigan, United States

The aryl hydrocarbon receptor(AhR) is a ligand-activated transcription factor that responds to chemical carcinogens. An endogenous AhR agonist, 6-Formylindolo[3,2-b]carbazole, is produced following ultraviolet light(UV) absorption by tryptophan. Epidemiological studies show a correlation between exposure to AhR agonists and non melanoma skin cancer(NMSC). As an example, AhR activation promotes metabolism of pro-carcinogens to carcinogens such as the metabolism of benzo(a)pyrene to benzo[a]pyrene diol epoxide(BPDE). There are differing epidemiological studies which explain the relationship between obesity and NMSC—some demonstrate a positive association while others demonstrate a negative association. However, evidence supporting an inverse correlation is relatively weak and heavily confounded by UV exposure among body mass index groups. Our objective was to determine the role of AhR in adipose tissue-stimulated malignant transformation. RNA-seq analysis demonstrated that secretions from adipocytes induced AhR-regulated phase I metabolizing enzymes in a non-tumorigenic, mouse epidermal cell line, JB6 P+, including CYP1A1 which metabolizes B[a]P to BPDE. Phase II detoxifying enzymes remained unchanged or reduced suggesting xenobiotic metabolism by adipocyte secretions. Together, B[a]P and adipocyte secretions induce malignant transformation, assessed by the JB6 P+ soft agar clonogenic assay. Primary human keratinocytes, cultured with adipocyte-conditioned medium for 24 hours, demonstrated elevated AhR protein levels and induction in CYP1A1 and CYP1B1 mRNA(3.6 and 10.2-fold)compared to the media control cells. Understanding how adipocytes modulate AhR activity in skin with or without environmental AhR ligands will lead to new therapeutic strategies to prevent epidermal cell transformation.

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***In vivo* tracking of clonal dynamics shows three phases of UV-induced skin carcinogenesis**

S. Avdieiev[1, 2], L. Tordesillas[3], O. Chavez Chiang[3], Z. Chen[4], L. Silva Simoes[2], Y. Chen[4], N. Andor[2], R. Gatenby[1, 2], E. Flores[5], J. Brown[1, 2], K. Y. Tsai[1, 3]
[1]Cancer Biology and Evolution Program, Moffitt Cancer Center, Tampa, Florida, United States, [2]Integrated Mathematical Oncology, Moffitt Cancer Center, Tampa, Florida, United States, [3]Tumor Biology Department, Moffitt Cancer Center, Tampa, Florida, United States, [4]Department of Biostatistics and Bioinformatics, Moffitt Cancer Center, Tampa, Florida, United States, [5]Department of Molecular Oncology, Moffitt Cancer Center, Tampa, Florida, United States

We hypothesize that skin cancer emerges from a combination of mutations and tissue disruption. We characterize clonal dynamics and transcriptional signatures during skin carcinogenesis using lineage tracing. For 3 months, we UV-irradiated fluorophore expressing K14Cre-ERT2 Confetti mice. Clone volumes were computed from 3-D digitized confocal z-stacks. scRNAseq was used to compare UV-exposed (EXP) epidermis, non-exposed (NON), and tumors. Over 6 months from the UV initiation, we generated 914 serial images of the EXP/NON skin. We analyzed 16,135 clones from the EXP and 21,506 clones from the NON skin. Median clone size did not differ between UV treatments and did not change with time. However, the mean size of EXP clones were 50% larger than NON ones, with a >6-fold increase in variance. The number of large EXP clones increased dramatically by months 3-4, plateauing at months 5-6. scRNAseq of EXP/NON epidermis and tumors revealed 16 clusters corresponding to different differentiation states. We observed dynamic changes to the clusters when progressing from normal to chronically exposed skin, and then to tumors. Development of large clones preceded high penetrance mutations. EXP clusters were associated with expression of cystatins (Scfa 3, BC100530), and alarmins/proliferative keratins (Krt16, Krt6a). Flr-expressing keratinocytes from large EXP clones exhibited altered keratinocyte differentiation, inflammation, and upregulation of metabolic regulators. These results show an initial disruption of tissue (2-3 months) permitting the emergence of unusually large clones (phase 1), followed by inter-clonal competition and mutational buildup (phase 2) and the emergence of lesions (phase 3).

083

Defining the immune response in basal cell carcinoma spontaneous regression

K. N. Wong[1, 2, 3], S. Atwood[1, 2, 3]
[1]University of California Irvine Department of Developmental and Cell Biology, Irvine, California, United States, [2]Center for Multiscale Cell Fate Research, Irvine, California, United States, [3]University of California Irvine Cancer Research Institute, Irvine, California, United States

In the US, more people are diagnosed with basal cell carcinoma (BCC) than any other cancer, but treatment of advanced tumors is frequently complicated by drug resistance. Interestingly, 20-29% of human BCC tumors are reported to shrink spontaneously in the absence of treatment through an unknown mechanism. With case studies suggesting a role for immune activation in this tumor regression, we hypothesize that T cells are recruited to growing BCCs to promote spontaneous tumor shrinkage. The inducible Gli1CreERT2; Ptch fl/fl transgenic BCC mouse model shows microtumor growth in the skin followed by significant reduction ($p<0.001$) in tumor area after 10 weeks. Using this model of regression in the absence of treatment, we assess the role of immune system interactions in promoting this mechanism and as a potential therapeutic target. Analysis of immune infiltration, bulk and single-cell RNA-sequencing of tumor-bearing skin shows evidence of an inflammatory neutrophil response during tumor growth followed by a transient cytotoxic T cell response. By assessing T cell necessity and the sufficiency of upregulated cytokines in inducing anti-tumor responses, we aim to define the immune response against BCC tumors and determine the role of effector T cells in regulating regression. This study will identify the cell populations, pathways, and interactions critical to the tumor regression mechanism and BCC pathogenesis. This will provide candidate therapeutic targets to enhance tumor regression, thus facilitating the development of alternative therapies to treat advanced BCCs.

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mRNA methylation in skin tumorigenesis and therapeutic resistance

Y. Cui[1], S. Yang[1], J. Wei[2], C. R. Shea[1], W. Zhong[1], F. Wang[1], P. Shah[1], M. Kibriya[1], X. Cui[2], H. Ahsan[1], C. He[2], Y. He[1]
[1]University of Chicago Division of the Biological Sciences, Chicago, Illinois, United States, [2]University of Chicago Division of the Physical Sciences, Chicago, Illinois, United States

Analogous to DNA and histone modifications, RNA molecules are chemically modified, the study of which gives rise to the field called epitranscriptomics. Among these modifications, N6-methyladenosine (m6A) RNA methylation is the most abundant internal modification in messenger RNA (mRNA) and non-coding RNA in eukaryotic cells, which regulates RNA metabolism, including RNA decay, translation and nuclear processing. However, the regulatory and functional role of m6A RNA methylation in skin cancer remain poorly understood. Recently we demonstrated that the m6A mRNA demethylase FTO promotes tumorigenesis and resistance to immunotherapy in melanoma. In addition, using skin-specific conditional knockout mouse models, in combination with in vitro cellular models, recently we have demonstrated a pivotal role of m6A RNA methylation in skin cancer development. FTO as an N6-methyladenosine (m6A) RNA demethylase is degraded by selective autophagy, which is impaired by low-level arsenic exposure to promote tumorigenesis. We found that in arsenic-associated human skin lesions, FTO is up-regulated, while m6A RNA methylation is down-regulated. In keratinocytes, chronic relevant low-level arsenic exposure up-regulated FTO, down-regulated m6A RNA methylation, and induced malignant transformation and tumorigenesis. Moreover, in mice, epidermis-specific FTO deletion prevented skin tumorigenesis induced by arsenic and UVB irradiation. Targeting FTO genetically or pharmacologically inhibits the tumorigenicity of arsenic-transformed tumor cells. We identified NEDD4L as the m6A-modified gene target of FTO. Finally, arsenic stabilizes FTO protein through inhibiting p62-mediated selective autophagy. Our study reveals FTO-mediated dysregulation of mRNA m6A methylation as an epitranscriptomic mechanism to promote arsenic tumorigenicity and may open up new opportunities to reduce skin cancer burden.

085

Senescent dendritic cells drive ROS-induced DNA damage in CTCL

A. Klosowicz, J. Crouch, Q. Zhan, I. Kim, A. Gehad, J. Teague, T. Kupper, R. A. Clark
Brigham and Women's Hospital, Boston, Massachusetts, United States

Progressive DNA damage and worsening aneuploidy are associated with the development of highly aggressive CTCL. We immunostained MF skin lesions for the phosphorylated DNA double-strand break repair genes γH2AX and 53BP1 and phosphorylated/activated ATM (pATM) and found that ongoing double-strand DNA breaks were frequent within the malignant T cells even in clinically stable MF skin lesions. IL-6, a product of senescent cells, is the second most highly expressed cytokine in MF. Immunostaining demonstrated the senescence marker p21Cip1 was increased in a stage specific manner in MF and localized to OX40L+CD40L+ dendritic cells. These DC also expressed thioredoxin (TXN), indicating production of reactive oxygen species (ROS) and were found clustered closely together with malignant and benign T cells. ROS production by senescent cells is known to drive ROS-induced DNA damage in neighboring cells. We therefore immunostained T cells near senescent DC for evidence of oxidative stress (TXN) and double-strand DNA breaks (γH2AX). We found a stage specific increase in T cells experiencing oxidative stress and ongoing DNA damage in T cells neighboring senescent DC. Malignant T cells were primarily affected but some CD7+ benign infiltrating T cells showed evidence of DNA damage in later stage MF (IIA, IIB). Our results suggest that ROS produced by senescent DC induces DNA damage in T cells and may drive T cell transformation and tumor progression. These studies suggest a possible role for senolytic therapies in the treatment of CTCL.

086

Decreased clonal diversity within the skin T-cell repertoire, but not blood, distinguishes large cell transformed mycosis fungoides from its non-transformed counterpart

L. Gleason, N. Nikbakht
Department of Dermatology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States

Mycosis fungoides (MF) is a T-cell lymphoma that originates in the skin and can progress in late stages to involve blood, lymph nodes, or viscera. An aggressive subtype of MF is large cell transformed mycosis fungoides (LCT-MF), defined by the presence of large cells comprising greater than 25% of the lesion infiltrate. LCT-MF is associated with a higher likelihood of disease progression and mortality. LCT-MF is solely defined by the size of the T-cells in the infiltrate; however, the clonal attributes of the T-cells within the infiltrate in large cell transformation (LCT) are not known. To investigate the changes of T-cell repertoire in LCT-MF, we sequenced T-cell receptors in lesional skin and blood in patients with LCT-MF and compared them to patients with MF and healthy subjects. We sequenced the antigen-binding region of T-cell receptor beta in lesional skin biopsies and blood samples from 25 healthy subjects and 25 patients with skin-limited MF (stages IA-IIB). Within the MF subset, 15 patients had LCT-MF. We quantified clonal diversity by Simpson's clonality score. Additionally, we identified the frequency of the malignant T-cell clone within the T-cell infiltrates as maximum frequency. Histologically defined LCT-MF skin biopsy specimens had significantly higher clonality scores and maximum frequency than MF (p-value < 0.0001). Blood from patients with MF or LCT-MF showed significantly higher clonality scores and maximum frequency compared to healthy blood (p-value < 0.0001). However, there was no significant difference in clonality and maximum frequency when blood samples from LCT-MF and MF patients were compared. Our data demonstrate LCT-MF T-cell infiltrates in the skin exhibit a less diverse clonal repertoire and a higher frequency of malignant T-cell clones in lesional specimens when compared to MF samples. However, these findings do not extend to blood T-cell repertoires. Our results suggest that the pathogenic events leading to LCT in MF may originate in the skin rather than the blood.

087

Tumor assembly of the spatially organized self-propagating myeloid niche

D. Haensel[1], B. Daniel[2], T. Fabo[1], S. Gaddam[1], J. Bjelajac[1], C. Pan1, T. Patel[1], S. Aasi[3], A. Satpathy[2], A. Oro[1]
[1]Program in Epithelial Biology, Stanford University School of Medicine, Stanford, California, United States, [2]Pathology, Stanford University School of Medicine, Stanford, California, United States, [3]Dermatology, Stanford University School of Medicine, Stanford, California, United States

Cancer immunotherapies have revolutionized treatment, but disappointing results in single-agent regiments reveal the need to understand the origin and assembly of the tumor immune niche to identify further targets in the highly heterogeneous tumor microenvironment. We have previously focused on tumor resistance mechanisms primarily driven by tumor epithelial heterogeneity in skin basal cell carcinoma (BCC), identifying discrete subpopulations resistant to canonical therapies. Here, we have used a combination of single-cell and imaging-based spatial analysis to define the extensive but specific and supportive microenvironmental heterogeneity surrounding the BCC tumor epithelia. We identify a key spatially organized population of TREM2+ skin cancer-associated myeloid cells (SCAMs), which support tumor proliferation through an oncostatin M mediated signaling pathway. Interestingly, SCAMs are dramatically distinct from TREM2+ cells found in normal homeostatic skin or during wound healing and express unique surface markers that can distinguish them. A key unique feature of SCAMs is their ability to actively proliferate within the tumor and propagate themselves even during serial tumor passaging. In turn, we find that SCAM development is driven by the tumor, with intratumoral injections demonstrating that the environment is sufficient to instruct naive monocyte maturation into a functional self-propagating SCAM. Overall, this work suggests that targeting SCAMs in conjunction with other immunotherapeutics may prove a useful strategy.

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Deciphering the molecular signals of EGFR pathway activation in Dlx3 deficient skin in cSCC

D. Bajpai[1], S. Mehdizadeh[1], A. Uchiyama[2], Y. Inoue[2], A. Sawaya[1], S. Nayak[1], S. Brooks[1], M. Kellett[1], E. Palazzo[1], S. Motegi[2], C. Cataisson[3], M. I. Morasso[1]
[1]National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, United States, [2]Gunma Daigaku, Maebashi, Gunma, Japan, [3]National Cancer Institute, Bethesda, Maryland, United States

Cutaneous squamous cell carcinoma (cSCC) is the second most common form of skin cancer. Emerging evidence suggests that homeoproteins can act as modulators of tumor initiation and progression in cSCC. DLX3 is a homeobox transcription factor which plays pivotal roles in embryonic development and epidermal homeostasis. Previous work from our lab, using both human samples and a Dlx3 knock-out (Dlx3cKO) mouse model, demonstrated DLX3's tumor suppressive role in epithelial tumor development and tumor progression. Molecular evaluation of the Dlx3cKO tumor-permissive properties identified specific mechanisms of Dlx3-mediated skin carcinogenesis via upregulation of EGFR ligands and activation of EGFR/ERBB2/MAPK pathway. Here, we have investigated the direct DLX3 genomic targets and signal pathways mediating DLX3's tumor suppressive function. Epigenetic studies using chromatin immunoprecipitation (ChIP) and next-generation DNA sequencing demonstrated that DLX3 is directly recruited into the regulatory regions of EGFR ligand genes including Areg, Ereg, Tgfα and Btc. Further, transcriptomic profiling using RNA sequencing identified STAT3 as a downstream mediator of EGFR/ERBB2 pathway activation in Dlx3cKO skin. Thus, our preliminary results support that STAT3 is a key downstream modulator of DLX3's tumor suppressive activity in cSCC. Future work will functionally evaluate the in-silico findings to elucidate the downstream events critical to gene activation important in skin tumorigenesis.

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Desmoglein 2 promotes tumor development through miR-146a/IRAK1/IL-8 signaling axis

B. L. Hill[1], A. Calder[5, 2], J. Flemming[1], S. Gilmore[1,] Y. Guo[1], L. Harshyne[3], A. Linnenbach[1], U. Martinez-Outschoorn[4], J. Curry[5], A. P. South[1, 5], A. Luginbuhl[5], M. Mahoney[1, 5]

[1]Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [2]College of Medicine, Drexel University, Philadelphia, Pennsylvania, United States, [3]Cancer Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [4]Medical Oncology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [5]Otolaryngology–Head and Neck Surgery, Thomas Jefferson University, Philadelphia, Pennsylvania, United States

The cadherin Dsg2 plays critical roles in cell growth and survival and is up-regulated in many cancers including skin and head and neck. We recently showed that Dsg2 down-regulates the tumor suppressor, miR-146a, and up-regulates the pro-inflammatory chemokine, IL-8. Furthermore, Dsg2 enhances small extracellular vesicle (sEV) release, which serve as a carrier of IL-8 on their surface potentially for receptor transactivation. In this study, bulk RNAseq analysis of HNSCC tumor biopsies from patients treated with the PD-1 inhibitor, nivolumab, revealed that Dsg2 was significantly higher in HPV (+) as compared to HPV(-) patients before treatment and was down-regulated in response to treatment. Visium spatial transcriptomic and single cell RNAseq confirmed that Dsg2, IRAK1, and IL-8 are co-expressed in malignant, aggressive tumor cells. HPV(+) non-responders had higher levels of IL-8 in their plasma as determined by ELISA, and analysis of blood-borne sEV showed that miR-146a was significantly lower in non-responders, indicating that the miR-146a/Dsg2/IL-8 signaling axis may influence clinical trial outcome. To assess the role of miR-146a in SCC, we used the XMIRXpress system to overexpress and facilitate the loading of miR-146a into sEV. Treatment of cells with miR-146a loaded sEV decreased the levels of IRAK1 and IL-8 and reduced proliferative capacity. Using a xenograft model, cell lines expressing miR-146a showed decreased tumor burden as compared to control. Overall, elevated miR-146a levels in sEV may serve as a favorable prognostic marker in HNSCC, which functions mechanistically by its regulation of IL-8.

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Inhibition of HMGB1 attenuates the inflammatory response in keratinocytes

K. G. Bui[1], A. Keith[1], C. Ebens[2], A. Bielinsky[3], J. Tolar[1, 2]

[1]Department of Genetics, Cell Biology, and Development, University of Minnesota Twin Cities, Minneapolis, Minnesota, United States, [2]Department of Pediatrics, Blood and Marrow Transplantation, University of Minnesota Twin Cities, Minneapolis, Minnesota, United States, [3]Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota Twin Cities, Minneapolis, Minnesota, United States

Recessive dystrophic epidermolysis bullosa (RDEB) is a blistering skin disease caused by mutations in COL7A1, the gene encoding type VII collagen (C7). RDEB is characterized by chronic wounds and a high incidence of aggressive squamous cell carcinoma (SCC), the leading cause of death in this patient population. High mobility group box 1 (HMGB1) is a serum biomarker of disease severity in RDEB patients that may have a mechanistic role in promoting SCC carcinogenesis. HMGB1 is a chromatin-associated protein that has a dual role as a mediator of DNA repair in the nucleus and a damage-associated molecular pattern that stimulates the innate immune response when secreted extracellularly in response to inflammatory stimuli or cellular damage. We hypothesize that chronic nuclear depletion and excessive secretion of HMGB1 in RDEB drives carcinogenesis by promoting a positive feedback loop that further exacerbates inflammation and genomic instability. Inhibition of HMGB1 secretion using a small molecule drug, inflachromene, in keratinocytes stimulated with lipopolysaccharide results in a 5-fold reduction of pro-inflammatory cytokine IL-6 secretion ($p < 0.0001$). We conclude that inhibiton of HMGB1 in keratinocytes attenuates the inflammatory response and warrants further mechanistic study to determine whether HMGB1 is a potential therapeutic target in RDEB SCC. Future directions are aimed at interrogating keratinocyte response to DNA damaging agents (psoralen, cisplatin, γ-irradiation or UV-B radiation) in the presence and absence of HMGB1 inhibitor. Additionally, we have generated an in vitro model of RDEB using CRISPR/Cas9 gene-editing to knock out C7 in immortalized keratinocyte cell lines that will be used to elucidate the effects of HMGB1 inhibition in RDEB keratinocytes.

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Role of insulin-like growth factor 2 mRNA-binding protein 1 in basal cell carcinoma development

F. Noubissi[1], C. Harris[1], M. Hajahmed[1], C. Yedjou[2]

[1]Biology, Jackson State University, Jackson, Mississippi, United States, [2]Biology, Florida Agricultural and Mechanical University, Tallahassee, Florida, United States

An estimated 3.6 million cases of Basal Cell Carcinoma (BCC) are diagnosed in the U.S. each year with the annual cost of treatment about $4.8 billion. BCC is caused mostly by long term sun exposure. However, patients with mutation in PTCH gene and immunocompromised patients are more susceptible to developing BCC. Constitutive activation of the Hedgehog (Hh) signaling pathway is a key factor driving the development of BCC. Activation of GLI1 which is the transcription factor through which the Hh signaling is mediated is a key step in the initiation of BCC development. We previously showed that GLI1 was also regulated by Wnt signaling. The insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) which is a direct target of the Wnt/β-catenin signaling was critical in Wnt-dependent regulation of GLI1 and the regulation of GLI1 by the Hh upstream signal was IGF2BP1-dependent as well. We hypothesized that the regulation of GLI1 by IGF2BP1 was important in driving BCC development. To test our hypothesis, we used the CRISPR/Cas9 approach to knock down IGF2BP1 in UW-BCC1 cells. Two million UW-BCC1 cells were injected subcutaneously in the back of 20 immunocompromised mice (Foxn1nu). Tumor growth was monitored weekly for a total of eight weeks. Knockdown of IGF2BP1 in UW-BCC1 cells significantly reduced tumor growth in xenograft mice compared to controls ($P<0.05$). We also generated a skin specific IGF2BP1 knockout mouse model in a BCC background (Ptch1tm1Mps/J) using the Cre/LoxP system. Experimental mice (IGF2BP1-loxP+/-; K5-cre-ERT2+/-; Ptch1+/- or IGF2BP1-loxP-/-; K5-cre-ERT2+/-; Ptch1+/-) received 75mg of tamoxifen/kg for five days to inhibit IGF2BP1. They were subsequently exposed to 240 mJ/cm2 UVB irradiation, three times a week for 44 weeks. Inhibition of IGF2BP1 significantly reduced BCC development compared to the control mice that did not receive tamoxifen. IGF2BP1 appears to contribute to BCC development and might represents a novel target in the treatment of basal cell carcinoma.

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Subtype-dependent differences in genomic profiles of acral and cutaneous melanoma

D. Y. Kim[1, 3], E. I. Buchbinder[2], R. Hartman[3]

[1]Harvard Medical School, Boston, Massachusetts, United States, [2]Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States, [3]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States

Acral melanoma is commonly diagnosed in people of color and has worse prognosis than cutaneous melanoma, even when controlling for tumor stage. Here, we seek to assess differences in tumor mutational profiles of acral and cutaneous melanomas, which could potentially impact prognosis and treatment outcomes.

Mutational profiles of 1273 melanoma patients (118 acral and 1155 cutaneous) from the American Association for Cancer Research Project Genomics Evidence Neoplasia Information Exchange (v10.0) were examined by melanoma subtype (acral or cutaneous) and tumor stage (primary or metastatic). Mutation count distributions were analyzed through Wilcoxon rank sum tests and gene-specific comparisons were assessed using Fisher's exact test. For both primary and metastatic tumors, cutaneous melanoma patients had significantly more mutations than comparable acral melanoma patients ($P < 0.001$). Similarly, cutaneous melanoma patients had higher mutational rates in BRAF ($P < 0.001$) and GRIN2A ($P < 0.001$) than acral melanoma patients. In patients with metastatic disease, NRAS mutations were more prevalent in cutaneous melanoma patient than those with acral melanoma ($P < 0.05$). Cutaneous melanoma patients were also significantly more likely to have mutations in actionable ($P < 0.001$) and DNA repair genes ($P < 0.001$). Cutaneous melanoma patients had higher total mutation counts and were more likely to have mutations in DNA repair genes, both of which have been shown to predict immunotherapy response. Similarly, cutaneous melanoma patients had higher mutational rates in actionable genes, such as BRAF and NRAS, that can be targeted by FDA-approved therapies. Our results provide insight into how treatment responses and clinical outcomes could differ based on melanoma subtype.

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Tumor mutational differences of cutaneous melanoma by age

D. Y. Kim[1, 3], E. I. Buchbinder[2], R. Hartman[3]
[1]Harvard Medical School, Boston, Massachusetts, United States, [2]Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States, [3]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States

Melanoma incidence and survival are age-dependent, with older patients having both higher incidence rates and worse survival outcomes. Here, we seek to examine age-dependent differences in mutational profiles of cutaneous melanoma, which may impact melanoma prognosis and treatment-related outcomes. Mutational profiles of 2263 cutaneous melanoma patients were extracted from the American Association for Cancer Research Project Genomics Evidence Neoplasia Information Exchange (v10.0) and examined by age (< 30 years, 30-65 years, or > 65 years) and tumor stage (primary or metastatic). Mutation count distributions were compared using Wilcoxon rank sum tests and mutational frequencies of specific genes were assessed using Fisher's exact test. Patients older than 65 years had significantly more mutations than patients aged 30-65 years (Primary: P<0.05; Metastatic: P<0.001) and patients younger than 30 years (Primary: P < 0.01; Metastatic: P<0.001). Patients older than 65 years were also less likely to have mutations in BRAF than patients aged 30-65 years (Primary: P<0.05; Metastatic: P<0.001) and patients younger than 30 years (Metastatic: P<0.001). For metastatic tumors, patients older than 65 years had significantly higher mutational rates in DNA repair genes than patients aged 30-65 years (P < 0.001) and patients younger than 30 years (P < 0.01). Patients older than 65 years had high mutational burdens and mutational rates in DNA repair genes, which may influence immunotherapy outcomes. Patients older than 65 years were also less likely to have mutations in BRAF, which can be targeted by vemurafenib. By identifying differences in genomic profiles by age, our results provide insight into which therapies may be most effective for certain age groups.

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BUB1B germline variant predisposes to cutaneous melanoma and multiple other primary malignancies

M. Kazmi[1, 2], J. Terrell[2], D. Martiniuc[4], J. D. McPherson[2], M. Kiuru[2, 3]
[1]University of California Davis School of Medicine, Sacramento, California, United States, [2]Dermatology, University of California Davis Health System, Sacramento, California, United States, [3]Pathology and Laboratory Medicine, University of California Davis Health System, Sacramento, California, United States, [4]Comprehensive Cancer Center, Hereditary Cancer Program, University of California Davis, Davis, California, United States

Although several inherited melanoma predisposition syndromes have been identified, a subset of melanoma families lack pathogenic variants in known highly penetrant predisposition genes, including CDKN2A, CDK4, and BAP1. We describe a patient with multiple melanomas and other primary tumors with a germline pathogenic variant in BUB1B. A 76-year-old woman presented with a history of multiple, distinct primary tumors, including childhood thymoma, thyroid cancer, two melanomas, two breast cancers, a solitary fibrous tumor, and metastasized lung cancer. Family history included brothers with breast cancer, prostate cancer and cutaneous melanoma, maternal relatives with breast and colon cancer, and father with urinary tract cancer. Germline testing of 75 cancer risk genes, including hereditary melanoma and breast cancer genes, and additional whole-exome sequencing (WES) revealed only one germline pathogenic variant, c.2316C>G, in BUB1B. WES of melanoma revealed somatic variants, including in CDKN2A and NRAS.Immunohistochemistry of BUB1B of normal skin, nevus, and melanoma samples did not reveal significant differences in BUB1B expression levels in patient versus control tissues. BUB1B encodes a kinase involved in spindle checkpoint function, a process dysregulated in many cancer types. To date, germline pathogenic variants in BUB1B have rarely been implicated in inherited tumor predisposition. We propose that heterozygous pathogenic BUB1B variants may increase susceptibility to multiple primary tumors, including melanoma, and may explain this patient's significant history of multiple cancers. Identification of novel genes associated with hereditary cancer risk is integral for optimal genetic counseling and appropriate cancer screening in at-risk individuals.

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Fibroblast alterations during cutaneous skin cancer development

L. N. Seldin[1, 2, 3], A. Hanlon4
[1]Cell Biology, Emory University School of Medicine, Atlanta, Georgia, United States, [2]Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States, [3]VA Medical Center Atlanta, Decatur, Georgia, United States, [4]Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States

Cutaneous basal (BCC) and squamous cell carcinomas (SCC) are the most commonly occurring cancers in humans. These skin cancers are driven by DNA damage to the epidermis, the body's outermost protective barrier. Our previous studies demonstrate that DNA damaging agents activate inflammasome signaling in dermal fibroblasts, which enhances epithelial cell proliferation and plasticity in wild-type epidermis, characteristics that are associated with tumorigenesis. Strikingly, however, the role of dermal fibroblasts in cutaneous skin cancer development has not been adequately addressed. Cancer-associated fibroblasts (CAFs) are predominant in tumor stroma and have been proposed to drive the progression of many epithelial cancers, including BCC and SCC. However, the direct role of CAFs in cutaneous skin cancer initiation and progression is currently unknown. In this study, we collected primary human skin specimens to analyze alterations in CAFs of BCC and SCC tumors compared with dermal fibroblasts from normal tissue. We noted striking morphological changes in both BCC and SCC CAFs, which had redistributed and accumulated near the epithelial interface. Furthermore, CAFs showed evidence of inflammasome activation, and the overlying epithelium exhibited stem cell fate mis-specification. Notably, these characteristics are similar to those of normal skin when exposed to DNA damaging agents. These data suggest that normal fibroblasts may be coaxed into a pre-CAF-like state upon DNA damage exposure, and that CAF inflammasome signaling may promote BCC and SCC tumor progression. Future work will test the necessity and sufficiency of CAF inflammasome signaling in skin tumorigenesis using both human tumor specimens as well as cancer mouse models.

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Deconvolution of adult T-cell leukemia/lymphoma with single-cell RNA-seq using frozen archived skin tissue reveals new subset of cancer-associated fibroblast

H. Kim[1], E. Joo[2], J. Bae[3], J. Park[2], Y. Bang[4], C. Park[5], N. Gulati[6], W. Park[2]
[1]Genomic Medicine Institute, Seoul National University College of Medicine, Seoul, Korea (the Republic of), [2]Samsung Medical Center, Gangnam-gu, Seoul, Korea (the Republic of), [3]Samsung Medical Center, Gangnam-gu, Seoul, Korea (the Republic of), [4]Seoul National University Graduate School Department of Biomedical Science, Seoul, Korea (the Republic of), [5]Seoul National University College of Medicine, Seoul, Korea (the Republic of), [6]Icahn School of Medicine at Mount Sinai, New York, New York, United States

Adult T-cell Leukemia/Lymphoma (ATLL) is a rare aggressive T-cell malignancy associated with human T-cell leukemia virus type 1 (HTLV-1) infection. However, little is known about the underlying activated molecular pathways at the single cell level. Moreover, the intercellular communications between the tumor microenvironment (TME) and tumor cells in this malignancy are currently unknown. Difficulties in harvesting fresh tissue in a clinical setting has hampered our deeper understanding of this malignancy. Herein, we examined ATLL using archived fresh frozen tissue after the biopsy by single-cell RNA sequencing (scRNA-seq) with T-cell receptor (TCR) clonal analysis. Highly clonal tumor cells showed multiple activating pathways, suggesting dynamic evolution of the malignancy. Dissecting diverse cell types comprising the TME led to the identification of a novel subset of cancer-associated fibroblast which showed enriched epidermal growth factor receptor (EGFR)-related transcripts. These findings suggest a potential targeted therapeutic pathway to better treat this neoplasm.

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Epidermal integrin α3β1 is a regulator of cytokine, CSF-1, and of crosstalk from keratinocytes to dermal macrophages

W. Longmate[1], L. Wu[1], A. Martinez[1], C. DiPersio[1,2]
[1]Surgery, Albany Medical College, Albany, New York, United States, [2]Molecular & Cellular Physiology, Albany Medical College, Albany, New York, United States

As the major cell surface receptors for the extracellular matrix, integrins regulate adhesion and migration, and have been shown to drive tumor growth and progression. Our earlier findings have identified integrin α3β1 as a key regulator of the keratinocyte secretome and of the skin tumor microenvironment, indicating its potential efficacy as a therapeutic target. Previous mass spectrometric analysis of conditioned medium from immortalized keratinocytes indicated that α3β1 positively regulates colony-stimulating factor 1 (CSF-1), a secreted cytokine that is a primary regulator of macrophage differentiation, proliferation, and survival. In our current studies, we first validated keratinocyte α3β1 as a regulator of CSF-1 in vitro, as well as in two cutaneous remodeling contexts *in vivo*: skin tumorigenesis and wound healing, using epidermis-specific α3 knockout mice. Additionally, we have previously shown that the number of tumor-associated macrophages were reduced in stroma following α3 deletion from epidermal tumor cells, citing an increase in macrophage apoptosis. Consistently, in a current co-culture study we used RAW 264.7 macrophages to demonstrate that macrophage proliferation is reduced when cultured in media conditioned by keratinocytes that lack α3β1 compared to α3β1-expressing keratinocytes. Overall, our data indicate that integrin α3β1 on keratinocytes regulates the expression of CSF-1, as well as crosstalk from keratinocytes to macrophages that is likely to impact the macrophage population in wounds and tumors.

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Intrinsic anti-tumorigeneic properties of the skin mediate resistance to chemically-induced carcinogenesis in naked mole-rats

A. Mardaryev[1], I. Fatima[1,2], N. Botchkareva[2], G. Chen[2], A. Sharov[2], V. Botchkarev[2]
[1]Center for Skin Sciences, University of Bradford, Bradford, West Yorkshire, United Kingdom, [2]Dermatology, Boston University, Boston, Massachusetts, United States

Naked mole-rats (NMRs, Heterocephalus glaber) are unique long-lived mammals that possess marked resistance to cancer and other age-related pathologies maintaining a sustained healthy life-span for over 30 years. Despite remarkable longevity, there is a lack of any reported spontaneous skin cancer incidents in NMRs, including basal/squamous cell carcinoma and melanoma. To explore the suitability of the NMR skin for studying the mechanisms of epithelial carcinogenesis and cancer resistance, we applied chemical skin carcinogenesis (DMBA/TPA) protocol to NMRs and FVB mice used as controls. In contrast to mice, NMRs showed remarkable resistance to DMBA/TPA and did not develop papillomas for up to 25 weeks after treatment. Furthermore, skin grafts of the NMRs transplanted onto nude mice did not develop epithelial tumors after DMBA/TPA treatment, suggesting that NMR skin possess the unique tumor resistance properties in a tissue-autonomous manner. In contrast to mice, epidermis of the NMRs showed markedly elevated levels of the DNA repair machinery-associated genes including XPA and faster elimination of the gamma-H2AX-positive cells. In addition, RNAseq analyses demonstrated that expression levels of a number of tumor suppressor genes (BTG1, HSPB7, DACH1) show significant upregulation in the NMR epidermis after DMBA/TPA treatment, compared to mice. These data provide novel insights into fundamental mechanisms underlying cancer resistance in the skin, and enhance the innovative potential of securely establishing a place for the NMR as a model organism for studying the biology of human skin and disease resistance.

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Aspirin protects against UVB-induced DNA damage through activation of AMP kinase

H. Rahman, T. Liu, D. Grossman
University of Utah Health Huntsman Cancer Institute, Salt Lake City, Utah, United States

The anti-inflammatory and chemopreventive activities of aspirin (ASA) may be mediated through its cyclooxygenase (COX) inhibitor function. We have previously shown that ASA can protect against ultraviolet (UV) radiation-induced skin inflammation and DNA damage, but the role of inflammation in UV-induced DNA damage and the mechanism underlying ASA protection are poorly characterized. Using immunodeficient NSG mice and immunocompetent C57BL/6 mice treated with immune cell-depleting antibodies, we found that inflammation was not required for UVB-induced 8-oxoguanine (8-OG) and cyclobutane pyrimidine dimers (CPD) *in vivo*. Unlike ASA, neither its immediate metabolite salicylate nor the COX inhibitor indomethacin reduced UVB-induced 8-OG or CPD in melanocyte Melan-a or keratinocyte HaCat cells in vitro. Moreover, addition of prostaglandin-E2 (PGE2) did not reverse the protective effect of ASA on UVB-treated cells. Phosphorylation of the 5' AMP-activated protein kinase (AMPK), observed in ASA-treated cells, could be blocked by the AMPK inhibitor Compound C (Comp C). Cells treated with ASA and Comp C, however, were not protected against UVB-induced DNA damage. Finally, injection of Comp C partially reversed the protective effect of ASA on UVB-treated mouse skin *in vivo*. These studies suggest that ASA confers protection against UVB-induced DNA damage through activation of AMPK rather than COX inhibition.

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Novel chromatin-associated activity and substrates for the polarity kinase aPKC in BCC drug resistance

F. Gonzalez[2], T. Patel[1], S. Ha[3], C. Pan[2], S. Gaddam[2], A. Mirza[4], A. Oro[2]
[1]University of California Los Angeles, Los Angeles, California, United States, [2]Program in Epithelial Biology, Stanford University School of Medicine, Stanford, California, United States, [3]Harvard University, Cambridge, Massachusetts, United States, [4]University of San Francisco, San Francisco, California, United States

Basal cell carcinomas (BCCs) are GLI transcription factor dependent and one of the most common and accessible human tumors making them ideal for studying the molecular and cellular basis of tumor evolution and resistance. We previously identified a GLI nuclear resistance mechanism involving the interaction between polarity kinase atypical PKC (aPKC) and the deacetylase HDAC1. While we showed that HDAC1-dependent GLI deacetylation drives GLI away from the nuclear envelope to chromatin through the LAP2 isoform-dependent Gli1 nuclear chaperoning system, how nuclear aPKC accumulates and functions remains mysterious. Here, we find that nuclear aPKC activity is present in a subset of sensitive BCCs cells and the majority of resistant BCCs cells and requires key modifications for nuclear entry. Using nuclear phosphorylation proteomics and proximity labeling proteomics we identify the nuclear intermediate filament Lamin A/C as an aPKC nuclear target. Phosphorylated aPKC-Lamin A/C colocalize at promoter chromatin sites with Gli1-Lap2a complex and loss of phosphorylated Lamin A/C correlates with decreased transcriptional activity, while a Lamin A/C phospho-mimetic partially reverses aPKC inhibitor effects. Interestingly, inhibition of aPKC leads to decreased chromatin-associated phosphorylated Lamin A/C- and decreased Hedgehog target gene transcription, but little change in GLI or LAP2 chromatin binding, reinforcing the independence of the GLI-LAP2 and aPKC-LaminA/C signaling pathways. This work shows for the first time the chromatin-associated activities and substrates of the polarity kinase aPKC and implicates Lamin A/C as a GLI coactivator in driving tumor resistance.

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Basal-to-mesenchymal transition, a distinct BCC therapy resistance trajectory

N. Li, D. Haensel, S. Gaddam, A. Oro
Program in Epithelial Biology, Stanford University School of Medicine, Stanford, California, United States

Tumor evolution and stable resistance state identification remains the main challenge for successful targeted tumor therapies. Previously our group has identified the distinct cell state trajectories in patient advanced basal cell carcinomas (BCCs) and have identified the molecular basis, surface markers for, reversibility of, and transcription factor driver of basal to squamous cell carcinoma transition (BST). In addition to the basal and differentiated tumor states, other trajectories and stable states may also exist. Here, we define a novel molecularly and phenotypically distinct epithelial resistant trajectory characterized by co-expression of a subset of mesenchymal-like cell markers called basal-to-mesenchymal transition (BMT). BMT shares features of the previously identified tumor-specific keratinocyte (TSK) population in squamous cell carcinomas, with unique aspects representing distinct collective tissue migratory behavior. Spatial transcriptomic, transcription factor analysis, and single cell integration define how the trajectory relates to BST. Findings from this study enhance our knowledge of tumor cell state plasticity and further provide a refined roadmap outlining different transitional trajectories that could be undertaken by BCC.

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Citrullinated histone H3, a marker for neutrophil extracellular traps, is associated with poorer prognosis in recessive dystrophic epidermolysis bullosa patients with cutaneous squamous cell carcinoma.

H. Ragot[4], S. Gaucher[4, 2], M. Titeux[4], M. Bonnet des Claustres[4], J. Basset[4], M. Battistella[1], E. Bourrat[3], A. Hovnanian[4, 2, 5]
[1]Department of Pathology, Saint Louis Hospital, Paris, France, [2]Universite de Paris, Paris, France, [3]Reference Center for Genodermatoses, Saint Louis Hospital, Paris, France, [4]Laboratory of genetic skin diseases, Imagine Institute, Paris, France, [5]Department of Genetics, Necker Hospital, Paris, France

Recessive dystrophic epidermolysis bullosa (RDEB) is a severe hereditary skin disease characterized by skin and mucosa fragility with blister formation due to type VII collagen deficiency. The major complication in RDEB patients is the development of squamous cell carcinoma (SCC) leading to premature mortality. Our study aims to correlate the immune profiles of SCCs with clinico-histopathological features in a cohort of RDEB patients.
The immune cell profile was assessed by immunohistochemical analyses on 33 SCCs from 19 RDEB patients included between March 2016 and December 2021. RDEB-SCCs were subdivided into 4 groups according to the first occurrence (primary) or recurrence status, and their grading determined by clinico-histopathological aggressiveness criteria. The tumor microenvironment of high-grade primary SCCs was associated with a higher proportion of infiltrated neutrophils to lymphocytes in comparison to low-grade primary SCCs and recurrent SCCs. The analysis of neutrophils in high-grade primary SCCs revealed the presence of fibers expressing myeloperoxidase and citrullinated histone H3, a neutrophil extracellular trap marker (NET), suggesting the induction of NETosis. Finally, circulating citrullinated histone H3 levels were increased in the serum of RDEB patients with high-grade primary SCCs. This study revealed a defect in the immune response in the tumor microenvironment of RDEB-SCCs with poorest prognosis, associated with an increased infiltrated neutrophils to lymphocytes ratio and enhanced NET marker in the serum.

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JAK-STAT activation in anti-PD-1 therapy-associated granuloma annulare

R. Choi, J. Wang, W. Damsky, A. Wang, A. Galan, J. S. Leventhal
Dermatology, Yale School of Medicine, New Haven, Connecticut, United States

Granuloma annulare (GA) is a cutaneous condition that can arise idiopathically or in association with risk factors such as diabetes, microvascular damage, and more recently, the use of anti-programmed cell death protein-1 (PD-1) agents. It can be difficult to treat as the pathogenetic mechanism is still unknown, but recent research has shown Janus kinase-Signal transducer and activator of transcription (JAK-STAT) pathway activation in idiopathic GA. Furthermore, tofacitinib has been seen to induce dramatic clinical improvement in patients with sarcoidosis and generalized GA. Thus, in this case series, we investigated the clinicopathological characteristics of 3 cases of anti-PD-1 agent-associated GA. Clinically, the patients included a 67 year-old (yo) female who developed GA after 3 months of nivolumab for lung cancer, a 55 yo male who developed GA after 6 months of pembrolizumab for head and neck cancer, and a 64 yo female who developed worsening GA lesions after pembrolizumab initiation for lung cancer. All patients' cancers responded well to immunotherapy, with two achieving complete response and one with significant resolution. In all 3 cases, immunohistochemistry of the biopsy specimens showed focal activation of STAT1 and STAT3. In conclusion, we suggest that anti PD-1 agent-associated GA is a cutaneous immune adverse event that may be driven by the JAK-STAT pathway, similar to has been suggested for idiopathic GA. We provide further evidence suggesting that development of GA in the setting of anti-PD-1 agent use may portend a favorable response to immunotherapy. Future research should focus on determination of whether PD-1 blockade results in activation of helper T-cells leading to IFN- γ production and macrophage activation; other JAK-STAT dependent cytokines may play a role as well.

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Loss of Kdm6a and Trp53 drives the development of squamous cell skin cancer in mice

L. Shea[5], N. Akhave[4], L. Sutton[2], L. Compton[1], C. York[3], S. Ramakrishnan[3], C. Miller[3], L. Wartman[3], D. Chen[2]
[1]Pathology and Immunology, Washington University in St Louis School of Medicine, St Louis, Missouri, United States, [2]Medicine, Division of Dermatology, Washington University in St Louis School of Medicine, St Louis, Missouri, United States, [3]Medicine, Division of Oncology, Washington University in St Louis School of Medicine, St Louis, Missouri, United States, [4]The University of Texas MD Anderson Cancer Center, Houston, Texas, United States, [5]Medicine, The University of Alabama at Birmingham School of Medicine, Birmingham, Alabama, United States

Cutaneous squamous cell carcinoma (cSCC) has among the highest mutation burdens of all cancers, reflecting its association with the mutagenic effects of ultraviolet light. Though mutations in established, cancer-relevant genes like TP53 and NOTCH1 are prevalent in cSCC, they are tolerated in clinically normal skin and are insufficient to cause skin cancer. Whether epigenetic regulators cooperate in the development of squamous cell carcinoma is just starting to be defined. KDM6A encodes a histone H3K27me2/me3 demethylase that is frequently mutated in cSCC and other cancers. We found that 60.7% (68/112) cSCC tissues have low or absent KDM6A expression compared to 100% (15/15) of normal skin with moderate to strong expression by immunohistochemistry, consistent with its potential role as a tumor suppressor in cSCC. To test this, we generated mice with keratinocyte-directed deficiency of both Kdm6a and Trp53, which developed cSCC with complete penetrance. Epidermal Trp53 deficiency alone rarely generated tumors, and Kdm6a-deficient mice never developed skin tumors. Male mice with combined Kdm6a and Trp53 deficiency tended to develop squamous cell carcinoma more rapidly than female mice (median latency 276 vs 362 days, p=0.054). Resulting tumors were subjected to whole exome sequencing, revealing recurrent mutations in Ncstn and Vcan, both of which are frequently mutated in human cSCC (7% and 26% respectively) and may be cooperating events in tumorigenesis. The results of this study establish the role of the epigenetic regulator Kdm6a as a relevant tumor suppressor in cutaneous squamous cell carcinoma pathogenesis.

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POF1B: A potential novel squamous cancer biomarker with implications in cell adhesion and migration

H. Matar[1], E. Guven Maiorov[1, 2], K. Mahmood Hameed[1], W. Wilson[1, 2], R. Guo[1, 2], R. Ponnamperuma[1], N. Sakakibara[1], K. King[1], W. C. Weinberg[1]
[1]*US Food and Drug Administration, Silver Spring, Maryland, United States,* [2]*IOTF, National Cancer Institute, Bethesda, Maryland, United States*

ΔNp63α, a p53 family member, is overexpressed in squamous cell carcinomas (SCC) of the skin, lungs, and head and neck. Mimicking this overexpression in an *in vivo* skin grafting model, we previously reported that long-term lentiviral over-expression of ΔNp63α in v-rasHa transduced primary murine keratinocytes results in 100% malignant conversion to SCC. We have since established that ΔNp63a-enhanced expression of c-Rel, a member of the NF-κB/Rel transcription factor family, is critical to malignant conversion in this model. Using microarray to identify downstream mediators of c-Rel in the context of ΔNp63α/v-rasHa transduced keratinocytes (+ sh-c-Rel vs control shRNA), we identified premature ovarian failure 1B (POF1B), a novel gene with limited characterization. We confirmed the regulation of POF1B by c-Rel in primary keratinocytes at the mRNA and protein levels. POF1B expression is associated with many cancers, including head and neck SCC, and is enhanced in colorectal cancers [see Human Protein Atlas/data compiled from The Cancer Genome Atlas (TCGA)]. It is reportedly expressed almost exclusively in epithelial tissues and has been shown to interact with non-muscle actin filaments and to co-localize with desmoplakin at cell-cell contacts. We utilized an in-silico method, PRISM, to model the 3D structures of POF1B interactions with actin and desmoplakin. To gain greater insight into its potential functions we utilized an in-silico method, HMI-PRED, that predicts protein-protein interaction partners based upon interface mimicry using known crystal structures and models these into pairwise interaction complexes. HMI-PRED revealed >100 unique POF1B interaction partners. Ongoing work is incorporating POF1B knockdown/overexpression in functional assays to elucidate POF1B's role in adhesion, migration and proliferation, as it pertains to HMI-PRED predicted interactions.

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Luminate - a non-invasive, high-throughput genomic test for assessment of UV damage in human skin

P. Tripathi, Z. Dhali, H. Sokkam, J. Rock, T. Holscher, J. Whitaker, M. D. Howell
DermTech Inc, La Jolla, California, United States

Extensive ultraviolet (UV) exposure has been shown to damage DNA leading to genetic mutations, immune suppression and photoaging. Moreover, non-melanoma skin cancer such as basal, and squamous cell carcinoma arise from the colonization of cells bearing mutations caused by UV exposure. Luminate combines non-invasive skin sample collection using the DermTech Smart StickerTM and the high-throughput, ultrasensitive, multiplexed detection of low-frequency mutations based on MALDI-TOF mass spectrometry (MS). The test enables detection of twenty-five low-frequency UV-signature mutations across four genes (TP53, CDKN2A, NOTCH1, and NOTCH2) to assess UV exposure and potential skin damage in normal-appearing skin. This study evaluated UV-induced mutations in skin samples collected from different sites on the face (sun-exposed), including the right and left temple, cheek, forehead, central forehead, and nose in a cohort of forty-five subjects with varying race, age, sex, and skin types. Of the different facial sites tested, the central forehead and nose exhibited the highest frequency of mutations, followed by the left then right side of the face. The mutation detected among these facial sites strongly correlates variant allele frequency (VAF) with age. Our assay demonstrated that the average number of mutations on facial skin sites increased with both age and cumulative UV exposure. Additionally, patients' demographics as well as both a personal and family history of cancer were utilized along with the VAF to provide a "Risk Score" to quantify the UV damage of the "healthy-looking skin." The study was performed with 1ng of gDNA, establishing the utility of the 'Smart Sticker TM' to non-invasively collect skin to assess mutation detection and skin damage. This sensitive and multiplexed non-invasive genomic test not only facilitate early detection of UV damage but will provide valuable clinical treatment guidance to mitigate skin damage in normal-appearing skin.

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DermTech smart stickers can non-invasively detect RNAs that are associated with non-melanoma skin cancer

L. Vo, R. Ai, M. Lee, T. Holscher, J. Rock, B. Jansen, L. Clarke, M. D. Howell, J. Whitaker
DermTech Inc, La Jolla, California, United States

The most common types of skin cancers are non-melanoma skin cancers (NMSC), including both: basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). NMSC's primarily form on sun exposed skin areas and are responsible for >15,000 deaths each year in the US alone. Current diagnosis of NMSC relies on an in-depth visual assessment of suspicious lesions followed by a surgical skin biopsy for histopathologic review. This study investigated whether non-invasive collection of skin tissue and subsequent genomic analysis could properly classify NMSC. Adhesive skin collections kits were used to collect suspicious lesions from 94 patients with BCC, 87 patients with SCC, and 173 patients with non-cancerous skin diseases (NCSD). Whole transcriptome RNA sequencing identified differentially expressed genes which were determined by comparing BCC and/or SCC with NCSD using multiple comparisons. Machine-learning identified a subset of 161 genes that captures the full whole transcriptomes classification power to differentiate NMSC from NCSD. Targeted RNA AmpliSeq analysis of these 161 genes was then performed on a fresh set of 108 patients with BCC, 89 patients with SCC, and 169 patients with NCSD. Of these, 30 genes were significantly (fold change >2; multiple-testing adjusted P<0.05) increased in NMSC compared to NCSD while 16 genes were lower in NMSC (fold change >2; multiple-testing adjusted P<0.05). The validated non-invasive identification of RNAs that are differential expressed between NMSC and NCSD, creates the opportunity for the development of a multi-gene machine-learning algorithm that uses "smart stickers" to diagnose NMSC without the need for surgical biopsy.

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Defining a bi-stable network switch that governs stem cell self-renewal and differentiation in squamous cell carcinoma

S. Hoang-Phou[1], M. Abbruzzese[1], A. Sastre-Perona[1, 3], Z. Ying[4], S. Beronja[4], M. Schober[1, 2]
[1]*The Ronald O. Perelman Department of Dermatology, NYU Langone Health, New York, New York, United States,* [2]*Department of Cell Biology, NYU Langone Health, New York, New York, United States,* [3]*Experimental Therapies and Novel Biomarkers in Cancer, Hospital Universitario La Paz, Madrid, Madrid, Spain,* [4]*Fred Hutchinson Cancer Research Center, Seattle, Washington, United States*

Stem cell – like tumor propagating cells self-renew to drive clonal expansion or differentiate into post-mitotic cells without tumorigenic potential in squamous cell carcinomas. This fate choice is governed by a transcriptional network comprised of SOX2-PITX1-TP63 driven self-renewal and KLF4 dependent differentiation circuits. Yet, how stem cell – like tumor propagating cells switch from self-renewal to differentiation remains elusive. Here, we report that this cell fate choice is governed by a bi-stable Klf4 enhancer that is occupied by the transcription factor SOX2 in self-renewing or KLF4 in differentiating squamous cell carcinoma cells, dependent on whether SOX2 is phosphorylated. We will present proteomic, transcriptomic, chromatin immunoprecipitation sequencing, chromatin conformation capture sequencing, and conditional gene targeting data leading to the discovery of a molecular mechanism that governs self-renewal, differentiation, squamous cell carcinoma promotion and growth. The identified mechanism provides a novel path towards the future development of differentiation therapies that could inhibit squamous carcinogenesis in patients.

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Mutations in the T-cell receptor antigen-binding region of malignant cells in mycosis fungoides coincide with clinical and histological progression

L. Gleason, N. Nikbakht
Department of Dermatology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States

Chronic T-cell antigen stimulation has long been suggested to contribute to carcinogenesis in mycosis fungoides (MF), but evidence of this phenomenon is lacking at the genetic level. Furthermore, mutations that alter malignant T-cells' antigen specificities have not been previously described in MF. Here, we report evidence from two patients whereby the presence of single base substitutions (SBS) in the antigen-binding regions of the malignant clones' T-cell receptors (TCR) coincide with changes in clinical behavior. A review of longitudinal high-throughput sequencing data obtained from lesional skin biopsies identified two patients with unique SBS in the antigen-binding region of malignant T-cell clones. In all biopsies, each predominant TCRβ clone encoded an uninterrupted chain of amino acids. While the SBS in both cases resulted in a single amino acid change, the V, D, and J family gene assignments remained identical. These observations imply mutations altering the antigen specificities of the malignant clones. The mutations coincided with different clinical and pathological behavior in both cases, including new-onset tumor formation with large cell transformation in the first case, and resistance to localized radiation and topical treatment in the second case. Furthermore, all deducted SBS mutations were identified within the ultraviolet (UV) signature according to the COSMIC Mutational Signatures collaboration. These cases provide evidence for UV-induced alterations in the antigen-binding regions of the malignant TCRs that parallel clinical and histological progressions in MF. Our observations can generate hypotheses about the role of stimulation by antigen in lymphomagenesis.

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Non-invasive molecular analysis for CTCL detection and differentiation from other skin conditions with adhesive smart sticker skin sample collection.

R. Cedeno, J. Rock, T. Allen, L. Clarke, B. Jansen, Z. Yao
DermTech Inc, La Jolla, California, United States

Cutaneous T cell lymphoma (CTCL) is a complex and heterogenous disease with a range of pathogenic, diagnostic, prognostic and therapeutic courses. Broadly, CTCL is characterized by cutaneous infiltrates of aberrant monocolonal T-lymphocytes. Clinically and histopathologically, CTCL often mimics psoriasis, eczema and other benign dermatoses. Diagnosis of CTCL is often delayed by 4-6 years from the time lesions initially appear, and typically requires multiple skin biopsies and blood tests. In this study, we employed non-invasive adhesive patches to collect samples from lesional skin of CTCL (n=12) and psoriasis (n=10) patients as well as non-lesional skin from healthy (n=12) volunteers. From these samples, we derived total RNA, generated cDNA, and subjected this material to qPCR gene expression analysis for 14 genes for purposes of detecting CTCL and distinguishing it from psoriasis. Of the 14 genes in our panel, 6 differentiated CTCL from psoriasis, as defined by statistically significant ($p < 0.05$) differential gene expression. Four of 14 genes involved in T-cell signaling and function in our panel (including 2 of the 6 in the CTCL vs. psoriasis statistically significant subset) reliably differentiated CTCL from healthy non-lesional skin ($p < 0.05$), and an additional 4 showed trends approaching statistical significance, highlighting the importance of repeating this study on larger cohorts. Overall, these results indicate the potential of applying a non-invasive genomic test for CTCL detection and differentiation from inflammatory dermatoses such as psoriasis. Such a test could obviate the need for biopsies and other invasive testing, allow earlier detection of CTCL, and make interventions and treatments more cost-effective.

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Cellular and molecular profiling of early-stage mycosis fungoides in comparison to parapsoriasis and atopic dermatitis reveals disease-specific biomarkers

N. Alkon[1], M. Drach[1], C. Bangert[1], H. Kurz[1], L. Shaw[1], G. Stingl[1], W. Weninger[1], M. Farlik[1], C. Jonak[1], P. M. Brunner[1, 2]
[1]*Medizinische Universitat Wien, Wien, Wien, Austria,* [2]*Icahn School of Medicine at Mount Sinai, New York, New York, United States*

Primary cutaneous T-cell lymphomas (CTCL) are a group of incurable extranodal non-Hodgkin lymphomas that usually develop from skin-homing CD4+ T cells, with mycosis fungoides (MF) as their most frequent entity. Due to clinical and histopathological similarities to benign inflammatory conditions, it takes a median of 3 years from the initial appearance of a skin lesion until a definitive diagnosis of MF can be made, and there are currently no specific diagnostic biomarkers available. To better define cellular and molecular characteristics of CTCL in comparison to its potential imitators, we profiled skin biopsies of early MF vs. atopic dermatitis (AD) and small-patch parapsoriasis patients by using single-cell RNA sequencing. MF was characterized by a single expanded T-cell clone, constituting 17.3-53.6% of all TCR+ cells. These clones were deficient in CD7 expression, in line with a lymphomatous phenotype, and overexpressed LGALS3, PGK1, IL26, OSTF1, and LDHA in comparison to polyclonal T cells. By contrast, parapsoriasis, similar to HC and AD, showed a generally polyclonal T-cell receptor repertoire. As a distinguishing feature from MF and parapsoriasis, AD harbored so-called "Th2A" cells, an CD3+CD4+IL17RB+CRTH2+ IL13-expressing cell type previously associated with allergic sensitization and tissue-resident disease memory formation in atopic individuals. By contrast, early MF lesions showed an overall type 1 immune skewing, while parapsoriasis samples did not reveal consistent gene regulation across samples. In sum, we found Th2A cells to specifically distinguish AD from early MF and parapsoriasis samples, with the presence of noticeable clonal T-cell expansion only in MF lesions.

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Gene expression changes in sezary syndrome patients following mogamulizumab treatment refines biomarker and immune profile

A. Davis, H. K. Wong
Dermatology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States

Sezary syndrome (SS) is the leukemic variant of CD4+ cutaneous T-cell lymphoma. Mogamulizumab is an FDA-approved monoclonal antibody targeting surface CCR4 on SS cells. We've previously described highly expressed SS biomarker genes and altered cytokine gene expression that favors a Th2-immune phenotype. Here we evaluated mogamulizumab's efficacy in reducing malignant T-cells, decreasing SS biomarker gene expression, and modulating cytokine genes to gain insight into cytologic and functional immune changes following treatment. We analyzed peripheral blood mononuclear cells (PBMC) from 11 healthy donors (HD) and 6 SS patients that met CD4+/CD26- and CD4+/ CD8+ diagnostic criteria. After mogamulizumab treatment, CD4+/CD26- T-cells decreased and the CD4+/CD8+ ratio normalized in all patients. Analysis of SS biomarker genes in pretreatment PBMC by qRT-PCR showed significant overexpression of PLS3, TWIST1, KCNK1, TOX, DNM3, and GATA3 compared to HD. Post-treatment, 100% of SS patients showed significant reduction in all 6 biomarker genes and expression levels were similar to normal in most genes. Only PLS3 and KCNK1 remained significantly overexpressed compared to HD. Most SS patients showed significantly increased expression in STAT4 and IFNG following treatment (83% and 67%, respectively). Cytokines IL17A, IL4, and IL10 showed reduced expression in 67% of patients following treatment. IL13 expression was reduced in 50% of patients. IL2 expression was increased in 33% of patients and significantly correlated with STAT4 expression ($r = 0.8217$, $p = 0.0010$). In conclusion, mogamulizumab demonstrated effective reduction of the number of malignant T-cells in CTCL, rapidly downregulated SS biomarker and Th2-associated cytokine gene expression, and increased expression of IFNG and STAT4. This study provides further detailed molecular responses during mogamulizumab treatment and identifies cytokine changes that may reflect meaningful functional immune alterations. Together, these markers could be important in classification of patient disease and response.

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The endogenous deaminase APOBEC3A readily localizes to the nucleus of cultured keratinocytes

S. Poojan, T. Chellappagounder, P. H. Park, A. P. South
Dermatology & Cutaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States

Members of the APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide) family of deaminases have been identified as potent mutagens in squamous cell carcinoma arising across all body sites. Previous work has shown that tissue damage-driven squamous cell carcinoma (SCC) arising in the skin of patients with the rare blistering genodermatoses recessive dystrophic epidermolysis bullosa (RDEB) are dominated by APOBEC-driven mutations as well as high mRNA levels of APOBEC3A. Current literature reports APOBEC3B (A3B) and not APOBEC3A (A3A) is capable of localizing to the nucleus of mammalian cells resulting in the notion that A3B and not A3A generates somatic mutations in SCC. In the current work, we overexpressed A3A and A3B and compared nuclear localization in HEK293 and HeLa cells as well as primary epidermal keratinocytes. Immunofluorescent microscopy of HEK293 and HeLa cells showed predominantly cytoplasmic A3A and nuclear A3B after transfection. In contrast, localization to the nucleus was readily detected for both A3A and A3B in primary keratinocytes. In related work, we were unable to demonstrate in vitro deaminase activity using purified A3A protein alone while total cell lysates from overexpressing cells showed clear deaminase activity using a fluorophore-based oligonucleotide assay, suggesting that A3A requires co-factors for deaminase activity. Mass spectrometry to understand the A3A interactome in HEK293 cells revealed a predominantly cytoplasmic repertoire of binding partners with a few exceptions: a hnRNP, a ribonuclear protein with stress-induced nuclear translocation and described effects on splicing and translation in other cancer settings, was identified in the top 50 proteins enriched for APOBEC3A binding. Overall, these data demonstrate APOBEC3A is capable of nuclear localization in primary keratinocytes and a likely mediator of mutagenesis in SCC.

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Blood-borne exosomes as biomarkers in head and neck squamous cell carcinoma (HNSCC)

A. N. Calder[2, 4], B. L. Hill[1], Y. Guo[1], A. Linnenbach[1], U. Martinez-Outschoorn[5], J. Curry[4], A. P. South[1, 4], A. Luginbuhl[4], L. Harshyne[3], M. Mahoney[1, 4]
[1]*Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States,* [2]*Drexel University College of Medicine, Philadelphia, Pennsylvania, United States,* [3]*Cancer Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States,* [4]*Otolaryngology-Head and Neck Surgery, Thomas Jefferson University, Philadelphia, Pennsylvania, United States,* [5]*Medical Oncology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States*

The strongest risk factors for developing HNSCC are human papillomavirus infection and alcohol/tobacco use. The latter gives rise to a clinically aggressive tumor with a poor prognosis, creating an urgent need for effective screening strategies. Exosomes, or small extracellular vesicles (sEV), and their content are emerging as potential cancer biomarkers. Here, CD9/63/81 positive sEV were isolated from circulating plasma of HNSCC patients and healthy volunteers by immunoaffinity purification and characterized by Western blot and Nanoparticle Tracking Analysis. sEV were subjected to Luminex Multiplex profiling of 12 well-established cancer-specific antigens. Several known cancer biomarkers were detected at high levels on the surface of HPV(-) HNSCC sEV, including CA125, ß-HCG, and HE4, of which HE4 was confirmed by Western blotting. Interestingly, HE4 is a protease inhibitor that may serve to protect sEV from degradation. miRNet target analysis revealed that several miRNAs target HE4, such as miR-146a-5p. KEGG pathway analysis determined that these miRNAs had the highest number of shared targets in pathways in cancer. Additional GO:BP pathway analysis showed that these miRNAs also had numerous shared targets in regulation of DNA binding. qPCR and miRNAseq of blood-borne sEV showed low levels of miR-146a in HPV(-) HNSCC. Collectively, this data demonstrates a correlation between HE4, miR-146a, and the aggressiveness of HPV(-) HNSCC. These combined studies suggest sEV and their components, including HE4, could be a source of potential cancer biomarkers for HNSCC that could provide improvements in diagnosis and treatment.

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The impact of aging on murine basal cell carcinoma development

M. Grachtchouk[1], E. A. Pedersen[1], P. W. Harms[1, 2], A. Hoover[1], D. Pyrozhenko[1], A. Alam[1], N. Lingam[1], A. A. Dlugosz[1, 3]
[1]*Dermatology, University of Michigan, Ann Arbor, Michigan, United States,* [2]*Pathology, University of Michigan, Ann Arbor, Michigan, United States,* [3]*Cell & Developmental Biology, University of Michigan, Ann Arbor, Michigan, United States*

Basal cell carcinoma (BCC) is the most common skin malignancy and most frequently occurs in patients over 70 years of age. Dysregulated Hedgehog (Hh) signaling and consequent activation of GLI transcription factors is a molecular hallmark of human BCC and drives BCC-like tumor development in genetically engineered mice, which are typically studied at a young age. We examined the impact of aging on BCC tumorigenesis in young versus aged cohorts of Lgr6-CreER;R26-LSL-rtTA;tetO-GLI2A mice, in which topical 4-OHT treatment activates Cre function and rtTA expression in Lgr6+ cells and their progeny, and treatment with doxycycline leads to expression of a tetO-driven activator form of GLI2. Transgene expression was induced in 37 young (7 weeks) and 97 aged (22-24 months) mice, and tumors arising on ears were monitored using weekly imaging for up to 8 weeks after first tumor appearance. Young mice developed significantly more tumors than aged mice (58.3% of young mice developed >20 tumors compared to 4.0% of aged mice, P<0.0001), and the time from transgene activation to tumor detection was significantly shorter in younger mice (mean 31.7 +/- 1.6 (SEM) days in young mice vs 42.6 +/- 1.5 (SEM) days in aged mice, P<0.0001); however, once tumors appeared there was no detectable difference in average tumor growth rate. Intratumor heterogeneity was detected histologically in nodular BCCs arising in both groups of mice, and preliminary immunostaining did not reveal notable differences in transgene expression, proliferation, or apoptosis between young and aged mice. Taken together, our findings did not uncover an age-related enhancement of BCC tumorigenesis in this animal model. These results are in keeping with the notion that the high incidence of BCCs in elderly patients likely involve factors, such as chronic UV exposure, that are beyond the intrinsic changes that occur during chronological aging.

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Identification of germline pathogenic mutations in patients with high-frequency basal cell carcinomas

A. Chiang[1], G. Swaminathan[1], V. Hua[1], W. Chan[1], H. Do[1], I. Bailey[1], K. Rieger[1], C. Curtis[2], A. Oro[1], J. Tang[1], K. Y. Sarin[1]
[1]*Dermatology, Stanford University School of Medicine, Stanford, California, United States,* [2]*Medicine and Genetics, Stanford University School of Medicine, Stanford, California, United States*

Basal cell carcinoma (BCC) is the most common malignancy worldwide with more than 4 million new cases annually in the US. Though BCCs develop from UV radiation, host genetics also play a role, as demonstrated by patients with Basal Cell Nevus Syndrome (BCNS), who carry germline PTCH1 mutations. Genome-wide association studies have also identified common genetic polymorphisms associated with BCC. Interestingly, frequent BCC development has been associated with risk of solid tumor malignancy. This study investigates germline variants that contribute to development of high-frequency BCCs (hfBCCs) in patients without BCNS. We performed germline whole genome sequencing in 88 patients with hfBCCs, defined as ≥6 in a 10-year period (mean 14.9 BCCs, range 6-98). 27.7% of hfBCC patients reported an internal malignancy. On average 2.2 pathogenic or likely pathogenic mutations per patient (range 0-11) were identified by ClinVar or InterVar. Recurrent mutations were seen in DNA repair, tumor suppressor, immune regulation, and pigmentation genes. Highly penetrant mutations included an EPHB2 variant, associated with prostate and brain cancer, in 5.7% of our cohort (enriched by 10.7-fold in comparison to Exome Aggregation Consortium Non-Finnish European (ExAC NFE) population frequency). In addition, 3.4% of patients had mutations in PADI3 (2.2-fold increase compared to ExAC NFE), associated with uncombable hair syndrome. Notably, 80% of patients with EPHB2 variant and 67% of patients with PADI3 mutations had a history of internal malignancy. Additional gene mutations identified include TYR, PRKN, MITF, EYS, JAK2, MLH3, and CHEK2. Our findings identify germline pathogenic mutations, some present in several patients with internal or hematopoietic cancers. We are currently exploring the functional implications of these genes in cell line assays to elucidate how they play a role in cancer susceptibility and development.

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Determinants of epithelial morphogenic change during oncogenic transformation

S. Yun, V. Greco
Yale School of Medicine, New Haven, Connecticut, United States

Aged, healthy skin contains cells with cancer-associated mutations yet remains aphenotypic, suggesting flexible management of oncogenic stress during homeostasis. Our lab found oncogenic β-catenin expression in follicular epidermis induced aberrant growths that regressed. Although a hallmark of epithelial derived tumors is loss of homeostatic tissue organization, the tissue not only resisted malignant transformation, but also returned to equilibrium. However, the molecular and cell state determinants of epithelial morphogenic change during cancer initiation due to oncogenic β-catenin remain unclear. As such, we sought to track β-catenin function in mutant cells by directly visualizing its localization to the cortex as a junctional component and the nucleus as a Wnt effector. This required longitudinally resolving mosaic expression of tagged oncogenic β-catenin in live animals. Thus, we established a system that inducibly expressed GFP tagged β-cateninΔ90 in adult murine skin, imaging over time with two-photon microscopy. As expected, aberrant growths from mutant interfollicular epidermis invaginated into dermal collagen before becoming eliminated. β-catenin intensity in stem cell nuclei positively correlated with growth formation, linking this molecular function to morphogenic change. As recent studies show differentiated cells also mediate epidermal disruption, we investigated whether differentiating stem cells, roughly 40 percent of all stem cells, could provoke a similar phenotype. These cells failed to yield growth even 30 days post-induction, suggesting the cell state context of mutation determines architectural outcome. Mutant differentiating stem cells also had little to no nuclear β-catenin. Attempting to rescue the growth phenotype, we knocked down E-cadherin, reasoning that cell-cell adhesion loss promotes tumor development and decreasing the cortical sink would increase nuclear β-catenin. Curiously, though nuclear levels rose, no growths formed. Collectively, these findings indicate coordination between molecular function and cell identity are necessary to mediate tissue disruption due to oncogenic β-catenin.

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Single-cell transcriptomics links cellular origins of malignant T cells to the tumor immune landscape in cutaneous T cell lymphoma.

X. Liu[1], S. Jin[2], S. Hu[2], F. Bai[2], Y. Wang[1]
[1]*Peking University First Hospital, Beijing, Beijing, China,* [2]*Peking University, Beijing, Beijing, China*

Cutaneous T cell lymphoma (CTCL) represents a heterogeneous group of non-Hodgkin lymphoma distinguished by the presence of clonal malignant T cells. The heterogeneity of malignant T cells and the complex tumor microenvironment remain poorly characterized. With single-cell RNA analysis, paired V(D)J sequencing and bulk whole-exome sequencing on 19 skin lesions from 15 CTCL patients, we deciphered the intra-tumor and inter-lesion diversity of CTCL patients and proposed a multi-step tumor evolution model. The activation/proliferation status of malignant T cells determined the heterogeneity of CTCL lesions, which was found to be related to patient prognosis. We identified the mono-clonal nature of malignant T cells and proposed a model of a multi-step subclonal evolutionary process, deciphering the inter-lesion diversity of CTCL patients. We further established a subtyping scheme based on the molecular features of malignant T cells and their pro-tumorigenic microenvironments: the TCyEM group, demonstrating a cytotoxic effector memory T cell phenotype, showed more M2 macrophages infiltration, while the TCM group, featured by a central memory T cell phenotype and adverse patient outcome, was infiltrated by highly exhausted CD8+ reactive T cells, B cells and Tregs with suppressive activities. Distinct molecular signatures, clinical behaviors, and tumor microenvironments were identified in the two groups. Our results establish a solid basis for understanding the nature of CTCL and pave the way for future precision medicine for CTCL patients.

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Defining the role of innate immune cells in cancer immunoediting in epidermal neoplasms via longitudinal, intravital imaging

X. Fan, D. Roop
Dermatology, University of Colorado Anschutz Medical Campus Bookstore, Aurora, Colorado, United States

Cancer immunoediting is a dynamic process whereby the immune system can both constrain and promote tumor development, which proceeds through three phases termed elimination, equilibrium, and escape. Although the cancer immunoediting process has set the foundation for understanding the immune system's dual roles on cancer cells and establishing the basis for revolutionary cancer immunotherapies, very few studies have captured immunoediting in its early stages due to lack of proper mouse models. To address this deficiency, we developed a novel mouse model for fluorescent tracing of somatic, epithelial transformation to study the role of immunoediting in epithelial cancer development. For the first time, our model provided the direct visualization of epithelial cancer development and allowed the observation of all three phases of cancer immunoediting. Using this model, we observed that innate immune deficiency permits rapid tumor growth whereas early transformed cells undergo elimination or equilibrium in immunocompetent mice, indicating innate immune cells contribute to the immunoediting process in the skin. To further understand the mechanisms driving innate immunoediting in early transformed epithelial cells, we utilized a high-throughput RNA sequencing (RNA-seq) technology in our studies to measure genome-wide gene expression changes. Our preliminary data showed that the transition from equilibrium lesions to escaped tumors involves the upregulation of TGFβ signaling in escaped tumors. The increased levels of TGFβ signaling convert activated NK cells into intermediate type 1 innate lymphoid cells that cannot inhibit tumor growth. Further studies will be performed to identify the key immune cells that are required for innate immunoediting in early epithelial transformation. Altogether, our work provides novel insights into innate immunoediting in early transformed epithelial cells and opens up the possibility in cancer treatment including targeting TGFβ signaling to limit cancer development.

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Epidermis-intrinsic transcription factor Ovol1 promotes epidermal and immune homeostasis against atopic dermatitis-like skin inflammation

Z. Chen, M. Dragan, X. Dai
Biological Chemistry, University of California, Irvine, Irvine, California, United States

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease characterized by skin barrier dysfunction and over-activated immune response. Studies using mouse disease models and human patient samples have prompted clinical trials leading to finding new treatment (e.g., biologics targeting IL-4) for AD. However, many patients do not respond well to the current treatment, and a comprehensive understanding of AD pathogenesis is still lacking. OVOL1, encoding a transcriptional repressor, has been identified by GWAS studies as an AD risk locus, but the function of OVOL1 in AD is unclear. In this study, we show that skin epithelia-specific deletion of Ovol1 in mice results in exacerbated AD-like skin inflammation induced by house dust mites and staphylococcal enterotoxin B, two agents highly relevant to human AD pathogenesis. Ovol1 deficiency in keratinocytes compromises skin barrier robustness and promotes the accumulation of dermal γδT cells, which then enhance the skin infiltration of neutrophils and dendritic cells and aggravate Th2 and Th17 immune responses in skin. Mechanistically, we identify Krt14 (epidermal basal keratin) and Aqp3 (transporter of hydrogen peroxide, a reactive oxygen species) as potential direct targets of Ovol1, and detect elevated Keratin 14 expression and hydrogen peroxide levels in Ovol1-deficient epidermis. Furthermore, we provide functional evidence that elevated IL-1 signaling due to barrier dysfunction is required for the Ovol1 deficiency-induced exacerbation in skin inflammation and dermal γδT cell accumulation. Collectively, our study uncovers a crucial transcription factor that promotes both epidermal and immune homeostasis against AD-like inflammation, and implicates the utility of targeting OVOL1-associated cellular and molecular components in the therapeutic intervention of AD.

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Using in vitro human skin equivalents to understand the complexities of skin ageing and identify therapeutic interventions to restore skin health

L. Smith[1], L. Costello[1], E. Low[2], S. Miwa[2], G. Alimohammadiha[2], T. Von Zglinicki[2], C. Bascom[3], S. Przyborski[1, 4]
[1]Department of Biosciences, Durham University, Durham, Durham, United Kingdom, [2]Ageing Biology Laboratories, Newcastle University Biosciences Institute, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom, [3]The Procter & Gamble Company, Cincinnati, Ohio, United States, [4]Reprocell Europe Ltd, Glasgow, Glasgow, United Kingdom

The ageing global demographic constantly reinforces the need for more age focussed research, in order to better support the population to live healthier for longer. As the interface between the human body and the external environment, the experience of skin is unique, being subjected to both internal and external ageing influences, leading to pathological changes in structure/function. Currently, there are few in vitro ageing skin equivalents to undertake this research, and even fewer which account for both ageing factors. The presence of senescent cells within the dermis is well established, but what is their role in influencing epidermal behavior and morphology and how do they interact with intrinsically aged cells? Modifying an established in vitro human skin model which utilizes scaffold technology, we have combined senescent cells with various primary skin cell populations to recapitulate the structure and function of aged human skin and better understand the role of senescent cells. Adding senescent cells at varying frequencies to young models results in altered cytokine, MMP and TIMP production as expected, indicating a senescence associated secretory phenotype, and a decrease in epidermal markers such as lamin B1 and p63. More recent work has involved adding senescent cells to models created entirely from populations from aged donors, in order to more accurately recapitulate the complexity of aged human skin. These models represent a more physiologically relevant platform for use in a range of age-related applications, such as exploring the mechanisms involved during skin ageing and assessing compounds for senotherapeutic activity in skin, with the aim of alleviating some effects of the ageing process and restoring some skin health.

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Profiling immune network in a case of regressing Rosai-Dorfman disease, a rare histiocytic dermatosis

K. Chung, J. Hwang, D. KIm
Department of Dermatology and Cutaneous Biology Research Institute, Yonsei University College of Medicine, Seodaemun-gu, Seoul, Korea (the Republic of)

Rosai-Dorfman disease (RDD) is a rare and heterogeneous histiocytic disorder characterized by myeloid cell proliferation, accompanying infiltrates of mixed inflammatory cells. The pathophysiology of this chronic inflammatory disease remains poorly understood presenting therapeutic challenges. Here, we report the immune profile of a case of a 47-year-old female patient previously diagnosed as RDD using single cell RNA sequencing(scRNA-seq). The patient first presented with disfiguring nodular skin lesions with leonine face. Workup, including immunohistochemical staining for S100 and CD68, confirmed RDD without extensive nodal involvement. Systemic and topical glucocorticoids failed to improve symptoms, but dapsone rapidly improved subjective symptoms and flattened the lesions. Nonetheless, she had residual nodular lesions without further improvement even after half a decade. To investigate factors leading to persistent inflammation, we thoroughly profiled the infiltrated cells using scRNA-seq by comparing the lesional (n=7,965 cells) and non-lesional (n=1,027) skin. Increased number of inflammatory cells, fibroblast and vascular cells were found in the lesion. Subset of CD45+ expressing cells further identified that infiltrates consisted of B cells, CD4 cells and cytotoxic lymphocytes with scarce infiltration of macrophages in the regressing condition. Cell-to-cell interaction analysis revealed that CXCL12-CXCR4 was a significant communication pathway ($P < 0.01$). Fibroblasts, endothelium, and pericytes were important sources of CXCL12, and most T/B lymphocytes and myeloid cells expressed CXCR4. Immunofluorescence confirmed CXCR4+ immune cells surrounding CXCL12+ stromal cells. Therefore, this study suggests that targeting this axis may be an important strategy for controlling persistent lymphoplasmacytic inflammation in chronic granulomatous inflammation.

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Impact of local iron overload on crosstalk and phenotypes of immune and tissue cells in skin

M. Torregrossa[2], J. C. Simon[1, 2], S. Franz[2]
[1]Universitatsklinikum Leipzig Klinik und Poliklinik fur Dermatologie Venerologie und Allergologie, Leipzig, Sachsen, Germany, [2]Department of Dermatology, Universitat Leipzig, Leipzig, Germany

This project focuses on elucidating the pathogenic effects of iron overload due to erythrocyte extravasation in the skin on the immune-tissue cell crosstalk leading to lipodermatosclerosis and leg ulcer in patients with chronic venous insufficiency. We generated a new mice model with local iron-overload in the skin, via ID-injection of iron-dextran (Fedx). Prussian blue staining proved that iron accumulates in the dermis and in the dWAT of these mice, like in patients' skin. Skin of Fedx mice shows signs of lipodermatosclerosis; an increase of total cell count ($p=0.001$) in the dermal layer, particularly of F4/80+ macrophages ($p=0.019$) and PDGFRa+ fibroblasts ($p<0.001$), accompanied with less collagen in the deeper dermis and a reduction in the size of the dWAT ($p<0.001$) and of the adipocytes ($p<0.001$). Microscopic and gene expression analysis highlight lipolysis, the consequent reduction of lipids droplets ($p=0.04$), and loss of plin-1 ($p=0.004$) on adipocytes membrane, associated with downregulation of pro-adipogenic genes in the dWAT of Fedx mice. FACS analysis reveals a shift in F4/80+ Macrophage subtypes with the induction of TNFa ($p=0.0163$) and reduction of CD301b and Relm-a (both $p<0.001$). Upregulation of CCL2 ($p=0.02$), IL1ß, HMOX-1 ($p=0.0001$ dermis, $p=0.01$ dWAT) and CCR2 ($p=0.01$) genes, indicate for a pro-inflammatory activation in the dermal compartment. Mechanistic analysis with human cells in vitro shows that erythrocyte-uptake induces ROS and a pro-inflammatory phenotype in Macrophages. Fibroblasts in response to erythrocyte-fed macrophages resemble those phenotypes found in Fedx mice. The impact of erythrocytes on the differentiation and activation of adipocytes and their crosstalk to Macrophages and Fibroblasts is ongoing. Our data suggest that local iron-overload causes a complex skin phenotype with a maintained inflammatory status in the dermis, proliferative and less fibrogenic fibroblasts, impaired adipogenesis, and increased lipolysis.

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Wnt signaling activation causes ATGL-dependent lipolysis in skin fibrosis

A. R. Jussila[1], B. Zhang[1], S. Kirti[1], R. Wyetzner[1], C. Reynolds[1], M. Steele[1], E. Hamburg-Shields[1], V. Horsley[2], R. Atit[1, 3, 4]
[1]Biology, Case Western Reserve University, Cleveland, Ohio, United States, *[2]Molecular, Cellular, and Developmental Biology, Yale School of Medicine, New Haven, Connecticut, United States, [3]Genetics, Case Western Reserve University School of Medicine, Cleveland, Ohio, United States, [4]Dermatology, Case Western Reserve University School of Medicine, Cleveland, Ohio, United States*

Fibrosis is the pathological accumulation of extracellular matrix and can occur in all soft tissues. In many organs, including skin, fibrosis is also associated with a loss of fat or lipid-filled cells. In the skin, dermal adipose tissue has many critical roles which are likely impacted by fibrotic remodeling. Despite identifying numerous dysregulated signaling pathways common to fibrotic disease, the mechanism of fibrotic fat loss remains poorly understood. Wnt signaling is widely dysregulated across fibrotic diseases and known to impact adipocyte cell fate commitment, but a causal role of Wnt signaling in lipid breakdown in mature dermal adipocytes hasn't yet been demonstrated. We found Wnt activation in dermal adipocytes in vitro and *in vivo* results in lipid depletion. Lipolysis is a process by which adipocytes breakdown stored lipid by a sequence of lipases beginning with adipose triglyceride lipase (ATGL). We hypothesized that Wnt activation in the dermis stimulates excessive ATGL-dependent lipolysis in dermal adipocytes. Using an inducible murine model of Wnt activation in the dermis, we first demonstrate activation of the ATGL/lipolysis axis and visible breakdown of lipid droplets by electron microscopy. Second, we show that Wnt-induced dermal adipocyte lipid depletion is dependent on ATGL *in vivo*. Third, we found that genetic ablation of Atgl in the dermis confers protection against early collagen remodeling in Wnt-induced skin fibrosis, suggesting that fibrotic fat loss has implications for fibrotic ECM accumulation. Our results demonstrate the involvement of ATGL-dependent lipolysis in Wnt-induced fibrotic remodeling, highlighting a new target for fibrosis therapies with the potential to impact both fibrotic fat loss and ECM remodeling phenotypes.

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Dermal adipocyte precursor immune fibroblastic cells (IFCs) drive neutrophil recruitment in response to bacterial infection

K. Cavagnero, T. Dokoshi, A. O'Neill, J. Seidman, M. Liggins, R. Gallo
Dermatology, University of California San Diego, La Jolla, California, United States

Immune fibroblastic cell (IFC) subpopulations of dermal fibroblasts differentiate into adipocytes and provide skin immune defense in response to infection. To better understand the role of these fibroblast cell subsets, we have undertaken a systematic analysis of dermal fibroblasts in models of skin inflammation. First, communication from fibroblasts to mesenchymal, epithelial, and bone-marrow derived cells in the skin was investigated using unbiased machine learning of single-cell RNA sequencing (scRNAseq) data from mice infected with Cutibacterium acnes. This network analysis revealed that fibroblasts communicate more with myeloid cells than any other cell lineage. Further analysis of scRNAseq data showed that lipocalin 2 (LCN2/NGAL)—a myeloid cell chemoattractant and antimicrobial protein—was a major gene upregulated by IFCs (17-fold change, $p<0.0001$), and LCN2/NGAL was most highly expressed by an IFC subset that expressed genes related to fat cell differentiation. Next, LCN2/NGAL+ IFCs were visualized in situ in the skin of mice infected with Staphylococcus aureus and modeled in vitro with the 3T3-L1 preadipocyte IFC cell line. 3T3-L1 IFCs showed massive induction of LCN2/NGAL mRNA following initiation of adipogenesis by bulk RNA sequencing (340-fold change, $p<0.0001$) and qPCR (59-fold change, $p<0.0001$). This response was further increased by TLR2 stimulation (5-fold change, $p<0.0001$). Functionally, conditioned media from TLR2 activated 3T3-L1 IFCs demonstrated robust in vitro chemotactic activity for neutrophils compared to unstimulated 3T3-L1 IFCs (4-fold change, $p<0.0001$), and LCN2/NGAL−/− mice showed reduced C. acnes induced inflammation. These findings show for the first time that dermal adipocyte precursor IFCs express LCN2/NGAL and likely promote host defense through interaction with myeloid cells.

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Free radicals and fast action to counteract oxidative damage in skin cells

N. Pernodet[1, 2], J. Trivero[1], D. Layman[1], A. Rella[1], E. Goyarts[1]
[1]Estee Lauder Companies, New York, New York, United States, [2]Estee Lauder Research Laboratories, Melville, New York, United States

Oxidative free radicals are one of the most toxic molecules produced in our body. Oxidative damage accumulates over time, accelerates aging, and contributes to many age-related diseases such as cancer, heart disease and Alzheimer's disease. Our skin is directly exposed to damaging environmental factors such as UV light and pollution, which trigger the production of free radicals (FR). Combined with reactive oxygen species formed by skin's own metabolism, these damaging molecules can overwhelm skin's natural antioxidant defenses, especially as skin ages and skin's natural antioxidant defenses are weakened. FR attack structural molecules in skin via oxidation of 1) proteins, resulting in an accumulation of dysfunctional proteins, 2) lipids, resulting in impaired barrier and cell membranes, and 3) DNA, resulting in strand breaks and DNA-protein crosslinks. In addition, FR activate damaging mechanisms, such as collagen degradation by collagenases and increased inflammation, resulting in accelerated skin aging. Recently, a new source of FR has been identified: blue light. Although blue light from sunlight is beneficial to synchronize our circadian rhythm with daytime, it is also a source of environmental damage by generating FR. Skin's exposure to this FR damage continues at night as it is exposed to blue light from LEDs for interior lighting and devices. Nighttime blue light exposure also contributes to skin damage by causing desynchronization of skin's natural circadian rhythm and a loss of repair at night, as we have previously shown. We show that blue light exposure generates a rapid rise in the level of FR in skin cells. Since FR are highly reactive, it is essential to act quickly and fight them via two actions: minimize their production and eliminate those that are generated. Therefore, we developed an exclusive lavender extract, which helps to fight FR, especially those from blue light exposure. This new technology helps skin cells defend against FR production and oxidative damage, resulting in healthier skin cells.

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IL-17 acts as the master regulator for metabolic rewiring in skin inflammation and drives keratinocytes towards a hyperproliferative phenotype

B. Dhamija, V. V. Sawant, M. Basu, D. Attrish, S. S. Marathe, R. Purwar
Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, Maharashtra, India

Immune system, especially T cells, and their cytokines play a vital role in the progression and maintenance of skin inflammation in many skin disorders. Increased frequency of IL-17 producing Th17 cells are known to be present in skin lesions of psoriasis and atopic dermatitis patients. However, the roles and mechanisms of IL-17 in metabolic reprogramming in human skin remain unexplored. Here, we employed multi-omics and system biology approach to examine the IL-17 mediated metabolic alterations in primary human keratinocytes (HPKs). 1H NMR metabolomics data revealed IL-17 mediated dysregulation of metabolites (glucose and malate) involved in carbohydrate metabolism. Moreover, lipidomics study revealed increased levels of sphingolipid and intracellular neutral lipids in IL-17 stimulated HPKs. System biology approach using MitoCore model confirmed our findings that IL-17 upregulated three major pathways: glycolysis, glutaminolysis, and lipid synthesis (p-value < 0.05); all resulted in a cumulative increase of nutrient pool for HPKs. Finally, stable isotope resolved metabolomics (using U-13C glucose and glutamine) and biochemical assays validated the roles of IL-17 in metabolic rewiring of central carbohydrate and lipid metabolism. Functionally, IL-17 modulated the redox status and increased Reactive Oxygen Species (ROS) levels (p-value < 0.01) inside the cells which in turn triggered a signaling cascade that resulted in metabolic perturbations and rendered cells towards a more proliferative phenotype. Overall, this study highlights for the first time, the roles of IL-17 in modulating metabolic reprogramming of keratinocytes and regulation of redox homeostasis which drives the cells towards hyperproliferation. Our study presents a novel approach to study immuno-metabolism cross-talk and the extracellular microenvironment that affects skin inflammation and its pathogenesis.

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TSG-6 protein delays the invasion of fungal dermatophytes in reconstructed human epidermis

C. Evrard, C. Matthys, C. Lambert de Rouvroit, Y. Poumay, E. Faway
Universite de Namur, Namur, Belgium

TSG-6 is an anti-inflammatory protein interacting with hyaluronan (HA) in the extracellular matrix (ECM) of epidermis and overproduced in pathological contexts such as in fungal infection. Dermatophytosis are widespread superficial infections of keratinized structures, especially produced by Trichophyton rubrum in humans. However, involvements of TSG-6 protein in pathophysiology of this dermatophytosis are not yet fully understood. The development of T. rubrum infection was characterized on TSG-6+/+ and TSG-6-/- reconstructed epidermis (RHE). Results support a quicker development of infection in TSG-6-/- RHE compared to TSG-6+/+ RHE as fungal elements reach the living layers of TSG-6-/- epidermis within three days of infection while they remain confined in the cornified layer of TSG-6+/+ tissues. However, barrier efficacy appears only slightly affected by this more rapid invasion of fungal elements. While the response of TSG-6+/+ and TSG-6-/- keratinocytes to fungal elements appears similar, more HA is released into the culture medium of TSG-6-/- RHE without any change in the expression of enzymes involved in HA metabolism suggesting a loss of HA amount between keratinocytes. Interestingly, a delay in the phosphorylation of ERK, which is notably a component of the Toll-Like Receptor (TLR) signaling pathway known to be involved in the recognition of fungal elements and in the implementation of the keratinocyte response, is observed during the infection of TSG-6-/- RHE compared to TSG-6+/+ RHE. This suggests a delay in the activation of certain signaling pathways in the absence of TSG-6 and thus when HA is not retained in the ECM possibly leading to a slower immune response implementation and a rapid invasion of the tissue by dermatophytes. Finally, HA maintained in the ECM by the TSG-6 protein is likely involved in matrix organization and can be critical for interaction of Pathogen-associated molecular pattern (PAMP) and Damage associated molecular pattern (DAMP) with TLR receptors.

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Pervasive immune dysfunction characterizes photoaged skin

A. Billi, F. Ma, M. Gharaee-Kermani, X. Xing, O. Plazyo, A. Schuler, R. Wasikowski, W. R. Swindell, M. Nakamura, Y. Helfrich, J. M. Kahlenberg, J. Lee, L. C. Tsoi, J. Voorhees, G. J. Fisher, J. E. Gudjonsson
University of Michigan Medical School, Ann Arbor, Michigan, United States

Long-term sun exposure markedly accelerates the skin changes of chronological aging, leading to deterioration of skin function and appearance in a process termed Photoaging. The molecular mechanisms underlying photoaging remain incompletely defined, due largely to the limitations of *ex vivo* studies. To interrogate this phenomenon *in vivo*, we performed single-cell RNA-seq of sun-protected and sun-exposed skin from four ~20-year-old (yo) and four ~90yo donors. Clustering and annotation of the resulting cells revealed pervasive immune dysfunction in photoaged skin. Relative to all other skin types, photoaged (sun-exposed, 90yo) skin showed decreased resident memory T cells, increased regulatory T cells, and accumulation of IL10+ dendritic cells, which were reported to dampen excessive inflammation. Inflammatory responses were also perturbed in non-immune cells of photoaged skin, including keratinocytes. Fibroblasts and other stromal cell types showed photoaging-associated shifts in expression of genes related to the extracellular matrix and fibrosis. These widespread changes led to dramatically altered cell-cell communication networks in photoaged skin. Most notably, predicted myeloid cell interactions with keratinocytes, fibroblasts, endothelial cells, and other myeloid cells were twice as abundant in photoaged skin as chronologically aged (sun-protected, 90yo) skin. For functional validation, we performed immune challenges including candida injection and imiquimod application to sun-protected and sun-exposed skin from young and aged volunteers. Treated sites were biopsied and RNA-seq performed, further corroborating immunological differences in photoaged skin. Together, our data capture photoaging *in vivo* at unparalleled resolution and reveal profound immune alterations in photoaged skin. These alterations may underlie not only the functional deterioration of photoaged skin but also its increased susceptibility to infection and neoplasm.

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Hepatocyte growth factor pathway drives enhanced skin tumor formation in a humanized mouse model of accelerated dermal aging

T. Quan, T. He, Z. Qin, Y. Liu, Y. Yan, A. Ermilov, J. Voorhees, A. A. Dlugosz, G. J. Fisher
Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, United States

Keratinocyte skin cancer affects many light-skinned elderly persons, yet knowledge of the detailed mechanisms remains unclear. Emerging evidence highlights the key role of the underlying connective tissue microenvironment in epithelial tumor formation. We have investigated the impact of age-associated alterations in the dermal microenvironment on keratinocyte skin cancer formation in a novel humanized mouse model of accelerated dermal aging. To mimic a key age-related change in human dermis, we engineered Col1a2-CCN1 transgenic mice to express human CCN1, a secreted extracellular matrix (ECM) associated protein, in dermal fibroblasts. CCN1 accumulates in the dermal ECM and by the sixth month of age Col1a2-CCN1 mice display key features of the aged human dermis, most notably loss and fragmentation of the dermal ECM. Importantly, six-month-old Col1a2-CCN1 mice exhibit significantly enhanced skin tumor development in two complementary models of skin cancer: two-stage chemical carcinogenesis (N=5, P<0.001) and HRas-oncogene-driven tumors (N=4, p<0.01). Notably, these models produced no tumors in two-month-old Col1a2-CCN1 mice, before developing the features of the aged dermis. RNA-seq and Gene Ontology (GO) analyses revealed that the hepatocyte growth factor (HGF) pathway is highly upregulated in six-month-old Col1a2-CCN1 mice. HGF was elevated 9.5-fold (N=6, P<0.05), compared to age and sex-matched control littermates. HGF expression was also elevated in chemically induced (N=4), and oncogenic HRas-induced (N=3) skin tumors. Inhibition of HGF signaling in Col1a2-CCN1 mice with the HGF receptor (c-Met) inhibitor PHA665752 (10μg/μl) reduced squamous tumor development by 70% (N=3, P<0.01). These data demonstrate that age-related alterations of the dermal microenvironment enhance squamous tumorigenesis, at least in part through upregulation of HGF, and provide new mechanistic insights into the high prevalence of skin cancer in the elderly.

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Mediators of capillary-to-venule conversion in psoriasis

Y. He[1], J. Kim[1], C. Tacconi[1], J. Shin[2], C. Hon[2], M. Detmar[1]
[1]*Eidgenossische Technische Hochschule Zurich Departement Chemie und Angewandte Biowissenschaften, Zurich, Zürich, Switzerland,* [2]*Rikagaku Kenkyujo Yokohama Campus, Yokohama, Kanagawa, Japan*

Psoriasis is a chronic inflammatory skin disease characterized by epidermal hyperplasia and hyperkeratosis, immune cell infiltration and vascular remodeling. Despite the emerging recognition of vascular normalization as a novel strategy in managing psoriasis, an in-depth delineation of the remodeled dermal vasculature has been missing. In the present study, we exploited 5' single-cell RNA-sequencing (scRNA-seq) to investigate the transcriptomic alterations in different subpopulations of blood vascular and lymphatic endothelial cells (ECs) directly isolated from psoriatic and healthy human skin. Individual subtypes of ECs underwent specific molecular repatterning associated with cell adhesion and extracellular matrix organization. Blood capillaries, in particular, showed upregulation of the melanoma cell adhesion molecule (MCAM) as well as its binding partners and adopted post-capillary venule-like characteristics during chronic inflammation that are more permissive to leukocyte transmigration. We also identified psoriasis-specific interactions between cis-regulatory enhancers and promoters for each EC subtype, revealing the dysregulated gene regulatory networks in psoriasis. Together, our results provide new insights into the specific transcriptional responses and epigenetic signatures of ECs lining different vessel compartments in chronic skin inflammation.

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Spatiotemporal interplay between epidermal keratinocytes and neutrophils in inflamed skin

Y. Xu, C. Parent, P. Coulombe
University of Michigan Medical School, Ann Arbor, Michigan, United States

Neutrophils are the first immune cells to reach inflamed sites and have been reported to contribute to the pathogenesis of many inflammatory skin diseases such as psoriasis, skin cancers, hidradenitis suppurativa and others. Yet, little is known about (i) the spatiotemporal pattern of neutrophil infiltration in inflamed skin *in vivo* after acute and recurrent irritation, and (ii) the source and identity of the signals responsible for neutrophil recruitment to stressed skin. We hypothesized that epidermal keratinocyte-derived signals mediate the recruitment of neutrophils to skin after topical exposure to irritants, and that the pattern of neutrophil infiltration in skin differs upon repeated exposure due to changes in signal(s) from stressed keratinocytes. Here, we use phorbol ester TPA to induce mouse dermatitis. We show that one topical irritation with TPA results in infiltration of neutrophils in the dermis, which resolves within 24h. When a second TPA irritant is applied 24h but not 48h later to pre-stimulated skin, the neutrophil influx is significantly accelerated and amplified, suggesting a transient "memory" of the initial insult. We refer to this phenomenon as TAR (Transient Amplification Response). Additional experiments suggest that the TAR is mediated by local signals originating in epidermal keratinocytes. Using mice carrying null or mutant Krt17 gene which encodes keratin 17 (K17), an intermediate filament protein that is inducibly expressed in epidermis in response to various environmental stressors, we find that TAR depends on the cytoplasmic pool of K17 in epidermal keratinocytes. This K17-dependent neutrophil chemotaxis is replicated *ex vivo* using conditioned media harvested from cultured keratinocytes. Finally, we show that K17 regulates protein kinase C α (PKCα), a direct target of TPA, in its activity level and membrane translocation. These results reveal a crucial role for K17 in regulating inflammation and immune response in skin, and may provide novel therapeutic targets to improve the treatment of neutrophilic skin diseases.

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Multimodal analyses of vitiligo skin identifies tissue characteristics of stable disease

J. Shiu[1], L. Zhang[2], G. Lentsch[3], J. Flesher[4], S. Jin[2], C. Polleys[5], I. Georgakoudi[5], Q. Nie[2], M. Balu[3], A. K. Ganesan[1]
[1]Dermatology, University of California Irvine, Irvine, California, United States, [2]Mathematics, University of California Irvine, Irvine, California, United States, [3]Beckman Laser Institute and Medical Clinic, Irvine, California, United States, [4]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, 5Biomedical Engineering, Tufts University, Medford, Massachusetts, United States

Vitiligo is an autoimmune skin disease that is characterized by the progressive destruction of melanocytes by autoreactive CD8+ T cells. Melanocyte destruction in active vitiligo is mediated by CD8+ T cells but why white patches in stable disease persist is poorly understood. The interaction between immune cells, melanocytes, and keratinocytes in situ in human skin has been difficult to study due to the lack of proper tools. We combine non-invasive multiphoton microscopy (MPM) imaging and single-cell RNA sequencing (scRNA-seq) to identify distinct subpopulations of keratinocytes in lesional skin of stable vitiligo patients. We show that these keratinocytes are enriched in lesional vitiligo skin and differ in metabolism, an observation corroborated by both MPM and scRNA-seq. Systematic investigation of cell-cell communication show that CXCL is the prominent signaling change in this small population of keratinocytes, which secrete CXCL9 and CXCL10 to create local inflammatory cytokine loops with T cells to drive stable vitiligo persistence. Further MPM imaging of patients undergoing punch grafting treatment showed that metabolically altered keratinocytes persist in non-responders but normalize in treatment responders. In summary, we couple advanced imaging with transcriptomics and bioinformatics to discover cell-cell communication networks and keratinocyte cell states that persist in stable disease and potentially prevent repigmentation.

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Macrophage migration inhibitory factor restriction of HIV-1 trans-infection from dendritic cells to CD4+ T-cells via regulation of autophagy

S. Caucheteux[1], R. Bayliss[2], J. Wheeldon[2], V. Piguet[1,3]
[1]University of Toronto Temerty Faculty of Medicine, Toronto, Ontario, Canada, [2]Cardiff University School of Medicine, Cardiff, Cardiff, United Kingdom, [3]Women's College Hospital, Toronto, Ontario, Canada

At mucosal surfaces, immature resident Dendritic Cells, Langerhans Cells and their subsets are the first cell types to encounter HIV-1 virus. During the early stages of infection, dendritic cells sense the virus through innate immune mechanisms inducing maturation and activating adaptive immune responses. HIV-1 subverts the immune system by hijacking dendritic cells, impairing cellular function to promote survival and propagation by trans-infection to target CD4 T-cells across the virological synapse. In a well-established model of cell-to-cell HIV-1 transfer from monocyte-derived DCs to CD4 T-cells, we have evaluated the molecular contribution of cytokines and cytokine receptors during the transfer of HIV-1 virus from DCs to CD4 T cells using a high-throughput siRNA library. Here we show that when macrophage migration inhibitory factor (MIF) is disrupted, the dynamics of HIV-1 trans-infection from DCs to T cells are greatly affected, making it a restrictive cytokine. We report a critical link between MIF expression in dendritic cells and autophagy. We show that the formation of LC3+ autophagosomes is a MIF-dependent process, required for the clearance of intracellular virus by selective degradation. Thus, the regulation of MIF expression in dendritic cells is critical in the induction of autophagy during HIV-1 infection, thereby reducing HIV-1 transmission from DC to CD4+ T cells, one of its earliest steps of entry in the host.

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Transcription coactivators YAP and TAZ are essential for postnatal maturation of the dermal extracellular matrix in mouse skin

A. Ermilov, J. Kim, Z. Qin, K. Calderone, J. Voorhees, G. J. Fisher, T. Quan
Dermatology, University of Michigan, Ann Arbor, Michigan, United States

YAP and TAZ are critical downstream effectors of the hippo signaling pathway, which play pivotal roles in controlling organ size and maintaining tissue homeostasis. Although dysregulation of YAP/TAZ has been linked to various human diseases their role in dermal extracellular matrix (ECM) regulation remains largely unknown. Here, we describe the role of YAP/TAZ in mouse dermal ECM maturation during early postnatal development We find that from birth (P0) to postnatal day 20 (P20), skin surface area increases 6-fold (N=6). This rapid expansion occurs at a linear rate, from approximately 500mm2 to 3000mm2. Interestingly, the total number of dermal fibroblasts remained nearly constant with 0.9x108 fibroblasts/mouse at P0 and P20 (N=15), resulting in decreased density of dermal fibroblasts from 1.5x106/mm3 to 0.3x106/mm3, between P0 and P20 (N=15). To explore the role of YAP/TAZ in dermal maturation, we generated mice that allowed conditional deletion of YAP/TAZ in dermal fibroblasts, the major ECM producing cells in skin, by Cre recombinase, under the control of the platelet-derived growth factor receptor-alpha promoter (pdgfra-CreER;Yapf/f;Tazf/f). To knockout Yap/Taz in dermal fibroblasts, P3 pdgfra-CreER;Yapf/f;Tazf/f mice and control sex-matched littermates were topically treated with 4-OH tamoxifen (10 mg/ml) for five consecutive days, and back skin was harvested at P20. Fibroblast-specific deletion of Yap/Taz significantly impaired dermal ECM collagen fibril production, deposition, and maturation. Dermal ECM density and type I collagen gene expression were reduced 69%, (N=6). RNA-seq/Gene Ontology (GO) analysis of whole skin revealed knockout of YAP/TAZ in fibroblasts altered expression of 2668 genes (1466 upregulated, 1202 downregulated). Notably, hippo signaling pathway was negatively enriched, whereas cytokines inflammatory response pathway was positively enriched. These data indicate that YAP/TAZ plays a critical role in postnatal dermal ECM maturation and homeostasis.

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Infiltrative BCC TILs show increased clonality and are associated with greater TCR repertoire overlap between cytotoxic and exhausted subtypes

N. Frazzette, N. Doudican, J. Carucci
Dermatology, NYU Langone Health, New York, New York, United States

Background: TCR repertoire of TILs is increasingly used as a fingerprint for identifying unique immune responses to cancer and as a biomarker for prognosticating clinical course and response to immunotherapy. Methods: CD8+ tumor infiltrating lymphocytes (TILs) obtained from fresh basal cell carcinoma ("BCC") tumor specimens from nodular subtype ("nBCC", n=6) versus infiltrative subtype ("iBCC", n=6) were subject to single-cell VDJ profiling. Data were analyzed using iCellR and ImmunArch. Results: Pooled CD8+ TILs from nBCC and iBCC generated a significantly more diverse TCR repertoire than CD8+ T cells from healthy skin, but a significantly less diverse repertoire than CD8+ from healthy PBMCs. TCR gene bias analysis revealed that nBCC and iBCC TILs expressed a differential preference for certain V genes on both the alpha and beta CDR3 chain. Clonotype analysis revealed the top 10 most expanded clonotypes among iBCC TILs represented 25% of the TCR repertoire versus 20% of the nBCC TIL repertoire; also, iBCC TIL singletons represented 37% of the TCR repertoire while nBCC TIL singletons represented 50% of the TCR repertoire, indicating a greater degree of clonality among iBCC TILs. Additionally, cytotoxic TILs recovered from nBCC possessed a more diverse TCR repertoire than those from iBCC. Moreover, cytotoxic TILs from nBCC possessed a distinct TCR repertoire with little overlap with exhausted TILs; cytotoxic TILs from iBCC possess a TCR repertoire that highly overlapped with exhausted TILs. Conclusion: TILs from nBCC generally possessed a less clonal TCR repertoire than iBCC TILs, possibly contributing to observed relative clinical aggressiveness of these tumors. Additionally, TCR repertoire in exhausted TILs in iBCC overlapped more with cytotoxic TILs, informing clinical decision-making with regards to check point inhibitor immunotherapy.

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Molecular investigation of secondary syphilis in the skin characterizes the nature of the B-cell promoting inflammatory environment.

J. Kirma[1], X. Xing[1], A. Billi[1], J. M. Kahlenberg[1, 2], L. C. Tsoi[1], J. E. Gudjonsson[1]
[1]*Department of Dermatology, University of Michigan, Ann Arbor, Michigan, United States,* [2]*Division of Rheumatology, Dept of Internal Medicine, University of Michigan, Ann Arbor, Michigan, United States*

Syphilis is a sexually transmitted disease with prominent cutaneous manifestations. A frequent histologic feature of syphilis is the presence of B-cells and plasma cells, which are infrequently observed in other acute inflammatory diseases of the skin. To determine the regulation of this unique inflammatory environment in syphilis, we performed bulk RNA-seq on formalin-fixed, paraffin-embedded biopsies obtained from 9 patients with secondary syphilis and 8 healthy controls. Using a threshold of a twofold increase or decrease and a false discovery rate (FDR) of 0.05, we found 917 genes to be differentially expressed in secondary syphilis in skin. Principal component analysis showed separation of syphilis samples from healthy controls. Enriched biologic processes included cellular response to interferon gamma and interferon alpha/beta signaling, and enriched adaptive immune response related to immunoglobulin superfamily domains. Enriched cytokine responses included IL-36 (FDR<10-15), type I IFNs (FDR<10-25), and IL-17A (FDR<10-10). The most enriched cell signature was for B memory cells (p<10-10), with increased expression of the B-cell chemokine C-X-C Motif Chemokine Ligand 13 (80-fold, p<0.01), and the B-cell activator Interleukin 21 (15-fold, p<0.05). Epidermal production of IL-21 and infiltration of B-cells (CD20) and plasma cells (CD138) was verified by IHC. These data provide a detailed outline of the nature of the inflammatory network in cutaneous manifestations of secondary syphilis and provide insights into the mechanisms involved in B-cell chemotaxis into the skin and local B-cell and plasma cell activation.

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Single-cell RNA-seq of dorsal root ganglion reveals neuroinflammatory process in atopic dermatitis

Y. Kim[1], J. Ryu[2], Y. Jang[2], D. Kim[2], Y. Bang[4], S. Choi[4], J. Moon[5], H. Kim[3], J. Shin[2]
[1]*Seoul National University College of Medicine, Seoul, Korea (the Republic of),* [2]*Dermatology, CHA University College of Medicine, Seongnam, Korea (the Republic of),* [3]*Genomic Medicine Institute, Medical Research Center, Seoul National University College of Medicine, Seoul, Korea (the Republic of),* [4]*Seoul National University Graduate School Department of Biomedical Science, Seoul, Korea (the Republic of), 5Samsung Genomic Institute, Samsung Medical Center, Seoul, Korea (the Republic of)*

Atopic dermatitis is a chronic inflammatory skin disease, the pathology of which is characterized by barrier dysfunction, microbiome shift, immune dysregulation initiated by a severe itching sensation. Because of its pathophysiological and clinical significance, research on understanding the mechanism of itch and finding new therapeutic targets on it has been highlighted recently. To dissect the cellular/molecular changes associated with itch, we obtained a total of six mouse dorsal root ganglion tissues; three from atopic dermatitis-induced NC/Nga mice and another three from control mice, and performed single-cell RNA sequencing. Among non-neuronal cells, proportions of neutrophil and macrophage increased in atopic dermatitis, which implies the presence of neuroinflammation. Non-peptidergic neurons, which have nociceptors transmitting itching sensation, occupy a larger proportion among neurons in atopic dermatitis, compared to the control. As the first single-cell transcriptome analysis of atopic dermatitis mouse DRG, our study provides insight into how itching sensation manifests itself on a single cell level in atopic dermatitis.

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Pathogenic CD8+ T cell infiltration and immune synapse formation in alopecia areata hair follicles

R. Gund[2], E. M. Mace[1], A. M. Christiano[3]
[1]*Pediatrics, Columbia University Irving Medical Center, New York, New York, United States,* [2]*Dermatology, Columbia University Irving Medical Center, New York, New York, United States,* [3]*Dermatology, Columbia University Irving Medical Center, New York, New York, United States*

Alopecia Areata (AA) involves CD8+ NKG2D+ T cell-mediated hair follicle (HF) damage that causes hair loss. Despite the identification of CD8+ T cells as primary pathogenic cells involved in AA, the molecular mechanisms used by these cells to kill their target cells within HF are not known. CD8+ T cells form immune synapses (IS) to contact, recognize and destroy their target cells, therefore we postulated that CD8+ T cells employ a cytolytic IS for target cell killing in AA. Here, we aimed to directly visualize the interactions of CD8+ T cells with HF target cells in a C3H/HeJ mouse model of AA using high-resolution confocal microscopy. We found that CD8+ T cells infiltrating the skin and HF were proliferative, and a subset of T cells expressed CD103 marker of tissue-resident memory cells. We tracked the in situ locations of skin infiltrating CD8+ T cells and determined the spatial localization and recruitment of key proteins that define immune synapses (IS) in AA. We found that CD8+ T cells penetrated through the dermal sheath to enter the HF, invaded multiple epidermal layers and assembled a cytolytic IS upon reaching the HF inner root sheath. IS formation was associated with the killing of target cells within Henle's layer K71+ cells of the inner root sheath. IS assembly involved the colocalization of polarized Lck signaling kinase and the microtubule organizing center (MTOC), along with converged cytolytic granules at the site of contact with HF target cells. In contrast, although CD8+ T cells located in the dermis exhibited granzyme convergence at MTOC, they did not show Lck polarization, consistent with the lack of T cell target engagement within the dermis. Taken together, these data demonstrated the direct visualization of in situ cytotoxicity of pathogenic CD8+ T cells within AA-affected skin and elucidated the mechanism of CD8+ T cell-mediated HF damage in AA via the formation of an immune synapse.

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Dissecting the cellular landscape of human skin across anatomical sites and in cutaneous malignancy through single cell transcriptomics and in situ sequencing

C. Ganier[1], X. Du-Harpur[1,3], J. Gabriel[1], N. Harun[1], F. M. Watt[1], M. D. Lynch[1,2]
[1]*Centre for Stem Cells and Regenerative Medicine, Guy's Hospital, King's College London, London, United Kingdom,* [2]*St John's Institute of Dermatology, London, United Kingdom,* [3]*Bioinformatics and Computational Biology Laboratory, The Francis Crick Institute, London, United Kingdom*

The Human Cell Atlas project has previously catalogued the cell types in human skin derived from adult, fetal skin and inflammatory skin diseases. However, we lack a comprehensive understanding of how these cell types are organized spatially within the tissue and how they differ qualitatively and across anatomical sites and in cutaneous malignancy. To address these questions, we have analyzed single cell RNA sequencing of 120,000 cells from diverse healthy anatomical sites and compared to 60 000 cells from basal cell carcinoma (BCC) - the most common malignancy in humans. We have integrated this with spatial transcriptomic data across 24 samples to map the spatial patterns of cellular localization at 50 micron resolution. Subsequently, in situ sequencing of 12 samples using a panel of 200 genes allowed us to define cellular-resolution maps of identity for these tissue states. We find that whilst cellular identities are broadly conserved across anatomical sites, cells retain a memory of embryological origin. We find T cells to be more abundant in the skin of the head compared to the trunk and limbs with differential expression of T cell markers. Additionally, we find that fibroblast subpopulations differ subtly in their identity across anatomical sites and exhibit distinct patterns of spatial localization within the dermis. In BCC, we observe a characteristic spatial arrangement of fibroblast populations at the interface of the epithelial and mesenchymal elements of the tumor. We dissect the relative importance of intrinsic transcriptional programs and spatial context in the specification of fibroblast identity through RNA FISH analysis in the immediate transition to cell culture conditions. Our findings provide key insights into the cellular landscape of skin from different healthy anatomical locations and BCC and suggest that the interface between epithelial and mesenchymal elements of the tumor may offer new therapeutic targets for the treatment of cutaneous malignancy.

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A noncanonical role for IRF6 in promoting cellular adhesions

A. Antiguas, M. Dunnwald
Anatomy and Cell Biology, The University of Iowa, Iowa City, Iowa, United States

We recently demonstrated that Interferon Regulatory Factor 6 (IRF6) is a transcription factor essential for keratinocyte adhesion. Specifically, it is required for the recycling and delivery of E-cadherin to the plasma membrane. Interestingly, the total E-cadherin levels were not altered in the absence of IRF6, and under culture conditions used to test keratinocyte adhesion behavior, IRF6 was not detected in the nucleus. IRF6 protein includes a DNA binding domain (DBD), a protein binding domain (PBD), and a serine rich autoinhibitory region (SRR). To determine which IRF6 domains are required for proper cellular adhesions, we designed multiple IRF6 constructs lacking the DBD, PBD or SRR. Adding back constructs with intact PBD and SRR in IRF6 deficient keratinocytes rescued the adhesion pattern to a wild-type phenotype. However, introduction of the DBD alone failed to rescue the adhesions. To further test a model in which protein-protein interactions between IRF6 and effector proteins mediate delivery of E-cadherin at the plasma membrane, we investigated whether IRF6 colocalized with NME1 and NME2, two proteins known to internalize E-cadherin towards recycling endosomes and regulate the secretory pathway, respectively. We found that NME2 does not colocalize with IRF6. However, by immunofluorescence, IRF6 and NME1 colocalize and their mutual coimmunoprecipitation suggest they are part of the same complex. Because NME1 is necessary for E-cadherin recycling, we tested the hypothesis that IRF6-NME1 regulates recycling endosome function by evaluating the interaction between E-cadherin, IRF6 and Rab11A, a required promoter of recycling endosome. Our results show that Rab11A colocalizes with all these proteins in recycling endosomes, and that IRF6, Rab11A and NME1 coimmunoprecipitated with each other, suggesting they belong to the same complex. Overall, our results suggest that the PBD of IRF6, and not the DBD, is required for proper E-cadherin-mediated cell adhesions, further supporting a noncanonical function for IRF6.

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The phenotype of dermal fibroblasts in young vs. aged human skin: Adaptation to dermal extracellular matrix deterioration and cell autonomous responses

Y. Cui[1], C. Worthen[1], R. Haas[1], S. Grill[1], M. Shi[2], L. C. Tsoi[1], J. Nandakumar[1], J. Voorhees[1], Y. Zhao[2], G. J. Fisher[1]
[1]*University of Michigan, Ann Arbor, Michigan, United States,* [2]*Tsinghua University, Beijing, Beijing, China*

The dermis is largely composed of collagen-rich fibrils that form a dense extracellular matrix (ECM). Dermal fibroblasts produce and are embedded within the dermal ECM. During aging, the ECM becomes fragmented. This fragmentation is associated with deleterious alterations of fibroblast function. We have investigated the degree to which these alterations stem from adaptation to dermal ECM deterioration versus cell-autonomous age-driven responses. Primary dermal fibroblasts from young (20-30 years old, 6 females, 6 males) and aged (>80 years old, 6 females, 6 males) individuals were placed in standard monolayer culture and either harvested at 70% confluence (P1) for telomere quantitation and RNAseq transcriptome analysis or cultured through multiple passages. At P1, the average telomere length was 1kb shorter for fibroblasts from aged versus young skin ($p < 0.01$). The growth rates of fibroblasts from young and aged skin did not statistically differ for at least 18 population doublings; however, the final number of population doublings was significantly less for fibroblasts from aged skin (43 for aged, 53 for young, $p=0.001$). RNAseq transcriptome analysis identified 477 differentially expressed genes (DEGs); 300 upregulated and 177 genes downregulated in P1 fibroblasts from aged versus young skin. Interestingly, upregulated DEGs were enriched in cytokine and chemokine-mediated signaling, including CCL2, CCL5, CXCL1, and CXCL6 pathways. Downregulated DEGs were mostly enriched in biosynthetic processes. Notably, P1 fibroblasts from young and aged skin expressed similar levels of genes directly involved in ECM homeostasis. This study supports the concept that aberrant ECM homeostasis observed in aged skin *in vivo* largely reflects fibroblasts' adaptation to dermal ECM deterioration, while upregulation of pro-inflammatory mediators largely involves cell-autonomous mechanisms.

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Structure of a novel endoplasmic reticulum-desmosome complex and its role in skin disease

N. Bharathan[1], W. Giang[1], J. Aaron[2], S. Khuon[2], T. Chew[2], A. Kowalczyk[1]
[1]*Dermatology, Penn State College of Medicine, Hershey, Pennsylvania, United States,* [2]*Howard Hughes Medical Institute Janelia Farm Research Campus, Ashburn, Virginia, United States*

Desmosomes are adhesive intercellular junctions that provide strength and integrity to the epidermis and other epithelia. Dysfunction of the endoplasmic reticulum (ER) resident calcium pump, SERCA2, causes Darier's disease (DD), an epidermal disorder characterized by compromised desmosomal adhesion and abnormal keratinocyte differentiation. However, the mechanism by which ER dysfunction impairs desmosome formation is unclear. Using electron microscopy and live-cell fluorescence imaging, we found that peripheral ER tubules are in close proximity to desmosomes and form mirror image arrangements at desmosomes. ER tubules also exhibit less mobility at desmosomes relative to non-desmosomal regions, suggesting that desmosomes stabilize ER associations. Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) and 3D reconstructions revealed intricate nanoscale associations of ER tubules with keratin filaments and the desmosome inner dense plaque. Knockout of a key desmosomal cadherin, desmoglein 2, resulted in altered peripheral ER morphology at cell-cell contacts, indicating that desmosomes regulate peripheral ER tubule organization. Keratin intermediate filaments, which anchor to desmosomes, aligned and intertwined with ER tubules on either side of the desmosome plaque. To test if keratins regulate ER morphology, we expressed a keratin 14 mutant that forms aggregates and causes the epidermal blistering disease, epidermolysis bullosa simplex. Virtually all keratin aggregates exhibited persistent ER association and shifted ER morphology from tubular to sheet-like. Our results reveal a unique tripartite structural complex comprising the ER, desmosomes, and keratin filaments. Furthermore, this architectural arrangement of the ER is altered when desmosomes or keratins are perturbed, thus revealing the ER as a previously unappreciated therapeutic target for skin diseases.

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Clinical epidemiology of Masson tumor

K. Yang[1], C. X. Pan[2], E. Russell-Goldman[3], V. Nambudiri[3]
[1]Tufts University School of Medicine, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States, [3]Brigham and Women's Hospital, Boston, Massachusetts, United States

Intravascular papillary endothelial hyperplasia (IPEH), also known as Masson tumor–a rare, benign vascular lesion typically presenting in the skin as a subcutaneous nodule – may be clinically mistaken for other neoplasms such as hemangiomas and lipomas. IPEH is classically categorized into three types:1) pure type arising in dilated endovascular spaces; 2) mixed type developing from preexisting vascular abnormalities; 3) extravascular type. While the prognosis of IPEH is excellent, it must be differentiated from malignant tumors such as angiosarcomas, which may require intensive treatments. Because the literature on IPEH is limited, we sought to characterize clinical and pathological features of IPEH. Subjects were identified using the Mass General Brigham (MGB) Research Patient Data Registry and included individuals with pathologically proven diagnosis of IPEH from 1/1980 to 8/2021 at Massachusetts General Hospital, Brigham and Women's Hospital (BWH), and the BWH Faulkner Hospital. Demographic information, clinical documentation, and pathology reports were reviewed for data extraction. 261 individuals were diagnosed with IPEH, with the majority being women (60%) and white (74%). The average age at diagnosis was 53 years old [4-98 years old]. The most frequently involved anatomic sites were the upper (29%) and lower (24%) extremities. Common initial clinical diagnoses of lesions were cysts, hemangiomas, and lipomas. The pure subtype of IPEH was the most common (50%), followed by the mixed (46%) and extravascular subtypes (4%). Extravascular IPEH occurred more frequently in women (5%) compared to men (1%). We found that most clinicians' initial impressions prior to biopsy did not include the final diagnosis of IPEH -- often using vague terms such as "soft tissue mass" -- indicating a potential need for greater awareness of this condition. Given the differential diagnosis of IPEH often includes conditions such as melanoma or angiosarcoma, clinicopathologic correlation is of utmost importance for this uncommon vascular lesion.

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Erythema multiforme in COVID-19 patients and following COVID-19 vaccination

F. Etaee[1], M. Basiri[1], T. Naguib[2], S. Daveluy[3]
[1]Yale University, New Haven, Connecticut, United States, [2]Texas Tech University Health Sciences Center School of Medicine Amarillo, Amarillo, Texas, United States, [3]Dermatology, Wayne State University, Detroit, Michigan, United States

Background: During the Severe acute respiratory syndrome coronavirus 2 pandemic, dermatologic complications have been reported in the setting of coronavirus disease 2019 (COVID-19) infection as well as its treatment. The aim of this systematic review is to assess the published cases of EM associated with COVID-19 infection and vaccination. Methods: We searched Google Scholar, PubMed, Springer, Ovid, and Science Direct. Results: Regarding studies related to EM after COVID-19 vaccination, 6 articles were initially identified in the literature search, of which 2 were duplicates, and 4 studies were ultimately included that described 8 cases of EM after COVID-19 vaccines, 3 after Moderna (37.5%), 4 after Pfizer (50%), and one report after CoronaVac (12.5%). In terms of studies related to EM in patients with COVID-19, 113 articles were initially identified in the literature search, of which 31 were duplicates. After screening for eligibility and inclusion criteria, 23 publications were ultimately included that reported 36 cases of EM in patients with COVID-19 infection, with 19 males (53%). Five of 36 patients (13.9%) presented with EM before any classic COVID-19 symptoms as a first presentation of the disease. Three patients (8.3%) presented with EM and COVID-19 symptoms simultaneously. However, in most of the patients (78%), EM started after COVID-19 symptoms. Eight patients (22.2%) did not take any medications before skin rash and therefore presented with COVID-19 associated EM. However, 78% (28/36) patients took medications before EM. Conclusions: Since some patients did not take any drugs, we believe that the underlying mechanism could be a delayed immune response to the COVID-19 infection as a sole reason for EM in some cases. Accordingly, EM may result from the interaction between the virus itself, antiviral immune response, and drugs.

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Prevalence of rosacea in transgender and gender diverse populations: A retrospective cohort study

J. Sanz[1, 3, 2], J. L. Gao[5, 3, 2], D. S. King[2], A. M. Modest[6, 4], E. D. Dommasch[3, 4, 2]
[1]New York Institute of Technology College of Osteopathic Medicine, Old Westbury, New York, United States, [2]Fenway Institute, Boston, Massachusetts, United States, [3]Dermatology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [4]Harvard Medical School, Boston, Massachusetts, United States, [5]The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, United States, [6]Obstetrics and Gyenecology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States

The prevalence of rosacea has not been well studied in the transgender and gender-diverse (TGD) populations. We sought to determine the prevalence of rosacea among TGD patients receiving masculinizing gender-affirming hormone therapy (mGAHT) and feminizing GAHT (fGAHT) compared to cisgender patients. We conducted a retrospective cohort study using electronic health records from TGD and cisgender adult patients seen at Fenway Health between August 1, 2014 and August 1, 2020. Adjusted risk ratios (aRR) and 95% confidence intervals (CI) were calculated using log binomial regression and adjusted for age, race, smoking status, hyperlipidemia, hypertension, and HIV. We compared TGD patients receiving mGAHT and TGD patients receiving fGAHT each to comparison groups of cisgender men, cisgender women, and TGD patients not on GAHT. Of the 46,507 patients identified, there were 1,394 TGD on fGAHT, 1,576 TGD on mGAHT, 25,594 cisgender men, 16,961 cisgender women, and 982 TGD patients not on GAHT. In the multivariate analyses adjusting for relevant demographic and clinical factors, TGD patients on fGAHT had a decreased prevalence of rosacea compared to cisgender women (aRR: 0.22 (95% CI: 0.08,0.58)), cisgender men (aRR: 0.32 (95% CI: 0.12,0.87)), and TGD patients not on GAHT (aRR: 0.23 (95% CI: 0.081, 0.79)). TGD patients on mGAHT did not have a significant difference in prevalence of rosacea compared to cisgender women (aRR: 0.92 (95% CI: 0.54,1.56)), cisgender men (aRR: 1.37 (95% CI: 0.81,2.32)), or TGD patients not on GAHT (aRR: 0.84 (95% CI: 0.38,1.84)). TGD patients on fGHAT had a lower prevalence of rosacea compared to cisgender patients and TGD patients not on GAHT, suggesting that estrogen or anti-androgenetic agents may be protective.

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Development and validation of a caregiver-reported numeric rating scale for measuring pruritus in children aged 6 months to <6 years with atopic dermatitis

A. Paller[1], E. Siegfried[2], S. E. Marron[3], M. Clark[4], D. B. DiBenedetti[5], L. Nelson[5], J. Chao[6], A. Bansal[6], Y. Sun[6], C. Chuang[7], Z. Wang[6]
[1]NU Feinberg School of Medicine, Chicago, Illinois, United States, [2]Cardinal Glennon Hospital, Saint Louis, Missouri, United States, [3]University Hospital Miguel Servet, Zaragoza, Spain, [4]RTI Health Solutions, Ann Arbor, Michigan, United States, [5]RTI Health Solutions, Research Triangle, North Carolina, United States, [6]Regeneron, Tarrytown, New York, United States, [7]Sanofi, Cambridge, Massachusetts, United States

Pruritus is the most burdensome symptom of atopic dermatitis (AD). A novel 11-point caregiver-reported worst scratch/itch numeric rating scale (WSI NRS; from 0 [no itching] to 10 [worst itching possible]) to assess pruritus in young patients with moderate-to-severe AD was developed and evaluated. Qualitative interviews were conducted with 24 caregivers of children with AD aged 6 months to <6 years to evaluate content validity. Caregivers understood the WSI NRS and were able to select a response without difficulty. Caregivers endorsed "scratching/itching" as optimal phrasing for their observation of behaviors and representation of their child's itch severity. Psychometric evaluations of the instrument were performed using data from a Phase 3 study of dupilumab in 161 children (aged 6 months to <6 years) with moderate-to-severe AD (NCT03346434). The test-retest reliability intraclass correlation coefficient (95% CI) was 0.94 (0.89, 0.96), above the recommended 0.70 threshold. The WSI NRS showed moderate to strong correlations with assessed caregiver/patient/clinician-reported clinical outcome assessments (COAs), supporting the convergent and divergent validity of the instrument. The discriminating ability of the WSI NRS was shown by significant differences in WSI NRS scores between patients grouped into COA-based bands. Anchor-based methods supported the use of at least a 2 to 4–point change in WSI NRS as clinically meaningful. These results indicate that the caregiver-reported WSI NRS is a valid, reliable and responsive instrument to assess pruritus in young children with moderate-to-severe AD.

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Management of the heightened risk for clinical events from atherosclerotic cardiovascular disease (ASCVD) in an established cohort of lupus erythematosus patients

M. Zhao[1, 2], K. J. Williams[3], D. Jacoby[4], R. Feng[5], V. Werth[1, 2]
[1]Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]Dermatology, VA Medical Center Corporal Michael J Crescenz, Philadelphia, Pennsylvania, United States, [3]Cardiovascular Sciences, Temple University Health System Inc, Philadelphia, Pennsylvania, United States, [4]Cardiology, Penn Medicine, Philadelphia, Pennsylvania, United States, [5]Biostatistics, Penn Medicine, Philadelphia, Pennsylvania, United States

Lupus erythematosus (LE) patients are at heightened risk of clinical events, chiefly heart attacks and strokes, caused by ASCVD. To address this problem, we recently proposed new guidelines for categorization of levels of risk for ASCVD events in LE patients, with corresponding recommendations for management of conventional risk factors, chiefly hypercholesterolemia, hypertension, smoking, and diabetes mellitus (Keyes E et al. 2021). Here, we performed a single-center study of our established cohort of cutaneous LE patients without or with concurrent systemic LE (n=370). Our goal was to assess how current management compares with the newly proposed guidelines. Of our LE cohort, 336/370 (90.8%) had a designated primary care physician. By the newly proposed guidelines, the most recent plasma low-density lipoprotein cholesterol (LDLc) levels for 254/370 (68.6%) of the LE cohort were above goal. Of those 254 LE patients with above-goal LDLc, the following were not on any LDL-lowering medications: 13/15 (86.7%) classified at high ASCVD event risk, 121/177 (68.4%) at very high event risk, and 24/62 (38.7%) at extreme ASCVD event risk. The American College of Cardiology calculator for the 10-year risk of an ASCVD event could be used on 248/370 (67.0%) of the LE cohort. Of those 248 LE patients, the following were not on LDL-lowering medications: 109/129 (84.5%) who had a calculated 10-year event risk <5%, 34/49 (69.4%) with 5-<10% risk, 23/43 (53.5%) with 10-<20% risk, and 11/27 (40.7%) with ≥20% risk. Of LE patients with clinically evident ASCVD, 36/82 (43.9%) were not on LDL-lowering medications. We conclude that LE patients are undertreated for conventional ASCVD risk factors. Efforts to improve the problem are underway.

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Proposing a standardized assessment of COVID-19 vaccine cutaneous reactions

R. Singh, R. Ali, S. Prasad, K. Blumenthal, E. Freeman
Massachusetts General Hospital, Boston, Massachusetts, United States

Introduction: COVID-19 vaccine skin reactions are increasingly well characterized. However, no standard grading scale exists for the spectrum of cutaneous reactions after vaccination. COVID-19 vaccine clinical trials used the U.S. FDA's Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. Only local injection site cutaneous reactions were categorized on this scale, with little granularity to grading of other cutaneous reactions. This incomplete picture restricts proper severity grading, assessment, and treatment of dermatology patients. Methods: A literature review of severity grading scales was conducted for MeSH terms: allergic reactions, drug reactions, and dermatological conditions using a standardized PubMed/Medline database search strategy, and their relevancy to grading COVID-19 vaccine cutaneous reactions was assessed by study authors using a standardized data extraction tool. Results (Proposal): Out of 30 articles assessed for inclusion, we extracted 10 relevant severity grading scales for drug and vaccine reactions. The FDA's toxicity grading scale contains relevant details on local reactions but lacked detail on other rashes seen after vaccination. The Brighton Collaboration criteria, Ring and Messmer scale, and NIAID/FAAN criteria were useful for anaphylaxis; however, they are unable to account for delayed or chronic cutaneous reactions after vaccination. The scale that could capture the broadest spectrum of COVID-19 vaccine cutaneous reactions was the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), which has been previously adapted for drug-induced cutaneous reactions. We therefore mapped known COVID-19 vaccine cutaneous reactions to the FDA's toxicity grading scale (local reactions) and the NCI's CTCAE scale (distal/generalized reactions). Conclusion: Adopting standardized terminology and grading for COVID-19 vaccine cutaneous reactions will assist researchers and clinicians in better characterizing vaccine reactions and providing appropriate counseling for patients.

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Thinking beyond race: No racial differences found in access to biologics among US psoriasis patients

R. Reddy[1], D. Yee[2], C. Zagona-Prizio[3], S. Khan[2], S. Khan[4], N. Maynard[2], M. D. Mehta[2], V. Chat[2], K. Wu[2], A. W. Armstrong[2]
[1]The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]University of Southern California, Los Angeles, California, United States, [3]University of Colorado, Denver, Colorado, United States, [4]The University of Texas Health Science Center at San Antonio Joe R and Teresa Lozano Long School of Medicine, San Antonio, Texas, United States

Multiple socio-demographic factors, such as age and English proficiency, have been associated with differential access to biologics among psoriasis patients. Although race plays a significant role in healthcare access, conflicting data exists regarding its influence on biologics access. This study compared biologic use among psoriasis patients of different races. A cross-sectional population-based study of US psoriasis patients was conducted using the 2003-2018 Medical Expenditure Panel Survey national database. Among 31,525,500 adults and children with psoriasis (weighted, mean age 49.4 years), 3,026,578 (9.6%) were on biologics. Among psoriasis patients, 27,464,864 (87.1%) identified as white, 2,033,802 (6.5%) identified as black, 1,173,435 (3.7%) identified as Asian or Pacific Islander, and 853,399 (2.7%) identified as other races, including Alaska Native, American Indian, and multiple races. Among those on biologics, 2,778,239 (91.8%) identified as white, 84,971 (2.8%) identified as black, 89,452 (3.0%) identified as Asian or Pacific Islander, and 73,917 (2.4%) identified as other races. No significant differences were found in access to biologics among psoriasis patients of different races compared to whites after adjusting for age, sex, ethnicity, insurance status, education level, poverty level, income, employment status, number of visits, and region of care (OR for blacks: 0.347 [0.118, 1.021], p=0.055; OR for Asians: 0.616 [0.240, 1.579], p=0.311; OR for other races: 0.850 [0.216, 3.336], p=0.814. This study suggests that race may not play a role in access to biologics among US psoriasis patients. Future studies are necessary to evaluate factors independently associated with biologics access among adults and children with psoriasis in the US.

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Accuracy of non-melanoma skin cancer clinical diagnosis compared to single-read histopathologic diagnosis among three independent dermatology practices

A. Aeruva, J. Rock, L. Clarke, A. Nguyen, A. Xayavong
Clinical Affairs, DermTech Inc, La Jolla, California, United States

Non-melanoma skin cancers (NMSC) are the most common malignancies affecting the global population, with basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) accounting for over 99% of NMSC. Although often curable, NMSC impose a substantial burden on healthcare systems for diagnosis and treatment. Lesional skin samples were collected non-invasively with adhesive patches from patients who presented to three dermatology practices with suspected NMSC under an IRB-approved protocol and with informed consent. After the clinical impression was recorded and non-invasive sampling was completed, a biopsy was performed, and histopathologic diagnoses were compared to the clinical impression. A total of 600 lesions were analyzed, 200 from each of 3 sites. Sites had concordance rates for clinical versus single-read histopathologic diagnoses for SCC from 29.1% (50/170), 68.5% (50/73) and 91.7% (22/24), while concordance for BCC ranged from 23.3% (7/30), 66.1% (84/127) and 46% (81/176), respectively. The average concordance for SCC was 40.7% and for BCC 57.3%. Overall, the clinical impression and histopathologic diagnosis were concordant in 49% for NMSC. The most common discordant histopathologic diagnoses for suspected SCC were actinic keratosis (AK) (43/200; 21.5%), BCC (20/200; 10%), and seborrheic keratosis (SK) (17/200; 8.5%). The most common discordant histopathologic diagnoses for suspected BCC were AK (43/200; 21.5%), SCC (42/200; 21%), and SK (6/200; 3%). The data suggest that AKs and SKs are common non-malignant simulators of NMSC. The non-invasively collected skin samples are being analyzed for genomic markers that might differentiate NMSC from common benign simulators.

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Differences in chronic venous disease and its associated risk factors amongst hispanics and non-hispanics from a diverse Population in south Florida

S. Stratman[1], G. Rouhani[2], R. Kirsner[1], H. Lev-Tov[1]
[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States, [2]Department of Public Health Sciences, University of Miami School of Medicine, Miami, Florida, United States

The objective of our study was to describe the characteristics of patients with chronic venous disease (CVD) from an ethnically diverse population of South Florida. We hypothesized that differences exist between Hispanic and non-Hispanic people with CVD. A prospective cohort of subjects was selected from a population of patients at the University of Miami Hospital and Clinics. Any adult age 18 – 95 who had venous reflux detected on duplex ultrasound of the lower extremities was contacted and questioned about study participation. Participants were divided into groups based on self-reported Hispanic/Latin ethnicity. Most of the 538 participants were women (66.7%) and, overall, 64.5% were Hispanic and 35.5% were non-Hispanic. Most of the participants were classified as overweight with 32% being Hispanic and 28.3% being non-Hispanic. We observed significant associations with ethnicity and number of pregnancies, history of varicose veins, and venous procedures. No significant association was found between Hispanic ethnicity and patient-reported history of deep venous thrombosis, smoking history, limitations in mobility, or anticoagulant medication use. There were significant associations observed between ethnicity and amount of physical activity, both days per week and minutes per week. Finally, Hispanics were more likely to have venous procedures performed. Clinicians should educate Hispanics females at early age about the risks of CVD associated with pregnancy and consider earlier screening for Hispanic multiparous females who may be at higher risk for CVD compared to their non-Hispanic counterparts, regardless of age and weight. Clinicians should also counsel Hispanic people with CVD early and aggressively about the benefit of physical activity. Future studies will randomize Hispanic people to these interventions to determine the effect on the prevalence of CVD.

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Wildfire air pollution is associated with increased eczema, acne, and itch queries on Google

R. P. Fadadu, M. Wei
University of California San Francisco Department of Dermatology, San Francisco, California, United States

Wildfires are increasing in intensity and frequency, and wildfire smoke has been shown to exacerbate atopic dermatitis and itch for patients at one tertiary care medical center in San Francisco, California.We hypothesized that the effects of wildfire air pollution on the skin could impact a larger population, as detected by trends in online searches. Information seeking behaviors on the internet can reflect shifts in population-level interests related to short-term events, such as episodes of poor air quality during California wildfires.We obtained daily environmental data, including particulate matter (PM2.5) concentration and smoke plume density score (0-3), for San Francisco during the wildfire season from June through November 2020 and the same months in 2016, as a negative control.We also collected search data for common skin symptoms and conditions from Google Trends, which provides a daily Search Volume Index (SVI) ranging from 0 to 100 (most interest) for each term. Multivariable linear regression was used to examine associations between air pollution and SVI, while adjusting for temperature, relative humidity, and year. Mean (standard deviation) weekly PM2.5 concentration was 13.5 µg/m3 (12.9) and smoke plume density was 0.79 (1.0). In adjusted analyses, a 10 µg/m3 increase in mean weekly PM2.5 was associated with an increased mean weekly SVI of 2.8 (95% CI: 0.9-4.7) for "eczema," 2.1 (0.1-4.2) for "acne," 2.8 (95% CI: 0.8-4.8) for "itchy," 1.8 (0.2-3.4) for "itchy skin," and 2.7 (0.7-4.7) for "red skin." No statistically significant results were found in adjusted models for "psoriasis." Wildfire air pollution in San Francisco had significant associations with public search interest for specific skin symptoms and diseases. Therefore, during this time, individuals may have experienced pollution-related skin exacerbations or increased questions about how air quality affects the skin. These results can inform skin health counseling and education that clinicians and public health practitioners provide during wildfires as well as suggest avenues for further study.

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Increasing incidence of cutaneous t-cell lymphoma in the United States: A SEER population data analysis

Z. Cai[1], M. Chen[1], M. Weinstock[2], R. Novoa[1], Y. H. Kim[1], E. Linos[1]
[1]Stanford University, Stanford, California, United States, [2]Brown University, Providence, Rhode Island, United States

Recent incidence patterns of cutaneous T-cell lymphoma (CTCL) in the United States are not well described. Our goal was to evaluate incidence trends of CTCL by subtype and examine associations to sex, age, race/ethnicity, and socioeconomic status (SES). Using data from the Surveillance, Epidemiology, and End Results (SEER) Registry, we calculated age-adjusted incidence rates and annual percentage change (APC) in incidence of CTCL. We identified 14,942 patients with a new diagnosis of CTCL between 2000 and 2018. CTCL incidence was higher in men compared to women across most subtypes and non-Hispanic Black patients had the highest incidence rate at 11.68 per million persons. Individuals in higher SES quintiles and metropolitan counties were more likely to be diagnosed with CTCL. The overall incidence of CTCL increased over the study period, (APC, 0.61%; 95% CI, 0.12 to 1.10). Among CTCL subtypes, Sézary syndrome had the highest APC (3.83%; 95% CI, 2.00 to 5.69), followed by mycosis fungoides (APC, 1.34%; 95% CI, 0.89 to 1.79). In stratified analyses, the groups with significant increases in incidence included women (APC, 0.92%; 95% CI, 0.34 to 1.50), < 40 years olds (APC, 2.87%; 95% CI, 1.57 to 4.19), non-Hispanic Black patients (APC, 1.63%; 95% CI, 0.80 to 2.47), patients in the lowest SES quintile (APC, 1.87%; 95% CI, 0.53 to 3.23), and individuals in metropolitan counties (APC, 0.68%; 95% CI, 0.16 to 1.20). Our findings suggest that the incidence of CTCL continues to increase, and high SES and metropolitan counties were associated with higher incidence of CTCL. In light of the known challenges involved in CTCL diagnosis, this difference may be due to lack of access to medical specialists in patients from lower SES quintiles and nonmetropolitan counties. Prospective data collection efforts for these diseases should gather data on socioeconomic status, geographic location, and healthcare access in order to better understand these differences.

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Atopic dermatitis is associated with cardiovascular risk factors in pediatric patients: A systematic review and meta-analysis

C. Kern[1], M. Johanis[2], M. Johanis[1], P. Tahir[1], M. Ye[1], A. Mulick[3], I. Allen[4], C. McCulloch[4], S. Langan[3], K. Abuabara[1]
[1]Dermatology, University of California San Francisco, San Francisco, California, United States, [2]Dermatology, UPMC, Pittsburgh, Pennsylvania, United States, [3]London School of Hygiene and Tropical Medicine, London, United Kingdom, [4]Epidemiology & Biostatistics, University of California San Francisco, San Francisco, California, United States

Atopic dermatitis (AD) is now recognized as a systemic inflammatory condition and has been associated with cardiac risk factors and outcomes, especially among adults with severe AD. Guidelines recommend enhanced cardiovascular (CV) screening for children with chronic inflammatory diseases but do not currently include AD. Thus, we performed a systematic review and meta-analysis to determine whether AD is associated with CV risk factors and outcomes in patients ≤ 18 years of age. We searched PubMed, Web of Science, CINAHL, and EMBASE through April 7, 2021 and included all observational studies with AD as an exposure and at least one risk marker or CV disease outcome. Studies on BMI have been analyzed previously and were therefore excluded from this review. Ten of 3,088 publications, with data from a total of 577,148 individuals, met inclusion criteria. Random effects meta-analyses revealed a statistically significant average association between AD and insulin resistance (aOR, 1.29; n = 4 studies; 95% CI, 1.09-1.53; I2 = 0.0%), and ischemic heart disease (OR, 1.68; n = 3 studies; 95% CI, 1.29-2.19; I2 = 0.0%), but not for lipid disorder (OR, 1.01; n = 7 cohorts; 95% CI, 1.01-1.02; 95% PI, 0.96-1.58, I2 = 96.8%) or hypertension (aOR, 1.11; n = 5 studies; 95% CI, 0.98-1.25; PI, 0.91-1.35, I2 = 0.0%). A sub-analysis of lipid disorder yielded a higher standardized mean difference for triglyceride levels (0.33 mg/dL; n = 3 studies; 95% CI, 0.16-0.51) only. Overall, studies exhibited a low risk of bias but high degree of heterogeneity. Further investigation into how AD course and severity impact CV risk will inform whether enhanced screening and treatment guidelines should be implemented to reduce CV morbidity and mortality in children with AD.

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Cutaneous spectrum of VEXAS syndrome

S. Ahmad[2], M. Ferrada[3], D. B. Beck[1], L. L. Wilson[4], P. C. Grayson[3], E. W. Cowen[2]
[1]Center for Human Genetics and Genomics, NYU Langone Health, New York, New York, United States, [2]Dermatology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, United States, [3]Rheumatology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, United States, [4]National Human Genome Research Institute, Bethesda, Maryland, United States

VEXAS (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic) syndrome is a newly described disease due to somatic mutations in UBA1, encoding ubiquitin-activating enzyme 1. Patients with VEXAS syndrome have hematologic features, such as macrocytic anemia and myelodysplastic syndrome, and systemic inflammation, including cutaneous involvement. Our aim was to characterize the spectrum of skin manifestations in a large cohort of patients with VEXAS. 87 males and 1 female with genetically confirmed VEXAS syndrome were included in this retrospective study. Mean age at disease onset was 64 years (range 39-78). Most patients (n=71, 81%) had skin involvement. Initial disease presentation was confined to skin in 16 patients (18.2%) or to skin with systemic features in 19 (21%) patients. Skin lesions were painful/tender (n=15, 21.1%) or pruritic (n=13, 18.3%), characterized by erythema (n=20, 28.2%) or nodules (n=17, 23.9%), and occurred most frequently in an acral distribution (n=25, 35.2%). 110 skin biopsies were performed in 45 patients. Skin biopsies were interpreted as small or medium vessel vasculitis (n=29, 26.4%), Sweet syndrome (n=24, 21.8%), connective tissue disease (n=9, 8.2%), or erythema nodosum (n=7, 6.4%). Histologic review of 11 cases identified a neutrophilic predominance (n=6, 54.5%) in the epidermis (n=2, 18.2%), papillary dermis (n=6, 54.5%) and reticular dermis (n=5, 45.5%). Skin lesions improved with glucocorticoids in 85.5% of patients (n=47/55), but relapse after tapering was common (60%, n=33/55). Severe injection site reactions to anakinra were frequent (64.3%, n=9/14). Skin lesions are a common, early feature of VEXAS syndrome and share similarities in clinical and histologic presentation with other inflammatory skin disease. Appreciation of the spectrum of skin findings in VEXAS will facilitate early diagnosis.

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Severity of COVID-19 in patients with dermatomyositis: A single center, retrospective observational cohort study

J. S. Johnson[1], A. Nowacki[2], J. Narang[1], S. Young[3], A. Fernandez[3]
[1]Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, Ohio, United States, [2]Cleveland Clinic Department of Quantitative Health Sciences, Cleveland, Ohio, United States, [3]Department of Dermatology and Pathology, Cleveland Clinic, Cleveland, Ohio, United States

Although dermatomyositis (DM) patients have been included in studies evaluating COVID-19 risk and severity in large cohorts of patients with chronic immune-mediated diseases, there is little data specifically evaluating these in DM patient cohorts. We performed a single-center, retrospective cohort study to evaluate the severity of COVID-19 in DM patients compared to patients with other dermatologic-immune-mediated diseases (DIMDs), and to assess for risk factors related to severe COVID-19 disease courses. Our DIMD cohort included 7,758 COVID-19 positive patients, 30 of whom had DM and 7,728 who had an alternative DIMD at time of diagnosis. COVID-19 was severe enough to require hospitalization in 7/30 DM patients (23.3%), one of whom required ICU care (3.1%) and another who died (3.1%). In an unadjusted chi-square analysis, there was a marginally significant increase in hospitalization rate among DM patients compared to other DIMD patients (23% vs. 12%, p=0.09). When controlling for age, sex, corticosteroid use, biologics use, and comorbidities, the weighted hospitalization rate was 23% in the DM cohort vs. 15% in the DIMD cohort (OR =1.73 (95% CI, 0.74-4.06)). Our study suggests that DM patients have an increased risk of developing severe COVID-19 compared to patients with other DIMDs, even after controlling for comorbidities and corticosteroid use. Although a relatively high rate of corticosteroid use (20%) and comorbidities likely contributed to severe COVID-19 in some DM patients, our results suggest other risk factors contribute to COVID-19 risk/severity in DM patients. Awareness of this risk is important for clinicians caring for DM patients in order to optimize their care and protection from a severe COVID-19 disease course.

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Skin cancer risk in people living with HIV during the antiretroviral therapy era

Y. T. Luu, Q. Luo, M. Horner, M. Shiels, E. Engels, M. Sargen
Division of Cancer Epidemiology and Genetics, National Institutes of Health, Bethesda, Maryland, United States

Background: Chronic immunosuppression is an important risk factor for non-keratinocyte skin cancers (NKSCs). Prior studies showing elevated risk of NKSCs in people living with HIV (PLWH) have focused on pre-1996 data prior to widespread antiretroviral therapy (ART). Objective: To quantify risk of NKSCs in PLWH during the ART-era. Methods: Using linked data from HIV and cancer registries in 12 U.S. regions (1996-2018), we calculated standardized incidence ratios (SIRs) for 23 NKSCs comparing risk in PLWH with and without AIDS to the general population. Poisson regression was used to assess risk factors for NKSCs. Results: Among PLWH (n=585,706) there were 2,743 NKSCs diagnosed during 4,575,794 person-years (median follow-up 7.5 years, interquartile range [IQR] 3.6-11.5), most commonly Kaposi sarcoma (KS, 82%), melanoma (12%), and lymphomas (2.6%). PLWH had elevated risk for KS (SIR 147; 95% CI 141-153), diffuse large B-cell lymphoma (DLBCL; 5.19, 3.13-8.11) and Merkel cell carcinoma (MCC; 3.15, 1.93-4.87). Adnexal cancer risk (SIR 2.01; 95% CI 1.12-3.31) was elevated for people with AIDS (n=359,823 individuals; median follow-up 7.2 years). KS risk was elevated in non-Hispanic black individuals (IRR 1.30; 95% CI 1.18-1.45), men who have sex with men (2.69; 2.38-3.05), and those with AIDS (3.60; 3.23-4.02). Melanoma risk in PLWH was decreased among non-Hispanic Black (IRR 0.06; 95% CI 0.04-0.09) and Hispanic (0.13; 0.09-0.19) individuals and increased with age (1.94; 1.73-2.18). Combined NKSC risk (all types, excluding KS) was not elevated for PLWH overall or among those with a prior AIDS diagnosis. Conclusion: Oncoviruses contribute importantly to skin carcinogenesis in PLWH, including KS (KS-associated herpesvirus), MCC (Merkel cell polyomavirus) and DLBCL (Epstein-Barr virus). The highly elevated risk for KS suggests that PLWH may benefit from routine skin surveillance. Excluding KS, PLWH with or without AIDS have similar risk for NKSCs compared to the general population during the ART-era.

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Diagnostic overlap of drug-induced acute interstitial nephritis with cutaneous involvement and drug-induced hypersensitivity syndrome.

S. M. Collier[1], J. Wongboonsin[3], S. Ahuja[1], S. Briggs[1], L. Bu[2], N. Goldfarb[3]
[1]University of Washington, Seattle, Washington, United States, [2]Mayo Clinic Minnesota, Rochester, Minnesota, United States, [3]University of Minnesota, Minneapolis, Minnesota, United States

A common clinical dilemma is distinguishing maculopapular exanthem (MPE) with systemic symptoms from severe cutaneous drug reactions, such as drug-induced hypersensitivity syndrome (DIHS). Drug-induced acute interstitial nephritis (DI-AIN) with MPE can be especially challenging to differentiate from DIHS with renal involvement. It is crucial to distinguish DI-AIN from DIHS as the prognosis and treatment differ. Our goal was to estimate the percentage of published DI-AIN cases meeting RegiSCAR criteria for DIHS and characterize the outcomes. We conducted a systematic literature search in MEDLINE (1946-2020) to find studies of DI-AIN with MPE, including DI-AIN with DIHS. Two data collectors performed independent review and data abstraction, including age, sex, RegiSCAR criteria, mortality, and drug culprit. Thirty-seven studies with 43 cases met the selection criteria. Of 28 published DI-AIN cases, 35.7% met RegiSCAR criteria for possible DIHS and 7.1% for probable/definite DIHS. Among these cases, mortality was 23.1% (possible) and 21.4% (probable/definite), respectively, compared to 6.25% for cases not meeting DIHS criteria. This review highlights that many DI-AIN cases meet RegiSCAR criteria for possible DIHS. A DIHS diagnosis may have been missed in some cases due to the diagnostic overlap between these conditions. Since there was a trend toward higher mortality in DI-AIN cases that met RegiSCAR criteria for possible DIHS, research is needed to clarify whether these cases are DIHS and whether treatment with oral corticosteroids improves outcomes.

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Halved incidence of scrub typhus after travel restriction to confine a surge of COVID-19 in Taiwan in 2021

E. Lin[2], H. Tu[4], C. Hong[1, 2, 3]
[1]Dermatology, National Yang Ming Chiao Tung University, Hsinchu, Taiwan, [2]Dermatology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, [3]Dermatology, National Sun Yat-sen University, Kaohsiung, Taiwan, [4]Kaohsiung Medical University College of Medicine, Kaohsiung, Taiwan

Scrub typhus is a rickettsial disease that is usually transmitted by mite exposure. Infected patients may present with a fever, fatigue, headache, and muscle pain. A blackish skin lesion, called eschar, is pathognomic. The mortality rate in untreated cases is high. The first case of scrub typhus in Taiwan was reported in 1908 during the Japanese colonization. In this article, using the National Infectious Disease Statistics System (NIDSS) from the Taiwan CDC, we analyzed the dynamic incidence of scrub typhus from 2016 to 2021, both seasonally and geographically. In addition, we asked whether the recent travel restrictions and social distancing policy in Taiwan (19 May to 27 July 2021), implemented due to the COVID-19 outbreak, would change the incidence of scrub typhus. The results showed that scrub typhus was most common in summer, with an incidence almost twofold greater than that in winter or spring. Most cases were identified in rural regions. Interestingly, there was a significant 52% reduction in the summer incidence in 2021, compared to the average summer incidence of the past 5 years. This reduction coincided with the countrywide lockdown measures and travel restrictions. The restricted measures for outdoor activities may have contributed to the reduced incidence of scrub typhus.

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Association of bullous pemphigoid and hypertension: A systematic review and meta-analysis

K. W. Lu[1], H. Perera[1], W. Guo[2], S. Na[1], R. Clark[2]
[1]Stony Brook University Renaissance School of Medicine, Stony Brook, New York, United States, [2]Dermatology, Stony Brook University Hospital, Stony Brook, New York, United States

Bullous pemphigoid (BP) is a chronic, autoimmune skin disorder characterized by subepidermal blistering in various regions of the body. Blister formation is caused by the production of autoantibodies to hemidesmosomal BP antigen 1 (BP230) and BP antigen 2 (BP180). Observational studies have suggested a possible association between BP and hypertension, with hypertension reported as the most frequent comorbidity in BP patients in several studies. We conducted a systematic review and meta-analysis to better elucidate the relationship between BP and hypertension. Ovid MEDLINE, Embase, Web of Science, and Cochrane databases were searched for clinical studies on bullous pemphigoid patients with hypertension, from inception to February 28, 2021. PRISMA guidelines were followed. 11 case-control studies were included in the meta-analysis, with a total of 71,812,699 study participants. Among them, 22,814 were patients with BP. The mean ages of BP patients and controls were 75.4 and 76.1, respectively. The proportion of females among the BP group was 49.9%. Using random effects modeling, the odds ratio (OR) for hypertension in patients with BP was found to be significant when compared with controls (OR=1.28 [95% confidence interval (CI) 1.04-1.56]. Subgroup analyses showed a significant association between BP and hypertension in studies with greater than 1000 BP cases (OR=1.56 [95% CI 1.19-2.04]). Whereas no association between BP and hypertension was observed among studies conducted in Europe (OR=0.94 [95% CI 0.52-1.68]), significant associations were found among studies conducted in Asian countries (OR=1.31 [95% CI 1.09-1.58]) and the United States (OR=1.96 [95% CI 1.90-2.03]). These findings suggest that BP is significantly associated with hypertension. Patients with BP may need to be closely monitored for comorbid hypertension. Despite our effort to not select papers that bias the data, there could still be bias in the selection process.

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Validation of a patient-reported outcome measure in adults with morphea

A. Walker[1], N. Teske[2], C. Zigler[3], H. Jacobe[1]
[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Dermatology, Oregon Health & Science University, Portland, Oregon, United States, [3]Population Health Sciences, Duke University, Durham, North Carolina, United States

Morphea is an inflammatory skin disease resulting in sclerosis of underlying tissues which causes functional limitations and cosmetic sequalae that negatively impact quality of life. Previous health-related quality of life (HRQoL) and patient reported outcome (PRO) studies have focused on children in which a validated measure, the Localized Scleroderma Quality of Life Instrument (LoSQI), has been developed. No validated PRO measure exists for adults with morphea. We aimed to validate the first adult morphea specific PRO using qualitative and quantitative methods. We hypothesize that the adult LoSQI will have support for validity in capturing patient-reported quality of life. Qualitative measures included focus groups to identify key domains affected in adults with morphea and cognitive interviews to validate items selected for the adult LoSQI. Quantitative analysis from field testing are forthcoming. Focus groups revealed items within content domains from the pediatric LoSQI unique to adults including worry about disease prognosis, impact on family planning, and effects on intimate relationships. Adults emphasized the impact of stiffness and limited mobility, rather than itch emphasized by pediatric patients. Feedback from cognitive interviews resulted in the removal of items not resonant with adults, including the feeling of embarrassment from morphea treatment as well as minor changes to instructions. We conclude that adults with morphea experience unique HRQoL impact compared to children and the items selected for the adult LoSQI accurately capture the adult patient's experience with morphea. The adult LoSQI represents the first morphea-specific PRO measure and an important development for the improvement of clinical trials and improved patient outcomes.

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Repeated occurrences of actinic keratoses in Medicare patients: A retrospective cohort study

N. Khalfe[1], Y. Li[2], L. Navsaria[2], C. Hinkston[2], S. Giordano[2], S. Shete[3], M. Wehner[2]
[1]Baylor College of Medicine, Houston, Texas, United States, [2]Health Services Research, The University of Texas MD Anderson Cancer Center, Houston, Texas, United States, [3]Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, Texas, United States

Actinic keratoses (AKs) are common premalignant skin lesions. Population-based research on AKs is limited, however. Anecdotally, many patients with AKs have new AKs at future visits, but evidence for this is lacking. We aimed to evaluate the number and timing of repeated occurrences of AKs in individual patients. We performed a retrospective cohort study using a Medicare dataset of 4,999,999 patients aged ≥65 from 2009 to 2018. We included patients who had 1 year of Medicare enrollment prior to an initial AK encounter (identified using validated ICD codes). We evaluated the cumulative risk of future AK encounters using a mean cumulative function and evaluated risk factors for future AK encounters with a Prentice-Williams-Peterson gap time model with robust sandwich variance.We identified 850,135 patients who met inclusion criteria. Patients were followed for a mean of 4.43 years (SD 2.67) after their initial AK encounter. The cumulative mean number of AK encounters was 2.36 (95% CI 2.36-2.37) at 2 years after initial AK, 4.09 (95% CI 4.08-4.10) at 4 years, and 6.11 (95% CI 6.09-6.13) at 6 years. Risk factors for future AK encounters included being male (HR 1.22; 95% CI 1.22-1.22), being non-Hispanic white (HR 1.05; 95% CI 1.04-1.06), and having a history of skin cancer at initial AK encounter (HR 1.12; 95% CI 1.11-1.12); adjusted for age and geographic region).This study reports that Medicare patients with AKs have approximately one AK encounter per year, indicating that AKs may behave as a chronic intermittent skin condition. Limitations include that an AK diagnosis code may have been present when a patient did not have an AK or did not have a new AK. However, approximately 80% of AK encounters had a premalignant destruction procedure. Understanding patterns of AK encounters and course of disease in individual patients can guide clinical and preventative care for patients with AKs.

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Association between atopic dermatitis and celiac disease: A systematic review and meta-analysis

U. Khan[1], K. Tang[1], W. Guo[1,2], H. Perera[1], S. Na[1], R. Clark[1,2]
[1]Stony Brook University Renaissance School of Medicine, Stony Brook, New York, United States, [2]Dermatology, Stony Brook University Renaissance School of Medicine, Stony Brook, New York, United States

Atopic Dermatitis (AD) is one of the most prevalent complex chronic inflammatory skin diseases worldwide and has been associated with many different autoimmune disorders. Celiac disease (CD), an immune-mediated gluten-enteropathy linked with dermatitis herpetiformis (DH), has recently been among these disorders. To understand the relationship between these diseases, we performed the first meta-analysis looking at the association of CD in AD patients. A systematic review of PubMed, Cochrane Central, Embase, and Web of Science was performed on all studies prior to February 26, 2021 for keywords related to AD and CD. Observational studies with data that examined the association between the two were included. A meta-analysis was then conducted using Review Manager, version 5.4. 9 of 1904 articles screened were included in this meta-analysis. Analysis demonstrated a significant association for adult AD patients having increased odds of having CD (OR: 2.25 [95% CI, 1.69-2.99], p < .05). Pediatric AD patients also had increased odds of having CD (OR: 1.80 [95% CI, 1.48-2.19], p < .05). The association was significant when stratifying by sex, men (OR: 2.54 [95% CI, 2.29-2.80], p < .05) and women (OR: 2.00 [95% CI, 1.63-2.47], p < .05) with AD had increased odds of having CD; individuals with AD also had increased odds of having DH (OR 9.77 [95% CI, 8.21-11.61], p < .05). A bi-directional association was found, as individuals with CD had increased odds of AD (OR: 2.25 [95% CI, 1.42-3.58], p < .05). This is the first meta-analysis that shows a strong association of CD in AD patients by age and sex, DH in AD patients, and AD in CD patients. It is possible that papers that found no connection of AD with bowel complaints under a review of systems were not included, however the positive relationships suggest a potential shared pathophysiological basis to these disease processes that should be the focus of future studies.

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Risk of skin cancer in individuals with neurofibromatosis type 1 in the United States: A retrospective market claims analysis

P. Trinh, S. Li, K. Y. Sarin
Dermatology, Stanford University School of Medicine, Stanford, California, United States

Neurofibromatosis type 1 (NF1) patients are susceptible to developing cancers of the central and peripheral nervous system due to loss of function mutations in neurofibromin, leading to hyperactivation of the RAS proto-oncogene and downstream signaling pathways. Although skin cancers are reported in patients with NF1, it is unknown whether NF1 patients have increased susceptibility to skin cancer compared to the general population. To evaluate the association between NF1 and skin cancers, we conducted a retrospective study of adult NF1 patients between 2009 to 2021 using the OptumInsight ® Clinformatics Data Mart claims database. NF1 patients were 1:10 propensity matched to controls without NF1 on age, sex, race, region, and length of enrollment. Conditional logistic regressions were employed to calculate odds ratios for associations between NF1 and melanoma, basal cell cancer, and squamous cell cancer compared to controls. Our study included 4,125 patients with a mean age of 47 (SD 18) and 56% female. NF1 patients displayed increased odds of melanoma (OR=2.27; 95% CI, 1.75-2.93, p<0.001) and non-melanoma skin cancers (OR=1.31; 95% CI, 1.15-1.51, p<0.001). Specifically, NF1 patients had increased odds for basal cell carcinoma (OR=1.30; 95% CI, 1.10-1.53, p=0.002) and squamous cell carcinoma (OR=1.32; 95% CI, 1.07-1.63, p=0.008). When stratified by race, there were increased odds of melanoma skin cancers among Black (OR=4.44; 95% CI, 1.37-14.43, p=0.013), Hispanic (OR=2.93; 95% CI, 1.08-7.93, p=0.035), and white NF1 patients (OR=2.16; 95% CI, 1.64-2.84, p<0.001). This study population is limited to patients with commercial insurance. In conclusion, patients with NF1 have increased odds of developing basal cell, squamous cell, and melanoma skin cancers, highlighting the role of germline RAS pathway hyperactivation in skin carcinogenesis.

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Itch and skin inflammation at insulin pump sites in patients with type 1 diabetes

M. Shinohara[1,2], J. Baran[3], D. Khakpour[3], I. Hirsch[3], A. Kalus[1]
[1]Division of Dermatology, Department of Medicine, University of Washington School of Medicine, Seattle, Washington, United States, [2]Division of Dermatopathology, Department of Laboratory Medicine and Pathology, University of Washington School of Medicine, Seattle, Washington, United States, [3]Division of Metabolism, Endocrinology, and Nutrition, Department of Medicine, University of Washington School of Medicine, Seattle, Washington, United States

Skin reactions are common with insulin pump use and can impact adherence and the ability to sustain pump therapy. Little is known about the skin changes with pump use. We investigated itch and mediators of itch at pump sites in a cohort of patients with type 1 diabetes (n=31). Skin biopsies from current and recently used pump sites were compared to control sites where a pump had never been used. Samples were analyzed by histology and tissue histamine. Ninety-four percent (29/31) of patients described itch at pump sites. Eosinophilic inflammation was significantly more common at the current (73% p<0.01) and recently used sites (75% p<0.01) compared to control, with eosinophils located deep in the dermis near the interface with the fat. The number of eosinophils at pump sites ranged from 0-31/hpf (high power field), with a median of 4/hpf. There was no significant association with the type of insulin (rapid-acting) or infusion set brand and number of eosinophils. Higher eosinophil counts were seen in patients using pumps for <10 years compared to those using pumps >20 years (p=0.02). Tissue histamine levels measured by metabolomics showed a 17% relative higher abundance at current pump sites compared to control (p=0.01). Eosinophils and histamine are likely mediators of itch with insulin pump use. Lower level of eosinophilic inflammation in long term users may suggest that early users stop pump therapy due to allergic reactions. The deep location of the inflammation suggests reaction to the infusion set materials or infused drug (preservative or trace chemicals) rather than tape used to adhere the device. Disclosure: Irl Hirsch research funding from Medtronic Diabetes, OmniPod, Beta Bionics and consultant for Abott Diabetes Care, Roche, Bigfoot and GWave.

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Clinicopathologic integration of data from skin biopsies histologically classified as "Spongiotic Dermatitis" with the EMR facilitates identification of atopic dermatitis cases

P. G. Sockler[1], D. J. Margolis[1,2], J. T. Seykora1
[1]Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]Biostatistics & Epidemiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States

Atopic dermatitis (AD) is a chronic skin condition with significant disease burden across populations. This clinical entity is often typified by a spongiotic inflammatory process, but this cutaneous pattern is not pathognomonic of AD. In this vein, AD research benefits from available tissue samples, which are not easy to obtain from patients with AD. As such, we sought to retrospectively identify patients with AD whose data, including previous biopsy specimens, can then be used for future studies, by integrating preexisting dermatopathologic information from skin biopsies with relevant clinical characteristics from the electronic medical record (EMR). In this validation study, 263 biopsies histologically classified as "spongiotic dermatitis" (SD) were cross-referenced with their corresponding EMRs. Guided by physician notes and the UK Working Party's (UKWP) diagnostic criteria for AD, clinical characteristics useful in determining the AD status of individuals were abstracted from the EMR, including history of other atopic diseases and AD comorbidities. Two groups of biopsy-EMR pairs were created, both SD+/AD- and SD+/AD+. Comparisons using logistic regression showed that the SD+/AD+ group had significantly greater proportions of relevant AD conditions, such as seasonal allergies (4.62 [2.72, 7.79], P < 0.001) and asthma (3.97 [1.90, 6.73] P < 0.001). This suggests that accurate AD diagnoses can be attributed to previous biopsy specimens, now making them usable for future studies of AD. Using similar approaches, it may be possible a comparable process could be used for other cutaneous conditions.

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COVID-19 mRNA vaccine booster cutaneous reactions reported to the AAD/ILDS dermatology registry

S. Prasad[1], D. E. McMahon[1], A. Tyagi[1], R. Singh[1], R. Ali[1], H. W. Lim[3], L. P. Fox[2], K. Blumenthal[1], G. Hruza[4], L. E. French[5], E. Freeman[1]
[1]*Massachusetts General Hospital, Boston, Massachusetts, United States,* [2]*University of California San Francisco, San Francisco, California, United States,* [3]*Henry Ford Health System, Detroit, Michigan, United States,* [4]*Saint Louis University, Saint Louis, Missouri, United States,* [5]*Ludwig-Maximilians-Universitat Munchen, Munchen, Bayern, Germany*

Background:In summer 2021, several countries including the U.S. authorized COVID-19 mRNA vaccine booster doses >6 months after completion of a patient's primary vaccine series. The aim of this study was to characterize vaccine cutaneous reactions following a booster dose of mRNA vaccine reported to the American Academy of Dermatology (AAD) & International League of Dermatologic Societies (ILDS) COVID-19 Dermatology registry. Methods:In December 2020, the AAD/ILDS registry was adapted to include COVID-19 vaccine skin reactions. In September 2021 the registry also solicited COVID-19 vaccine booster reactions either as new cases or updates to existing entries. Results:From Dec 2020-Jan 2022, 994 cases of vaccine skin reactions were entered in the registry, of which 44 records indicated the presence or absence of cutaneous reactions following a booster dose. Of 44 records, 31(71%) developed a cutaneous reaction to the booster dose and 29% developed a reaction to the 1st and/or 2nd dose but not the booster. Of the 31 patients who developed a reaction to the booster dose, 22 reacted to the booster alone, 1 reacted to the 1st & booster, 3 reacted to the 2nd & booster, and 5 reacted to all three doses. The most common morphologies among all booster reactions were local injection site reactions (n=31), delayed large local reaction (n=7), erythromelalgia (n=3), and vesicular reactions (n=3). Conclusion:Booster reactions represent a small portion of COVID vaccine reactions in the registry. Infrequent reporting could be due slow booster uptake, reporter fatigue, and/or booster reactions may truly be less frequent than reactions to the initial series. Dermatologists should be aware that cutaneous reactions to boosters are possible, even when reactions to dose 1 & 2 did not occur;none of the reactions were life-threatening.

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Poor validity of hidradenitis suppurativa diagnostic criteria in pediatric patients

J. C. Williams, N. W. Kittler, M. V. Kudlinski, J. Lester, K. Abuabara, H. B. Naik
Dermatology, University of California San Francisco, San Francisco, California, United States

Although HS commonly presents in adolescence, diagnostic criteria have not been validated in children. This study aimed to determine the proportion of pediatric patients who met HS diagnostic criteria at time of diagnosis. We conducted a single-center retrospective chart review of pediatric patients identified by HS ICD-9/10 code from January 1, 2012–July 1, 2021. We collected data on demographics and fulfillment of major diagnostic criteria: (1) typical lesion, 2) typical location, 3) recurrence (>2 flares in past 6 months)). We present descriptive statistics as proportions and fisher's exact tests. 296 patient records were included in the study. The majority were female (78.1%), Latinx (39.2%), and Black (18.9%). Median age at disease onset was 14 years (IQR 12-16 years). 55.1% (N=163) of patients were documented to have met all three diagnostic criteria at diagnosis. 96.0% (121/126) of patients who did not meet all criteria failed to meet the recurrence criterion. 79.3% (96/121) of patients who did not meet the recurrence criterion at diagnosis had additional visit records that were examined for flare frequency. Additional lesions consistent with HS were documented in 68 patients. Of 28 patients who had ≥1 lesion consistent with HS before their diagnostic visit, 10 had a documented lesion within a 6-month interval. With these additional data, diagnostic criteria were actually fulfilled in 58.4% (173/296) of patients at the time of diagnosis. Of 40 patients who had ≥1 lesion consistent with HS after their diagnostic visit, 33 (82.5%) had lesions within a 1-year period. Patients who did not meet major diagnostic criteria were less likely to have ever received a diagnosis by dermatology (34.1% vs 55.0% p < 0.001). Over 40% of pediatric HS patients do not meet all major diagnostic criteria. Many pediatric patients experience disease recurrence at a longer interval, suggesting that this criterion may be too strict for the pediatric population.

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Non-melanoma skin cancer and hereditary hemochromatosis: A retrospective cohort study

C. X. Pan[1, 2], K. Yang[1, 3], C. B. Lau[1, 4], G. Zhou[1], V. Nambudiri[1, 2]
[1]*Brigham and Women's Hospital, Boston, Massachusetts, United States,* [2]*Harvard Medical School, Boston, Massachusetts, United States,* [3]*Tufts Medical Center, Boston, Massachusetts, United States,* [4]*Boston University, Boston, Massachusetts, United States*

Hereditary hemochromatosis (HH) is a genetic disease of altered iron homeostasis. HH causes skin hyperpigmentation and various diseases, such as hepatocellular carcinoma via oxidative stress. Although oxidative damage also plays a role in the pathogenesis of non-melanoma skin cancer (NMSC), the link between HH and NMSC remains unknown. Data on HH patients with skin neoplasms and controls from 1980 to 2021 matched by age, sex, and race were extracted. HH carriers without disease and/or those without pathology-proven skin neoplasms were excluded. Univariate and multivariate regression analyses (adjusted for age, sex, skin type, smoking history, and NMSC risk factors) were conducted. Among 321 HH patients and 374 controls, HH patients (92%) had significantly higher rates of NMSC than controls (83.4%) (adjusted odds ratio (OR), 2.08 [95%CI, 1.26, 3.43], P= 0.004). When stratified by NMSC type, HH patients had significantly greater odds of having basal cell carcinoma (BCC) than controls (adjusted OR, 1.93 [95%CI, 1.39, 2.69], P<0.001). There were no significant differences in age at diagnosis (P= 0.086), mean number of NMSCs (P=0.129), or rates of squamous cell carcinoma (SCC) (P= 0.077). A subset analysis of HH patients with NMSC by phlebotomy treatment status showed that treated patients had significantly higher rates of NMSCs (P<0.001) and were diagnosed at a younger age compared to the untreated group (P<0.034). Notably, there were no significant differences in rates of BCCs and SCCs when stratified by NMSC type. Our findings suggest that HH may be a risk factor for NMSCs, particularly BCCs, potentially warranting increased skin cancer surveillance among HH patients. Phlebotomy treatment does not appear to be protective against NMSCs and may be associated with confounders, such as greater interaction with the healthcare system, requiring further analysis.

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International survey: Effects of cumulative exposure to corticosteroids in patients with eczema, including topical steroid withdrawal syndrome (TSWS).

K. Z. Tullos[2], K. Barta[1], P. Lio[3], T. Winders[4, 5]
[1]*Advocacy, Allergy and Asthma Network, Vienna, Virginia, United States,* [2]*International Topical Steroid Awareness Network, Dacula, Georgia, United States,* [3]*Dermatology and Pediatrics, NYU Langone Hospital - Long Island, Mineola, New York, United States,* [4]*President, Allergy and Asthma Network, Vienna, Virginia, United States,* [5]*Global Allergy and Airways Patient Platform, VIENNA, Virginia, United States*

Patients with eczema have a lifetime of exposure to corticosteroids (topical (TCS), oral (OCS), inhaled, nasal sprays etc). The survey examined cumulative effects of corticosteroid exposure, sociodemographic characteristics, disease attributes, history of eczema treatment, and experience and knowledge of topical steroid withdrawal syndrome (TSWS). Surveyed total lifetime corticosteroid use of all types. 91% reported TCS use with an average duration of 15.3 years, applying 1-2 times daily, for 15-30 days a month, on an average body surface area (BSA) of 24%. Adult and child body diagram was used to assess eczema symptom location and severity from the PO-SCORAD tool, and to determine % of BSA where TCS were used most often. OCS for eczema or other conditions was 36%, with an average of 8 OCS courses during their lifetime. Adults (83%) and children (64%) had new and/or worsening symptoms overtime. A description of TSWS was included - 79% of adults and 43% of children had symptoms consistent with TSWS. Body diagram assessed TSWS BSA, symptoms, and severity. Over 90% of all adults with TSWS report burning, skin shedding/profuse flaking, and flushing or darkening depending on skin tone. Survey distribution was by email or social media to patients by patient organizations and panels between 11/2020–1/2021, and completed by those 18 years of age and older diagnosed with eczema or a caregiver of a child with eczema. All participants (n=2,160) completed the survey; 87.4% were adults while 12.5% were caregivers of children. Respondents were from 70 countries; 85% of adults were female; 55% of the children were male. Cumulative corticosteroid exposure over a lifetime of eczema is substantial and associated with development of new conditions, including TSWS.

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Epidemiology and survival of merkel cell carcinoma by sex in the United States

I. H. Moseley[1], S. D. Ragi[1], A. Lombardi[2]
[1]Brown University Warren Alpert Medical School, Providence, Rhode Island, United States, [2]Skin Cancer & Cosmetic Surgery Center of New Jersey, Edison, New Jersey, United States

Introduction: Merkel Cell Carcinoma (MCC) is a rare, aggressive skin cancer with a known male predominance. However, it is unknown whether tumor presentation, age of presentation, or prognosis varies by sex. We analyzed data from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEERs) Program to investigate these questions. Methods: Data for MCC were collected from the SEER-18 (2000-2018) database. The total number of cases, incidence, age, race, ethnicity, primary tumor location, staging, cause-specific death, and first malignancy status were collected in SEER*Stat. We used traditional chi-squared or Fisher exact tests to compare the aforementioned variables between sexes. We calculated relative survival by the Kaplan–Meier method. Rates are per 100,000 and age-adjusted to the 2000 US Std Population. Results: In the SEER data set, MCC had an incidence rate of 0.616 (95% CI 0.604-0.628), 0.943 (95% CI 0.919-0.967) in men, and 0.390 (95% CI 0.377-0.403) in women. Significant differences between sexes (p <0.05) were observed for: age, race, ethnicity, location, staging, first malignancy status, and disease-specific death. Survival rates at 1, 3, & 5 years also differed significantly by sex. 76.80% of men & 72.88% of women presented before age 85. MCC was the first malignancy diagnosed in 66.23% of men & 75.44% of women. The most common location was the face (23.77% of men & 29.37% of women), and most common stage at presentation was localized (46.69% of men & 51.67% of women). Relative survival rates at 1, 3, and 5 years were 83.8%(95% CI 82.40-85.10), 63.40%(95% CI 61.50-65.30), & 56.20%(95% CI 53.90-58.40) in men & 87.20%(95% CI 85.60-88.70), 74.40%(95% CI 72.00-76.50), & 70.10%(95% CI 67.30-72.70) in women. Death due to MCC occurred in 29.44% of men & 21.08% of women. Conclusion: Our results elucidate differences in MCC survival between the sexes and reveal that women may have more favorable prognoses with significantly higher relative survival rates at 1, 3, and 5 years.

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Analyzing the impact of COVID-19 on trends in the dermatology literature

R. Ali, S. Prasad, R. Singh, L. Abdelrahman, R. Kankaria, S. Singh, S. Mehta, V. Mroz, E. Freeman
Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Intro: For the past two years, the COVID-19 pandemic has significantly impacted scientific publishing across medical specialties. We sought to characterize the rising body of evidence related to COVID-19 within the dermatology literature since March 2020, the WHO-declared start of the pandemic. Methods: We extracted all publications indexed in PubMed from March 11, 2020 to Sept 31, 2021, from the four highest impact factor journals in dermatology: Journal of the American Academy of Dermatology (JAAD), JAMA Dermatology (JAMADerm), British Journal of Dermatology (BJD), and Journal of Investigative Dermatology (JID). Rapid communications were excluded. Titles & abstracts of all publications were reviewed to determine relevance to COVID-19; articles were then classified by publication type and category of COVID-19 related content. Results: Of 3,649 publications, 335 were COVID-19 related (9%). Of these 335 publications, content focused on: cutaneous manifestations of COVID (32%), other diseases in the time of COVID (28%), practice management (11%), PPE/occupational health (10%), medical education (8%), vaccines (5%), pathophysiology (3%), impact of COVID on research (2%), and disparities (1%). In the first three-month period of the pandemic, 95 COVID related articles were published, the majority of which were research letters (18(19%)) followed by editorials (12(13%)). In the following three-month period (June-Aug 2020), 93 articles were published, primarily research letters (23(25%)) followed by original articles (3(3%)). Conclusion: The rapid rise in COVID-related dermatologic publications within the first few months of the pandemic mirrors the trend seen in other specialties. COVID-19 related articles now comprise a significant share of the dermatologic literature and cover a range of topics in pandemic response beyond characterization of SARS-CoV-2 itself.

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Oblique earlobe crease as a physical examination finding in drug reaction with eosinophilia and systemic symptoms

T. Gilkey[3], M. Amigo[3], S. Himed[3], N. Rojek[1], N. Milani-nejad[2], A. Korman[3], J. Trinidad[3], B. H. Kaffenberger[3]
[1]Department of Dermatology, UC Irvine Douglas Hospital, Orange, California, United States, [2]Department of Dermatology, University of California Los Angeles David Geffen School of Medicine, Los Angeles, California, United States, [3]Department of Internal Medicine, Dermatology, The Ohio State University College of Medicine, Columbus, Ohio, United States

Introduction: While drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome is known to be associated with facial swelling, this edema can be challenging to identify. This study evaluates the utility of a physical examination finding, the oblique earlobe crease, to support the diagnosis of DRESS. Design: This is a retrospective case-control study in which dermatologists evaluated for the presence of an oblique earlobe crease in patients diagnosed with DRESS syndrome compared to unmatched controls with morbilliform drug reactions, Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN), or acute generalized exanthematous pustulosis (AGEP). Ear images were available in the chart of 17 DRESS, 18 morbilliform, 5 SJS/TEN, and 5 AGEP patients and were included in the study. Results: The patients included were an average age of 56 and 51% were male. When comparing DRESS vs. all other eruptions (low-risk morbilliform, SJS/TEN, and AGEP), the presence of an oblique earlobe crease had a sensitivity of 81% (CI 64-93%) and specificity of 71% (CI 57-83%) for diagnosis of DRESS. When comparing DRESS vs. low-risk morbilliform drug eruptions in particular, the presence of an oblique earlobe crease had a sensitivity of 81% (CI 64-93%) and specificity of 65% (CI 46-80%) for diagnosis of DRESS. Conclusions: This study demonstrates that the presence of an oblique earlobe crease is a non-invasive indicator for the potential diagnosis of DRESS syndrome when compared to morbilliform, as well as all other drug eruptions. The presence of an oblique earlobe crease in a patient with a suspected drug eruption is a strong indication to complete a validated DRESS scoring assessment.

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Impact of childhood atopic dermatitis on cognition and achievement

J. Wan[4], J. M. Gelfand[1], E. Ma[3], D. B. Shin[1], S. Hooper[2]
[1]University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina, United States, [3]University of Maryland School of Medicine, Baltimore, Maryland, United States, [4]Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Atopic dermatitis (AD) often arises in early childhood during a critical period of cognitive development and has been linked to attention deficit hyperactivity disorder, sleep disruptions, and neuropsychiatric comorbidities. However, less is known about the impact of AD on cognition and academic achievement. In a birth cohort of U.S. children followed in the Study of Early Child Care and Youth Development from 1991 to 2007, we conducted a retrospective cohort study comparing 116 children with AD and 1,104 children without AD with respect to the Wechsler Abbreviated Scale of Intelligence in 4th grade and Woodcock-Johnson-Revised (WJ-R) tests of achievement in 3rd and 5th grades. AD was assessed at 36 months old, 54 months old, and 1st grade; caregiver report of AD at ≥2 points was used to define the presence of AD. Mean (SD) full-scale IQ was 108.8 (15.3) and 106.6 (14.3) in AD and non-AD groups, respectively (p=0.15). Adjusted for sex, race, ethnicity, parental education and family income, AD was not significantly associated with a bottom-quartile IQ<90 (OR 1.30, 95% CI 0.58-2.91). Mean standard scores for WJ-R Broad Math and Broad Reading did not differ between AD and non-AD groups in 3rd or 5th grades. Using generalized estimating equations adjusted for sociodemographics and parental education, AD was not statistically significantly associated with low achievement in math (OR 1.24, 0.49-3.12) or reading (1.73, 0.71-4.20). Sensitivity analyses broadening AD definitions to include caregiver-reported AD at any single timepoint led to similar results. While we did not observe any statistically significant differences in general cognition or academic achievement in this population of children with likely predominantly mild AD, future studies examining larger populations including children with more severe AD and using concurrent outcome measurements are needed.

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A non-invasive genomic assay for pigmented lesions to rule out primary cutaneous melanoma: Interim analysis of a national registry database

C. Rubin[1], M. Skelsey[2, 3], L. Clarke[4], J. Rock[4], B. Jansen[4], T. Arnold[4], J. Wood[4]
[1]Dermatology, Oakland University William Beaumont School of Medicine, Rochester, Michigan, United States, [2]Dermatology, Dermatologic Surgery Center of DC, Chevy Chase, Maryland, United States, [3]Dermatology, Georgetown University, Washington, District of Columbia, United States, [4]Clinical Affairs, DermTech Inc, La Jolla, California, United States

Purpose: A non-invasive genomic test that detects over-expression of PRAME and/or LINC00518 RNA has been shown to rule out melanoma in uncertain pigmented lesions with a negative predictive value of >99%. Detection of TERT promoter mutations (TERTpm) was recently shown to further enhance the assay's sensitivity, and TERTpm analysis is now offered as an optional add-on to the assay when sufficient DNA is present. A clinical registry was initiated to better characterize TERTpm analysis and its effect on test performance. Materials and Methods: An interim analysis was initiated when registry enrollment reached approximately 50%. All lesions with positive test results (abnormalities of PRAME, LINC00518, and / or TERTpm) were identified and test results were compared to the histopathologic diagnoses within submitted pathology reports. Results: Between April to December 2021, the registry enrolled 4244 lesions tested at 59 different clinical sites from across the United States. Analysis of TERTpm was requested in 2761 (65%). 51/2761 (1.9%) were positive for TERTpm, sixteen of which (16/2761; 0.9%) were also positive for LINC and PRAME over-expression. Corresponding pathology reports were available for 15/16 'triple-positive' cases and showed that eleven were diagnosed as melanoma (5 in situ and 6 invasive, thickness range 0.3mm - 0.9mm), two as dysplastic nevi with moderate-to-severe atypia, one as a pigmented basal cell carcinoma, and one as a seborrheic keratosis. Conclusions: Of 16 lesions positive for the 3 genomic abnormalities detected non-invasively by the assay, subsequent biopsies confirmed that 14 were either malignant or high-risk lesions, indicating the high specificity of the test result. Further analyses will be performed upon completion of registry enrollment in mid-2022.

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Associations between atopic dermatitis and arthritic conditions: A systematic review and meta-analysis

R. C. Williams, M. Brako, H. Usmani, W. Guo, S. Na, R. Clark
Stony Brook University Renaissance School of Medicine, Stony Brook, New York, United States

Atopic dermatitis (AD) is an inflammatory skin disease with a strong immune component. Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes synovitis and destruction of small joints. Researchers have attempted to quantify an association between both diseases with mixed conclusions. Studies on associations between AD and psoriatic (PsA), osteo (OA), and juvenile idiopathic arthritis (JIA) also have mixed conclusions. This systematic review and meta-analysis will study the association between AD and various arthritic conditions. Medline, Web of Science, Cochrane, and EMBASE databases were searched for relevant studies from inception to March 2021. Observational studies examining relationships between AD and arthritis were selected. Risk of Bias analyses were done using the Newcastle-Ottawa Scale and statistical analyses were done using RevMan. 2539 studies were screened and 9 were found suitable for quantitative analysis. Patients with RA were not at significantly increased odds of having comorbid AD (OR 1.10, 95% CI 0.82-1.46, p=0.52). These findings were consistent for both case-control (OR 1.28, 95% CI 0.81-2.02, p=0.29) and cross-sectional (OR 1.20, 95% CI 0.72-1.46, p=0.89) studies. However, an association was found for RA in AD patients (OR 1.28, 95% CI 1.07-1.53, p<0.001). This finding was consistent for both cross-sectional (OR 1.37, 95% CI 1.16-1.61, p=0.0002) and case-control (OR 1.15, 95% CI 0.72-1.82, p=0.56) studies. Three cohort studies also found significant risk of RA in AD patients. There were not enough studies to perform a meta-analysis between AD and other forms of arthritis. Two studies, one on JIA and one PsA, found no association with AD. Two studies on AD and OA had conflicting results. The present study provides definitive evidence of an increased probability of comorbid RA in AD patients, but not PsA, OA or JIA. No such association exists for AD in arthritis patients. These results highlight the unique inflammatory aspect of RA as opposed to other arthritic conditions.

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The association between bullous pemphigoid and schizophrenia: A systematic review and meta-analysis

B. Cannata, F. Noor, W. Guo, K. W. Lu, S. Na, D. Lozeau
Stony Brook University Renaissance School of Medicine, Stony Brook, New York, United States

Background: Bullous pemphigoid (BP) is a common autoimmune disorder characterized by pruritic, tense blisters. BP is often seen in the elderly and is associated with BP180 and BP230 autoantibodies against the epidermal basement membrane zone (BMZ) which result in blister formation via the separation of the dermis and epidermis. Earlier studies demonstrated an association between BP and various neurologic diseases such as stroke, Parkinson's disease, and dementia, but few have explored the relationship between BP and schizophrenia specifically. Objective: This systematic review aimed to evaluate the relationship between BP and schizophrenia. Methodology: This study used a comprehensive database search that included OVID Medline, EMBASE, Cochrane Central, and Web of Science. The case-control studies that met specific search criteria were selected to calculate a pooled odds ratio. The Newcastle-Ottawa scale was used to evaluate risk of bias for each included study. Three case-control studies were included in the meta-analysis. Results: Three studies were reviewed. The pooled meta-analysis identified a significantly increased odds of having schizophrenia in BP patients OR, 2.36; 95% CI 1.84, 3.04. One study by Forsti found a significant bi-directional association between BP and schizophrenia. This study also found a significant association between BP and schizotypal and delusional disorders. One study by Chen stratified by gender and found that BP was not significantly associated with schizophrenia in males but there was a positive association with schizophrenia in females. Conclusion: The results show that there is a positive association between BP and schizophrenia. These findings are in line with previous studies that found significant associations between BP and other neurological diseases. Medications and autoantibodies are two factors theorized to contribute to this outcome. Clinicians should be aware of the potential association between BP and neurologic diseases.

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Merkel cell carcinoma recurrence risk is lower in patients with autoimmune disease than in those with other types of immune suppression

S. Y. Park[1], D. Hippe[1], L. Zawacki[1], M. Bierma[1], S. Bhatia[1], L. Zaba[2], P. Nghiem[1], N. Singh[1]
[1]University of Washington, Seattle, Washington, United States, [2]Stanford University, Stanford, California, United States

Merkel cell carcinoma (MCC) is twice as likely to recur in immunosuppressed (IS) patients (pts) as in immunocompetent (IC) pts. Iatrogenic IS due to autoimmune disease (AD) may influence prognosis differently than intrinsic IS such as due to hematologic malignancy. Moreover, modification of IS medication may improve prognosis. Here, we assess data from 762 MCC pts in an IRB-approved registry. We categorized pts into 3 groups: IS due to AD (ISAD), IS from other causes (ISnon-AD), or immune competent (n=31, 70 and 661 respectively). ISAD pts were subcategorized into rheumatoid arthritis (ADRA, n=13) vs. AD except for RA (ADnon-RA,n=18). Pts with ISAD had lower stage disease (local disease: 58% vs. 36%, p = 0.003) and smaller primary tumors than ISnon-AD (≤ 2 cm: 83% vs. 57%, p=0.023). After adjusting for age, sex, and stage, ISAD pts (ADRA and ADnon-RA) overall had a 54% higher recurrence rate (HR: 1.54, p=0.21) than IC pts. In comparison, ISnon-AD group had a 165% higher recurrence rate (HR: 2.65, p<0.001) than IC pts. When considered separately, ADRA pts appeared to have a similar recurrence rate as IC pts (HR: 1.19, p=0.76) while ADnon-RA pts had a higher recurrence rate (HR: 1.83, p=0.16) relative to IC pts. At the time of MCC diagnosis, 80% (n=24) of AD pts were on IS medication including conventional disease modifying medications, biologics, or oral steroids. After MCC diagnosis, 22% (5 pts) stopped all medication. Among patients on biologics, 89% (8/9 pts) elected to stop the drug. Eleven pts with AD experienced recurrences. Our study was underpowered to demonstrate associations regarding particular IS medications and recurrence. In this cohort, pts with AD appeared to have a better prognosis than intrinsic IS, with RA conferring very little risk above that for immune competent pts.

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FFA and personal care products: a systematic review and meta-analysis

O. Kam[1], S. Na1, W. Guo[2], C. Tejeda[2], T. Kaufmann[2]
[1]Stony Brook University Renaissance School of Medicine, Stony Brook, New York, United States, [2]Dermatology, Stony Brook University Renaissance School of Medicine, Stony Brook, New York, United States

Background: Frontal fibrosing alopecia (FFA) is a scarring, immune-mediated follicular scalp disease. Researchers have postulated that there is a hormonal and genetic etiology since FFA most commonly affects postmenopausal Caucasian women. Recently, dermatologists have reported cases of FFA as being potentially caused by using skin and hair products. Therefore, this systematic review and meta-analysis intends to be the first to analyze the relationship between FFA and personal care products. Methods: The Cochrane, PubMed, EMBASE, and Medline databases were searched for relevant studies from the date of inception to June 2021. Case-control, cross-sectional, and cohort studies examining the effects of personal care product use on FFA, available in English full-text, were included. Analyses were performed using Review Manager, version 5.4. Results: Five studies were included in our quantitative analyses, totaling 683 FFA patients and 755 controls. There were significant positive associations found for FFA and sunscreen (OR 2.82, 95% CI 1.21-6.57; p<0.05) and facial moisturizer (OR 1.93, 95% CI 1.46-2.56; p<0.05). Analyses did not demonstrate any association with facial cleanser (OR 1.35, 95% CI 0.85-2.12; p<0.05), shampoo (OR 0.63, 95% CI 0.16-2.45; p<0.05), hair conditioner (OR 0.77, 95% CI 0.41-1.46; p<0.05), hair mousse (OR 1.37, 95% CI 0.75-2.51; p<0.05), hair gel (OR 0.90, 95% CI 0.48-1.69; p<0.05), hair dye (OR 0.59, 0.27-1.30), hair perming (OR 1.23, 95% CI 0.68-2.24; p<0.05), hair styling aid (OR 0.96, 95% CI 0.59-1.55; p<0.05), facial toner (OR 0.28, 95% CI 0.04-1.84; p<0.05), cosmetic foundation (OR 1.09, 95% CI 0.75-1.59; p<0.05), and aftershave (OR 2.08, 95% CI 0.08-50.85; p<0.05). Conclusions: This meta-analysis concluded that leave-on facial products, namely facial sunscreen and moisturizer, are associated with FFA. No relationship was found for hair products. These findings suggest an environmental etiology in the development of FFA, particularly UV-protecting chemicals.

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Development of systemic lupus in patients with cutaneous lupus: A comparison of three classification criteria

A. Walker[1], S. Black[1], F. Walocko[1], X. Li[2], B. F. Chong[1]
[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Population and Data Sciences, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

Studies investigating the progression of patients from cutaneous lupus (CLE) to systemic lupus (SLE) have used the American College of Rheumatology (ACR) diagnostic criteria more than newer criteria including the European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) criteria or the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria. Our study aimed to compare rate of progression from CLE to SLE within each criteria set in a cohort of CLE patients. We also compared baseline risk factors and criteria gained. We conducted a retrospective, single-center chart review study of CLE patients seen in outpatient dermatology clinics at University of Texas Southwestern Medical Center and Parkland Health and Hospital System between December 2008 to July 2021. 2.8% (3/107) of patients progressed from CLE to SLE using EULAR/ACR criteria (median time: 3.5 years). 13.1% (14/107) and 8.4% (9/107) CLE patients progressed to SLE under ACR criteria (3.5 years) and SLICC criteria (3.6 years), respectively. The progression rate under EULAR/ACR criteria was significantly different from ACR (p=0.0016) and SLICC (p=0.034) criteria. Patients who progressed to SLE under ACR and SLICC criteria most frequently developed hematologic abnormalities and positive ANA, while those under the EULAR/ACR criteria all acquired arthritis. Baseline risk factors for SLE progression under the EULAR/ACR criteria included ANA titer ≥1:80 (100% vs 35.2%, p=0.049), and low C3 (33.3% vs 0%, p=0.03). Decreased CLE to SLE progression under EULAR/ACR criteria may be due to the hierarchal design allowing one criterion to count for each domain and ANA not counting toward total points. This lower rate of progression under EULAR/ACR criteria may better reflect the general trend of CLE patients developing mild SLE disease.

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Evaluation of skin stiffness in cutaneous fibrosing disorders: A novel constructive shear wave interference ultrasound technology

M. E. Belina[1], T. Patel[1], B. Liu[2], C. Green[2], P. Sener[3], P. Hollender[3], A. Cardones[4]
[1]Duke University School of Medicine, Durham, North Carolina, United States, [2]Biostatistics and Bioinformatics, Duke University, Durham, North Carolina, United States, [3]Microelastic Ultrasound Systems, Durham, North Carolina, United States, [4]Dermatology, Duke University Medical Center, Durham, North Carolina, United States

There is a critical need for objective and reproducible methods to evaluate skin sclerosis in individuals with fibrotic skin conditions. Constructive Shearwave Interference (CSI) is a novel ultrasonic method for noninvasive characterization of skin elasticity; the tool uses a cylindrical source to generate constructively interfering shear waves in the target medium. The speed at which the shear waves propagate is directly proportional to local tissue stiffness, with stiffer areas of tissue exhibiting faster shear wave speed (SWS). In previous studies, shear wave measurement has been found to be a sensitive, reproducible method of evaluating skin sclerosis. We report here preliminary data from an Institutional Review Board-approved, noninterventional, prospective study to evaluate the utility of CSI in quantifying skin stiffness in patients with cutaneous fibrosing disorders as well as healthy volunteers. We obtained CSI measurements from 37 individuals with chronic graft-versus-host disease (16), systemic scleroderma (3), morphea (3), other fibrosing disorders (2), patients with prior bone marrow transplant but no cutaneous disease (3), and healthy volunteers (10). Prior to imaging, each patient was evaluated by a dermatologist with expertise in fibrosing skin disorders, and each skin site imaged received a Clinical Sclerosis Score (CSS) ranging from 0 to 3. Preliminary analysis demonstrated that average SWS score increased with increasing CSS. Average dermal thickness, determined by standard B-mode ultrasound and further evaluated on histology for 15 patients, was also found to correlate positively with CSS. Our findings suggest that CSI is a promising new technology for portable, noninvasive, and objective assessment of skin sclerosis in patients with cutaneous fibrosing disorders.

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Multi-center, retrospective analysis of patients with drug reaction with eosinophilia and systemic symptoms (DRESS)

D. A. Emge[1], B. Liu[2], C. Green[2], L. Banezl[3], M. Mauskar[3], C. Ziemer[4], R. Micheletti[5], F. Nutan[6], K. DeNiro[7], A. Mostaghimi[8], J. Keller[9], B. Nardone[10], C. Nguyen[10], L. Seminario-Vidal[11], J. Curtis[12], L. Madigan[12], R. deShazo[12], A. Cardones[1]
[1]Dermatology, Duke University School of Medicine, Durham, North Carolina, United States, [2]Biostatistics & Bioinformatics, Duke University School of Medicine, Durham, North Carolina, United States, [3]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [4]Dermatology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina, United States, [5]Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [6]Virginia Commonwealth University Medical Center, Richmond, Virginia, United States, [7]Dermatology, University of Washington School of Medicine, Seattle, Washington, United States, [8]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, [U]nited States, [9]Dermatology, Oregon Health & Science University School of Medicine, Portland, Oregon, United States, [10]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [11]Eli Lilly and Company, Indianapolis, Indiana, United States, [12]Dermatology, The University of Utah School of Medicine, Salt Lake City, Utah, United States, [13]Agency for Healthcare Research and Quality, Rockville, Maryland, United States

We conducted a multi-center, 10-year retrospective study of 11 institutions across the USA to identify and describe characteristics, management, and outcomes among DRESS patients. There were a total of 391 pediatric and adult inpatients with final diagnosis of DRESS on discharge. The majority (332/391, 84.9%) had a RegiSCAR score qualifying as either possible, probable, or definite DRESS. Only 110/391 (28.1%) would be classified as typical or atypical DRESS by J-SCAR criteria. Morbilliform eruption or BSA >30% (each in 325/391, 83.1%) were the most common skin findings. Liver involvement (244/391, 68.5%) was the most common extracutaneous finding. Median time from admission to complete resolution of DRESS (defined as not requiring oral or topical medication) was 52 [IQR 32,89] days among 234 patients with this data. Few (9/391, 2%) patients died during admission. This is the largest cohort of DRESS patients from the USA that has been described. Our data reflects a prolonged course, but low mortality for DRESS patients.

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Is prurigo pigmentosa simply a "keto" rash?

A. Shen[1], N. Vecerek[2], S. Worswick[2], M. Hogeling[1]
[1]University of California Los Angeles David Geffen School of Medicine, Los Angeles, California, United States, [2]University of Southern California Keck School of Medicine, Los Angeles, California, United States

Prurigo pigmentosa (PP) is an inflammatory skin disease that has increasingly been associated with the ketogenic diet and is even nicknamed "keto rash." However, when Nagashima first described PP in 1971, ketosis was not the proposed cause but rather friction from clothing. PP is an uncommon diagnosis, even more so in Western countries. As a result, current knowledge has been limited to case reports. A retrospective chart review of PP patients over a 10-year period including several academic centers in the United States was designed to study a larger number of PP patients. Data on the demographics, diet, location of the rash, season of onset, comorbidities, blood test results, and treatments were extracted. Preliminary data revealed ten PP patients from a single center. Interestingly, only two of the ten patients were on a ketogenic diet prior to the onset of symptoms. The others denied ketogenic diet and had normal hemoglobin A1c levels and no prior diagnosis of diabetes mellitus or other metabolic disorders. Notable characteristics include a large female predominance (female to male ratio 7:3) with the mean age of 44 (age range from 18-72). All but one patient had BMI >25. Interestingly, five of the ten patients had onset of symptoms in the Fall, between October to November. The most commonly affected site was the back, followed by the extremities. Treatment-wise, all patients treated with antibiotics had resolution of PP. Topical steroids provided temporary relief but did not lead to complete resolution. Our data shows there may be several triggers for PP, which involve more than just ketogenic diet and ketosis. In particular, the effectiveness of treatment with antibiotics may provide insight into PP's pathogenesis.

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Fracture risk in adult and pediatric patients with atopic dermatitis -a population-based cohort study

M. Syed[1], D. B. Shin[1], J. Wan[1], A. Lemeshow[2], J. M. Gelfand[1]
[1]University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Pfizer Inc, New York, New York, United States

Atopic dermatitis (AD) is a chronic, relapsing skin disease associated with significant morbidities. Emerging evidence suggests that AD may be a risk factor for major fractures due to a decrease in bone mineral density resulting from concurrent chronic corticosteroid exposure and/or systemic inflammation. However, previous studies were limited by design and generalizability and pediatric data are scarce. We aimed to quantify the association between AD and fractures using a U.K. population-based electronic health records data from 1994 -2015. 625,083 adults and 409,431 children with AD were identified using a previously validated algorithm and matched on age, practice, and index date with 2,678,888 adult and 1,809,029 pediatric controls. Hazard ratios (HRs) were calculated using Cox regression and stratified by age and severity, which was defined as a time-updated variable using proxy measures of treatments and/or dermatologist referral. We found an overall small but increased fracture risk in children with AD aged 6-11 y (HR:1.07; 95% CI 1.04 -1.09) and 12-17y (HR: 1.04, 95% CI 1.00-1.07) compared to children without AD but no difference in risk among those aged <6 y (HR:1.00; 95% CI 0.98 -1.02). In children with severe AD, there was no difference in risk between AD and controls across all age groups. In comparison, select age groups of children with moderate (12-17 y HR:1.10; 95% CI 1.02 -1.18) and mild AD (6-11 y HR:1.07; 95% CI 1.05 -1.10) were more likely to develop fractures compared to controls. Among adults, those with AD had an increased overall risk of fractures versus controls (18-64 y HR:1.04; 95% CI 1.02 -1.05; ≥65y HR: 1.06, 95% CI 1.04-1.08). These associations were strongest among adults with severe AD (18-64 y HR:1.22; 95% CI 1.15 -1.29; ≥65y HR: 1.26, 95% CI 1.16-1.36). In summary, AD is associated with an overall small but increased risk of fractures, suggesting the need for additional research to establish biological plausibility.

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Improved survival of multiple vs single primary melanomas

M. M. Sarver[1], J. D. Rames[1], G. M. Beasley[2], J. Gao[3], S. Jung[3], S. C. Chen[4]
[1]Duke University School of Medicine, Durham, North Carolina, United States, [2]Surgery, Duke University School of Medicine, Durham, North Carolina, United States, [3]Biostatistics, Duke University School of Medicine, Durham, North Carolina, United States, [4]Dermatology, Duke University, Durham, North Carolina, United States

Background: Patients with single primary melanomas have an increased risk of developing subsequent melanomas. Second tumors diagnosed within three months of the incident lesion are termed "synchronous", while "asynchronous" tumors are diagnosed outside of this window. Objective: Compare the tumor distributions and survival characteristics of patients with synchronous and asynchronous second primary melanomas to patients with single primary melanomas. Methods: Retrospective cohort study. Data were collected from an institutional database from 14,029 patients with a diagnosis of a primary melanoma seen between 1970 and 2004. Results: The synchronous and asynchronous cohort demonstrated a significantly improved survival probability compared to the single primary cohort (p=.04 and .002, respectively). After controlling for relevant covariates, only the asynchronous group demonstrated a significant survival advantage over the single primary cohort (HR: 0.76, p=.02). Single primaries (2.2mm, SD=2.3) were significantly thicker than synchronous (2.0mm, SD=1.7) and asynchronous lesions (1.7mm, SD= 1.3). Synchronous lesions were more likely to be concordant compared to asynchronous lesions (55.7% vs 38.2%, p<.001). Limitations: Limitations include a single-center study design, incomplete records specifically pertaining to Breslow depth data for second primary melanomas, and database-level lack of histopathologic characteristics. Conclusion: Patients with second primary melanomas demonstrated a significant survival advantage and thinner lesions compared to single primary melanomas. Our reported tumor distributions support the role of full-body skin exams (FBSE's), with attention to the region of initial diagnosis.

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Mental health comorbidities and alcohol use disorder in atopic dermatitis: A case-control study in the All of Us research program

R. Fan[2], A. Leasure[2], W. Damsky[1], J. M. Cohen[1]
[1]Dermatology, Yale School of Medicine, New Haven, Connecticut, United States, [2]Yale School of Medicine, New Haven, Connecticut, United States

Atopic dermatitis (AD) is an inflammatory skin disorder known to be associated with mental health disorders. We aim to determine the rates of depression, anxiety, suicidal ideation (SI), and alcohol use disorder (AUD) among AD patients in the All of Us study, a diverse US-based cohort. We performed a nested, matched, case-control study in All of Us. We identified AD cases using ICD-10 L20 and/or SNOMED 24079001 and age, sex, and race-matched controls for each case using nearest-neighbor propensity score matching with replacement. We used logistic regression to determine whether AD was associated with mental health comorbidities and AUD in multivariable analyses. Among the 214,206 All of Us participants, we identified 11,752 cases of AD (average age 59, standard deviation 16; 68% female) and 47,008 controls matched by age, sex, and race/ethnicity (all P>0.99). AD cases were more likely to have a diagnosis of depression (45.9% vs. 21.7%, P<0.001), anxiety (46.8% vs. 21.6%, P<0.001), SI (3.5% vs. 2.3%, P<0.001), and AUD (8.5% vs. 4.4%, P<0.001) compared to controls. In multivariate analysis after adjusting for age, race, and comorbidities such as AUD and hypothyroidism, AD remained significantly associated with depression (OR 2.80, P<0.001), anxiety (2.98, P<0.001), and SI (1.23, P=0.001). AD was also significantly associated with AUD in multivariate analysis after adjusting for demographic factors, depression and anxiety (1.39, P<0.001). We found that AD is significantly associated with mental health disorders and AUD in the adult US population, with individuals with AD having a 180%, 198%, 23%, and 39% increase in odds of having depression, anxiety, SI, and AUD respectively. Dermatologists treating AD patients should be aware of these relationships and consider mental health and alcohol use screening in this population.

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Patient-reported outcome measures for health-related quality of life in acne vulgaris: A systematic review of measure development and measurement properties

Z. Hopkins[3], D. Thiboutot[2], L. Perez Chada[1], H. A. Homsi[4], J. S. Barbieri[1]
[1]Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Penn State Health Milton S Hershey Medical Center, Hershey, Pennsylvania, United States, [3]Broward Healthcare System Inc, Fort Lauderdale, Florida, United States, [4]University of Waterloo School of Public Health and Health Systems, Waterloo, Ontario, Canada

While multiple patient-reported outcome measures (PROMs) for health-related quality of life exist for patients with acne vulgaris, little is known about the validity of the development and measurement properties of these PROMs. A systematic review was conducted following the Consensus-based Standards for the selection of health Measurement Instruments (COSMIN) framework. Data were extracted on PROM properties (target population, domains measured, recall period, development language, number of items), COSMIN-based development and pilot study grading, content validity score (relevance, comprehensiveness, comprehensibility, overall), and measurement properties (structural validity, internal consistency, reliability, construct validity, responsiveness, measurement error, and responsiveness). Quality of evidence was assigned for each measure and an overall recommendation rating was assigned based on content validity and measurement property scoring and evidence quality. We identified 47 relevant acne PROM validation or development studies pertaining to 10 acne-specific PROMs, 6 Dermatology-specific PROMs, and 6 generic PROMs. Acne-Q and CompAQ received "A" recommendations, the remaining acne-specific, dermatology-specific, and general HRQoL PROM's received "B" recommendations. The Acne-Q and CompAQ were found to be validated to a sufficient standard to support recommendation for measuring acne-associated quality of life. The AcneQoL and ASIS could also be considered if additional evaluation of content validity is performed. However, important measurement properties have not been studied, or have been studied insufficiently for all PROMs. Further research is needed to better define validity, measurement properties, and interpretability of PROMs for HRQoL in patients with acne.

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Association between psoriasis and sleep disturbance in the US: Results from National Health and Nutrition Examination Survey (NHANES)

T. Bhutani, K. Stone, A. Prather, M. Hakimi, M. Chung, S. Yeroushalmi, E. Bartholomew, W. Liao
University of California San Francisco, San Francisco, California, United States

Sleep deprivation can modulate the immune response and is a risk factor for cardiovascular, metabolic disease, and depression-the same comorbidities that are associated with psoriasis. Many psoriasis patients report subjective sleep disturbance characterized by difficulties initiating and maintaining sleep, as well as shortened sleep duration. However, no studies have compared the prevalence of sleep disturbance in individuals with and without psoriasis which is the aim of this study. This study was conducted using publicly available data from the 2011-2014 NHANES cycles. Participants were surveyed about psoriasis, sleep quantity ("How much sleep do you get (hours)?"), and quality ("Have you ever told a doctor you had trouble sleeping?"). All adults (≥20 years of age) who responded to questions regarding psoriasis were included. Two multivariable logistic regression models were constructed to examine the relationship between psoriasis and (a) sleep quantity (categorized into <7hrs and ≥7hrs) and (b) sleep quality after adjustment for age, sex, race, and BMI. Our final dataset comprised 11,999 patients including 329 cases (2.6%) of psoriasis. No significant association was found between psoriasis and sleep quantity. However, psoriasis patients were more likely to report trouble sleeping (OR, 1.87 [95% CI, 1.47-2.39]; p<0.001) compared to those without psoriasis. Older age (OR, 1.01 [95% CI, 1.01-1.02]; p<0.001), female sex (OR, 2.76 [95% CI, 1.9-4.0]; p<0.001), and higher BMI (OR, 1.05 [95% CI, 1.04-1.06]; p<0.001) were associated with greater odds of trouble sleeping. Psoriasis severity and psoriatic arthritis were not significant predictors of poor sleep quality in further multivariate models. Our results show that psoriasis patients report similar sleep hours to individuals without psoriasis, but they are more likely to have poor quality sleep. Further studies are needed to learn more about sleep health in psoriasis and its relationship to disease severity and comorbidities.

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Lack of association between dietary and supplemental vitamin E intake with skin cancer risk in postmenopausal women

J. So[1], S. Li[1], E. Linos[1], S. Swetter[1, 2], M. L. Stefanick[3], J. Tang[1]
[1]Department of Dermatology, Stanford Medicine, Stanford, California, United States, [2]Dermatology Service, Veterans Affairs Palo Alto Health Care System, Palo Alto, California, United States, [3]Prevention Research Center, Stanford Medicine, Stanford, California, United States

Risk factors for cutaneous melanoma (CM) and nonmelanoma skin cancer (NMSC) include fair skin and extensive sun exposure. But, incidence has risen despite efforts to promote sun protection. Thus, identifying methods for therapeutic prevention is needed. Antioxidants such as vitamin E are theorized to reduce cancer risk by scavenging reactive species to reduce oxidative stress and DNA damage, but associations between vitamin E intake and skin cancer risk in women remain unclear. To investigate this, we used data from >93,000 postmenopausal women from the observational arm of the Women's Health Initiative (WHI). The final cohort comprised of 78,881 White women across the US. WHI surveys captured baseline demographics, daily dietary vitamin E estimated from food frequency questionnaires, daily supplemental vitamin E, medical history, childhood and current sun exposure, and tendency to burn or tan. Outcomes were a confirmed diagnosis of melanoma in situ or invasive CM or a self-reported diagnosis of NMSC during study follow-up. The exposure was daily dietary and supplemental vitamin E intake at baseline stratified by tertile. Hazard ratios (HR) of CM by tertile of vitamin intake were estimated by proportional hazards models as the WHI recorded time-to-diagnosis for CM. Odds ratios (OR) of NMSC by tertile of vitamin intake were assessed by logistic regressions. Overall, 1,530 CM and 13,773 NMSC cases were identified over a median 19.3 years of follow-up. No associations between vitamin E intake and CM or NMSC were observed (CM: 2nd tertile HR=1, p=0.5; 3rd tertile HR=1, p=0.5. NMSC: 2nd tertile OR=1, p=0.9; 3rd tertile OR=1, p=0.2, versus the lowest tertile of intake). In summary, total vitamin E intake did not have a protective effect against NMSC or CM risk in postmenopausal women. Further study of potential therapeutic prevention agents for skin cancer in women is needed.

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Clinical characteristics associated with functional abnormalities in pediatric and adult morphea: A cross-sectional study

H. W. Chen[1], A. Walker[1], K. Schollaert-Fitch[2], K. Torok[2], H. Jacobe[1]
[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Rheumatology, Pediatrics, University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Morphea is an inflammatory skin condition leading to fibrosis of the skin and subcutaneous tissue which produces cosmetic and functional impairment (FI). Few studies have systematically examined the demographic and clinical features of morphea associated with FI, making it difficult to stratify patients for screening for FI based on risk. To address this gap, we performed a multicenter cross-sectional analysis of adults and children with morphea (n=1104) enrolled in the Morphea in Adults and Children registry at UT Southwestern (n=756) or the National Registry for Childhood-Onset Scleroderma at the University of Pittsburgh (n=348). Patients were classified as having FI if they had limited range of motion, contracture, limb length discrepancy, or inflammatory arthropathy at their visit by a domain expert (HJ/KT). Predictor variables included demographics and key clinical morphea features. Descriptive statistics and a priori multivariable logistic regressions were performed. FI was noted in 293 patients (26.5%). Analysis of body site involvement revealed increased frequency of large joints such as knees (48% vs 23%, p<0.001), hips (39% vs 14%, p<0.001), and shoulders (28% vs 14%, p=0.009) in children relative to adults while adults had more involvement of smaller joints such as toes (32% vs 18%, p=0.007). Demographic and clinical features associated with FI included deep involvement (OR 4.45 [3.01, 6.70]), eosinophilic fasciitis/morphea profunda (OR 2.37 [1.27, 4.47]), tenderness to palpation (OR 2.29 [1.40, 3.74]), and pediatric status (OR 1.85 [1.33, 2.59]). Plaque subtype (relative to linear OR 0.44 [0.09, 0.35]) and epidermal involvement only (OR 0.11 [0.01, 0.54]) were protective against FI. We found readily determined demographic and clinical features, such as pediatric patients, deep involvement, and tenderness to palpation, are associated with FI in morphea, informing the need for careful monitoring and treatment when present.

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Increased risk for wildfire smoke-associated atopic dermatitis and itch exacerbations in older adults

R. P. Fadadu[1], M. Green[2], N. Jewell[2], M. Wei[1]
[1]University of California San Francisco Department of Dermatology, San Francisco, California, United States, [2]London School of Hygiene & Tropical Medicine, London, London, United Kingdom

Aging affects the structure and function of human skin;as a result, aging skin, especially in people with existing skin disease, becomes less resilient to insults like air pollution. It has previously been shown that short-term exposure to wildfire smoke significantly exacerbated atopic dermatitis (AD), but not itch, in adults older than 18 years, and we hypothesized that adults particularly 65 years and older would be at greater risk for pollution-related skin exacerbations. We collected data on particulate matter (PM2.5) concentration and smoke plume density (SPD, ranging 0-3) in San Francisco and the number of outpatient dermatology visits for AD or itch at an academic medical center in the same city from October 2015-February 2019, which includes the time of the Camp Fire. Outcome data were segregated by age (18-64 or ≥65) and analyzed using generalized Poisson regression. Statistical models included 4 one-week exposure lags and were adjusted for temperature, humidity, patient age, year, and patient volume. A total of 5,453 visits for AD and 1,280 visits for itch were analyzed. In adjusted analyses, the rates of AD and itch clinic visits for adults ≥65 during a week with a wildfire were, respectively, 1.4 (95% CI: 1.1-1.9) and 1.6 (1.1-2.5) times the rate for non-wildfire weeks, for a 0-week lag. The corresponding rates for adults age 18-64 were 1.1 (1.0-1.3) and 1.1 (0.8-1.7). A 1-unit increase in mean weekly SPD score was associated with a 1.4 (1.1-1.9) times higher rate of itch visits for adults ≥65. We found that during a California wildfire, rates of clinic visits for both AD and itch were higher among adults ≥65 years of age compared to those younger. The increasing risk for pollution-induced skin exacerbations with older age may be due to age-related molecular processes weakening the skin barrier. As wildfires increase in frequency and intensity, it is important to provide clinical counseling and public health education on skin health that are targeted to older adults.

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Assessing dermatologic conditions in potential liver transplant candidates

E. Herringshaw, K. Cooper, A. Colletta, D. Devuni
University of Massachusetts Chan Medical School, Worcester, Massachusetts, United States

Background: Various skin findings (e.g., spider angioma, palmar erythema, xanthoma) are known sequelae of chronic liver disease (CLD). Such extrahepatic manifestations of disease can inform risk assessment in CLD patients, including transplant candidates. We examined the relationship between cutaneous findings and liver transplant evaluation in patients with CLD. Methods: Medical records for patients evaluated for liver transplant at this institution between 1/1 - 8/2/2021 were reviewed. Transplant work up including physical exam, laboratory data and past medical history were collected. Records were categorized by location of evaluation (inpatient vs. outpatient). Skin conditions were classified as acute (rash, cellulitis, dermatitis) and non-acute (chronic dermatologic disease). Comparisons were made using Chi squared and associations using logistic models. Statistical significance was assessed at p = 0.05. Results: One hundred and sixteen patients, mean age 56.1, were evaluated. About 1/3 of evaluations occurred inpatient (30%). Age, ethnicity, gender, nor etiology of CLD (56% alcohol, 34% NASH) differed by location. One in four patients had dermatologic history, which was associated with undergoing an inpatient evaluation (X2 = 4.2 p=0.04). When categorized by chronicity, non-acute dermatologic conditions (14.3% vs. 24.7%, p=0.31) and skin exam findings (31.4% vs. 28.4%, p=0.91) did not differ between inpatient and outpatient groups. Conversely, inpatient candidates were 12x more likely to have an acute skin presentation compared to outpatient candidates (40% vs. 5% p<0.0001); this remained significant after adjusting for BMI, MELD-Na, etiology, and history chronic skin disease (p=0.03). Conclusion: These findings suggest significant dermatologic history may be associated with expedited liver transplant evaluation. Further, acute skin conditions, such as rash and cellulitis, may be more strongly associated with this than “classic” skin findings of CLD, illustrating the importance of detailed skin exams in patients with CLD.

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Patient-reported disease burden in epidermolysis bullosa simplex (EBS)

J. So[1], S. Fulchand[1], C. Wong[1], S. Li[1], J. Nazaroff[1], E. Gorell[2], M. P. de Souza[3], D. Murrell[4], J. Teng[1], A. Chiou[1], J. Tang[1]
[1]Department of Dermatology, Stanford Medicine, Stanford, California, United States, [2]Department of Dermatology, University of Cincinnati, Cincinnati, Ohio, United States, [3]deSouzaTech, LLC, Berkeley, California, United States, [4]Department of Dermatology, University of New South Wales, Sydney, New South Wales, Australia

EBS is a group of genodermatoses with blistering above the dermal-epidermal junction and is the most common form of epidermolysis bullosa. Phenotypes vary widely, from mild acral blisters to severe generalized blisters and wounds. But, the impact of EBS severity on patient-reported outcomes and quality of life (QOL) remains unknown. Self-reported cross-sectional surveys were obtained from 2012-2016 via EBCare, an international online EB registry. We analyzed 203 subjects who self-reported a diagnosis of EBS. Self-reported disease severity is as follows: 21 (10%) severe, 100 (49%) moderate and 82 (40%) mild. Increased severity correlated with greater disease burden including increased presence of large wounds (severe 67% vs moderate 31% vs mild EBS, p<0.01), infections (62% vs 31% vs 34%, p<0.01), difficulty walking (48% vs 54% vs 33%, p=0.02) and worse pain in the past year (scale from 0-10: 7.4 vs 7.8 vs 5.5, p<0.01). Subjects reporting severe EBS also noted increased use of gastrostomy (G) tubes (24% vs 0% vs 4%, p<0.01), routine use of analgesics including opiates (71% vs 59% vs 41%, p=0.01), anti-itch medications (52% vs 28% vs 18%, p<0.01) and use of analgesics including opiates for dressing changes (67% vs 34% vs 18%, p<0.01). Among subjects who completed the validated QOL in EB (QOLEB) survey (n=66), poorer QOL (indicated by higher QOLEB score) correlated with worse EBS severity but was nonsignificant (16.6 vs 14.5 vs 13.6, p=0.7). Age-stratified analyses showed greater G-tube use in younger patients (age 0-9=15% vs age 10-20=9% vs age ≥21=1%, p<0.01), suggesting age-specific disease burden. EBS patients—particularly those with severe EBS—face significant disease burden including large wounds, infections, difficulty walking, pain and medication use. Further study with larger samples is needed to determine if EBS severity correlates with QOL.

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Cutaneous toxicities associated with immune checkpoint inhibitors: An observational, pharmacovigilance study

T. Le[1], I. Brown[1], M. Taylor[1], J. Deng[1], V. Parthasarathy[1], Z. A. Bordeaux[1], M. P. Alphonse[1], J. Alhariri[1], S. Kang[1], Y. Semenov[2], S. G. Kwatra[1]
[1]Department of Dermatology, Johns Hopkins Medicine, Baltimore, Maryland, United States, [2]Department of Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Cutaneous immune-related adverse events (cirAEs) are the most prevalent complication to arise from immunotherapy. We determined the spectrum, timing, clinical features, and outcomes of cirAEs by conducting an observational pharmacovigilance study (ClinicalTrials.gov, NCT04898751) employing Vigibase, the WHO's global database of individual case safety reports. Using a Bayesian confidence propagation neural network method, cutaneous toxicities were identified with a positive signal at the bottom of the information component 95% confidence interval (IC025 >0). This positive signal was validated utilizing reporting odds ratios (RORs), and characteristics of these cutaneous toxicities were compared using pair-wise chi-squared tests. Of the 91,323 adverse events reported for ICIs, there were 10,933 cutaneous toxicities within 51 distinct dermatologic types, with 27 specific eruptions with a IC025>0. Of these 27 eruptions, 8 cirAEs had n>100 reports including vitiligo (IC025 4.87, ROR 40.98 95% CI 37.19-45.16), bullous pemphigoid (4.08, 21.09, 18.96-23.47) lichenoid dermatitis (3.69, 16.37, 14.33-18.71), erythema multiforme (EM, 1.03, 2.72, 2.37-3.12), toxic epidermal necrolysis (TEN, 0.41, 2.60, 2.19-3.08), Stevens-Johnson syndrome (SJS, 0.41, 1.69, 1.49-1.92), drug eruption (0.11, 1.49, 1.27-1.73) and eczematous dermatitis (0.11, 1.42, 1.26-1.65). CirAEs co-occurred with GI and thyroid toxicities (p < 0.001). A Krukal-Wallis test with post-hoc pair-wise comparisons using Dunn’s test showed differences in time to onset after ICI initiation, with a median of approximately 1 month (EM, SJS, and TEN), 2 months (drug eruption and eczematous dermatitis), 4 months (lichenoid dermatitis), and 5-6 months (bullous pemphigoid and vitiligo). This global study finds diverse cirAEs that co-occur with other organ systems and have distinct onset times linked to cirAE subtype.

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Associations between dietary and supplemental vitamin A and skin cancer risk in postmenopausal women

J. So[1], S. Li[1], E. Linos[1], S. Swetter[1, 2], M. L. Stefanick[3], J. Tang[1]
[1]Department of Dermatology, Stanford Medicine, Stanford, California, United States, [2]Dermatology Service, Veterans Affairs Palo Alto Health Care System, Palo Alto, California, United States, [3]Prevention Research Center, Stanford Medicine, Stanford, California, United States

Nonmelanoma skin cancer (NMSC) and cutaneous melanoma (CM) are among the most common cancers in older women. While studies on the protective effects of vitamin A against NMSC have been mixed, a 2012 cohort study which included 36,096 women showed that supplementation with retinol—a form of vitamin A—correlated with reduced CM risk in women. To investigate associations of vitamin A and skin cancer in postmenopausal women, we used data from the Women's Health Initiative (WHI) observational study of 93,676 postmenopausal women. The final cohort included 78,881 White women nationally. We analyzed survey data at baseline including demographics, daily dietary vitamin A by food frequency questionnaires, daily supplemental vitamin A, medical history, tendency to burn/tan, and childhood and current sun exposure in minutes. Outcomes were self-reported diagnoses of NMSC or adjudicated diagnoses of melanoma in situ or invasive CM. The primary exposure was daily baseline dietary and supplemental vitamin A intake, stratified by tertile. We estimated odds ratios (OR) of NMSC by tertile of vitamin intake with logistic regressions, and hazard ratios (HR) of CM by tertile of vitamin intake with proportional hazards models as time to CM diagnosis was captured by the WHI. Overall, 13,773 NMSC and 1,530 CM cases occurred over a median 19.3 years of follow up. In multivariate analyses, higher vitamin A intake correlated with greater odds of NMSC (2nd tertile OR=1.1, p<0.01; 3rd tertile OR=1.1, p=0.05 compared to the lowest tertile). However, we observed no association between vitamin intake and risk of CM (2nd tertile HR=1.0, p=0.9; 3rd tertile HR=1.0, p=1.0). In contrast to prior work, our data do not show a protective effect of vitamin A on NMSC and CM, and instead suggest that increased vitamin A intake may contribute to greater risk of NMSC for postmenopausal women. Further investigation of these associations is warranted.

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Second primary cancer risk after diagnosis of melanoma by subtype

Y. Luu, A. Goldstein, M. Sargen
Division of Cancer Epidemiology and Genetics, National Institutes of Health, Bethesda, Maryland, United States

Background: Survivors of cutaneous melanoma (CM) have an increased risk for developing multiple cancers, including melanoma, breast cancer, prostate cancer, and non-Hodgkin lymphoma (NHL). Limited data exists comparing differences in second primary cancer (SPC) risk between CM subtypes. Purpose: To compare the spectrum of SPC risk between CM subtypes (superficial spreading, SSM; lentigo maligna, LMM; acral lentiginous, ALM; nodular, NM; desmoplastic, DM; amelanotic, AM). Methods: Using Surveillance, Epidemiology, and End Results (SEER) 18 cancer data (2000-2018), we calculated SPC risk by CM subtype. Analyses were restricted to individuals with a first primary cancer (no prior malignancies) diagnosis of CM. Results: Of 172,637 CM survivors, 20,696 (12 %) developed a SPC, corresponding to nearly a two-fold increased risk (standardized incidence ratio [SIR] 1.73, 95% confidence interval [CI] 1.71-1.76) compared to the general population. SPC risk was highest for NM (SIR 2.11, 95% CI 2.03-2.18) and lowest for LMM (1.55, 1.50-1.61). Across all subtypes, there was increased risk for developing NHL (SIR range: 1.38 [LMM]-2.19 [AM]) and melanoma (SIR range: 10.52 [LMM]-16.80 [AM]). Risk of thyroid cancer was increased for all subtypes (SIR range: 1.52 [LMM]-3.19 [NM]) except for AM. Prostate cancer risk was elevated after SSM (SIR 1.24, 95% CI 1.18-1.31), NM (1.15, 1.02-1.29), and LMM (1.21, 1.10-1.32). Risk of breast cancer was increased following SSM (SIR 1.13, 95% CI 1.06-1.20), but not for other subtypes. Kidney cancer risk was also increased following NM (SIR 2.09, 1.70-2.55) and SSM (1.33, 1.19-1.48). Conclusions: The spectrum of SPC risk varies according to melanoma subtype. Germline variation in cancer susceptibility genes may partially explain these patterns of SPC risk. Our study also supports current practice patterns of intensive skin surveillance for all melanoma survivors given the highly elevated risk for a second primary melanoma following each of the CM subtypes.

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Heterogeneity in cutaneous infection prevalence and frequency by timing of atopic dermatitis onset

S. Shah[2], D. J. Margolis[1], N. Mitra[1], J. Wan[3]
[1]University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [3]Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Cutaneous infections are common in atopic dermatitis (AD) due to impaired skin barrier and immunologic dysfunction. Early- and late-onset pediatric AD are distinct phenotypes that vary in disease persistence and risk of other atopic comorbidities. However, whether infection risk varies by the timing of AD onset is unknown. Using population-based, electronic medical records data from the UK, we examined a cohort of children enrolled within the first year of life and who had a diagnosis of AD before age 18. Among 154,022, 30,970, and 4,788 children with early- (≤ 2 years old), mid- (3-7 years old), and late-onset (8-17 years old) AD, those with late-onset AD were 20-25% less likely to have molluscum (OR 0.74, 95% CI 0.68-0.80), varicella/herpes zoster (0.75, 0.70-0.79), impetigo (0.78, 0.72-0.83) and herpes simplex virus (0.81, 0.69-0.96), after adjusting for age, sex, duration of follow-up, and other atopy. They also had 10% lower odds of cellulitis (OR 0.90, 0.83-0.97) and warts (0.91, 0.85-0.97). Mid-onset AD exhibited similar to slightly greater odds of infections compared to early-onset AD. However, when examining the frequency of infectious episodes among children with at least 1 episode of any given infection (cellulitis, herpes simplex virus, impetigo, molluscum, tinea, varicella/herpes zoster, or warts), late-onset AD did not differ from early-onset AD. Mid-onset AD was associated with slightly lower frequency of cellulitis (adjusted IRR 0.88, 0.81-0.95) and varicella/herpes zoster episodes (0.92, 0.86-0.98) compared to early-onset AD. Taken together, our findings suggest that while AD onset age is inversely related to the overall risk of several cutaneous infections, the timing of AD onset has less impact on one's susceptibility to repeated infection. Further work is thus needed to understand the heterogeneity in cutaneous infection risk among children with AD and distinguish the phenotypes of AD complicated by repeated infections.

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Using Skindex-16 scores to predict skin flaring in autoimmune blistering diseases

Z. Hopkins[2], A. Jimenez[1], V. Taliercio1, A. M. Secrest[1]
[1]Dermatology, University of Utah Health, Salt Lake City, Utah, United States, [2]Dermatology, Broward Healthcare System Inc, Fort Lauderdale, Florida, United States

Background: Chronic relapsing-remitting dermatologic diseases like autoimmune bullous diseases (ABDs) have significant impacts on quality of life (QOL). Monitoring these impacts regularly with patient-reported outcome (PRO) measures, like Skindex-16 can not only aid clinician-patient communication but also track treatment success and identify skin flares requiring intervention. Methods: Skindex-16 is a dermatology-specific QOL measure to assess skin disease impact on symptoms, emotions, and functioning (range 0-100, higher scores=greater impact). Demographic and clinical data retrieved via manual chart review were linked to PRO scores for each clinic visit, including autoimmune labs. Associations between self-reported flare ("Are you flaring today? Yes/No") and PRO score were performed for each disease subtype (pemphigoid, pemphigus, and dermatitis herpetiformis (DH)) and overall. Associations between Skindex-16 domain scores with flaring status were made using logistic regression and Somers' D test. Concordance between flares, indirect immunofluorescence, and serologic lab values versus PRO scores was also compared. Results: We included 193 patients with 317 visits seen between September 2016 and January 2020 where Skindex-16 was completed. The median Skindex-16 completion time was 2.1 minutes. Patients reported skin flaring at 29.0% of visits. All Skindex-16 domains were higher for all disease categories when flaring compared to non-flaring (p<0.001). Skindex-16 scores, but not serologic labs, were associated with flare (Pemphigoid OR=1.13, 95% CI 1.09–1.18; Pemphigus OR=1.23, 0.99–1.54; DH OR=1.11, 1.03–1.20). For all diseases, Skindex-16 better discriminated flare than lab values (c-index >0.80 for all). Conclusions: ABDs have high morbidity and QOL impact. Flares are a common reason for visits and negatively impact QOL. These findings highlight the potential clinical use of serial Skindex-16 monitoring for early responsiveness to flares to prevent adverse downstream consequences.

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Impact and burden of pityriasis rubra pilaris on quality of life: An exploratory focus group

R. C. Velasco[1, 2], B. Cutler[1, 3], T. Greiling[1]
[1]Dermatology, Oregon Health & Science University, Portland, Oregon, United States, [2]School of Medicine, University of Illinois at Chicago, Chicago, Illinois, United States, [3]School of Medicine, University of Utah Health, Salt Lake City, Utah, United States

Pityriasis rubra pilaris (PRP) is a severe inflammatory skin disorder with major impacts on patients' quality of life. Current treatment plans focus on dermatologic symptoms, however PRP may also cause severe deficits in other areas, including mobility, thermoregulation, and mental well-being. This qualitative study aimed to identify the diversity of symptoms experienced by PRP patients in order to capture a broader disease experience. To this end, we conducted four, two-hour focus group sessions (n=45 English-speaking participants with PRP from six different countries) mediated by a representative from the PRP Alliance, followed by a post-session 23-question survey (completed by n=45 (100%) participants). In regards to most impactful physical symptoms, participants reported severe skin itch and pain, as well as palmoplantar involvement that caused difficulty with performing daily activities and mobility. Non-skin impacts included body temperature dysregulation, anhydrosis, skin buildup in the ears that affecting hearing, rhinorrhea, and joint pain. Difficulty sleeping and excessive daytime fatigue was one of the most often cited systemic impacts. Furthermore, most participants agreed that the mental aspect of PRP was equal to or worse than the physical impact, citing depression, loss of self-worth, feelings of isolation, and passive suicidal ideation. Participants felt that their providers did not adequately address the mental health impacts of PRP. In conclusion, this study found that PRP has debilitating health effects from a broad variety of symptoms and quality of life impacts. These included skin and non-skin symptoms, as well as systemic symptoms and mental health. PRP patients are best treated by a multidisciplinary team that includes mental health management.

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What is the risk of Merkel cell carcinoma recurrence beyond pathologically clear margins? An analysis of 926 cases

A. Fu[1], N. Singh[2], K. Lachance[1], D. Hippe[1], P. Nghiem[1], S. Y. Park[1]
[1]University of Washington, Seattle, Washington, United States, [2]Virginia Tech Carilion School of Medicine, Roanoke, Virginia, United States

Early-stage melanoma, squamous and basal cell carcinomas have local control rates of >90% with wide excision after pathologically clear margins and >95% with Mohs micrographic surgery. Local control rates for these approaches are not well defined in Merkel cell carcinoma (MCC). Herein, we analyzed data from 80 patients (pts) in a Seattle-based IRB-approved registry who had surgical excision with pathologically clear margins, negative sentinel lymph node biopsy, and no local radiation therapy. We also performed a meta-analysis of 13 published studies (846 pts) based on a random-effects model. For the 80 pt cohort, local recurrence rate (LRR; ≤2 cm from the primary tumor) was 10%. In-transit recurrence rate (ITR; >2cm from the primary) was 1% and regional nodal recurrence rate (RRR) was 5%. Distant recurrence rate (DRR) was 4%. This cohort had mostly low-risk characteristics with small primary tumors (74% were ≤1 cm, 23% were 1-2, and 4% were >2 cm). There was no residual tumor in 60% of re-excisions while 29% had closest pathologic margins <1 cm. Lymphovascular invasion was found in 21% (15/70) of pts with available data. Meta-analysis of 9 published studies (745 pts) who underwent excision with clear pathological margins yielded LRR of 16.4% [95% CI 8.3-26.5], ITR of 9.5% [95% CI 5.4-14.6], RRR of 32.1% [95% CI 19.1-46.7], and DRR of 9.5% [95% CI 5.7-14.3]. Data from 4 studies (101 pts) who underwent Mohs yielded LRR of 3.6% [95% CI 0-16.3], ITR of 12.8% [95% CI 6.4-21.1], RRR of 20.7% [95% CI 13.8-38.3], and DRR of 1.8% [95% CI 0-12.9]. In each cohort, risk of local/in-transit recurrence following surgical excision with pathologically clear margins was >10%. These data suggest MCC is more likely to recur near the excision site than other skin cancers and may reflect a biological difference in the pattern of MCC local extension (discontinuous spread beyond pathologically clear margins). Even for pathologically negative excisions, higher risk tumors may benefit from adjuvant radiotherapy.

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Atypical masses associated with PIK3CA-related overgrowth spectrum (PROS)

K. K. Barry[1, 2], M. G. Liang[1, 2], W. Eng[1, 2]
[1]Vascular Anomalies Center, Boston Children's Hospital, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States

Background: PIK3CA-Related Overgrowth Spectrum (PROS) is a heterogeneous disorder caused by post-zygotic, mosaic, gain-of-function PIK3CA mutations. Patients with congenital lipomatous overgrowth, vascular malformations, epidermal nevi, and scoliosis/skeletal anomalies (CLOVES), which is a subset of PROS, are at higher risk for Wilms tumor; these patients are often screened with abdominal ultrasounds in early childhood. Methods: Four patients (one previously published, PMID 34854542) with a molecularly confirmed diagnosis of PROS who had a mass identified on magnetic resonance imaging underwent targeted next generation sequencing of lesional tissue using OncoPanel. Oncopanel is a genomic assay that detects variants in 447 genes implicated in cancer. Clinical and demographic information were recorded via chart review. Results: Masses were identified in the spleen, mandible, ovary, and brain; three were discovered incidentally: one on whole-body MRI prior to clinical trial enrollment; two on imaging for other indications. Histopathologic evaluation revealed a microcystic splenic lymphatic malformation, mandibular intraosseous venous malformation, intracranial purely venous malformation (iCVM), and ovarian mucinous cystadenoma. Lesional tissues were positive for PIK3CA mutations. Three patients received targeted medical therapy with an AKT inhibitor; two underwent complete surgical resection. Masses remain stable or have regressed after 1-4 (mean 2.3) years of follow-up. Conclusion: Despite enrichment of PIK3CA mutations in many solid tumors, malignancy is rare in PROS. In our study, masses were identified outside of routine ultrasound screening. Splenic lymphatic malformations are rarely identified in PROS; mandibular intraosseous venous malformation, ovarian mucinous cystadenoma, and iCVM have not been previously described in PROS. Understanding the incidence and significance of masses in PROS will be important as PROS screening guidelines are developed and clinical trials for PROS emerge.

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Your pores and the outdoors: Investigating the association between pollution and inflammatory dermatological diseases

M. D. Mehta[1], R. Reddy[4], D. Yee[1], C. Zagona-Prizio[2], S. Khan[1], S. Khan[3], N. Maynard[1], A. W. Armstrong[1]
[1]Dermatology, University of Southern California Keck School of Medicine, Los Angeles, California, United States, [2]University of Colorado Denver School of Medicine, Aurora, Colorado, United States, [3]The University of Texas Health Science Center at San Antonio Joe R and Teresa Lozano Long School of Medicine, San Antonio, Texas, United States, [4]The University of Texas Southwestern Medical Center Medical School, The University of Texas Southwestern Medical Center Medical School, Dallas, TX, US, academic/medsch, Dallas, Texas, United States

Particulate Matter 2.5 (PM 2.5) is a type of pollutant ~20 times smaller than human pores. Recent in-vitro and in-vivo studies have linked exposure to PM 2.5 to a variety of dermatologic conditions including atopic dermatitis, alopecia, and skin cancer. However, population based studies remain limited. This study investigates the association between PM 2.5 and 3 inflammatory conditions: atopic dermatitis (AD), hidradenitis suppurativa (HS) and psoriasis. We compared average PM 2.5 levels to Google Trends Search Volume Index (SVI) in U.S. states; SVI served as a proxy for disease prevalence. As a negative control, we compared CO2 concentrations by state to Google Trends SVI for all 3 conditions. After adjusting for precipitation, temperature, percentage of the population living in an urban environment, and density for each state, all 3 conditions had significant associations with PM 2.5 levels: AD (R2 = 0.41, p=0.0004); HS (R2 = 0.29, p=0.011); and psoriasis (R2 = 0.27, p=0.018). All 3 conditions did not have significant associations with CO2: AD (R2 = 0.02, p=0.36); HS (R2 = 0.0001, p=0.95); and psoriasis (R2 = 0.004, p=0.66). These novel findings contribute to our understanding of the pathogenesis of these inflammatory conditions. Further research is needed to quantitatively characterize the impact of air pollution on the prevalence or severity of these conditions. As air pollution rises globally, elucidating its role in the pathogenesis and exacerbation of these diseases is critical in order for clinicians to better care for their patients with these conditions.

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Adverse events associated with hydroxychloroquine use in cicatricial alopecia patients

M. Collins, S. Ali, I. Pupo Wiss, M. Senna
Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

The anti-malarial hydroxychloroquine (HCQ) is a common treatment for cicatricial alopecias (CA). Serious adverse events include prolonged QT interval, ventricular arrythmias and irreversible retinopathy. There is limited literature on the incidence of adverse events in CA patients taking HCQ. A retrospective analysis was performed of 60 CA patients, mean age 60 years, prescribed HCQ. Alopecia diagnoses included lichen planopilaris, frontal fibrosing alopecia, discoid lupus, central centrifugal cicatricial alopecia and folliculitis decalvans. Average length of HCQ treatment was 2.98 years. Dosing ranged from 100 to 400 mg daily. 83.3% of patients did not experience an adverse event. Adverse events that occurred include GI distress (n=5), tinnitus or other hearing-related changes (n=2), and allergic skin rash (n=1). One patient developed non-sustained ventricular tachycardia (NSVT) 17 months after starting HCQ 400 mg daily. During her hospitalization, no structural cardiac abnormalities were revealed, and the cause of the NSVT was not determined. She was prescribed daily estradiol-norethindrone hormone replacement therapy (HRT) for several years prior to the NSVT. Her medical history was significant for congestive heart failure and remote history of pulmonary hypertension and left ventricular hypertrophy. The patient permanently discontinued both medications. She has not developed another NSVT episode and remains healthy. Within our cohort, there were no other cardiac related events despite some patients taking concomitant medications known to increase risk of QT prolongation. These patients were monitored with regular electrocardiograms. The risk of HCQ-associated retinopathy increases with > 5 years of cumulative use. No patients included in our analysis developed retinopathy, including the 6.67% taking HCQ for > 5 years. We demonstrate that while adverse events may occur during treatment, HCQ is generally well tolerated by CA patients. We hope this will help support treatment discussions with CA patients.

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COVID-19 complications in vitiligo patients: A multicenter study

R. Raiker[1], S. Salingaros[2], H. Pakhchanian[3], M. Helm[4]
[1]West Virginia University School of Medicine, Morgantown, West Virginia, United States, [2]Weill Cornell Medicine, New York, New York, United States, [3]The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, United States, [4]Penn State College of Medicine, Hershey, Pennsylvania, United States

Vitiligo is an autoimmune disorder that leads to the destruction of melanocytes. It has been shown to be associated with comorbidities which may increase the risk of worse COVID-19 outcomes. Limited data on COVID-19 complications in vitiligo patients exists and as COVID-19 cases continue to rise worldwide, it is important to assess this. A retrospective analysis was done using TriNetX, a multicenter deidentified database of ~80 million records. COVID-19 patients were identified by validated ICD-10 and serology codes per CDC guidelines and then split into vitiligo and non-vitiligo cohorts. Patients who were vaccinated for COVID-19 prior to infection were excluded. An 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics, to calculate adjusted Risk Ratios (aRR) with 95% confidence intervals (CI) for COVID-19 related complications in 30-day follow up. Subgroup analysis for vitiligo patients with a 1-year history of systemic steroids was also performed. In a matched sample of 2009 patients in each cohort, vitiligo patients had a lower risk in hospitalization (aRR[95%CI]=0.764[0.65,0.88]) and mortality (0.62[0.39,0.99]) compared to non-vitiligo patients. No differences between cohorts was seen for acute respiratory distress syndrome (1.07[0.6,1.7]), sepsis (0.84[0.6,1.2]), thromboembolic events (0.89[0.5,1.5]), acute kidney injury (0.79[0.6,1.1]), and mechanical ventilation (0.9[0.6,1.3]). Subgroup analysis revealed 1-year systemic steroid use increased hospitalization risk (1.52[1.1,2.2]) compared to controls. Vitiligo may confer protective effects against COVID-19 complications, possibly due to increased interferon signaling found in vitiligo patients that is known to disrupt COVID-19 signaling and thus preventing worse outcome. However, additional studies are warranted to examine the long-term effects of COVID-19.

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Tetracyclines are associated with development of new hyperpigmentation in acne patients

K. Young[1], J. Yoon[1], E. D. Getachew[1], B. Leung[2], N. Nguyen[2], Y. Semenov[2, 1], N. Theodosakis[2]
[1]Harvard Medical School, Boston, Massachusetts, United States, [2]Department of Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Acne is the most common skin condition in the United States, affecting up to 90% of people at some point in their lives. It is frequently accompanied by post-inflammatory hyperpigmentation, which affects skin of color with greater severity. Tetracyclines are the most widely prescribed oral antibiotics for acne and have been proven effective against moderate to severe inflammatory subtypes, but previous studies have shown that tetracyclines are independently associated with hyperpigmentation. Given their antagonistic anti-inflammatory and pro-hyperpigmentation effects, it is important to characterize the risk of hyperpigmentation associated with tetracyclines in different skin types. Using retrospective data collected from 44 institutions in the TriNetX database, we identified 1,018,736 patients with a diagnosis of acne. From this cohort, we identified patients who were prescribed doxycycline (n=150,715), minocycline (n=43,975), and cephalexin (n=38,112) as an oral antibiotic monotherapy. Patients diagnosed with melasma or prescribed isotretinoin at any time were excluded. Patients with a prior history of hyperpigmentation were also excluded. In our study, patients given doxycycline (OR 1.66, p<0.0001) and minocycline (OR 1.58, p<0.0001) were more likely to have developed new hyperpigmentation compared to those given cephalexin. Among different racial groups, the odds of new hyperpigmentation associated with doxycycline versus cephalexin was highest in Hispanics (White: OR 1.19, p=0.018; Black: OR 1.54, p=0.001; Hispanic: OR 1.90, p=0.001; Asian: OR 1.35, p=0.337). The odds associated with minocycline versus cephalexin was highest in Blacks (White: OR 1.23, p=0.008; Black: OR 1.44, p=0.009; Hispanic: OR 1.00, p=0.991; Asian: OR 1.09, p=0.785). Our results suggest that doxycycline and minocycline are risk factors for new hyperpigmentation in acne patients of all racial groups. These associations should be taken into consideration when prescribing acne treatment regimens.

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The impact of childhood stressful life events on atopic dermatitis disease activity and severity: A prospective study

J. Tully[1, 2], N. Tomaszewski[2], S. Kidd[2], S. Langan[3], K. Abuabara[2]
[1]The University of Arizona College of Medicine Phoenix, Phoenix, Arizona, United States, [2]University of California San Francisco, San Francisco, California, United States, [3]London School of Hygiene and Tropical Medicine Faculty of Public Health and Policy, London, London, United Kingdom

Stress has been associated with atopic dermatitis (AD), however, longitudinal data on the association with AD course are limited. We aimed to examine whether stressful life events are associated with increased AD disease activity and severity throughout childhood using the Avon Longitudinal Study of Parents and Children prospective English birth cohort, comprised of 13,972 children with assessments from birth. The primary exposure was a standardized, age-appropriate scale of stressful life events repeated at 7 times points between ages 1.5 and 8.5. The primary outcome was a repeated measure of AD period prevalence, as defined by caregiver-reported symptoms of flexural dermatitis. The annual period prevalence of AD ranged from 18-21%. For each standard deviation (SD) increase in stressful life events across childhood there was an increased risk of AD activity (OR: 1.07; 95% CI 1.04-1.16), and the association was largest with severe disease (OR 1.13, 95%CI 1.02-1.23). There was no effect modification by the presence of filaggrin gene null mutations or history of asthma or rhinitis. Given the nature of the stressful life events measured, reverse causality is not likely to be an explanation for the results. In a large, prospective, population-based study, we found that stressful life events in childhood confer a small, but significant, risk in increased AD activity and severity. These findings suggest that the impact of stress-reducing interventions should be further investigated in those with AD.

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The burden of alopecia areata (AA) vs psoriasis (PsO) in the United States

J. Chung[1], L. Bartolome[1], D. Gruben[2], M. Ray[3], E. Masters[1], D. Mitra[1], A. Mostaghimi[4]

[1]Pfizer Inc, New York, New York, United States, [2]Pfizer Inc, New York, New York, United States, [3]Pfizer Inc, New York, New York, United States, [4]Brigham and Women's Hospital, Boston, Massachusetts, United States

The well-established disease burden of PsO can provide a benchmark for understanding the disease burden of other immune-mediated dermatologic disorders that may be associated with high health care resource utilization (HCRU), such as AA. The objective was to describe the economic burden of patients with AA vs PsO aged ≥12 years. A retrospective analysis using IBM MarketScan Commercial & Medicare databases was conducted to identify patients aged ≥12 years with ≥2 claims of either AA (ICD-10-CM: L63.x) or PsO (ICD-10-CM: L40.x) recorded between 1 Jan 2016–31 Dec 2019. The date of first recorded diagnosis for either AA or PsO (patients with both were excluded) was the index date, with ≥12 months continuous enrollment before and after the index date. Demographic and clinical characteristics were used to propensity match AA patients to PsO patients using a 1:2 ratio. Descriptive analyses were performed with the matched cohorts for demographic and clinical characteristics, treatment, and healthcare resource use in the pre-index period. Annualized mean all-cause costs were compared in both groups in the post-index period. The matched analysis included 17,081 and 33,687 patients with AA and PsO, respectively. Mean age was 40.8 and 41.3 years and females comprised 61.6% and 63.6% of patients, respectively. Mean (SD) medical costs during the post-index period were $7,457 ($31,992) and $10,310 ($33,392), respectively (standardized difference [stdiff]: 0.09). Mean (SD) pharmacy costs were $2,470 ($8,851) and $11,421 ($23,746) in the post-index period, respectively (stdiff: 0.5). This analysis of patients with matched demographic and clinical characteristics demonstrated the substantial economic burden of AA, with medical costs approaching those of PsO. Differences observed in pharmacy costs may be due to the lack of approved therapies for AA. As treatment options for AA are developed, it will be important to continue evaluating the impact of effective therapies on cost burden.

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Association of hidradenitis suppurativa with a Crohn's disease panel

A. Nosrati[1], M. E. Torpey[1], T. M. Andriano[1], P. Y. Ch'en[1], T. Dervieux[2], K. L. Campton[1], S. R. Cohen1

[1]Albert Einstein College of Medicine, Bronx, New York, United States, [2]Prometheus Inc, San Diego, California, United States

Hidradenitis suppurativa (HS) is a chronic, debilitating inflammatory skin disease. Crohn's disease (CD) is among the most reported comorbid disorders in HS patients. Concurrent HS and CD are frequently associated with perianal disease that requires immunosuppressive therapy and surgery. We sought to identify unrecognized IBD associated with HS using a commercially available panel of serologic, genetic, and inflammatory markers with high specificity and sensitivity for CD (CD+) (IBD-sgi™ Panel, Prometheus Laboratories, San Diego). This test has not been previously evaluated in an HS cohort. An IRB-approved retrospective chart review of patients receiving care at the Einstein/Montefiore HS Center (HSC) was conducted between August-December 2021. All participants (n=272) were screened with a standard of care IBD-sgi™ panel. Overall, 121 patients (44.5%) were CD+. Comparing CD+ and CD- participants, we found no differences regarding age or gender. By contrast, CD+ participants had elevated HS-PGA(3.8±1.2 vs. 2.9±1.2, p<.001) and pain scores(6.2±3.2 vs. 4.2±3.5, p<.001). Those found CD+ had a higher frequency of HS involving groin and buttocks (p<.001). Indicators of HS severity associated with CD positivity included a significantly higher frequency of treatment with anti-TNF biologics, IV antibiotics, as well as intralesional and intramuscular corticosteroid injections (p<.002). Moreover, CD positivity was associated with lower hemoglobin (p<.001), leukocytosis (p<.01), and increased inflammatory markers (erythrocyte sedimentation rate (p<.001), C-reactive protein (p<.001), interleukin-6 (p<.007)). There were no differences in rates of CD positivity when HS primarily involved axillae, breast, abdomen, and thighs. Our findings unexpectedly revealed 44.5% of HS patients were CD+. Those screening CD+ had more severe disease involving the groin and buttocks. The association of CD positivity and IBD remains unclear. Further investigation of IBD screening in HS patients is needed.

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How to serve those who serve us: Sun protection behaviors among veterans vs non-veterans in a nationally representative sample

S. Khan[1], N. Maynard[2], M. D. Mehta[2], R. Reddy[3], D. Yee[2], C. Zagona-Prizio[4], S. Khan[2], A. W. Armstrong[2]

[1]The University of Texas Health Science Center at San Antonio Joe R and Teresa Lozano Long School of Medicine, San Antonio, Texas, United States, [2]University of Southern California Keck School of Medicine, Los Angeles, California, United States, [3]The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [4]University of Colorado School of Medicine, Aurora, Colorado, United States

Military veterans are a unique, predominantly male population at increased risk for skin cancer due to factors such as occupational sun exposure and special environments. Little is known about the use of sun protective behaviors in this population. This study examined sun protective behaviors among male veterans (20-59 YO) using the National Health and Nutrition Examination Survey (NHANES) from 2003-2006. 23,864,665 male veterans and 125,365,819 male non-veterans were included (weighted sample). Only 16.20%, 18.84%, and 10.23% of those in the veteran group reported consistent sunscreen use, consistently seeking shade, and consistent long-sleeve shirt use, respectively, similar to rates among non-veterans (14.18%, 21.54%, 8.06%). A significantly higher percentage of veterans (28.46%) reported consistent hat use compared to non-veterans (19.44%, p= 0.005). Multivariable logistic regression showed that, compared to non-veterans, veterans were less likely to stay in the shade consistently (aOR= 0.67, p=0.004, 95% CI= .52-.87), likely due to prior occupational conditioning. However, veterans were more likely to wear a hat that shades the face and neck (aOR= 1.42, p= 0.044, 95% CI= 1.01- 2.01) compared to non-veterans after adjusting for age, race, income, education, marital status, BMI, skin reaction to the sun, and history of skin cancer. There was no significant difference in odds of sunscreen and long-sleeve shirt use between the two groups. In conclusion, this study highlights the need for greater sun-safety education among this susceptible population.

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Skin cancer prevalence among foreign-born people in the US from 2000-2018

A. Gangal[1], K. J. Supapannachart[1], H. Yeung[1, 2]

[1]Department of Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States, [2]Clinical Resource Hub, VA VISN 7, Decatur, Georgia, United States

Foreign-born populations in the US face poorer health outcomes when compared to US-born populations. Limited data describe skin cancer prevalence among foreign-born individuals; such data may inform preventative practices. We compared skin cancer prevalence among foreign-born and US-born adults of the same race/ethnicity using National Health Interview Survey data from 2000-2018. Skin cancer prevalence estimates were compared between US versus foreign-born using multivariable logistic regression adjusted for age, sex, demographics, and health behaviors. Among foreign-born participants, prevalence was compared by markers of acculturation (citizenship status, years in US, survey interview language) using multivariable logistic regression adjusted for age, sex, demographics, health behavior, and global region of birth. The study included 363,138 Non-Hispanic Whites (4.91% foreign born), 94,507 Hispanics (59.16%), and 26,906 Asians (78.33%). Skin cancer prevalence was lower among foreign-born Non-Hispanic Whites (weighted prevalence; 95% CI, 2.49%; 2.21-2.79) than US-born Whites (4.08%; 3.99-4.17), foreign-born Hispanics (0.27%; 0.22-0.32) than US-born Hispanics (0.44%; 0.36-0.53), and foreign-born Asians (0.16%; 0.10-0.25) than US-born Asians (0.85%; 0.61-1.18). Skin cancer prevalence among Non-Hispanic Blacks could not be reliably estimated due to low absolute numbers. Differences in skin cancer prevalence by foreign-born status remained significant in multivariable models across all races. Among foreign-born participants, non-US citizens had lower odds of skin cancer (0.32; 0.16-0.61). Study limitations included survey administration in predominantly English and Spanish and potential recall bias. Foreign-born individuals have lower skin cancer prevalence when compared to US-born individuals of the same race. Acculturation is positively associated with higher odds of skin cancer. Skin cancer in US-born individuals and highly acculturated immigrants warrants focused public health interventions and nuanced clinical counseling.

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Prevalence and risk factors of actinic keratosis: Results from the rotterdam study

C. George[1, 2], S. Tokez[1], L. Hollestein[1], M. Wakkee[1], L. Pardo-Cortes[1], T. Nijsten[1]
[1]Dermatology, Erasmus MC, Rotterdam, Zuid-Holland, Netherlands, [2]Dermatology, SUNY Downstate Health Sciences University College of Medicine, New York, New York, United States

Actinic keratoses (AKs) are pre-malignant skin lesions that occur secondary to extended ultraviolet radiation (UVR) exposure. Limited population-based studies are available to analyze the risk factors, severity and natural course of AK. The aim of this study was to calculate the prevalence of AK and investigate its risk factors and progression. Participants from the Rotterdam Study (RS), an ongoing prospective cohort study, who underwent full body skin examination (FBSE) were included. Univariable and multivariable multinomial logistic regression analyses were performed to study associations between selected risk factors and the number of clinically diagnosed AK lesions. A subset of participants underwent two FBSEs approximately 4 years apart for longitudinal assessment. Of the 8,239 included participants, 1,731 (21.1%) had at least one AK lesion, with the majority having 1-3 AKs (54.4 %). Male gender, older age, lighter hair color, Glogau scale, baldness, and a high tendency to develop sunburn were all significantly associated with AK, with the strongest associations detected for participants with ≥10 AKs. Light eye color was associated with severe AK, but not with mild to moderate AK. Current tobacco smokers had lower odds of mild and severe AK when compared to former and never smokers. 1,547 (18.8%) participants underwent two FBSEs. Of this group, 49% did not develop AK, 15.8% had an increase in AK number, 21.7% had a decrease in AK and 13.4% had approximately the same number. In this large-scale population-based study, we demonstrated that higher age, male gender, lighter hair color, baldness, and high Glogau scale score were associated with higher odds of AK. This effect was most prominent for subjects with severe AK. Conversely, smoking showed an inverse relationship with AK, which suggests there is a protective element that reduces the odds of cutaneous pre-malignancy. Over time, AK lesions are more likely to decrease in number rather than increase or remain unchanged.

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Sentinel lymph node biopsy in patients with clinical stage IIB/C cutaneous melanoma.

R. J. Straker, C. E. Sharon, E. Chu, J. T. Miura, G. C. Karakousis, M. Ming
University of Pennsylvania, Philadelphia, Pennsylvania, United States

Recent approval of adjuvant anti-PD1 therapy for patients with pathologic stage IIB/C cutaneous melanoma has led to questioning of whether sentinel lymph node biopsy (SLNB) is necessary for patients with clinical stage IIB/C disease. We sought to evaluate the prognostic significance of SLNB in patients with clinical stage IIB/C melanoma across T-stages using logistic regression and propensity matching, a technique that has not been utilized previously to examine this issue. Immune checkpoint inhibitor naïve patients with clinical stage IIB/C cutaneous melanoma were identified from the Surveillance, Epidemiology and End Results database (2004-2011). Patients with regional lymph node positivity (LN+) were compared to LN- patients using multivariable logistic regression. Propensity matching was performed, and 5-year disease-specific survival (DSS) was estimated. Of 9,018 patients evaluated, 6,219 (69.0%) underwent LN evaluation, of which 1,989 were LN+ (32.0%). Among patients who underwent SLNB, factors associated with LN+ were Asian American/Pacific Islander race (OR 2.83, p<0.001), truncal (OR 1.74, p<0.001) or extremity (OR 1.55, p<0.001) tumor location, acral lentiginous histology (OR 2.83, p=0.002), increasing tumor thickness (OR 1.00, p<0.001), ulceration (OR 1.73, p<0.001), and younger age (OR 1.02, p<0.001). After propensity matching of patients undergoing SLNB, LN+ was associated with significantly reduced 5-year DSS (52.6% LN+ vs. 77.5% LN-, p<0.001). Notably, 5-year DSS remained significantly different across T-stages: T3b (53.5% LN+ vs. 64.8% LN-, p=0.001), T4a (56.0% LN+ vs. 72.2% LN-, p=0.001), T4b (35.3% LN+ vs. 59.7% LN-, p<0.001). Performance of SLNB was not associated with a 5-year DSS difference in the matched cohort (62.7% SLNB performed vs. 60.3% no SLNB performed, p=0.394). For patients with clinical stage IIB/C cutaneous melanoma, sentinel lymph node status provides invaluable prognostic information and can be an important factor for collective decision-making regarding adjuvant therapy administration based on risk assessment.

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Pre-existing cutaneous autoimmune disease may improve survival in patients treated with anti-PD-1 or anti-PD-L1 therapy: A population level cohort study

K. Tang[1], B. C. Tiu[1], G. Wan[1], S. Zhang[1], N. Nguyen[1], B. Leung[1], A. Gusev[2], K. Reynolds[1], S. G. Kwatra[3], Y. Semenov[1]
[1]Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, United States, [2]Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, United States, [3]Johns Hopkins University, Baltimore, Maryland, United States

Immune checkpoint inhibitors (ICIs) are associated with immune-related adverse events (irAEs) that resemble autoimmune diseases (ADs). Despite similarities of irAEs and ADs, ICI clinical trials largely exclude patients with history of AD. This study explores the impact of baseline ADs on survival among ICI-treated patients using observational data. Using the TriNetX Diamond Network, 17,497 patients with history of ADs prior to anti-programmed cell death receptor/ligand 1 therapy for malignant neoplasms of digestive organs, bronchus/lung, melanoma, and urinary tract were matched to 17,497 ICI recipients without history of ADs on demographics, cancer type, and ICI target. A Cox proportional hazards model was used to determine the prognostic impact of pre-existing ADs on survival. Overall, patients with baseline ADs were not at significantly higher risk of mortality than non-AD controls (HR=1.03,p=0.052). Interestingly, there was a significant protective effect among several cutaneous ADs, including vitiligo (HR=0.52,p=0.003), lichen planus (HR=0.70,p=0.01), and alopecia areata (HR=0.61,p=0.04). History of Hashimoto's disease (HR=0.75,p=0.002) and celiac disease (HR=0.74,p=0.03) were also significantly protective of mortality. This is the first population-level study examining the impact of underlying ADs on survival of ICI recipients. Baseline ADs do not negatively impact mortality and can even be associated with a favorable prognosis, particularly in the setting of cutaneous ADs. The differential effect on mortality may relate to extent of systemic immunosuppression utilization, which can blunt the effect of ICIs. We show that baseline ADs need not be a contraindication to inclusion in ICI clinical trials and administration.

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Cutaneous immune-related adverse events predict longer patient survival in advanced cancer patients

C. Lu[1, 2], S. Zhang[1, 2], K. Tang[1], P. Ugwu-Dike[1], N. Raval[1], J. Seo[1], G. Wan[1, 2], N. Nguyen[1], N. Alexander[1], R. Jairath[1], J. Phillipps[1], B. Leung[1], N. Theodosakis[1, 2], L. Zubiri[1], G. Boland[1, 2], D. Liu[3, 2], S. Chen[1, 2], N. LeBoeuf[5, 3, 2], K. Reynolds[1, 2], K. Yu[2], H. Tsao[1, 2], S. Demehri[1, 2], A. Gusev[3, 2], S. G. Kwatra[4], Y. Semenov[1, 2]
[1]Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States, [3]Dana-Farber Cancer Institute, Boston, Massachusetts, United States, [4]Johns Hopkins University, Baltimore, Maryland, United States, [5]Brigham and Women's Hospital, Boston, Massachusetts, United States

Cutaneous immune-related adverse events (cirAEs) are the most common toxicities to occur in the setting of immune checkpoint inhibitor (ICI) therapy. However, their impact on mortality is largely understudied. Here, we investigate the impact of cirAEs on survival among ICI recipients using a robust multi-institutional clinical registry. 3,731 ICI recipients were identified from the Mass General Brigham Healthcare System and the Dana-Farber Cancer Institute, of which 676 developed cirAEs and 3,055 did not. Landmark survival analyses and time-varying Cox proportional hazards modeling were performed, adjusting for demographics, Charlson comorbidity index, cancer type, ICI target, and year of ICI initiation. Overall, patients who developed cirAEs were significantly protected from mortality (HR=0.87, p=0.036), particularly in the setting of melanoma (HR=0.67, p=0.007). Among individual morphologies, lichenoid eruption (HR=0.46, p<0.001), psoriasiform eruption (HR=0.43, p=0.004), vitiligo (HR=0.26, p=0.012), and non-specific rash (HR=0.69, p<0.001) were significantly protective of mortality after multiple comparisons adjustment. Our results demonstrate that CirAE development is associated with a 13% reduction in mortality among all cancers and a 33% reduction in melanoma patients. Furthermore, almost all cirAE morphologies are associated with better survival. Even after excluding vitiligo, cirAE development remains protective among all ICI recipients and melanoma patients. These findings are important as cirAEs tend to occur early after ICI initiation and may serve as predictive biomarkers of therapeutic response.

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Associations between itch and sleep disturbance in people living with HIV

C. D. Huebner[1], K. Lee[2], K. Abuabara[1]
[1]Department of Dermatology, University of California San Francisco School of Medicine, San Francisco, California, United States, [2]Department of Family Health Care Nursing, University of California San Francisco School of Nursing, San Francisco, California, United States

Sleep disturbance and daytime fatigue are two of the most prevalent symptoms reported by people living with HIV (PLHIV) but little is known about the causes of sleep disturbance in PLHIV. Because PLHIV have reduced skin barrier function, dry skin and eczematous rashes, we sought to investigate the associations between experiencing symptoms of itch and sleep disturbance. We performed a secondary analysis of data from the Symptoms and Genetics Study, a cohort of 317 participants with at least a 6 month history of HIV, who were followed for up to 4 years at UCSF. Sleep was measured using the Memorial Symptoms Assessment Scale and the Pittsburgh Sleep Quality Index, and sleep quality was measured by wrist actigraphy, over 72 hour periods. Itch was reported by 71 (22.4%) of 317 participants. After adjusting for relevant demographic and clinical characteristics in multivariable linear regression models, we found that PLHIV with itch slept shorter amounts of time and reported higher global PSQI scores, but there were no statistically significant differences found in actigraphy measured sleep onset latency (β = -0.0938, 95% CI [-0.2989 - 0.1021]), actigraphy measured total sleep time (β = -22.6704, 95% CI [-49.3888 - 4.0480]), or global PSQI score (β = 0.4126, 95% CI [-0.5508 - 1.3760]) between PLHIV experiencing itch and those who did not. Our results suggest that itch does not play a major role in sleep onset insomnia and insomnia in the general population of PLHIV, however future studies should investigate the role of itch in sleep disturbance in PLHIV with secondary skin diseases.

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Calciphylaxis arising following bariatric surgery

B. Cucka[1], B. Biglione[1], L. Ko[1], E. Nguyen[1], C. Khoury[2], S. Nigwekar[3], M. Robinson[4], D. Kroshinsky[1]
[1]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Nephrology, Washington University in St Louis School of Medicine, St Louis, Missouri, United States, [3]Nephrology, Massachusetts General Hospital, Boston, Massachusetts, United States, [4]Metabolic and Bariatric Surgery, Brigham and Women's Hospital, Boston, Massachusetts, United States

Calciphylaxis is a rare, highly morbid disease leading to vascular calcification and cutaneous necrosis, typically associated with end-stage renal disease (ESRD). While the pathogenesis and risk factors of calciphylaxis are poorly understood, some cases of calciphylaxis in patients who had previously undergone gastric bypass have been reported. The objective of this study is to report cases and describe an association between nutritional deficiencies in the context of bariatric surgery and calciphylaxis. Retrospective study in one large academic hospital that serves as a referral center for calciphylaxis in Boston, Massachusetts between 2012-2018 involving 11 patients who had bariatric surgery prior to calciphylaxis. Median time between bariatric surgery and calciphylaxis diagnosis was 2 years and 2 months, (range 7 months – 30 years). Parathyroid hormone levels were significantly higher in patients with ESRD compared to those with non-uremic calciphylaxis (p=0.036). Corrected calcium levels were significantly higher in patients who had malabsorptive compared to restrictive procedures (p=0.01). Limitations include retrospective nature, sample size, and missing weight loss data from remote bariatric surgical cases. Recognition of risk factors for calciphylaxis and careful evaluation of therapies for other comorbidities in patients who have undergone bariatric procedures is essential to improve outcomes for this rare but problematic disease.

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Updated results of 3,050 lesions in 1725 patients treated with novel technology using image guided superficial radiotherapy (IGSRT), a multi-institutional study

M. Moloney[2], D. Ladd[4], D. Serure[3], L. Yu[1]
[1]Director of Radiation Oncology, Laserderm Dermatology, Smithtown, New York, United States, [2]New York Institute of Technology College of Osteopathic Medicine, Old Westbury, New York, United States, [3]Chief of Dermatology, Laserderm Dermatology, Smithtown, New York, United States, [4]Medical Director, Tru-Skin Dermatology, Austin, Texas, United States

Image-guided superficial radiation therapy (IGSRT) is becoming an attractive non-surgical curative treatment option for nonmelanoma skin cancer (NMSC). We previously reported IGSRT treatment results from a multi-institutional study of 1632 patients with 2917 NMSC lesions (Yu et al. Oncol Ther 2021, https://doi.org/10.1007/s40487-021-00138-4) showing excellent local control(LC) of 99.3% with mean follow-up(f/u) of 69.8 weeks. This abstract analyzes an additional 93 patients, updates previous findings with longer f/u, and performs subgroup analysis. A total of 1725 patients with 3,050 Stage 0, I, and II NMSC lesions treated from 2017 to 2020 were retrospectively analyzed. Lesions received a median of 20 fractions of 50, 70, or 100 kilovoltage(kV) IGSRT using image guidance. Average f/u was 25.03 months (108.8 weeks) with a maximum follow up of 65.6 months (285.0 weeks) for the entire cohort. 68 patients expired from unrelated causes with no-evidence of disease(NED) at last f/u prior to death, thus Disease-Free-Survival (DFS) was 100%. Overall, 3,027 of 3,050 lesions achieved an absolute LC of 99.2%. Overall absolute LC for BCC, SCC, and SCCis was 98.9%, 99.2%, and 99.8%, respectively. No additional late complications were found to date as of January 2022. These updated results demonstrate that IGSRT continues to achieve a high level of LC with low complication rates. IGSRT should be considered an attractive first-line option for the non-surgical treatment of NMSC.

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A retrospective chart review of outcomes after hyperbaric oxygen therapy for the treatment of calciphylaxis

B. Cucka[1], B. Biglione[1], J. Locascio[2], J. Goldfarb[3], A. Gutium[3], D. Kroshinsky[1]
[1]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Neurology, Massachusetts General Hospital, Boston, Massachusetts, United States, [3]Massachusetts Eye and Ear, Boston, Massachusetts, United States

Calciphylaxis is a rare thrombotic vasculopathy characterized by high mortality with worse outcomes in nephrogenic(NC) compared to non-nephrogenic(NNC) calciphylaxis. Hyperbaric oxygen therapy(HBOT) may be used in the treatment of calciphylaxis, however, studies analyzing the effectiveness of HBOT for calciphylaxis are lacking. This study involved a retrospective medical record review of 185 adult patients with diagnosed calciphylaxis between 1/1/2006-12/31/21. For each patient, the main calciphylaxis lesion, defined as the largest or most documented lesion, was longitudinally scored based on dermatologist assessment from chart review and photographs. All-cause mortality was analyzed with traditional survival analyses and Cox proportional hazard models. Longitudinal wound outcomes were analyzed with mixed effects modeling. 145(78.4%) received standard care and 40(21.6%) received HBOT, of which 14(35.0%) received Full HBOT(at least 25 sessions) and 26(65.0%) received Partial HBOT(less than 25 sessions). Full HBOT was associated with significantly longer survival time than the other treatments(Hazard Ratio 0.221,p=0.0111). All-cause mortality for Full HBOT was 21% compared to 59% for standard care(p=0.0208). Both Full HBOT and Partial HBOT demonstrated significant improvement in wound healing over time(p=0.001), with Full HBOT doing better than Partial HBOT after the end of HBOT(p=0.0063). These findings suggest a role for HBOT in the treatment of calciphylaxis with benefits demonstrated in both mortality and wound healing.

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Clinical epidemiology of ecthyma gangrenosum

W. C. Lau, K. Yang, C. X. Pan, C. B. Lau, B. Kassamali, V. Nambudiri
Department of Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States

Ecthyma gangrenosum (EG) is an uncommon infectious cutaneous eruption. It typically presents with painless macules, transitions to hemorrhagic vesicles, and eventually evolves into necrotic ulcers. EG should be recognized early and treated aggressively as it is associated with significant morbidity. Because the literature on this disease is limited, we sought to characterize clinical and pathologic features of EG in a large, multi-institution academic center. Patients were identified using the Mass General Brigham Research Patient Data Registry and included individuals with a diagnosis of EG from 1/1980 to 9/2021 seen at Massachusetts General Hospital, Brigham and Women's Hospital (BWH), and the BWH Faulkner Hospital. Demographic information, clinical documentation, and pathology reports were reviewed for data extraction. 83 individuals were diagnosed with EG, with the majority male (65%) and white (89%). Lesions were most commonly found in the lower extremities (53%), followed by the truncal region (19%). Pseudomonas aeruginosa was the most common pathogen isolated from the lesions (70%). Fungal organisms were also seen as the causative pathogen in a small number of cases, including Candida albicans (5%). The majority of patients with EG were immunocompromised (80%) and 61% of patients also experienced sepsis. Our study highlights that EG is often caused by Pseudomonas aeruginosa, but in approximately one-third of cases, another causative pathogen may be identified. There were a variety of other fungal and bacterial etiologies including Candida, Fusarium, Citrobacter, Escherichia, Enterococcus, Klebsiella, Morganella, Serratia, Proteus, and Staphylococcus species. 20% of patients with EG were immunocompetent, suggesting that this condition may also manifest in otherwise healthy patients. Limitations of our study include its retrospective nature, allowing only for observations of association. Nevertheless, early detection of ecthyma gangrenosum and determining its etiology are critical for appropriate management.

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Distinguishing clinical features for pseudocellulitis in pediatric inpatients: A retrospective study

B. Biglione, B. Cucka, S. Chand, R. Rrapi, C. K. Gabel, S. Song, D. Kroshinsky
Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Cellulitis is a common skin infection presenting with expanding erythema, warmth, pain, and swelling of the affected area. While adult cellulitis is well-studied, there is a paucity of data on pediatric cellulitis and no objective tests to confirm a cellulitis diagnosis. There are also several diseases that clinically mimic cellulitis, known as pseudocellulitis. This study evaluates and compares the clinical presentation of inpatient pediatric pseudocellulitis to cellulitis to identify associated factors. A retrospective chart review was conducted of 893 pediatric inpatients admitted with cellulitis at a tertiary hospital from 2007-2019. Of the 588 cases included (65.8%), 559 patients (95.1%) had a discharge diagnosis of cellulitis, and 29 patients (4.9%) had an initial admission diagnosis of cellulitis that was changed to a different diagnosis. Within the 29 cases of pseudocellulitis, the most common alternative diagnoses were suppurative lymphadenitis (n=6;20.7%), hypersensitivity reaction (n=5;17.2%), and irritant or contact dermatitis (n=3;10.3%). Patients in the pseudocellulitis and cellulitis groups had comparable demographics, clinical presentation, and bloodwork (p>.05). Notably, dermatology and infectious disease consultations were significantly associated with higher rates of pseudocellulitis diagnosis (p<.001, p=.013, respectively). Within the 36 patients evaluated by dermatology, the clinical features associated with true cellulitis rather than pseudocellulitis were fever (p=.008), tachycardia (p=.002), and presence of purulence (p<.001). Bloodwork, such as leukocytosis, was not revealing in distinguishing between pediatric cellulitis and pseudocellulitis (p>.05). This study supports findings that dermatology and infectious disease consultation identify higher rates of inpatient pediatric pseudocellulitis. Absence of certain vital signs and laboratory abnormalities were associated with pseudocellulitis diagnosis among dermatology consultations, supporting findings that routine bloodwork is not recommended for diagnosis.

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Geographic distribution of primary discoid lupus erythematosus and environmental hazards in Massachusetts

N. Goldman, B. Kassamali, C. Nwankwo, J. Merola, G. Cobos, R. Vleugels, A. LaChance
Department of Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States

Primary discoid lupus erythematosus (pDLE) is a subset of cutaneous lupus erythematosus that occurs independently of systemic lupus erythematous (SLE). Prevalence of DLE has been estimated to range between 20-40 cases per 100,000. While the etiology of pDLE is not well understood, environmental exposures are thought to contribute. This project aims to identify correlations between areas of pDLE clusters and possible environmental triggers. Patients residing in Massachusetts (MA) with a diagnosis of pDLE were identified from the Mass General Brigham hospital system (n=1539). Addresses were then mapped to census tracts on ArcGIS geospatial software and period prevalence was calculated for the range of 1988-2021. These maps were then compared to environmental hazards in MA and statistical analysis was performed using linear regression. Overall, the average period prevalence by census tract was found to be 22.0 cases per 100,000 (IQR 0-36.8). Census tracts with a higher percentage of Black residents were associated with a higher prevalence of pDLE (p<0.001). Oil and hazardous material sites were most strongly associated with a higher pDLE prevalence for all census tracts (p<0.001) and for counties surrounding Boston Harbor (p=0.016). Census tracts containing hazardous waste recycling or discharge/storage facilities were also positively associated with disease prevalence when controlling for other environmental hazards (p=0.037). While these results are unable to determine causation, the geographic clustering of pDLE cases near oil and hazardous material sites is consistent with prior research suggesting a correlation between SLE and petroleum products. Previous research has already suggested that residents of minority communities face disproportionate exposure to environmental hazards, and this study may further support the role of environmental justice in protecting vulnerable populations.

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Evaluation of nailfold capillaries in dermatomyositis using a dermatoscope

J. Dan[1, 2], G. Sprow[1, 2], M. Afarideh[1, 2], J. Concha[1, 2], N. Kodali[1, 2], T. Vazquez[1, 2], D. Diaz[1, 2], V. Werth[1, 2]
[1]Derm, PSOM at UPenn, Los Angeles, Pennsylvania, United States, [2]Derm, Corporal Micheal J. Crescenz VAMC, Phil, Pennsylvania, United States

Nailfold capillary (NC) abnormalities are increasingly utilized in the evaluation of rheumatic conditions. Their presence can distinguish primary Raynaud's phenomenon from secondary etiologies and are used in the diagnostic criteria of scleroderma. Dermoscopy is a convenient method of evaluating NC changes with similar efficacy to capillaroscopy. We imaged all ten nailfolds of subjects with dermatomyositis (DM) using a dermatoscope with a smartphone adapter. Fingernails were cleaned with an alcohol swab prior to evaluation. Images were assessed for loop dilation (LD), abnormal morphology (AM), capillary bed disorganization (CBD), capillary hemorrhage (CH), capillary dropout (CD), decreased capillary density (DCD), and subpapillary plexus visibility (SP). Cutaneous Dermatomyositis Area and Severity activity (CDASI-A) scores and systemic activity (lung, muscle, or joint involvement) were collected. Analysis used Student's t-test and chi-square test. Subjects with hypertension, hyperlipidemia, diabetes, glaucoma, or other primary rheumatic disease were excluded. 44 subjects, 17 with amyopathic DM (ADM), were included. Mean (standard error) CDASI activity and damage scores were 13 (1.05) and 4 (0.39), respectively. 89% of subjects demonstrated capillary abnormalities. LD (77%), followed by CBD (73%), were the two most common findings. NC changes were most often found on 4th and 5th fingers. Thumbs demonstrated the fewest abnormalities. Mean CDASI-A was higher in patients with AM (CDASI 16 vs 10, p=0.003), CD (15 vs 10, p=0.016), DCD (17 vs 10, p<0.001), and trended towards significance for LD and CBD. There was a trend towards a higher percentage of CH in patients with ADM compared to those with muscle disease (no difference in CDASI-A). Subjects with skin limited disease (n=23) vs active systemic disease demonstrated more AM (74% vs 38%, p=0.02; no difference in CDASI-A). Given its prevalence, NC changes are useful in the evaluation of DM. AM, CD, and DCD may be markers of cutaneous disease activity.

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Risk factors and outcomes for sepsis in cutaneous T cell lymphoma compared to in other non-Hodgkin lymphomas

M. Hooper[1], F. L. Veon[1], T. LeWitt[1], C. Grimes[1], M. Nguyen[1], Y. Pang[1], T. Borders[1], J. Guitart[1], M. Burns[2], X. Zhou[1]
[1]*Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States,* [2]*Biology, Loyola University Chicago, Chicago, Illinois, United States*

Cutaneous T cell lymphoma (CTCL) patients often suffer from recurrent skin infections that progress to sepsis. However, it is unknown how risk factors and outcomes differ between sepsis in CTCL versus in other non-Hodgkin lymphomas (NHL). We analyzed a retrospective cohort of 97 CTCL (40% mycosis fungoides, 40% Sézary syndrome) and 89 non-CTCL NHL patients with sepsis and positive blood cultures at a single U.S. academic institution, 2000-2021. Median follow-up time was 159 months. CTCL patients with sepsis were on average older than other NHL patients with sepsis (mean age 62.3 versus 55.7 years, p=0.003), experienced more episodes (mean 1.9 versus 1.4 episodes, p<0.0001), and more often died of sepsis within 30 days (54% versus 25%, p=0.041). CTCL patients were primarily African American (AA) at 57% versus 38% in NHL (p<0.0001). At sepsis onset, 9% CTCL had stage I disease, 25% stage II, 5% stage III, and 61% stage IV (versus 73% NHL). Of CTCL culture-positive episodes, 85% were bacterial, 9% viral, 4% fungal, and 2% mixed. Staphylococcus aureus was the most common cultured organism in CTCL (26%); Escherichia coli predominated in NHL (14%). Kaplan-Meier analysis showed fungemia, viremia, mixed microbes, older age, late-stage disease, and extracutaneous disease were associated with shorter survival time in CTCL sepsis. Fungemia, male sex, metastases, and late-stage disease were associated with shorter survival in NHL sepsis. Prior stem cell transplant was associated with longer survival time in both CTCL and NHL sepsis. There were no survival differences in CTCL patients with gram-positive versus gram-negative bacteremia. Compared to other NHLs, CTCL sepsis is associated with more episodes, mortality within 30 days, S. aureus-positive blood cultures, older age, predominance of both stage IV and II (including IIB tumor-stage) disease, and AA race. Sepsis risk factors and outcomes differ between CTCL and other NHLs.

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Identification of meaningful aspects of health connected to the symptom of nocturnal scratching in patients with atopic dermatitis

L. Cesnakova, L. Clay
Digital Medicine Society (DiMe), Boston, Massachusetts, United States

Substantial work is underway across both pharmaceutical companies and digital product manufacturers to apply digital technologies to better measure symptoms in acute dermatologic conditions. To date, among the more advanced and promising applications of digital clinical measures as a drug development tool is nocturnal scratch. Novel digital clinical measures could provide an additional tool in answering the need for improved, precise, long-term quantitative measures of nocturnal scratching. In this work, a consortium of industry, patient advocacy and non-profit organizations came together to develop a mixed methods study to explore patient experience with nocturnal scratch. The research study utilized both qualitative and quantitative methods to understand the patient experience about life with eczema, symptom of night-time scratching and it's burden to everyday life. We performed qualitative one-on-one patient interviews that informed design of the quantitative survey distributed to a large number of patients and caregivers. In the qualitative patient interviews we recruited 12-20 patients from all age groups. In the quantitative part, that we distributed the survey to 600 representative patients.
As of date of this abstract submission, the study is underway, and we will amend the abstract with the results of the study in due time before the annual meeting.

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Evidence for a role of ambient temperature on skin aging: A cross-sectional analysis from three metropolitan cities of India.

N. Singh[1], P. Jahan[1], P. Vijay[2], H. Phuleria[2], J. Krutmann[1], T. Schikowski[1]
[1]*IUF-Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany,* [2]*Environmental Science & Engineering Department, Indian Institute of Technology Bombay, Mumbai, Maharashtra, India*

Skin aging is driven to a large extent by environmental factors such as air pollution. Air pollution is a major cause of global warming. It is currently not known if increasing ambient temperature (AT) per se contributes to skin aging and/or if it modifies air pollution-induced skin aging. In order to address this topic, we analyzed the association between skin aging traits and AT using an ordinal multiple logistic regression model. This study was conducted in three metropolitan Indian cities: Delhi, Mumbai, and Bangalore. All together 1,510 women aged between 20-91 years were investigated. The data on skin aging traits were collected between May 2018 to Feb 2019 using SCINEXA™, a validated visual score to measure extrinsic facial skin aging. Five-year mean exposure to average AT was calculated using gridded temperature data from India Meteorological Department (0.5 x 0.5 km resolution). The long-term exposure to traffic-related air pollutants including fine particulates (PM2.5) and NO2 was collected using on-ground measurements. We here report a positive and significant association between AT and skin aging parameters including wrinkles on the forehead (OR: 1.13, 95% CI; 1.06, 1.21), wrinkles under the eyes (OR: 1.11, 95% CI; 1.04 1.19), laxity on cheeks (OR: 1.10, 95%CI; 1.02, 1.18), hyperpigmentation on the forehead (OR: 1.28, 95% CI; 1.16, 1.42), and dark circles under eye (OR: 1.92, 95% CI; 1.76,2.09). The association of wrinkles under the eyes and dark circles under the eye with mean AT increased after adjusting for the confounding effect of PM2.5. Thus, the study provides epidemiological evidence that long-term exposure to high AT may contribute to skin aging and increase the effect of traffic-related air pollution on skin aging.

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Socioeconomic predictors of melanoma Breslow thickness at a Rhode Island academic center

F. Ahmed[1], R. Lim[1], I. H. Moseley[1], M. Hoang[2], O. Wisco[3], L. Robinson-Bostom[3], A. Qureshi[3], E. Cho[3]
[1]*Brown University Warren Alpert Medical School, Providence, Rhode Island, United States,* [2]*Brown University, Providence, Rhode Island, United States,* [3]*Dermatology, Brown University Warren Alpert Medical School, Providence, Rhode Island, United States*

Introduction: Breslow thickness is an important prognostic factor in malignant melanoma (MM). Lower socioeconomic status (SES) has emerged as a predictor of poor prognosis, though few US data relate SES to thickness. Furthermore, few studies investigated neighborhood-level income alongside insurance status and clinicopathological factors of MM. To examine relationships between SES and tumor thickness, we retrospectively reviewed medical and pathology records of MM patients at a Rhode Island academic center.
Methods: For patients diagnosed with MM from 10/1/16 to 5/31/20, addresses were extracted and geocoded to census tracts. Tract-level median annual household income was used as a proxy for SES (≤$65000; >$65000). In addition, health insurance type & tract-level estimates of educational attainment, vehicle access, age of housing structures, and relative distance to supermarkets were tested as predictors of Breslow thickness >1.00mm. We calculated adjusted odds ratios (aORs) using logistic regression. Covariates included age, sex, race/ethnicity, personal and family skin cancer history, smoking history, sunscreen use, tumor site, and histologic subtype.
Results: Of 264 MM patients (mean age: 68.9±14.4, female: 43.9%), 89 had tumors >1.00mm thick. Lower SES (n=70) was associated with increased odds of presenting with thicker tumors (aOR 3.17, 95% CI 1.21-8.28). Other tract-level factors were not significant in multivariable analysis; lower rates of high school completion and vehicle access predicted thickness >1.00mm in age-adjusted models only. Compared to privately insured patients, those with public or no insurance (n=47) had higher odds of thickness >1.00mm (aOR 3.50, 95% CI 1.40-8.77).Conclusion: In this study, lower SES and lack of private health insurance were associated with higher risk of thicker MMs at presentation, despite adjusting for several neighborhood- and individual-level factors.

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Disparities in global representation within dermatology publishing: A bibliometric analysis

S. Prasad[1], R. Ali[1], V. Mroz[1], S. Mehta[1], R. Singh[1], L. Abdelrahman[1], R. Kankaria[1], S. Singh[1], R. Dellavalle[2], E. Freeman[1]
[1]Massachusetts General Hospital, Boston, Massachusetts, United States, [2]University of Colorado Denver School of Medicine, Aurora, Colorado, United States

Intro: Low- and middle-income countries (LMIC) represent a disproportionate share of the global burden of skin disease. Despite this, dermatologic research funding and agenda-setting have been largely skewed towards the disease burden in high income countries. Little is known about the global representation of authorship in dermatology literature, especially with regard to LMICs. The aim of this study was to quantify geographic representation in recent academic publishing in dermatology. Methods: We extracted all publications indexed in PubMed from four academic journals with the highest impact factors in dermatology, (Journal of the American Academy Dermatology (JAAD), JAMA Dermatology (JAMADerm), British Journal of Dermatology (BJD) and Journal of Investigative Dermatology (JID)) from Oct 1, 2018 to Sept 31, 2021. Geographic representation of authorship was determined by location of either first or senior author's affiliation. Range of publication types were commentary, case reports, research letters and full-length articles. Results: Of 8,024 publications, the U.S. had the largest representation in academic publishing (48%). This held true for each journal individually, with the U.S. representing 68% of literature in JAAD, 22% BJD, 62% JAMADerm and 39% JID. Other frequently represented countries included the U.K. (8.6%), Germany (5.6%), France (4.7%) and China (4.5%). LMIC authors were underrepresented across all journals, with only 10.5% of all publications having any LMIC affiliation for first/senior author (135 countries). The most represented LMICs in the dataset besides China included India (2.4%), Brazil (1.1%), Chile (0.3%) and Mexico (0.2%). Conclusion: Academic publishing in dermatology is largely skewed to favor literature from the U.S. and other high-income countries. Greater global representation is needed in dermatology publishing to ensure research better matches global need.

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Pityriaris rubra pilaris has a significant psychiatric burden and impact on quality of life

A. Ji-Xu[1], D. Lei[2], S. Worswick[3], N. Maloney[4], M. Kim[5], L. Cutler[3]
[1]Department of Dermatology, University of California Davis, Sacramento, California, United States, [2]Mount Sinai Health System, New York, New York, United States, [3]Department of Dermatology, Keck Hospital of USC, Los Angeles, California, United States, [4]Department of Dermatology, Stanford University School of Medicine, Palo Alto, California, United States, [5]Department of Dermatology, Wayne State University School of Medicine, Detroit, Michigan, United States

Pityriasis rubra pilaris (PRP) is an inflammatory condition that has debilitating complications and is frequently refractory to treatment. Despite this, the psychiatric burden of PRP remains largely uninvestigated. Our aim was to quantify comorbid psychiatric disorders and identify disease factors associated with worsened quality of life in this condition. We conducted an online Qualtrics survey study of PRP patients. We recruited participants through a mailing list from the PRP Alliance. Inclusion criteria were diagnosis of PRP by a dermatologist and age >18 years at survey completion. Univariate analyses comparing mean Dermatology Life Quality Index (DLQI) scores were performed using Kruskal-Wallis tests. In total, 307 patients met inclusion criteria. The mean DLQI score for PRP patients was 11.5 ± 8.4. Overall, 61.9% of patients reported depression, 23.1% anxiety, 69.4% mobility issues, and 58.6% significant impact on their ability to work or attend school. Most (60.9%) patients had a >50% reduction in sleep. Factors associated with a higher DLQI included alopecia, joint pain, photosensitivity, and history of hospitalization (p<0.001). Only 9.1% reported that their dermatologist referred to a psychologist or psychiatrist. Patients referred to psychiatrists or psychologists had mean DLQI scores of 16.4, compared to 11.0 in those without a referral (p=0.0012). Our findings reveal that psychiatric comorbidities and impaired daily living are highly prevalent in PRP patients. Despite this, only a small proportion of patients are referred to psychiatry or psychology, with those who are referred exhibiting higher DLQI scores.

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The clinical definition and classification of field cancerization in patients with actinic keratosis

K. A. Kelly[1], V. Ranpariya[1], M. Shah[1], L. Mohney[1], S. R. Feldman[1, 2, 3]
[1]Center for Dermatology Research, Department of Dermatology, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States, [2]Department of Pathology, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States, [3]Department of Social Sciences & Health Policy, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States

Background: Actinic keratoses (AKs) present as red, scaly, hyperkeratotic papules that arise from chronic sun exposure. Field treatments treat multiple AKs in a given area, but since they also treat subclinical lesions, the benefit of a topical treatment may depend on the size of the field. In a systematic review of 39 studies, the defining features of a field of cancerization (FoC) varied considerably with differences in both numbers of AKs and overall size, highlighting the discordance among researchers surrounding the clinical criteria of a FoC.1 Objective: To clearly define the clinical criteria for determining the size of the FoC. Methods: This is a prospective, pilot study of 10 patients with AKs from Atrium Health Wake Forest Baptist dermatology clinics. We recorded the number of AKs as well as the presence of dyspigmentation, dryness, and scaling on a specific anatomical area. We quantified the size of the FoC in this area with cardboard cutouts to determine if the FoC met the FDA-approved topical treatment guideline (less than or equal to 25 cm2). Results: 70% of patients presented with a FoC greater than 25 cm2 (50-725 cm2). The average number of AKs in this patient cohort was 20.7 (5-57). Skin dryness, dyspigmentation, and scaling were present in the field among 100% of patients. Conclusions: Presence of skin dryness, dyspigmentation, and scaliness may be a defining feature of FoC. The majority of patients presented with a field greater than 25 cm2, suggesting the FDA guidelines may not align with clinical presentation of FoC.

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Use of biologics in transplant patients: A retrospective cohort study

C. Madden[1, 2], W. F. Dean[3], I. T. Smith[3], J. Ike[4], B. Hall[5], L. Wheless[2, 6]
[1]SUNY Downstate Health Sciences University College of Medicine, New York, New York, United States, [2]Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States, [3]Vanderbilt University, Nashville, Tennessee, United States, [4]Meharry Medical College School of Medicine, Nashville, Tennessee, United States, [5]Vanderbilt University School of Medicine, Nashville, Tennessee, United States, [6]Medicine, Division of Epidemiology, Vanderbilt University Medical Center, Nashville, Tennessee, United States

Organ transplant recipients can develop various dermatological conditions that require treatment with biologic therapies. Biologics have been associated with risks, such as infection and drug interactions, and may have increased negative immunomodulating effects in transplant patients on immunosuppressive therapies. The purpose of this study is to evaluate the complications of biologic use in transplant patients in regard to rejection and infection. Using the Synthetic Derivative, a de-identified database of Vanderbilt's electronic health record, we identified 122 organ transplant recipients who were or were not treated with a biologic post-transplant. The biologics group included 81 patients (mean[SD] age 58.9[15.3]); 37% female) with varying types of transplantation: heart (1.2%), lung (3.7%), kidney (14.8%), liver (24.7%), pancreas (1.2%), and bone marrow/stem cell (55.6%). The non-biologics group included 41 patients (mean[SD] age 64.5[11.2]; 43.9% female) with the following distribution of transplantation: heart (14.6%), lung (7.3%), kidney (24.4%), liver (9.8%), and bone marrow/stem cell (43.9%). When assessing rejection, there was a significant association between biologic use and rejection with an 11.1% rate of rejection in the biologics group vs 29.3% in the non-biologics group (p = 0.01). Infection rate was 45.7% in the biologics group vs 75.6% in the non-biologics group, which indicated a significant association between biologic use and infection (p = .002). These findings suggest that biologic therapy use does not carry an increased risk of rejection or infection in organ transplant recipients post-transplant.

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Voriconazole metabolism is associated with the number of skin cancers per patient

J. Ike[1], I. T. Smith[2], W. F. Dean[2], C. Madden[3], A. Lewis[5], L. Bastarache[5], L. Wheless[4, 6]

[1]Meharry Medical College School of Medicine, Nashville, Tennessee, United States, [2]Vanderbilt University, Nashville, Tennessee, United States, [3]SUNY Downstate Health Sciences University College of Medicine, New York, New York, United States, [4]Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States, [5]Biomedical Informatics, Vanderbilt University Medical Center, Nashville, Tennessee, United States, [6]Medicine, Division of Epidemiology, Vanderbilt University Medical Center, Nashville, Tennessee, United States

Voriconazole is an antifungal medication often used in organ transplant recipients. Despite its widespread use, it is associated with an increased risk of skin cancer that is thought to be due to its primary metabolite voriconazole N-oxide (VNO), however it is unclear how this impacts the total number of skin cancers a patient will develop. We hypothesized that patients who metabolize voriconazole more rapidly would accumulate more VNO and develop an increased number of skin cancers. Using Vanderbilt's Synthetic Derivative database, a de-identified mirror image of its electronic health record, we identified 1,778 self-reported white organ transplant recipients with known metabolizer phenotypes for CYP2C19, voriconazole's primary metabolizer. Of these, 135 recipients were treated with voriconazole. There were 1,154 (64.9%) normal metabolizers, 588 (33.1%) rapid, and 36 (2.0%) poor. Patients with voriconazole exposure tended to be transplanted at an earlier age (49.0 years vs 52.0, p < 0.01), had fewer years of follow-up (4.0 vs 5.6, p < 0.01), and had fewer skin cancers overall (0.6 vs 1.0, p = 0.03). Both groups were 61.5% male. Among those with voriconazle exposure, metabolizer status was significantly associated with the total number of skin cancers per patient (mean [standard deviation]. poor metabolizers: 0 [0], normal: 1.0 [3.4], rapid: 4.0 [16.3]), but not among those without voriconazole exposure (poor: 2.6 [9.4], normal: 1.4 [5.5], rapid: 1.4 [6.5], 2-way ANOVA p = 0.03). Our findings indicate that skin cancer risk from voriconazole exposure is mediated by CYP2C19 metabolizer status, likely via the photosensitizing metabolite VNO.

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Risk of herpetic and non-herpetic cutaneous infections in adult patients with atopic dermatitis exposed to oral janus kinase inhibitors or dupilumab.

E. A. Michelen-Gómez[2], Z. C. Chiesa Fuxench[1]

[1]Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]Dermatology, Universidad de Puerto Rico Escuela de Medicina, San Juan, Puerto Rico, United States

Introduction: Atopic dermatitis (AD) is a chronic, inflammatory skin disease characterized by highly pruritic episodes that recur in a relapsing and remitting pattern. Patients with AD have a higher baseline risk of developing cutaneous infections than non-AD patients. Moreover, their exposure to multiple immunosuppressive agents can potentially increase this risk. Objective: To examine the risk of herpetic and non-herpetic skin infections among adult patients with AD treated with oral Janus Kinase Inhibitors (JAKIs) or dupilumab. Methods: Following PRISMA guidelines, studies that examined the efficacy and safety of oral JAKi and/or dupilumab in adults with AD were systematically searched for in Medline, EMBASE, PubMed and clinical trial registries; 359 references were screened. Studies that reported data on herpetic and non-herpetic cutaneous infections were included. Results: 27 placebo-controlled randomized clinical trials met our inclusion criteria (N=11-dupilumab, N=5-abrocitinib, N=7-baricitinib and N=4-upadacitinib). Baricitinib, 1mg, 2mg or 4mg, had a significantly increased risk of developing both herpetic and non-herpetic skin infections compared to the placebo (risk ratio [RR] of 1.87 [p=0.03], 2.1 [p=0.004], and 2.35 [p=0.002], respectively). Upadacitinib 15mg (p=0.00002) or 30mg (p=0.000067) had a significantly increased risk of developing herpetic skin infection only, when compared to the placebo (RR=2.35 [p<0.001] and 3.76 [p<0.001], respectively). Abrocitinib 100mg or 200mg did not show an increased risk of developing either herpetic or non-herpetic infection when compared to placebo. This was also the case for patients receiving dupilumab 300mgQ2W. Conclusion: With the advent of newer systemic agents for AD on the horizon, it is imperative that we develop a better understanding of the side effect profile of these agents, including risk of infections as this has implications in treatment selection.

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Male genital lichen sclerosus, micro incontinence and occlusion: Mapping the disease across the prepuce

G. Kravvas[1], A. Muneer[2], R. Watchorn[1], F. Castiglione[2], A. Haider[3], A. Freeman[3], P. Hadway[2], H. Alnajjar[2], M. D. Lynch[4], C. Bunker[1]

[1]Dermatology, University College London Hospitals NHS Foundation Trust, London, London, United Kingdom, [2]Urology, University College London Hospitals NHS Foundation Trust, London, London, United Kingdom, [3]Histopathology, University College Hospital, London, London, United Kingdom, [4]Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, London, United Kingdom

Introduction: Male genital lichen sclerosus (MGLSc) can lead to sexual dysfunction and urological morbidity and is a risk factor for penile cancer (PeCa). Although the precise cause of MGLSc remains controversial, accumulated evidence indicates a relation to chronic, occluded exposure to urine. We mapped the presence of MGLSc across the prepuce to better understand its spatial distribution and correlate the clinical and histological extents of the disease. Methods: Preputial samples were collected from ten patients with clinically-diagnosed MGLSc undergoing circumcision. Samples were divided into a grid and punch biopsies were obtained to determine the spatial distribution of the disease. Results: All patients reported having urinary micro-incontinence, and all were histologically confirmed to have MGLSc. The proximal aspect of the prepuce was found to be universally affected by LSc, whereas the distal part was overwhelmingly shown to be the least affected. Of the 63 LSc-affected regions, 62 were in direct physical contiguity with one another. The histological extent of the disease was not found to be congruent with the severity of the symptoms and examination findings. Discussion: In uncircumcised men with urinary micro-incontinence, after the prepuce has been replaced post micturition, small amounts of urine pool between the juxtaposed epithelial surfaces. The proximal aspect of the prepuce is subjected to the maximum amount of occlusion and contact with accumulated urine, and the distal prepuce is subjected to the least. Our findings suggest that accentuated contact between urine and susceptible tissues can lead to MGLSc. This is the first study examining the spatial distribution of MGLSc in the prepuce and contributes to our understanding of disease aetiology and progression.

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Phenotypes of cognitive dysfunction in adults with atopic dermatitis

L. Jackson-Cowan[2], J. I. Silverberg[1]

[1]Dermatology, George Washington University Medical Faculty Associates, Washington, District of Columbia, United States, [2]AU/UGA Medical Partnership, Augusta University, Augusta, Georgia, United States

Importance: Atopic Dermatitis (AD) was recently shown to be associated with cognitive dysfunction in adults. Yet, little is known about the patterns of cognitive deficits experienced by patients suffering from AD. Objective: To investigate the phenotypes of cognitive deficits and their predictors in adults with AD. Methods: A prospective dermatology practice-based study was performed using questionnaires and evaluation by a dermatologist (n=195). Cognitive function was assessed using the Patient-Reported Outcomes Measurement Information System (PROMIS) Cognitive Function 8-item Short-Form. Results: Latent class analyses identified 4 major cognitive subtypes in adults with AD: normal cognition (71.88%), concentration deficit (14.64%), moderate cognitive impairment (CI; 7.71%), and severe CI (5.77%). Patients with severe CI at baseline were more likely to have persistent CI over time (60.06%) than those with moderate CI (25.39%) or concentration deficit (0.00%). Patients with a concentration deficit, moderate, and severe CI were more likely to have moderate-severe SCORing AD (SCORAD; 77.78%, 90.48% and 75.00% vs 59.05%; p=0.03), moderate depression (Patient Health Questionnaire [PHQ]-9; 35.71%, 50.00% and 83.33% vs 8.57% PHQ9; p<0.0001), and severely impacted quality of life (Dermatology Life Quality Index [DLQI]; 57.14%, 57.14% and 66.67% vs 27.86%; p= 0.008) compared to those with normal cognitive function. Patients with moderate and severe CI additionally reported more severe skin pain (38.10% and 83.33% vs 19.39%; p<0.0001). Conclusions: Three distinct phenotypes of cognitive dysfunction were identified in adults with AD. The occurrence of depression, a severely impacted quality of life and skin pain increased with increasing levels of cognitive dysfunction.

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The importance of inflammatory markers in generalized pustular psoriasis (GPP): An immunohistochemical analysis

R. E. Schopf
Dermatology, Johannes Gutenberg Universitat Mainz, Mainz, Rheinland-Pfalz, Germany

GPP is a rare potentially life-threatening disease characterized by the presence of sterile pustules on erythematous skin. Although clinically different from psoriasis vulgaris (PV) it shares some features of PV such as the spongiform pustule. The cause of GPP is unknown; triggers include infections, corticosteroid withdrawal and genetic factors such as a mutation of the IL-36 RN antagonizing IL36-G and -A. Antibody treatment with the IL-36R antagonist spesolimab has shown efficacy. The role of other inflammatory factors is not well understood. To further elucidate pathogenesis we performed an immunohistochemical study in 13 patients with PPP and in corresponding skin of 6 healthy control individuals. Skin biopsies were stained with antibodies against HLA-DR, IL-8 recruiting neutrophils, IL-17A, IL-17F, IL-23, IL-36 gamma, the chemokine CXCR3 (CD183) in IFN-induced inflammation, tyrosine kinase (Tyk) phospho-diesterase (PDE) 4B, and the transcription factor FOX-P3 in T-cells and JAK-3. The stained slides were photographed in 20x enlargement with the Leica DFC 295 microscopic system and digitally analyzed with the ImageJ Plugin-IHC Profiler scanning 82364 square micrometers. Stained cells ranged from 50 to 365 per visual field. The two-sided t-test served for statistical analysis. In GPP compared to healthy controls, we found the following significance of staining in decreasing order: FoxP3 ($p<0.00001$), IL36 gamma ($p<0.001$), IL-17F ($p<0.004$), IL-8 ($p<0.03$), CXCR3 ($p=0.16$), JAK-3 ($p=0.16$), IL17A ($p=0.20$), Tyk ($p=0.33$) HLA-DR (0.58), PDE 4B ($p=0.76$). These results clearly indicate IL36 gamma to be a dominant cytokine in GPP. Moreover, the transcription factor FoxP3, the cytokines IL-17 F, IL-23, and IL-8 also exhibited a significantly higher staining compared to healthy controls. Our results may provide a basis for successful new treatment in generalized pustular psoriasis.

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Hidradenitis suppurativa interferes with socialization in the majority of patients regardless of objective disease severity: A cross-sectional survey study

P. O. Perche[1], R. Singh[1], A. Senthilnathan[1], S. R. Feldman[1, 2, 3], R. O. Pichardo[1]
[1]Dermatology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States, [2]Pathology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States, [3]Social Sciences & Health Policy, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States

Background: Hidradenitis suppurativa (HS) can severely impact quality of life. However, its specific impact on participation in social activities is not well studied. Objective: To assess HS's interference with social activities in relation to disease severity. Methods: We recruited patients with a clinical diagnosis of HS (ICD-10 code L73.2) from clinic (n=30) and mail (n=123) to complete a survey. Respondents reported the extent to which HS interfered with social activities (none, a little, a lot). Disease severity was assessed using a validated self-assessment tool. Differences in disease severity were compared to degrees of interference with social activities using Chi-squared analysis. Results: A total of 67 completed responses were received and analyzed. Respondents were 22% Hurley Stage 1, 35% Hurley Stage 2, and 43% Hurley Stage 3 disease severity. Most reported HS interfered with their ability to go out (53%), engage in hobbies (57%), participate in sports or recreational activities (68%), go out socially or to a special event (65%), and go to parties (52%), with no significant differences by Hurley stage ($p=0.31$-0.68), though going out socially or to a special event ($p=0.12$) approached significance. Conclusion: Most patients in our cohort, regardless of disease severity, reported HS interfered with their social activities. HS is a physically and socially debilitating disease. Given social distancing policies during the COVID-19 pandemic, HS patients may be even more prone to isolation. Interventions to help HS patients with their social support structure may be helpful in improving their quality of life.

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Cutaneous dermatomyositis area and severity index activity score (CDASI-A) and associated patient-reported outcomes in a phase 2 clinical trial in dermatomyositis

J. Dan[1, 3], J. Concha[1, 3], G. Sprow[1, 3], R. Feng[2], M. Afarideh[1, 3], N. Kodali[1, 3], T. Vazquez[1, 3], D. Diaz[1, 3], B. White[4], V. Werth[1, 3]
[1]Derm, PSOM at UPENN, Phil, Pennsylvania, United States, [2]Biostats and Epi, PSOM at UPenn, Phil, Pennsylvania, United States, [3]Derm, Corporal Micheal J. Cresenz VAMC, Phil, Pennsylvania, United States, [4]Corbus Pharmaceuticals, Norwood, Massachusetts, United States

Retrospective reviews of clinical databases from two sites have identified strong associations between skin activity in dermatomyositis (DM), as measured by CDASI-A, and patient-reported quality-of-life (QoL). We hypothesized that associations between CDASI-A and QOL outcomes could also be demonstrated in the clinical trial setting. We evaluated correlations between CSADI-A and PROMIS Short Form domain scores, Skindex domain scores, Patient Global Assessment (PtGA) scores, and Physician Global Assessment (PGA) scores in a double-blind, randomized, placebo-controlled clinical trial. Data were from a 5-visit, 16-week Phase 2 trial of lenabasum, a cannabinoid receptor type 2 agonist, in 22 subjects with DM. CDASI-A scores and QOL outcomes collected at each study visit were correlated using linear mixed effect models to account for within-subject variability and repeated measures. As our goal was to correlate CDASI and QoL throughout the trial, analysis was performed without regard to treatment. Improvement in CDASI-A significantly correlated with PROMIS social role ($p\leq0.05$). No other PROMIS domain scores correlated with the CDASI. The CDASI-A also significantly correlated with all Skindex scores (symptoms, functioning, emotions, and itch), all PtGA scores (global disease, itch, pain, and global skin), and all PGA scores (global disease, skin activity, and global skin), $p<0.001$ each. All reported correlations were in the appropriate direction. Our findings support that CDASI-A reflects both skin disease and QOL and is an appropriate outcome in DM clinical trials. Most PROMIS domains did not correlate with skin activity suggesting that Skindex and PtGA scores may better relate to skin activity as measured by the CDASI.

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Incidence and severity of itch and pain in bullous pemphigoid

J. Dan[1, 2], M. Afarideh[1, 2], G. Sprow[1, 2], J. Concha[1, 2], N. Kodali[1, 2], T. Vazquez[1, 2], D. Diaz[1, 2], V. Werth[1, 2]
[1]Derm, PSOM at UPenn, Phil, Pennsylvania, United States, [2]Derm, Corporal Micheal J. Cresenz VAMC, Phil, Pennsylvania, United States

Itch is a common manifestation of Bullous Pemphigoid (BP) that may indicate blister onset or recurrence. Recent studies characterized the effect of itch on quality of life (QoL) in BP, though the literature regarding pain in BP is sparse. We compare itch and pain reported by patients diagnosed with BP. Data from the initial study visits of 38 subjects enrolled in our prospective blistering disease database was retrospectively reviewed. Bullous Pemphigoid Disease Area Index (BPDAI) activity scores, Physician Global Assessment (PGA) activity scores, and Patient Global Assessment (PtGA) itch and pain were completed at the study visit. PGA itch 3-7cm and pain 3.5-7.5cm were considered moderate. Questions regarding itch and pain were extracted from completed QoL forms (Skindex q1 and q10; ABQOL q1 and q2). Spearman's correlations and Mann-Whitney tests were performed. Median (IQR) BPDAI and PGA activity scores were 7 (20.7) and 0.95 (3.2), respectively. In our study, 82% and 53% of patients reported itch and pain. Median scores were as follows: PtVAS itch 2.9 (6.2), pain 0.05 (0.42); Skindex-29 itch 2 (1), pain 2 (1); ABQOL itch 2 (1), pain 1 (2). BPDAI activity scores correlated with PtGA itch and ABQOL itch ($p\leq0.05$), with PtGA pain, Skindex pain and itch, and ABQOL pain trending towards significance ($p\leq0.07$). PGA activity scores correlated with all PtGA scores, all Skindex scores, and ABQOL itch ($p\leq0.05$), with ABQOL pain nearing significance ($p=0.054$). Patients with blisters or erosions had higher average PtGA pain scores than those without (mean 2.8 vs 0.8). This relationship neared significance ($p=0.052$). Even with mild disease, moderate itch and mild pain are present in the majority of our population and are correlated with disease activity. However, subjects reported more severe itch, which demonstrated a stronger correlation with disease activity vs pain. This suggests that itch plays a larger role than pain in our mild population.

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Absence of human polyomaviruses in angiolymphoid hyperplasia with eosinophilia (ALHE)

E. Lee[2], T. Vandergriff[2, 3], G. Hosler[2, 1], R. C. Wang[2, 4]
[1]ProPath, Dallas, Texas, United States, [2]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [3]Pathology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [4]Harold C. Simmons Center, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

Angiolymphoid hyperplasia with eosinophilia (ALHE) is an uncommon skin disease that presents as papules or nodules in the head and neck region. The pathogenesis of the vascular proliferation that characterizes ALHE is unclear, and neoplastic or reactive etiologies have both been proposed. Several human polymaviruses (HPyV), including HPyV6, HPyV7, trichodysplasia spinulosa polyomavirus (TSPyV), and Merkel Cell polyomavirus (MCPyV) contribute to specific skin diseases, but can also be detected on healthy skin. HPyV6 was detected in the lymph node of a patient with Kimura Disease (KD), a lymphoid and vascular proliferative disease related to ALHE. HPyV6 was also reported to be present in 4/5 cases of ALHE, suggesting a possible link between HPyV6 infection and the development of Kimura Disease and/or ALHE. We identified 12 cases of formalin-fixed, paraffin-embedded (FFPE) ALHE. Endpoint PCR did not identify abundant HPyV6 in any cases. Expression of HPyV T antigen was not detected by immunohistochemistry. In our small case series, there was no evidence that HPyV infection contributed to the development of ALHE. Further studies may reveal whether HPyVs could contribute indirectly to ALHE.

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The association between sodium intake and atopic dermatitis in a US population-based cohort

M. Ye, B. Chiang, K. Abuabara
University of California San Francisco, San Francisco, California, United States

Diet may be an important environmental factor driving the onset and persistence of atopic dermatitis. Recent evidence showing that most of the body's exchangeable sodium is stored in the skin and that high sodium environments trigger and perpetuate local immune dysregulation support the hypothesis that excessive dietary sodium intake could be associated with atopic dermatitis. We aimed to investigate this hypothesis using data from 13,183 children and adults in the 1999-2000, 2001-2002, and 2003-2004 cycles of the National Health and Nutrition Examination Survey, which included validated dietary intake questionnaires and questions about participants' history of dermatitis, eczema, or rash. Normalized values for usual dietary sodium intake were estimated using the National Cancer Institute Method. The average dietary sodium intake was 3.30 grams (standard deviation 1.58), 809 (6%) participants reported current dermatitis at the time of the survey, and 1,518 (12%) participants reported dermatitis in the past year. After adjusting for potential confounders including age, sex, ethnicity, and poverty income ratio in logistic regression models, we found that a 1 gram increase in dietary sodium intake was associated with an increased risk of current dermatitis (AOR 1.22, 95% CI 1.02,1.45), and a marginally significant increase in dermatitis in the past year (AOR 1.15, 95% CI 0.98, 1.34). These data support for salt restriction as a low-cost, safe intervention for atopic dermatitis that could be offered in diverse settings, although additional research is needed using more specific measures of atopic dermatitis in a longitudinal population cohort.

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Peristomal ulcer as presenting feature of inflammatory bowel disease (IBD)

T. F. Abrantes[1], Y. Farid[1], A. Halim[2], S. Seo[1]
[1]Brown University Warren Alpert Medical School, Providence, Rhode Island, United States, [2]University of Maryland Baltimore, Baltimore, Maryland, United States

77-year-old female with a history of diverticulitis and bowel perforation with subsequent hemicolectomy presented with three weeks of abdominal pain, bloody output from ostomy and rectum, and an enlarging painful peristomal wound. She was initially seen by outpatient dermatology for a small ulcer; wound culture grew E. coli, and she was started on ciprofloxacin. Over the next week, the ulcer rapidly grew and inpatient examination revealed an 11.5 x 11 x 0.5 cm deep peristomal ulcer with violaceous borders. CT scan demonstrated findings concerning for colitis, and colonoscopy showed erosive inflammation, friability, and deep ulcerations in a continuous and circumferential pattern from the ascending colon to the cecum. Colonic biopsies confirmed a diagnosis of severe IBD. Biopsy of the skin ulcer showed a neutrophilic infiltrate and abscess formation consistent with pyoderma gangrenosum (PG). Gram, AFB, and fungal stains were negative for microorganisms. Tissue culture grew few gram-negative rods but was otherwise negative (suspected contaminant). The patient was started on IV hydrocortisone with significant improvement in ulcer size and depth. The discharge treatment plan included a prednisone taper and infliximab; due to the risk of pathergy, wound debridement was avoided. PG is a chronic cutaneous ulcerative disease associated with underlying IBD in about 30% of cases. Peristomal PG is an unusual variant almost exclusively associated with IBD. Lesions classically begin as a small papule or pustule that progresses to an enlarging ulcer with a necrotic center. Ulcers typically exhibit a purulent base with a ragged, undermined, violaceous border. We present this case due to the unusual presentation of peristomal PG prior to diagnosis of IBD. She did have a history of colectomy secondary to perforation but reported no previous diagnosis or treatment of IBD. Her subsequent cutaneous manifestation brought to light her underlying disease. Our case emphasizes the importance of systemic workup in patients with PG.

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Health-related quality of life in a Canadian atopic dermatitis cohort

D. M. Lebo[1, 2], C. Bouchard[1], N. Merati[1, 3], V. Hladky[1, 2], R. Habib[1, 2], R. Jeremian[1, 3], C. Jack[1, 3]
[1]Centre of Excellence for Atopic Dermatitis, McGill University Health Centre, Montreal, Quebec, Canada, [2]Faculté de médecine, Universite de Montreal, Montreal, Quebec, Canada, [3]McGill University Faculty of Medicine and Health Sciences, Montreal, Quebec, Canada

Adult patients ages 16 to 78 with a confirmed diagnosis of AD (N=151) as per Hanifin and Rajka criteria were consented at the McGill University Health Centre, Centre of Excellence for Atopic Dermatitis to the Dermatitis BioBank longitudinal cohort. We aimed here to identify host and treatment factors that contribute to health-related quality of life (HRQL) and disease. At baseline, we collected data on patient demographics, self-management, treatment, and healthcare utilization. White (55.6%) women (57.0%), aged 25 to 34 (27.8%) predominated this cohort, with 15.2% reporting adult-onset AD and 31.9% diagnosed with AD before the age of 2. 29.8% missed work or school because of AD; for 19.0% at least three times in the last year. Comorbid asthma (40.4%), allergic rhinitis (57.0%), and food intolerance (37.7%) were common. Despite comprising a wide range of ethnicities, 79.5% were born in Canada; 65.6% had completed a university or graduate degree. Pearson's correlations were calculated to explore factors associated with HRQL as assessed by: history of remission with clear skin in the past year (31.1%), use of systemic treatments at baseline (39.1%), and self-reported depression or anxiety secondary to AD (36.4%). HRQL outcomes did not correlate with ethnic groupings, nor were they associated with foreign birth or sex. However, patients with postsecondary education reported less depression or anxiety due to their AD (ρ = -0.188, p=0.023) and less missed work or school in the past year (ρ = -0.177, p=0.036). This cohort of AD patients is unique in its large size and diversity, providing an opportunity to explore the determinants of disease outcome in tertiary care AD patients. Further investigation is required into the impact of AD on HRQL and opportunities to identify patients who are at risk of poor HRQL as well as interventions to improve outcomes in diverse populations.

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Risk factors for post-operative surgical site infections: A case-control study

R. Christensen, V. Harikumar, M. A. Dirr, N. Anvery, J. Brieva, S. Yoo, M. Alam
Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Surgical site infection (SSI) is a common post-operative complication associated with patient morbidity, lengthened hospital stays, and high costs. While prior research on SSI risk factors is often specific to particular procedure types, the current study assessed risk factors for increased SSI risk regardless of surgery type or indication for surgery. The 2010-2014 Nationwide Readmission Database, a registry containing a representative sample of US readmissions, was searched. Patients with an ICD-9 primary diagnosis of post-operative infection within one month of a hospital admission for a surgical operation were included. For each case, four controls were matched based on primary hospital procedure. Patient and hospital characteristics were collected. 45,445 patients with post-operative SSI were identified and compared to 173,130 controls. Multivariate analysis found higher odds of SSI in patients who were obese (OR: 1.39, 95% CI: 1.28-1.51); were tobacco users (OR: 1.08, 95% CI: 1.02-1.15); were diagnosed with diabetes (OR: 1.16, 95% CI: 1.10-1.22); had a hospital length of stay 4-6 days (OR: 1.35, 95% CI: 1.29-1.42); or had an Elixhauser Comorbidity Index of two or greater (OR: 1.14, 95% CI: 1.09-1.20). Odds were decreased in patients who were 60-79 years (OR: 0.78, 95% CI: 0.73-0.84); 80 years or older (OR: 0.66, 95% CI: 0.59-0.73); female (OR: 0.95, 95% CI: 0.91-0.99), underweight (OR: 0.14, 95% CI: 0.03-0.59); served in a non-metropolitan hospital (OR: 0.83, 95% CI: 0.75-0.91); or whose procedure was self-paid (OR: 0.82, 95% CI: 0.74-0.91) or covered by Medicare (OR: 0.86, 95% CI: 0.80-0.91). In assessing a large, diverse cohort, the present study provides generalizable information related to post-operative SSI risk. Our study supports previous research in that risk of post-operative SSI may be increased by certain patient and hospital characteristics. By better understanding these risk factors, physicians can more effectively identify, counsel, and monitor vulnerable patients.

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Short term exposure to wild-fire associated air pollution does not significantly increase clinic visits for psoriasis

M. Green[1], R. P. Fadadu[1], D. Seth[1], N. Jewell[2, 3], M. Wei[1, 4]
[1]University of California San Francisco, San Francisco, California, United States, [2]University of California Berkeley, Berkeley, California, United States, [3]London School of Hygiene & Tropical Medicine, London, London, United Kingdom, [4]San Francisco VA Health Care System, San Francisco, California, United States

Previous work by our group has shown that short term exposure to air pollution due to wild-fires is associated with significantly increased healthcare utilization, as measured by clinic visits for atopic dermatitis or itch to dermatology clinics at a tertiary academic medical center in California. In this study, we assessed whether these results generalize to other types of skin disorders, such as psoriasis. We collected data on particulate matter (PM2.5) concentration and smoke plume density (0-3) in San Francisco and the number of adult outpatient dermatology visits for psoriasis at an academic medical center in the same city from October 2015-February 2019, the period of time before, during and after the Camp Fire in northern California. Wildfire-associated air pollution was assessed using three metrics: fire status, concentration of particulate matter (PM2.5), and satellite-based smoke plume density scores. Outcome data were analyzed on a weekly basis using generalized Poisson regression. Statistical models included 4 one-week exposure lags and were adjusted for temperature, humidity, patient age, year, and patient volume. A total of 986 visits for psoriasis were analyzed. In adjusted analyses, the rate of psoriasis clinic visits for all adults during a week with a wildfire was 1.1 (95% CI: 0.7-2.0) times the rate for non-fire weeks, for a 0-week lag. The corresponding result for adults age 65 yr or older was 1.3 (0.6-2.8). The rate of pediatric psoriasis clinic visits was 1.0 (95% CI: 0.2-3.8) times the rate for non-fire weeks, for a 0-week lag. We found that during a California wildfire, rates of clinic visits for psoriasis did not differ significantly from the rates during non-fire weeks, indicating that the effect of air pollution on skin health differs depending on the underlying skin disorder.

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Environmental injustice and risk for wildfire-associated skin flares

D. Seth[1], R. P. Fadadu[1], N. Jewell[2], M. Wei[1]
[1]Dermatology, University of California San Francisco, San Francisco, California, United States, [2]Medical Statistics, London School of Hygiene & Tropical Medicine, London, London, United Kingdom

Environmental inequities are ubiquitous; emerging data suggest they affect the distribution of skin disease. The CalEnviroScreen is a cumulative environmental inequity score based on environmental, health, and sociodemographic data, allowing for comparison across census tracts in California. Previously, we showed that short-term exposure to wildfire smoke can exacerbate atopic dermatitis (AD). Here, we hypothesize sociodemographic and environmental score differences between the patients seen before and during/after a wildfire. We collected sociodemographic data (gender, patient-identified race, and insurance type) for patients seen via outpatient dermatology visits for AD and itch symptoms at an academic medical center from August 2018 – December 2018, 3 months before, during, and 1 month after the Camp Fire in northern California. Differences between "non-fire visits" and "during fire visits" were assessed using t-tests and Pearson chi-square tests. The difference in mean CalEnviroScreen score between "non-fire visits" and "during fire visits" was assessed using Welch t-test of unequal variances. We analyzed 2,123 atopic dermatitis visits in the 3 months before the Camp Fire (control) and 1,560 visits in the period during and 1 month after the Camp Fire (exposed). There were no statistically significant differences in sex (Pearson chi-square, P=0.61) or age (t-test, P= 0.72) between the two groups. However, between the two groups, there was a statistically significant difference in race (Pearson chi-square, P=0.002) and insurance type (Pearson chi-square, P=0.021). The mean CalEnviroScreen score of patients during the fire period was higher than that of patients before the fire period (Welch t-test, $p < 0.01$). Our findings suggest that AD flares during a wildfire may vary based on baseline environmental injustice score, race, and insurance type. This suggests that there may be underlying inequities that predispose certain patients to AD flares during wildfires, informing avenues for future environmental policy.

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Type of immunosuppression matters: Efficacy of immunotherapy in immunosuppressed Merkel cell carcinoma patients

E. Gong, L. Zawacki, A. Remington, M. Bierma, K. Lachance, T. Akaike, P. Nghiem
University of Washington, Seattle, Washington, United States

Merkel cell carcinoma (MCC) has a high propensity for recurrence and distant metastasis. Persons with chronic immunosuppression have a higher risk of developing MCC and a more aggressive disease course. Immune-checkpoint inhibitors are associated with improved disease-specific survival. However, the effectiveness and side-effect profile of these agents in immunosuppressed MCC patients is not well categorized partly due to their ineligibility in prior clinical trials. This study addresses challenges such as: the risk-benefit profile of immunotherapy, the differences between forms of immunosuppression and the comparison of immunotherapy efficacy in immunocompetent versus immunosuppressed MCC patients. Data were abstracted from a prospective registry of 1,529 MCC patients from which 36 were identified to have been treated with immunotherapy and had chronic immunosuppression. Of these 36 patients, 13 patients (36%) had a complete response (CR), 3 patients (8%) had a partial response (PR), and 20 patients (56%) had progressive disease (PD). Progression of disease and survival status varied greatly among different types of immunosuppression. Five types of chronic immunosuppression were represented in these 36 patients and were evaluated for an objective response (CR or PR): chronic lymphocytic leukemia (CLL, 3/13, 23%), autoimmune disorders (AD, 3/9, 33%), solid organ transplant (SOT, 2/4, 50%), HIV/AIDs (2/3, 67%), and other hematologic malignancies (OHM, 6/7, 86%). In comparison, in the PD-(L)1 first-line study of immunocompetent patients, 28 of 50 (56%) had objective responses. Toxicities were also high in patients with immunosuppression as 9/36 (25%) patients stopped treatment due to toxicities. The efficacy of immunotherapy in patients with chronic immunosuppression appears to be dependent on the type of immunosuppression. While there is reason for optimism for patients with certain types of immunosuppression, MCC treatment for patients with CLL remains a major concern in which only one out of 13 patients experienced a CR.

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Functional and psychological outcomes of combined pulsed dye laser and fractional carbon dioxide laser treatment for scars in pediatric patients

E. Kleinman[1,2,3], D. Eichenfield[1,2], A. Krakowski[4,1], W. Tom[1,2], L. F. Eichenfield[1,2]
[1]*Pediatric and Adolescent Dermatology, Rady Children's Hospital San Diego, San Diego, California, United States,* [2]*University of California San Diego, La Jolla, California, United States,* [3]*Tel Aviv University Sackler Faculty of Medicine, Tel Aviv, Israel,* [4]*Dermatology, St Luke's University Health Network, Bethlehem, Pennsylvania, United States*

Scars can manifest with cosmetic disfigurement, functional impairment, and discomfort which can be particularly impactful in children and adolescents. Laser therapy has been recently developed as a useful modality to improve both the appearance of scars and the functional status of patients. Our study aims to determine an association between ratings of blind evaluators and patient reported outcomes in pediatric scar patients pre- and post-treatment with combined pulsed dye laser (PDL) and fractional carbon dioxide (CO2) laser therapy. A retrospective medical record review was performed of patients receiving laser intervention for scar management between 1/2001 and 9/2021. Pre-treatment and post-treatment clinical photographs were extracted from the medical record; cases with fewer than 2 clinical images of the scar during treatment were excluded. Demographic data, scar type, etiology, clinical images, treatment details, and adverse events were collected from the patients' medical records. A total of 306 pediatric patients with scars underwent laser therapy between 1/2001 and 9/2021; 136 patients had both a baseline and post-treatment photo of 165 scars. Average number of treatment per lesion was 4.5 (SD ± 3.04, range 1-16). Locations of scars were predominantly on the head/neck (33%), followed by the upper extremity (24%), trunk (19%), and lower extremity (19%). The type of scar treated was predominantly hypertrophic (90%). To date, this is the largest cohort of pediatric patients that have undergone laser therapy for scars of various etiologies. We hypothesize that objective appearance is not correlated with functional improvement and patient satisfaction. Thus, we aim to develop a patient-centered scar scale for pediatric patients that can be utilized to evaluate treatment progress.

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Development and validation of a caregiver-reported numeric rating scale for measuring sleep quality in children aged 6 months to <6 years with atopic dermatitis

A. Paller[1], E. Siegfried[2], S. E. Marron[3], M. Clark[4], K. Kosa[5], D. Whalley[6], J. Chao[7], A. Bansal[7], Y. Sun[7], C. Chuang[8], Z. Wang[7]
[1]*NU Feinberg School of Medicine, Chicago, Illinois, United States,* [2]*Cardinal Glennon Hospital, Saint Louis, Missouri, United States,* [3]*University Hospital Miguel Servet, Zaragoza, Spain,* [4]*RTI Health Solutions, Ann Arbor, Michigan, United States,* [5]*RTI Health Solutions, Research Triangle, North Carolina, United States,* [6]*RTI Health Solutions, Manchester, United Kingdom,* [7]*Regeneron, Tarrytown, New York, United States,* [8]*Sanofi, Cambridge, Massachusetts, United States*

Children with atopic dermatitis (AD) commonly have sleep difficulties and disturbance that could negatively impact their physical development. An 11-point caregiver-reported sleep quality numeric rating scale (NRS; from 0 [worst possible sleep] to 10 [best possible sleep] over past 24 hrs) to assess sleep quality in children aged 6 mo. to <6 yrs with moderate to severe AD was developed and evaluated. Caregivers (n=15) participating in qualitative interviews found the sleep quality NRS to be important and relevant to the impact of AD on their child's sleep, and the response scale clear and easy to use. Psychometric properties of the instrument were evaluated using data from a Phase 3 study of dupilumab in children aged 6 mo. to <6 yrs with moderate to severe AD (NCT03346434). The test-retest reliability intraclass correlation coefficient (95% CI) was 0.80 (0.69, 0.87), above the recommended 0.70 threshold. The sleep quality NRS was shown to discriminate well between patients grouped into bands according to the Caregiver Global Impression of Disease (CGID), Infants' Dermatitis Quality of Life Index (IDQOL), Children's Dermatology Life Quality Index (CDLQI) and Patient-Oriented Eczema Measure (POEM) ($P < 0.0001$). Evidence of responsiveness was demonstrated by the moderate correlations of change ($|r| \geq 0.30$) observed with many of the supportive measures, and significant difference across subgroups categorized as improved, unchanged, or worsened on CGID ($P = 0.0037$). These results indicate that the caregiver-reported sleep quality NRS is a valid, reliable, and responsive instrument to assess sleep quality in children aged 6 mo. to <6 yrs with moderate to severe AD.

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Development and validation of a caregiver-reported numeric rating scale for measuring skin pain in children aged 6 months to <6 years with atopic dermatitis

A. Paller[1], E. Siegfried[2], S. E. Marron[3], M. Clark[4], N. Harris[5], S. Quin[5], J. Chao[6], *A. Bansal[6], Y. Sun[6], C. Chuang[7], Z. Wang[6]*
[1]*NU Feinberg School of Medicine, Chicago, Illinois, United States,* [2]*Cardinal Glennon Hospital, Saint Louis, Missouri, United States,* [3]*University Hospital Miguel Servet, Zaragoza, Spain,* [4]*RTI Health Solutions, Ann Arbor, Michigan, United States,* [5]*RTI Health Solutions, Research Triangle, North Carolina, United States,* [6]*Regeneron, Tarrytown, New York, United States,* [7]*Sanofi, Cambridge, Massachusetts, United States*

Skin pain is a common symptom in patients with atopic dermatitis (AD). A novel 11-point caregiver-reported instrument to assess skin pain by numeric rating scale (NRS; from 0 [no pain] to 10 [worst pain possible]), in young patients with moderate-to-severe AD was developed and evaluated. Caregivers (n=24) of children aged 6 months to <6 years with AD provided feedback on the skin pain NRS during combined concept elicitation/cognitive debriefing interviews. The majority of caregivers (23/24; 96%) reported that skin pain or discomfort were AD-related symptoms for their child. All caregivers understood and were able to provide a response on the skin pain NRS. Psychometric properties were evaluated using data from a Phase 3 study of dupilumab in children (aged 6 months to <6 years) with moderate-to-severe AD (NCT03346434). The test-retest reliability intraclass correlation coefficient (95% CI) was 0.68 (0.50, 0.80). The ability to detect change was shown by significant correlation with changes in anchor clinical outcome assessments ($p<0.05$), including Caregiver Global Impression of Disease (CGID; correlation coefficient [95% CI], 0.64 [0.52, 0.73]), Children's Dermatology Life Quality Index (CDLQI; 0.60 [0.42, 0.73]), and Patient-Oriented Eczema Measure (POEM; 0.63 [0.51, 0.72]). Anchor-based methods suggest an at least 2 to 5-point change in skin pain NRS as clinically meaningful. The caregiver completed skin pain NRS is a valid, reliable, and responsive instrument to assess skin pain in children aged 6 months to <6 years with moderate-to-severe AD.

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Contact dermatitis adverse events: A review of contact dermatitis-associated reports from the FDA adverse event reporting system (FAERS)

J. S. Taylor
Dermatology, Cleveland Clinic, Cleveland, Ohio, United States

The FDA Adverse Events Reporting System (FAERS) is a computerized database of adverse events submitted by product manufacturers, consumers, and health care professionals. The repository is part of the agency's post-marketing safety surveillance program with relatively few published dermatologic-related reports. We analyzed the FAERS public data base using the search terms "dermatitis, contact" and sorted the data as a whole from 1969 and more specifically for the past two years. Daily Med (National Library of Medicine) was consulted to verify routes of exposure. There are 12,841 total cases of contact dermatitis since 1968, of which 4355 were submitted by health professionals. We sorted the non-serious cases further for 2020-2021 reviewing a total of 203 reports. Of these only 43 were reports of reactions to topical medications including anti acne, ophthalmic, antifungal, corticosteroid, and transdermal patches. The largest number of reports for single medications were the injectable medications treprostinil (27) and dupilumab (18) and the oral drug lenalidomide (18). Of the remaining 97 cases most were from other injectable drugs with some oral medications included. Other diagnoses were included in some of these reports, and one was occupationally related. Factors affecting FAERS reporting include media attention, litigation, nature of the event, and reporting regulations. There may be duplicative reports, the data is not verified, and the existence of a report does not necessarily establish causation. In the case of the topical medications some of the reports may be irritant rather than allergic contact dermatitis. Of the injectable medications reported here, many are administered subcutaneously with local reactions reported as contact dermatitis; some may have been systemic contact dermatitis, with other non-contact cutaneous reactions evident. FAERS includes all US marketed products and is ideal for identifying reporting trends and emerging safety concerns.

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West Nile Virus presenting as a bullous dermatosis with evidence for keratinocyte involvement in viral replication

E. Lee[1], L. Matthews[1], K. Shah[1], F. Lee[4], J. Schoggins[2], T. Vandergriff[1, 3], K. Yancey[1], R. C. Wang[1]

[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Microbiology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [3]Pathology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [4]Internal Medicine/Infectious Diseases, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

West Nile Virus (WNV) is a leading cause of viral encephalitis in the United States. Infected mosquitos deliver the virus to the skin before the virus moves to draining lymph nodes to establish a systemic infection. In vitro studies in human keratinocytes and *in vivo* studies in mice suggest a role for keratinocytes in viral replication, and rashes have been reported in half of WNV encephalitis patients, but the early stages of WNV infection in patients remain incompletely understood. We report a 58-year-old male patient who presented with altered mental status, rigors, fever, and rash. A morbilliform eruption on the back and abdomen evolved into a vesicular eruption. Pathology revealed an intraepidermal vesicle with acantholysis consistent with Grover disease. WNV encephalitis was diagnosed by the presence of WNV IgM antibodies in the serum and cerebrospinal fluid. Immunohistochemistry (IHC) excluded herpes simplex virus and varicella zoster virus infection, and direct immunofluorescence (IF) studies excluded autoimmune blistering diseases. Followup studies revealed the abundant presence of WNV non-structural glycoprotein 1 (NS1) by IF and IHC. The patient recovered fully with supportive care. This case reveals WNV to be present in human skin lesions and suggests that keratinocytes support WNV viral replication. WNV should be included as a rare cause of blistering skin eruptions.

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Clinical characteristics and treatment outcomes of sweet syndrome

V. E. Orfaly[1], H. Shakshouk[1], E. Latour[2], A. Ortega-Loayza[1]

[1]Dermatology, Oregon Health & Science University School of Medicine, Portland, Oregon, United States, [2]Knight Cancer Institute, Oregon Health & Science University School of Medicine, Portland, Oregon, United States

Background: Sweet syndrome (SS) is a rare inflammatory skin condition. Current available data is limited to case reports or small case series. Objective: To describe the clinical characteristics and treatment outcomes of published cases of SS. Methods: Search was conducted in OVID Medline in 2021 for all published case reports and case series. A total of 509 articles were included. Results: The 537 cases comprised of 55.4% (296) females & 44.6%(238) males, with a mean age of 49 years. Of all, 43.6 %(234) cases were classic/idiopathic SS; 28.9 %(155) cases had malignancy associated SS; and 27.6 %(148) cases of drug-induced SS. There was a significant difference in the proportion of females ($p < 0.001$) among types of SS. Hematological malignancy was the most common (74.2%,115), whereas solid organ tumors were found in 23.2 % (36) of cases. Acute myeloid leukemia was the most common malignancy 29.6% (34). Granulocyte-colony-stimulating factor was the most common culprit drug (18.9%, 28). Upper extremities were most commonly involved (75.9%, 406), followed by the head & neck (66.6%, 356) and lower extremities (56.8%, 304). Most cases (82.6%, 444) reported fever & 20.3% (109) reported arthralgia. The most common extracutaneous manifestations were ophthalmological in the form of conjunctivitis (7.1%) and episcleritis (2.1%). Corticosteroid therapy was reported in 81.6% (438). Only 64% (341) achieved marked improvement or remission within 1 month, whereas 10 % (55) had recalcitrant disease. There was a significant difference in remission responses among SS types ($P < 0.001$). In particular, malignancy-associated SS had lower response rates as 73.4% had remission compared to 94.1% in classic and 95.4% in drug-induced SS ($P < 0.017, 0.05$). Conclusion: This retrospective review of all published cases highlights female predominance in the classic/idiopathic subtype. Upper extremities were the most common location involved. Treatment outcome significantly varies among types of SS.

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Development and validation of the dysesthesia assessment questionnaire

K. Erickson[1], R. Tripathi[2], R. Klatzky[4], J. Bordeaux[3], B. T. Carroll[3]

[1]Case Western Reserve University, Cleveland, Ohio, United States, [2]Johns Hopkins University, Baltimore, Maryland, United States, [3]Dermatology, University Hospitals, Cleveland, Ohio, United States, [4]Psychology, Carnegie Mellon University, Pittsburgh, Pennsylvania, United States

Sensory changes such as dysesthesia and numbness are frequent postoperative symptoms of cutaneous surgery due to disruption of the underlying network of nerves. These sensory disturbances are an unpredictable phenomenon with widely variable estimates of incidence (2.3% to 43.4%) and longevity (1 to >12 months) in the literature. Questionnaires are vital for assessing dysesthesia, as neurocutaneous testing does not align with self-reported sensory deficits. This field of research is limited as there are few tools to evaluate cutaneous sensation change. Our objective was to design and validate the Dysesthesia Assessment Questionnaire (DAQ) to assess postoperative dysesthesia, numbness, and the impact on quality of life. Questions were written based on principles outlined in current psychophysical scale development research. In this pilot study, 35 patients undergoing Mohs surgery for a facial neoplasm completed the DAQ on the date of their surgery and two weeks into the post-operative period to evaluate interrater reliability. Thirty-four patients completed the questionnaire at two time points (97.1%, n=1 lost to follow-up). Overall performance of the questionnaire demonstrated adequate interrater agreement, with 8/9 question items identified as statistically significant with acceptable weighted kappa values (from 0.12 to 0.63; $p > 0.05$) and fair to substantial interrater reliability. All items illustrated consistent trends in response frequency across time. Consistent outcomes between raters establish the Dysesthesia Assessment Questionnaire (DAQ) as a reliable tool to assess post-operative dysesthesia. This study validates the DAQ as an instrument for clinical screening of cutaneous sensory abnormalities after cutaneous surgery.

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Vitamin C and cutaneous squamous cell carcinoma risk: Results from the genetic epidemiology research on adult health and aging cohort study 2007–2015 and mendelian randomization analyses

Y. Kim[1, 2], J. Yin[3], S. L. Breton[1], E. Jorgenson[4], H. Huang[5, 6], H. Choquet[3], M. M. Asgari[1, 2]

[1]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, Massachusetts, United States, [3]Division of Research, Kaiser Permanente Northern California, Oakland, California, United States, [4]Regeneron Pharmaceuticals Inc, Tarrytown, New York, United States, [5]Department of Medicine, Harvard Medical School, Boston, Massachusetts, United States, [6]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts, United States

Vitamin C has been shown in both in vitro and animal studies to prevent oxidative damage to keratinocytes, suggesting a putative role in the chemoprevention of keratinocyte carcinomas, including cutaneous squamous cell carcinoma (cSCC). However, epidemiologic studies examining the association between vitamin C and subsequent cSCC risk have shown conflicting results. We investigated the association between self-reported vitamin C intake and cSCC risk in 67,773 non-Hispanic whites from the Kaiser Permanente Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort using multivariable logistic regression models adjusting for age, sex, body mass index, and smoking status. To overcome the limitations of unmeasured confounding in an observational study design, we also examined the causal effect of serum vitamin C on cSCC risk using two-sample Mendelian randomization (MR) approach. Genetic instruments for serum vitamin C were obtained from a published genome-wide association study (GWAS) meta-analysis conducted in 52,018 Europeans. For cSCC risk (outcome), we used summary-level data from a published GWAS of cSCC performed on the GERA cohort. No association between vitamin C intake and cSCC risk was observed in the multivariable logistic regression analyses. Further, MR analyses did not support a causal association between genetic predisposition to a higher serum vitamin C level and cSCC risk (inverse-variance weighted model: odds ratio [95% confidence interval] = 0.90 [0.73–1.11]). Our findings do not support an association between vitamin C and subsequent cSCC risk.

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Steven johnsons syndrome/toxic epidermal necrolysis management in the burn intensive care unit

J. Rahesh, K. Holder, J. Griswold
Texas Tech University System, Lubbock, Texas, United States

Background: Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis comprise a spectrum of severe hypersensitivity skin reactions. Stevens-Johnson Syndrome is the least severe on the spectrum of mucosal erosions, with Toxic Epidermal Necrolysis being the most severe. Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis is a disease of keratinocytes and therefore any squamous cell epithelium is at risk. This includes the cornea, conjunctiva, oral mucosa, esophagus, urethra, and anal canal. This skin reaction is typically drug-induced and has a very poor prognosis. Methods: Study personnel identified potential subject records for the study using electronic medical records at University Medical Hospital. We obtained a spreadsheet of identifiable patient information from the medical records office by searching ICD-10 codes pertaining to causes necessitating treatment of SJS including L53.1 (Stevens-Johnson syndrome-toxic epidermal necrolysis overlap syndrome), L00-99 (Diseases of the skin and subcutaneous tissue), L49-54 (urticaria and erythema), L51 (erythema multiforme). Results: We present an analysis of mortality of Stevens-Johnson Syndrome patients who managed solely in the burn intensive care unit at our facility. The mainstay of treatment included supportive care with an emphasis on fluid and electrolyte replacement. Transfer of patients to the burn unit is not the current standard of care, however could decrease the mortality and morbidity of patients. As seen in our centers burn intensive care unit patients only had a mortality rate of 17% over 5 years. Management of Stevens-Johnson Syndrome in the burn intensive care unit with a comprehensive interdisciplinary wound care team rather than solely dermatological intervention may improve outcomes. Conclusion: Transfer of patients to the burn unit is not the current standard of care, however, could decrease the mortality and morbidity of patients. Management of Stevens-Johnson Syndrome in the Burn Intensive Care Unit with a comprehensive interdisciplinary wound care team decreases mortality.

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Understanding health disparities among patients with psoriasis: Results from National Psoriasis Foundation annual surveys 2019 – 2021

G. Gondo[1], L. Howard[1], R. Friesel[1], A. B. Gottlieb[2], J. Merola[3]
[1]National Psoriasis Foundation, Portland, Oregon, United States, [2]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [3]Harvard Medical School, Boston, Massachusetts, United States

The National Psoriasis Foundation conducts a cross-sectional study of the psoriatic patient community using an online survey conducted annually. Using survey data from 2019 – 2021, this study sought to understanding of health disparities among patients with psoriasis. Survey participants provided demographic information and provider diagnosis of psoriasis (PsO), psoriatic arthritis (PsA) or both. Severity was assessed using the Patient Reported Extent of Psoriasis Involvement (PREPI) for patients with PsO and established cut points for the Psoriatic Arthritis Impact of Disease (PsAID-9) for those with PsA. The Patient Health Questionnaire (PhQ-2) screened participants for depression. Quality of life and social participation was assessed using the Dermatology Life Quality Index (DLQI) and the PROMIS 'Ability to participate in social roles and activities SF-4a'. Chi-square tests were conducted to examine if outcomes differed based on race or ethnicity. Among the 4,019 patients with psoriatic disease who completed the surveys, 1,823 (45.4%) had PsO only, 216 (5.4%) had PsA only and 1,980 (49.3%) had PsO and PsA, 3,430 (87.4%) were White or Caucasian, 114 (2.9%) were Black or African American, 165 (4.2%) were Asian or Asian American, and 214 (5.5%) were some other race, and 327 (8.3%) identified as being of Latinx ethnicity. Mild PsO (BSA < 3%) was more common in Whites (54.7%; p<.001) and non-Latinx (53.8%; p<.01) patients. Asians reported higher rates of acceptable PsA symptoms (50.0%, p<.001). Rates of depression were higher among Blacks (p<.001) and Latinx (p<.01). Whites (p<.001) and non-Latinx (p<.001) reported higher rates of no or little effect on quality of life due to skin disease (59.8%; p<.001 and 58.9%; p<.001) and normal ability to participate in social roles (56.7%; p<.001 and 56.8%, p<.01). Results from the NPF's annual surveys suggest that considerable health disparities exist among patients with psoriatic disease.

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Prevalence of autosomal recessive genodermatoses: Determination based on pathogenic sequence variants in publicly available exomic and genomic databases

C. Huang, A. Saeidian, L. Youssefian, H. Vahidnezhad, J. Uitto
Thomas Jefferson University, Philadelphia, Pennsylvania, United States

Many genes harboring mutations in genodermatoses have been identified, and clinical trials have begun for treatment of some of these rare conditions. However, accurate and generalizable prevalence information for these diseases is not readily available due to their low incidence and the limited amount of current epidemiological data. We have developed a robust disease prevalence estimator utilizing publicly available exomic and genomic sequencing data from the general population; all variants for each gene were obtained from the Genome Aggregation Database (gnomAD), consisting of a total of 76,156 genomes from unrelated individuals. The variants were assessed for pathogenicity, and allele frequencies were used to calculate an estimation for the disease prevalence. This method was applied to four autosomal recessive genodermatoses known to be associated with distinct gene defects: recessive dystrophic epidermolysis bullosa (RDEB; COL7A1), epidermodysplasia verruciformis (EV; TMC6), keratitis-ichthyosis-deafness syndrome (KIDS; AP1B1), and pseudoxanthoma elasticum (PXE; ABCC6). The prevalence for these genodermatoses (based on a defined number of variants) was estimated to be 18.52 per million for RDEB (100 variants), 82.47 per million for PXE (69 variants), 0.07 per million for EV (33 variants), and 0.13 per million for KIDS (7 variants). The calculated prevalence of each of these diseases, determined by this method, was largely comparable with published estimates from prior epidemiological studies. Thus, this method will allow prevalence estimates on diseases for which no epidemiological data exist. Furthermore, the growing size of aggregated population variant databases on human genomes will allow for more refined and highly reproducible prevalence estimates for autosomal recessive genodermatoses, which is critical for the design and prioritization of clinical trials.

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Tissue-specific homing in cutaneous immune-related adverse events

B. Leung[1], S. Zhang[2], N. Nguyen[1], G. Wan[1], R. Jairath[1], N. Alexander[1], J. Phillipps[1], L. Zubiri[1], S. Demehri[1, 2], K. Yu[2], A. Gusev[3, 2], S. G. Kwatra[4], N. LeBoeuf[3, 2, 5], K. Reynolds[1, 2], Y. Semenov[1, 2]
[1]Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States, [3]Dana Farber Cancer Institute, Boston, Massachusetts, United States, [4]Johns Hopkins University, Baltimore, Maryland, United States, [5]Brigham and Women's Hospital, Boston, Massachusetts, United States

Cutaneous immune-related adverse events (cirAEs) are the most common toxicities to occur in the setting of immune checkpoint inhibitor (ICI) therapy. This multi-institutional retrospective cohort study examines the relationship between cancer type, tumor tissue type, and cirAE development. Using data from Mass General Brigham and Dana-Farber, we identified 3,711 ICI recipients, of whom 672 developed cirAEs and reviewed their medical history - cancer type, presence of cirAEs, and cirAE morphology. Cancer types were grouped by organ system (skin, gastrointestinal, gynecologic, genitourinary, head and neck, thoracic). Tumor tissue types were characterized into epithelial (squamous, transitional, adenoid) and non-epithelial (melanoma, others) tumors. A multivariable Cox proportional hazards model, adjusted for demographics, Charlson comorbidity index, ICI target, and year of ICI initiation, with an interaction term between cancer type and tumor tissue type was used. CirAE morphology was significantly different by cancer type (p=<0.001) and tumor tissue type (p=<0.001). Compared to other non-epithelial cancers (sarcomas, hematologic, and CNS malignancies), cutaneous squamous carcinoma (cSCC)(HR=2.76, p=0.003), melanoma (HR=2.19, p<0.001), head and neck adenocarcinoma (HR=2.18, p=0.006), breast adenocarcinoma (HR=2.06, p=0.002), and urothelial carcinoma (HR=2.04, p<0.001) were at significantly higher risk of cirAE development in multivariate analyses. These translated into adjusted survival benefits in the setting of melanoma (HR=0.37, p=<0.001) and cSCC (HR=0.55, p=0.025) by comparison to non-epithelial. The highest observed rate of cirAEs and survival benefits among cutaneous malignancies treated with ICI suggest a tissue homing mechanism behind these toxicities, which needs to be further explored in future studies.

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Responses following COVID-19 vaccination in patients with autoimmune skin disease

G. Sprow[1, 2], M. Afarideh[1, 2], J. Dan[1, 2], E. Keyes[1, 2], M. Grinnell[1, 2], J. Concha[1, 2], T. Vazquez[1, 2], D. Diaz[1, 2], N. Kodali[1, 2], V. Werth[1, 2]
[1]University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]VA Medical Center Corporal Michael J Crescenz, Philadelphia, Pennsylvania, United States

Vaccination against COVID-19 reduces the risk of severe COVID-19 disease and death. However, few studies have examined the safety of the COVID-19 vaccine in patients with autoimmune skin disease. We sought to determine the incidence of disease exacerbation in this population following COVID-19 vaccination as well as the associated factors. We performed a chart review of all patients seen in the autoimmune skin disease clinic of the principal investigator during the study period. All patients included for analysis were systematically and prospectively asked about COVID-19 vaccination status, manufacturers, vaccine dates, autoimmune symptoms after the vaccine, and timing of symptom onset using a standardized template as part of their visit. Demographics and autoimmune disease diagnosis were also collected. Analysis used Chi-square tests. 402 subjects were included for analysis. 86% of patients were fully vaccinated, with 13% unvaccinated and 1% partially vaccinated. 15% of fully vaccinated patients reported autoimmune symptoms after the vaccine. Fully vaccinated dermatomyositis (DM) patients were more likely to report autoimmune symptoms after the vaccine (23%) than fully vaccinated lupus (LE) patients (9%) (p=0.009). Patients fully vaccinated with the Moderna vaccine were more likely to report autoimmune symptoms after the vaccine (19%) than those with the Pfizer vaccine (12%) (p=0.038). Of the patients who had autoimmune symptoms after vaccination, 20% had symptoms after the 1st dose, 82% after the 2nd dose, and 4% after the 3rd dose with median onset (95% confidence interval) of 7 (2,14), 14 (14,21), and 18 (7,28) days later, respectively. More fully vaccinated DM patients had autoimmune symptoms after the vaccine than fully vaccinated LE patients. However, given the risks of COVID-19, clinicians should still promote vaccination in most patients with autoimmune skin disease.

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Examining the risk of new skin cancers among obese patients who undergo bariatric surgery: A multicenter analysis

R. Raiker[1], H. Pakhchanian[2], E. Hochman[3, 4], M. Deng[3, 4]
[1]West Virginia University School of Medicine, Morgantown, West Virginia, United States, [2]The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, United States, [3]MedStar Georgetown University Hospital, Washington, District of Columbia, United States, [4]MedStar Washington Hospital Center, Washington, District of Columbia, United States

Obesity has been shown to be a significant risk factor for several cancer types. However limited studies exist that examine the risk of skin cancer among obese patients, especially those who have undergone bariatric surgery. A retrospective cohort study was conducted using TriNetX, a multicenter real time database of ~80 million patient records. Patients were queried by ICD-10 and CPT codes to find variables of interest from 2006-2017 and divided into obese patients with and without any history of bariatric surgery. A 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics to calculate adjusted hazard ratios (aHR) with 95% CI at 5 year follow-up for new skin cancer risk between the cohorts. aHR was estimated using the Cox proportional hazard model. A matched cohort of 192,844 patients revealed obese patients who underwent bariatric surgery were at a significantly lower risk for developing most new skin cancers such as malignant melanoma (aHR[95%CI]=0.75[0.6,0.9]), basal cell carcinoma (0.53[0.5,0.6]), squamous cell carcinoma (0.45[0.4,0.5]), melanoma in-situ (0.56[0.5,0.7]), cutaneous carcinoma in situ (0.48[0.4,0.6]), and actinic keratosis (0.59[0.5,0.6]) when compared to obese patients without bariatric surgery. No differences were seen with Merkel cell carcinoma. These findings show bariatric surgery is associated with a lower risk of developing most skin cancers in obese patients, thereby lending additional support that a link between obesity and skin cancer risk may be present. Additional studies are warranted to strengthen this association.

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Safety and efficacy of the COVID-19 vaccine among fully vaccinated and boosted skin cancer patients

R. Raiker[1], H. Pakhchanian[2], M. Deng[3, 4]
[1]West Virginia University School of Medicine, Morgantown, West Virginia, United States, [2]The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, United States, [3]MedStar Georgetown University Hospital, Washington, District of Columbia, United States, [4]MedStar Washington Hospital Center, Washington, District of Columbia, United States

As new COVID-19 variants emerge, it's vital to understand how certain populations are affected. Sparse data exists on whether vaccinated patients with prior cutaneous malignancies are at higher risk for adverse events or breakthrough infections (BI) compared to the general population. Therefore, we examined the safety and efficacy of the COVID-19 vaccine in skin cancer patients. A retrospective study using TriNetX, a multicenter database of ~80 million records, was completed. Patients who completed a mRNA COVID-19 vaccine sequence or received an additional booster were identified up to November 15th 2021 and divided into two cohorts based on history of skin cancer. 1:1 propensity score matching was done, adjusting for comorbidities and demographics, to find adjusted risk ratios (aRR) and hazard ratios (aHR) with 95% CI for safety and efficacy outcomes respectively. Safety outcomes included 1-day anaphylaxis, 60-day follow-up for hospitalization, and FDA-defined adverse events of special interest (AESI). Efficacy included 60-day risk of BI. Subgroup analysis was conducted for basal cell carcinoma, squamous cell carcinoma, and melanoma patients. A matched cohort of 17,384 fully vaccinated skin cancer patients revealed no higher risk in 1-day anaphylaxis (aRR[95%CI]=1.01[0.4,2.6]) or 60 day hospitalization (0.98[0.7,1.3]) and AESI (1.2[0.9,1.8]). Skin cancer patients also were not at higher BI risk (1.61[0.9,2.8]). No differences were seen among subgroups compared to controls. In a matched cohort of 6,382 boosted skin cancer patients, similar findings were seen in overall skin cancer patients and subgroups with no higher risk in any assessed adverse events compared to controls. Overall, the COVID-19 vaccine is safe and effective for patients with a history of skin cancer, but further research is needed.

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Development of secondary neoplasms in patients with nevus sebaceous

A. Burli, F. Bawany, M. Cordisco
Dermatology, University of Rochester School of Medicine and Dentistry, Rochester, New York, United States

Introduction: Nevus sebaceous (NS) is a hamartoma composed of epidermal, dermal, follicular, and apocrine appendages found in the pediatric population. The current standard of treatment is full-thickness excision, but this is controversial as much is still unknown about the malignant potential of this benign neoplasm. Secondary lesions associated with nevus sebaceous include poromas, trichoblastomas, and syringocystadenomas. The purpose of this study is to investigate patient characteristics of patients with NS and study the incidence and characteristics of secondary neoplasms. Materials and Methods: We conducted a retrospective review of electronic medical records at our academic institution of all patients diagnosed with NS in the past 7 years. 617 patient charts were reviewed, and 73 patients were found to have a clinical/pathologic diagnosis of NS. Results: Our patient cohort had an average age upon first consultation of 10.18 +/- 5.23 years. 59 patients were white, 7 were African American, 2 were Asian American, 3 were Hispanic, and 2 were race unknown. The majority of the patients had NS on their scalp (N=50). 40 patients underwent surgical excision for their NS. 6 patients had secondary lesions (8.12%). The most common secondary lesion was a verrucous papule, with no poromas, trichoblastomas, or syringocystadenomas in our patient cohort. Discussion: Our study showed that the majority of patients received surgical excision for NS, but ultimately only 6 patients eventually developed secondary lesions. Further research must be conducted into the utility of surgical excision for NS to prevent development of primary/secondary neoplasms.

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Evaluating the risk of post-operative complications in obstructive sleep apnea patients undergoing Mohs micrographic surgery

R. Raiker[1], H. Pakhchanian[2], M. Deng[3, 4]
[1]West Virginia University School of Medicine, Morgantown, West Virginia, United States, [2]The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, United States, [3]MedStar Georgetown University Hospital, Washington, District of Columbia, United States, [4]MedStar Washington Hospital Center, Washington, District of Columbia, United States

Obstructive sleep apnea (OSA) is a sleep-related breathing condition that leads to increased sympathetic activity. Previous studies have shown OSA is associated with increased oxidative stress and inflammation along with decreased perfusion, all of which can contribute to impaired wound healing. It is currently unknown whether patients with OSA are at a higher risk for post-operative complications after Mohs micrographic surgery (MMS) compared to those without OSA, therefore we aimed to address this knowledge gap. A retrospective cohort study was done using TriNetX, a multicenter database of ~80 million patient records. CPT and ICD-10 codes were used to identify MMS patients and complications were determined apriori. Cohorts were stratified by history of OSA. 1:1 propensity score matching was conducted, adjusting for comorbidities and demographics, to calculate Adjusted Risk Ratios with 95% CI (aRR[95%CI]). A matched cohort of 16280 patients revealed OSA patients had a higher risk for developing 10 post-operative complications in 30-day follow up. These include cellulitis (1.93[1.21,2.99]), any cutaneous infection (1.51[1.17,1.96]), hematomas (2.11[1.01,4.49]), wound dehiscence (1.57[1.05,2.34]), pain (2.60[1.80,3.75]), anesthesia of skin (2.45[1.35,4.34]), paresthesia of skin (2.47[1.28,4.76]), rash (2.22[1.11,4.06]), dyspigmentation (2.18[1.33,3.57]), and localized swelling (1.77[1.07,2.92]). Patients with OSA have significantly higher risk of post-operative complications after MMS. Greater caution must be taken in these patients to mitigate the risk of negative outcomes.

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Evaluating the safety and effectiveness of the COVID-19 vaccination among pediatric atopic dermatitis patients

H. Pakhchanian[1], R. Raiker[2], K. Jenkins[3], L. Shen[3]
[1]The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, United States, [2]West Virginia University School of Medicine, Morgantown, West Virginia, United States, [3]Boston Medical Center, Boston, Massachusetts, United States

Atopic dermatitis (AD) is a systemic inflammatory disease and the most common skin disorder among children. It has been found to be associated with several comorbidities. As COVID-19 cases continue to surge worldwide, especially among children, we aim to study the safety profile and efficacy of the COVID-19 vaccine in AD children, a topic with little data thus far. A retrospective analysis was done using TriNetX, a multicenter database of ~80 million deidentified records. Children (<18 years) who were vaccinated with the BNT162b2 vaccine up to November 15th, 2021 were identified and split into AD and non-AD cohorts. A 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics, to generate adjusted risk ratios (aRR) and hazard ratios (aHR) with 95% CI for safety and efficacy outcomes respectively. Safety outcomes included 1-day anaphylaxis and 60-day follow-up for all-cause hospitalization, and FDA-defined adverse events of special interest (AESI). Efficacy was measured as 60-day risk of breakthrough infection (BI). From the 73,984 children who received a COVID-19 vaccine, 6% had AD. A matched cohort of 4505 AD children revealed no differences in safety outcomes for 1 day anaphylaxis (aRR[95%CI]=1.03[0.4,2.4]), 60-day hospitalization (1.25[0.6,2.7]) and AESI (1.02[0.3,2.4]) between cohorts. Additionally AD children did not have a higher risk of BI (aHR[95%CI]=1.7[0.8,3.3]) compared to controls. The results show that the COVID-19 vaccine is safe and effective for pediatric AD patients. Further research is warranted to examine if severity of AD could increase risk of adverse events.

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Comorbidity associations among pediatric hidradenitis suppurativa patients: A cross-sectional analysis

K. Jenkins[1], R. Raiker[2], H. Pakhchanian[3], L. Shen[1]
[1]Boston Medical Center, Boston, Massachusetts, United States, [2]West Virginia University School of Medicine, Morgantown, West Virginia, United States, [3]The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, United States

Hidradenitis Suppurativa (HS) is a chronic inflammatory skin disease that can cause painful abscesses/nodules in intertriginous areas with apocrine-gland bearing skin. Limited studies involving pediatric HS exist, especially those examining the association of comorbidities in large sample sizes. Therefore, the goal was to assess this. A retrospective cross-sectional analysis was done using TriNetX, a multicenter database of ~80 million deidentified records. Children (≤17 years) with HS were identified and compared with non-HS children. A 1:1 matched propensity score analysis was conducted (adjusting for age, sex, and race) to generate adjusted odds ratios with 95% CI (aOR[95%CI]) to determine association. From the 11,096,586 children in the database, 0.03% had HS. In a matched cohort of 3571 HS patients, HS children were more likely to have obesity (6.92[5.8,8.3]), hypertension (1.94[1.1,3.6]), diabetes mellitus (2.37[1.5,3.8]), metabolic syndrome (2.11[1.01,4.5]), dyslipidemia (4.03[2.1,8.1]), thyroid disorders (2.38[1.2,4.8]), polycystic ovarian syndrome (3.01[1.5,6.2]), asthma (1.43[1.1,1.9]), allergic rhinitis (2.15[1.3,3.5]), atopic dermatitis (5.52[3.3,9.2]), acne vulgaris (6.87[5.1,9.4]), and pilonidal cyst/sinus (2.51[1.2,5.2]) compared to controls. No significant association was seen for inflammatory bowel disease, inflammatory polyarthropathies, depression, anxiety disorders, or attention deficit disorder compared to controls. The findings from this large sample study show that overall, pediatric HS patients may be more associated with comorbid endocrine/metabolic, respiratory, and cutaneous disorders compared to non-HS children. Further research, especially those examining influence of HS severity on comorbid associations, is needed to support these findings.

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Impact of COVID-19 healthcare disruption on delays in melanoma treatment in rural and urban Iowa

A. Munjal[1], M. Fitzhugh[1], M. Walsh[2], P. Gorrepati[1], R. Tripathi[2], V. Liu[4], J. G. Powers[3]
[1]The University of Iowa Roy J and Lucille A Carver College of Medicine, Iowa City, Iowa, United States, [2]Internal Medicine, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States, [3]Dermatology, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States, [4]Dermatology and Pathology, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States

Previous studies have shown delays in skin cancer diagnosis and treatment due to disrupted care as a result of the COVID-19 pandemic. Diagnoses of melanoma in rural areas are associated with higher all-cause mortality when compared to metropolitan areas. Patients with melanoma treated after three months of diagnosis experience higher mortality than patients treated after one month. This study aimed to determine how COVID-19 shutdowns affected time to treatment of melanoma and patient outcomes between patients who lived in rural vs. urban areas. Patients with a pathologic diagnosis of melanoma at a large academic tertiary referral center were identified from 1/1/2019 to 12/31/2020 (n=408). Data from pathologic reports and demographic information regarding rurality (address and zip code) were collected from medical records. Documented telephone encounters were utilized to determine delays due to COVID-19. Average Breslow depth increased from 1.16 mm to 1.50 mm and proportion of clinical lymphadenopathy increased from 1.12% to 6.76% from 2019 to 2020. Conversely, average time to treatment from diagnosis decreased from 2019 to 2020 (29.55 vs. 23.76 days). These findings suggest that the COVID-19 pandemic resulted in a higher proportion of later stage tumors at presentation, but with no significant treatment delay once melanoma diagnosis was established. These results characterize the impacts of pandemic shutdowns on medical care and can aid health systems in designing optimal strategies to better serve patients from rural backgrounds.

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Depression and anxiety in atopic dermatitis vs bullous pemphigoid

T. DeGrazia[1], D. Mustin[2], Y. Liu[3], B. Bradley[1], T. Adkins[1], E. Cole[1], R. Feldman[1]
[1]Dermatology, Emory University, Atlanta, Georgia, United States, [2]Emory University School of Medicine, Atlanta, Georgia, United States, [3]Department of Biostatistics and Bioinformatics, Emory University School of Public Health, Atlanta, Georgia, United States

Atopic dermatitis (AD) and bullous pemphigoid (BP) are inflammatory skin diseases associated with intense pruritus and significant quality of life (QOL) impact. While they differ in classical presentation (eczematous eruption vs urticaria and blisters) and population characteristics (young-to middle-aged persons vs elderly), early stages of BP can appear similar to AD clinically and histologically. As part of this observational cohort study, we investigated whether there were differences in anxiety and depression between the cohorts at baseline and over time. This study included 93 patients with either AD (50) or BP (43) and a history of chronic pruritus. Our cohort was mostly female (61%), mean ages 47.2 (AD) and 72.2 (BP), with mean severity scores 35.9 (AD, SCORAD) and 19.1 (BP, BPDAI). The Hospital Anxiety and Depression Scale was used to measure depression (HADS-D) and anxiety (HADS-A). At baseline, BP patients had overall higher HADS-D scores, 6.14 ± 4.4 compared to AD 4.33 ± 4.4 (p = 0.023) indicating greater depressive symptoms, although the mean HADS-D and HADS-A scores in both cohorts were in the "Normal" range (score ≤7). Notably, 31% of BP and 16% of AD patients were in the "Borderline/Abnormal" range for depression and 38% of BP and 30% of AD patients for anxiety. While HADS-D scores were noted to be increased in BP patients at baseline, no differences were found at follow-up visits in AD or BP. There were no significant changes in HADS scores over time within each cohort. Both HADS-D (PCC 0.424, p<0.01) and HADS-A (PCC 0.235, p=0.038) scores significantly correlated with itch severity measured using the ItchyQuant. Anxiety and depression are important aspects of patient care to consider in diseases such as AD and BP that can greatly impact QOL. Clinicians should be particularly mindful of depression and anxiety as it relates to itch severity.

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10 year experience of pediatric kerion celsi in Costa Rica

B. Hidalgo-Matlock[1], D. Correa-Tovar[2]
[1]Dermatology Department, Hospital Nacional de Niños, Hospital Nacional de Ninos, San Jose, San José, CR, hospital/children, San José, Costa Rica, [2]Universidad de Costa Rica, San Jose, San José, Costa Rica

The Kerion Celsi (KC) is an inflammatory tinea capitis,. It is a public health problem listed by the World Health Organization as the second most common childhood dermatological infection. Proper diagnosis and treatment are crucial to prevent permanent sequelae. Aim: To determine the characteristics of children with KC in an Outpatient Clinic of a tertiary, pediatric, reference center in Costa Rica between January 01, 2009 to December 31, 2019. Methods: Retrospective, observational study based on chart review. Results: 107 patients were included, with 63.55% males and an average age of 5 years and 9 months Most patients came from low income and sub-urban areas. 30.8% of the patients registered a pet, majority dogs (24.2%). 14% had history of atopy. The most frequent symptoms were an alopecic lesion (86.9%), erythema (86%), desquamation (56.1%), pustules (53.3%) and regional lymphadenopathy (35.5%). Culture was done in 60.7% of cases; 25.1% had a positive fungal culture. M. canis (14%), T. rurbrum (4.7%) and M. gypseum (3,7%) were the most common. Fluconazole was the main antifungal used (dose 5-6mg/kg/d). Systemic glucocorticoids were used in 12% of the cases. 72% (n=77) resolved completely, 21.5% had persistent alopecia and 6% lost follow-up. Conclusions: Due to the little existing literature about the population characteristics of KC in Costa Rica, the epidemiological characteristics of the affected population from the last 10 years were collected in order to find early clinical manifestations that facilitate the identification of the pathology, determine the most striking diagnostic findings and the treatment used and its efficacy. In addition, to be able to establish these characteristics and thus, propose local guides on the management of this condition. However, there were limitations due to the underreporting of information in the existing medical record.

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Impact of COVID-19 infection on hidradenitis suppurativa activity

M. E. Torpey, A. Nosrati, K. L. Campton, S. R. Cohen
Albert Einstein College of Medicine, Bronx, New York, United States

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease manifest as painful nodules, abscesses, and draining sinus tracts ("tunnels"). Recent studies have shown increased inflammation and immune system hyperactivity are hallmarks of SARS-CoV-2 (COVID-19) infection and typically forecast disease progression. Coincidentally, chronic inflammation and immune dysregulation are characteristic of HS. We sought to examine if COVID-19 infection affected HS activity. An IRB-approved retrospective chart review and telephone survey of patients receiving care at the Einstein/Montefiore HS Center (HSC) was conducted between September-December 2021. Patient demographics, vaccine status, and prior COVID-19 infection status were documented. Of 66 patients who agreed to participate, the mean age was 35.2±14.0 years; 44 (67%) were female. Fifty-three (80%) patients were vaccinated. Of the non-vaccinated patients, 42% cited fear of worsening HS with vaccination. Sixteen patients (24%) reported clinical infection that was confirmed by testing positive for COVID-19. While the majority of HS patients infected with COVID-19 experienced no change in HS activity, 3 (18.8%) reported a marked increase in pain associated with new red nodules and drainage. Vaccination status at the time of infection could not be verified. Preliminary data suggest that HS patients are at risk for exacerbation of disease when infected with COVID-19. The effect of COVID-19 vaccination in reducing the HS disease progression warrants further study.

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The association between primary focal hyperhidrosis and psychiatric illnesses

R. Raiker[1], H. Pakhchanian[2], K. Phan[3]
[1]West Virginia University, Morgantown, West Virginia, United States, [2]The George Washington University, Washington, District of Columbia, United States, [3]Liverpool Hospital, Liverpool, New South Wales, Australia

Primary Focal Hyperhidrosis (PFH) is a debilitating condition that causes excessive sweating and is known to be associated with a lower quality of life and psychosocial distress. However, little is known on the clinical consequences of having PFH. Therefore, the goal was to examine the association between PFH and mental health (MH) disorders. A cross-sectional study was conducted using data from the National Inpatient Sample (2004-2014), a database consisting ~20% stratified sample of all US hospitalizations. Weighted multivariable logistic regression models were constructed to obtain adjusted odds ratios (aOR) with 95% CI controlling for demographics and socioeconomic factors. In a weighted sample of 11092 PFH patients, PFH was significantly associated with increased odds of 10 grouped MH disorders examined. PFH was associated with anxiety (aOR[95% CI]): 3.56[3.09-4.10], schizophrenia (1.57[1.13-2.18]), personality disorders (3.04[2.15-4.30]), depression & mood disorders (3.10[2.75-3.50]), attention deficit disorder & conduct disorders (6.18[4.70-8.11]), childhood mental illnesses (5.60[3.09-10.1]), substance disorder (1.79[1.44-2.21]), history of mental health disorders (1.84[1.61-2.10]), adjustment disorder (2.84[1.83-4.42]), and developmental disorders (2.42[1.63-3.60]). PFH is associated with higher odds of MH Disorders. These findings suggest the complex dysfunction of the autonomic nervous system that is theorized to play a role in PFH may also be linked to MH Disorders.

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Adiposis dolorosa and deoxycholic acid: An alternative therapeutic method

C. Silence[1], S. M. Rice[2], A. S. Kourosh[1, 3]
[1]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]University of Massachusetts Medical School, Worcester, Massachusetts, United States, [3]Harvard Medical School, Boston, Massachusetts, United States

Dercum disease, or adiposis dolorosa, is a rare disorder characterized by multiple painful lipomas distributed throughout the body. There are currently no approved treatments for Dercum disease and excision is often not feasible due to tumor burden and extent of disease. Here we present the use of deoxycholic acid (DCA) to reduce the size of lipomas associated with Dercum disease. Ultrasound was used to obtain 3-dimensional measurements of painful lipomas in three patients. Lesions were identified for treatment with patient and radiology input based on larger size, troublesome symptoms, and feasibility of location (e.g. accessibility for injection and avoidance of critical structures). Controls were chosen based on similar criteria and symmetry within the body (e.g. lesions of similar size and pain in left vs right forearm). Imaging was repeated, tracking changes in the size of treated and untreated lipomas and volumes were calculated. The treated lesions had a 34% (patient 1), 68% (patient 2), 41% (patient 2) and 30% (patient 3) decrease in volume, while the control lesions had a 111% (patient 1), 22% (patient 2), 93% (patient 3) increase in volume. The results show that all lipomas treated with DCA had a reduction in volume while untreated controls increased in volume. Interestingly, repeat imaging of the treated lipoma in patient 3 revealed an anechoic center, possibly highlighting cell death of adipocytes in response to injected DCA. DCA is naturally found in the body and assists with emulsifying and solubilizing fats. While initially used as a treatment method for submental fat, it may be a possible alternative treatment modality for individuals with extensive painful lipomas who are not candidates for surgical excision. Additional studies should be conducted to determine appropriate dosing and side effect profile.

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Long-term efficacy and safety of investigational autologous gene-corrected skin sheets (EB-101) for recessive dystrophic epidermolysis bullosa (RDEB)

J. So[1], V. Iwummadu[1, 2], J. Nazaroff[1], I. Bailey[1], D. McCarthy[2], M. Mirza[2], J. Tang[1], A. Chiou[1]
[1]Department of Dermatology, Stanford University School of Medicine, Stanford, California, United States, [2]Abeona Therapeutics Inc, Cleveland, Ohio, United States

RDEB is a rare, severe dermatosis caused by mutations in type VII collagen (C7), a major component of structural attachments between the basement membrane and dermis. Manifestations include large, chronic wounds and blisters, increased squamous cell carcinoma (SCC) and early death. Currently, there are no approved therapies. We report long-term outcomes of a Phase 1/2a trial to treat large, chronic RDEB wounds with 35cm2 autologous skin sheets (EB-101) gene-corrected with a retroviral vector (RV). 7 patients received 42 total sheets and were monitored for 4-8 years (mean 5.7). At last follow-up, 5 patients were alive. 1 died from SCC at a non-grafted site and 1 died from infection due to underlying disease; deaths were considered unrelated to EB-101. We saw long-term improvements in symptoms: 74% (31/42) of treated sites had ≥50% wound healing and 60% (25/42) had ≥75% wound healing. Subjects also noted improvements in quality of life including no pain or itch at 86% (36/42) of treated sites compared to no pain at 42% (19/42) and no itch at 38% (16/42) of sites prior to treatment, and improved skin durability at 73% (31/42) and decreased blistering at 71% (30/42) of treated sites compared to baseline. Regarding safety, no SCCs on grafted sites or replication-competent RV infections were identified. 14% (6/42) of sites developed infections—all within 1 year of grafting—that self-resolved or resolved with antibiotics. No subjects developed long-term systemic autoimmunity to EB-101 as defined by the presence of anti-C7 cytotoxic T cells, increased blistering or persistent circulating anti-C7 antibodies (Ab). One patient had tissue-bound anti-C7 Ab at treated sites past year 1 but did not exhibit signs of systemic autoimmunity, suggesting this response remained localized. Thus, EB-101 is a safe, effective treatment for large, chronic RDEB wounds in this Phase 1/2a trial. A confirmatory Phase 3 trial is ongoing.

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Complete clearance of actinic keratosis observed from day 8 of tirbanibulin treatment, along with good tolerability: post-hoc analysis of two Phase 3 studies

B. Berman[1], G. Gupta[2], L. Padullés[3], F. Hernández[4]
[1]Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, United States, [2]NHS Lothian, Edinburgh, United Kingdom, [3]Almirall, Barcelona, Spain, [4]Almirall, Sant Feliu de Llobregat, Spain

Tirbanibulin 1% ointment was approved for actinic keratosis (AK) of face or scalp based on two Phase 3 trials. Pooled rates of complete (100%) clearance (CC) and partial (≥75%) clearance (PC) in these trials were reported for Day (D) 57 of treatment, being 49% and 72% respectively. We present post-hoc efficacy and safety analyses evaluating earlier time points.
Participants (N=702) in the Phase 3 trials had 4-8 clinically visible AK lesions (25 cm2 area); 353 were randomized to tirbanibulin (self-applied once daily, 5 consecutive days). At study visits (D8-D15-D29-D57) efficacy was assessed through CC/PC, and safety through local skin reactions (LSR) including erythema, flaking/scaling, crusting, swelling, vesiculation/pustulation and erosion/ulceration. Each LSR was scored between 0-3 (absent-severe). Individual scores were added resulting in a LSR composite score between 0-18. The maximum LSR composite scores reached up to D57 were averaged for participants achieving CC at each visit.
CC rate was 13.4% at D8, increasing during treatment to 24.7%(D15), 36.4%(D29) and 49.3%(D57). PC rate was 20.2% at D8 and gradually rose to 41.2%(D15), 62.8%(D29) and 72.2%(D57). Among patients reaching CC at each visit, baseline characteristics were similar except for a trend to higher percentage of face treatments in those achieving CC at D8-D15 vs. D29-D57. The mean (±standard deviation) maximum LSR composite score reached during the follow-up was similarly low regardless if CC was obtained at D8(4.7±1.8), D15(4.8±2.2), D29(4.9±2.1) or D57(4.9±2.1).
Although the highest CC rate with tirbanibulin was observed at D57, this analysis confirms that AK patients can show much earlier responses (from D8). These were not accompanied by an increase in the severity of LSRs, thus showing a similarly good tolerability as compared to later responders.

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Effects of ruxolitinib cream on sleep and quality of life over 52 weeks in black patients with atopic dermatitis

L. F. Eichenfield[7], L. F. Stein Gold[1], K. K. Brar[2], Z. C. Chiesa Fuxench[3], J. I. Silverberg[4], M. E. Venturanza[5], H. Kallender[5], J. Gao[5], E. Simpson[6]
[1]Henry Ford Health System, Detroit, Michigan, United States, [2]New York Grossman School of Medicine, New York, New York, United States, [3]University of Pennsylvania, Philadelphia, Pennsylvania, United States, [4]George Washington University, Washington, District of Columbia, United States, [5]Incyte Corporation, Wilmington, Delaware, United States, [6]Oregon Health & Science University, Portland, Oregon, United States, [7]University of California San Diego, San Diego, California, United States

Atopic dermatitis (AD) is an inflammatory skin disease with phenotypic differences across race and can affect sleep and quality of life (QoL). In 2 phase 3 studies of identical design (TRuE-AD1/TRuE-AD2), patients (pts; ≥12 y with AD for ≥2 y; Investigator's Global Assessment score 2/3; 3%–20% affected body surface area) were randomized (2:2:1) to twice-daily 0.75% or 1.5% ruxolitinib (RUX; Janus kinase [JAK]1/JAK2 inhibitor) cream or vehicle for 8 wk (continuous treatment), followed by a long-term safety period (LTS; as-needed treatment) up to Wk 52. Pts randomized to RUX cream remained on their regimen during the LTS; pts on vehicle were rerandomized to either RUX cream strength. For Black pts who were initially randomized to the 0.75% RUX cream/1.5% RUX cream/vehicle to 0.75% RUX cream/vehicle to 1.5% RUX cream groups and continued in the LTS (n=91/97/25/22), sleep-related impairment and sleep disturbance scores per Patient-Reported Outcomes Measurement Information System at baseline (BL) were 16.3/16.4/15.0/17.5 and 18.9/19.7/17.9/19.8, respectively. Scores had decreased (less impairment) at LTS start in the RUX cream groups (Wk 8; 14.2/14.7/16.1/15.5 and 16.7/17.5/19.0/19.4) and were below BL at Wk 52 in all groups (14.3/14.8/13.9/14.4 and 18.0/18.0/17.4/16.3). Dermatology Life Quality Index (DLQI) scores were decreased at Wk 8 (mean change from BL, −7.4/−6.6/−3.8/−4.8); decreased scores were maintained to Wk 52 (−7.1/−6.5/−5.6/−8.8). Results were similar for children's DLQI (Wk 8, −4.0/−6.9/−4.0/−3.0 [n=12/9/1/3]; Wk 52, −5.6/−11.6/−12.0/−7.3 [n=9/7/1/4]). In summary, sleep and QoL improved with RUX cream; improvements were maintained for 44 wk with as-needed use in Black pts.

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LLLT and Minoxidil combination treatment in androgenetic alopecia: A review of the literature

M. A. Kaiser[1], S. M. Almeida[1], N. Issa[2], M. Rodriguez[1], N. T. Issa[1], J. J. Jimenez[1]
[1]*Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, University of Miami, Coral Gables, Florida, United States,* [2]*St. George's University School of Medicine, West Indies, Grenada*

Purpose: Low Level Light Therapy (LLLT) is a promising therapy for androgenetic alopecia (AGA). We analyzed randomized clinical trials (RCTs) evaluating the efficacy of combined therapy with LLLT and topical minoxidil. Methods: A literature search within PubMed identified RCTs evaluating hair regrowth following LLLT and minoxidil. Selection criteria were 600–1,100 nm wavelengths (optical window at red and near-infrared wavelengths), treatment time ≥16 weeks, and objective evaluation for hair regrowth. Results: Five RCTs compared LLLT with topical minoxidil (2% or 5%) to either no treatment, 5% minoxidil treatment, or LLLT treatment. One study found that combination therapy of LLLT and 5% minoxidil showed greater improvement of hair density and diameter than LLLT or 5% minoxidil monotherapy. Another found combination LLLT with 2% minoxidil induced hair regrowth equivalent to 5% minoxidil. Similarly, another study described LLLT with 5% minoxidil and 5% minoxidil alone to increase the total number of hairs with no statistical difference between the two groups. One trial found that combination group only showed statistically significant increase in hair regrowth in the first 2 months, but all groups showed increased hair growth at 4 months. The last study found a statistically significant increase in hair density of the combined LLLT and 5% minoxidil solution compared to minoxidil with sham device. Discussion: The studies describe either superiority of combination treatment or equivalence of combination therapy to minoxidil monotherapy for AGA. Early outcomes appear to support the superiority of combination therapy, but this advantage wanes at the end of the study periods. These findings suggest that more rigorous clinical trials with increased sample size and stratification of alopecia by severity are necessary to establish whether combination therapy is superior.

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Treatment of aphthous ulcers with montelukast

A. S. Hwang[1], E. H. Campbell[2], J. C. Sartori-Valinotti[2]
[1]Mayo Clinic School of Medicine - Scottsdale Campus, Scottsdale, Arizona, United States, [2]Mayo Clinic Minnesota, Rochester, Minnesota, United States

Aphthous ulcers are the most prevalent oral mucosal lesions, yet treatment is challenging. There is evidence that oral montelukast is efficacious in treating aphthous ulcers. A randomized placebo-controlled trial comparing montelukast against prednisone for recurrent aphthous stomatitis demonstrated montelukast has superior efficacy compared with placebo, decreased efficacy compared with prednisone, but decreased adverse effects compared to prednisone. Our single-center, retrospective study identified subjects with a minimum of 2 distinct encounters using the search term:(Singular or montelukast) AND (aphthous ulcers). 16 subjects met inclusion criteria and were found to have follow up after treatment. Over half (56.3%) of patients had positive clinical responses, measured by provider assessment. Among these, 44.4% had reduction in number of ulcers, 22.2% had decrease in frequency of ulcer eruptions, and remainder had unspecified improvement. Mean dosing of montelukast was 9 mg daily, with a range of 4-10 mg. 4-5 mg was prescribed to pediatric patients;10 mg for all others aged 15 and older. While 10% of patients in the aforementioned RCT reported side effect of diarrhea from montelukast, none in our study reported any adverse effects, despite a mean therapy duration of 455.4 days (measured by encounter dates). We were unable to determine an accurate time to response due to longer durations between encounters without specific documentation of time to response. The aforementioned RCT had an active treatment duration of 2 months. Our study demonstrates a longer lasting efficacy of montelukast, as response was measured at the last related encounter. Our study further strengthens support for montelukast as a safe and effective treatment option for recalcitrant aphthous ulcers with reduction in both number and frequency of ulcers. Montelukast has minimal adverse effects, even with long treatment duration, compared with other systemic medications used for management. Further studies to determine the recurrence rate after cessation of montelukast are warranted.

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Photo validation study using cutaneous dermatomyositis disease area and severity index in dermatomyositis patients

M. Grinnell[1, 3], J. Concha[1, 3], R. Feng[2], E. Keyes[1, 3], J. Okawa[1, 3], D. Diaz[1, 3], T. Vazquez[1, 3], V. Werth[1, 3]
[1]*Department of Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States,* [2]*Department of Biostatistics, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [3]*Corporal Michael J. Crescenz VA Medical Center, Philadelphia, Pennsylvania, United States*

The Coronavirus disease 2019 (COVID-19) pandemic revealed our need for reliable tools to evaluate patients with skin disease virtually. Thus far, there has not been a study that has attempted to score the Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI), a validated outcome measure of skin activity and damage, from photographs. In this study, patients were prospectively recruited during routine clinic visits and skin regions used in scoring the CDASI were photographed by research staff using two iPhone cameras (an iPhone 8 and iPhone 11). Two dermatologists served as the raters. The in-person CDASI assessment was scored by rater 1 at the clinic visit and the photographs were scored at a later date by both rater 1 and rater 2. Of the 34 patients participating in the study, 82.3% were female, 85.3% were Caucasian with a mean age of 54 years (SD=12). For the total activity score, the intraclass correlation coefficient (ICC) between rater 1's in-person assessment compared to photograph assessment was 0.806 (95% CI 0.649-0.898 p<0.001) and was 0.822 (95% CI 0.675-0.907 p<0.001) between rater 2 and the in-person assessment. For the total damage score, the ICC between rater 1 and the in-person assessment was 0.54 (95% CI 0.254-0.739 p=0.004) and was 0.601 (95% CI 0.338-0.778 p<0.001) between rater 2 and the in-person assessment. The reliability was interpreted as "excellent" for skin activity, an important measure in clinical trials for dermatomyositis. Photographs may be a useful tool for evaluating clinical trial patients in the future. More research is needed to determine innovations for improving our ability to evaluate skin activity through photographs such as the use of a color checker card or color correction algorithm.

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The use of PRP to target dysregulated pathways in androgenetic alopecia

R. Abdin[1], Y. Zhang[2], J. J. Jimenez[2]
[1]*Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, Florida, United States,* [2]*Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States*

Androgenetic alopecia (AGA) is the most common type of progressive hair loss; however, its treatment remains limited in scope, thus driving the need for alternative therapies for hair restoration. The pathogenesis of androgenetic alopecia is not completely understood but involves disruption of the Wnt/β-catenin signaling pathway, resulting in premature termination of the anagen growth phase in hair follicles. This manifests clinically as the transformation of terminal hair into thinner vellus-like hair, a process known as hair follicle miniaturization. Platelet rich plasma (PRP) has recently been regarded as a novel treatment for AGA. PRP is an autologous preparation of plasma that contains supraphysiologic concentrations of platelets and their associated growth factors, which have been implicated in regulating hair follicle growth. Nevertheless, the extensive variabilities in PRP preparation and administration protocols makes it difficult to interpret its clinical efficacy in treating AGA. This study follows a previous review from our group in 2018 by Cervantes et al. to analyze and discuss recent clinical trials which use PRP as treatment for AGA. We included those that assessed PRP in combination or in direct comparison with standard of care treatment for AGA, namely minoxidil and/or finasteride. We thoroughly examined and summarized the methodologies of fifteen original clinical trials published between 2018 and October 2021. Of the fifteen studies, five evaluated the combined and compared effects of PRP with standard treatment, seven established PRP as an effective treatment for AGA alone, and three concluded that PRP is ineffective in the treatment of AGA. By rigorously analyzing each clinical trial, we aim to provide an overall consensus on how PRP can be best used in the treatment of AGA.

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A phase 2 randomized clinical trial of serlopitant, a neurokinin-1 receptor antagonist for the treatment of chronic itch in patients with epidermolysis bullosa

U. Okata-Karigane, E. Gorell, K. Sum, K. Yekrang, M. Phung, M. Barriga, P. Udrizar, I. Bailey, S. Li, J. Tang, A. Chiou
Stanford University School of Medicine, Stanford, California, United States

Chronic itch is one of the most frequently reported symptoms in patients with epidermolysis bullosa (EB). We hypothesized that a neurokinin-1 receptor antagonist (NK1RA) which targets the substance P pathway can reduce EB-related itch. In 2019, we reported on the safety of a NK1R antagonist, serlopitant, in a pilot study. Here we report the phase 2 randomized, double-blind, placebo-controlled trial evaluating serlopitant 5mg PO daily for 8 weeks versus placebo for EB-related pruritus. The double-blind phase was followed by a 4-week washout and optional open label extension. Key inclusion criteria included age ≥13 yr, chronic itch lasting ≥6 wks and average 24-hour itch numerical rating scale (NRS) ≥5 at screening. The primary endpoint was the proportion of patients who achieved at least 3-point reduction in an 11-point NRS from baseline at 8 weeks as measured by daily NRS itch diaries. We enrolled 24 patients with a stratified randomization strategy to ensure equal distribution of participants with more severe EB subtypes. Two patients discontinued for non-compliance (n=1) and LFT elevation (n=1). Treatment arms were balanced in terms of EB subtypes and baseline itch; the mean (SD) of NRS was 5.3 (±2.2) in the placebo and 6.3 (±2.4) in the serlopitant group with the placebo group trending towards being older (mean 40.8 yo vs. 30.8 yo). At 8 weeks, 25% (n=3) of patients in the serlopitant group achieved at least a 3-point reduction compared with 8.3% (n=1) of placebo-treated patients although it was not statistically significance (p=0.59). In a linear mixed model analysis, the serlopitant group showed more NRS reduction relative to placebo (-0.64 point, p=0.16) at 8 weeks. No treatment-related serious adverse events were reported. This early phase study did not identify superiority of serlopitant but provides the basis for future studies in this rare disease.

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Antenatal vitamin D supplementation & offspring risk of atopic eczema in infancy.

S. El-Heis[1], S. D'Angelo[1], E. Curtis[1], E. Healy[2], R. Moon[1], S. Crozier[1, 3], H. Inskip[1, 4], C. Cooper[1, 4], N. Harvey[1, 4], K. Godfrey[1, 4]
[1]MRC Lifecourse Epidemiology Centre, University of Southampton, Southampton, Hampshire, United Kingdom, [2]Dermatopharmacology, University of Southampton Faculty of Medicine, Southampton, Southampton, United Kingdom, [3]NIHR Applied Research Collaboration Wessex, National Institute for Health Research, Southampton, United Kingdom, [4]NIHR Southampton BRC, National Institute for Health Research, Southampton, United Kingdom

Observational studies have led to speculation that antenatal vitamin D supplementation may reduce the risk of offspring atopic eczema, but currently there are no proven general population preventive interventions. In the Maternal Vitamin D Osteoporosis Study double-blind, randomized, placebo-controlled trial, we examined the link between maternal supplementation (from 14 weeks' gestation until delivery) with cholecalciferol 1000 IU/day or matched placebo with offspring atopic eczema risk at age 12 months (n=636), ascertained based on the UK Working Party Criteria for the Definition of Atopic Dermatitis. Mothers and offspring characteristics did not differ between the intervention and placebo groups with the exception of longer breast feeding duration in the intervention group. The offspring of mothers who received 1000 IU cholecalciferol had lower odds ratios (OR) of atopic eczema at age 12 months: OR (95%CI) 0.57 (0.33-0.98), p=0.04. Sensitivity analysis stratified by breastfeeding duration demonstrated a reduced risk of atopic eczema in the intervention group in infants who were breastfed for more than 1 month (OR 0.48 (0.24,0.94), p=0.03), but not in those breastfed for less than one month (OR 0.80 (0.29,2.17), p=0.66); however, interaction terms between intervention and breastfeeding duration were not statistically significant (p=0.41). Our data provide the first randomized controlled trial evidence of a protective effect of antenatal cholecalciferol supplementation on risk of infantile atopic eczema, with the effect only seen in infants that were breastfed for more than 1 month. The findings support a developmental influence on atopic eczema, and point to a potentially modifiable perinatal influences on atopic eczema.

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Modulation of inflammatory proteins in blood may reflect cutaneous immune responses in topical cancer immunotherapy

J. Han[1], J. Correa Da Rosa[1], S. Owji[1], Y. Estrada[1], J. Ungar[1], J. G. Krueger[2], N. Gulati[1]
[1]Dermatology, Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York, United States

Diphencyprone (DPCP), a hapten that causes delayed-type hypersensitivity reactions, has shown up to 84% efficacy in treating cutaneous metastases in melanoma patients. While a transcriptomic analysis of skin biopsies from melanoma metastases treated with topical DPCP revealed increases in Th1-related genes, a serum proteomic analysis of these patients has not yet been done. We evaluated the serum proteome of five patients with cutaneous melanoma metastases treated with DPCP twice weekly until day 112, assessing 96 proteins using the Olink immuno-oncology panel. All patients had at least partial regression of skin metastases. There was significant upregulation of proteins associated with promoting tumor immunity (TNFRSF4, TNFRSF9, CD83) and vascular/tissue remodeling (MMP12, PGF, ADGRG1) (P<0.05) upon DPCP treatment. Among the T-cell subsets, there was a significantly upregulated Th1 response (CXCL9, CXCL10, IL12) that progressively increased from day 63 to 112, when compared to day 0 (P<0.05). However, there was only a significant upregulation in Th2 (IL33) and Th17 (CCL20) markers on day 63 (P<0.05), but not day 112, in line with prior gene expression studies on skin samples. There was also significant and progressive upregulation of PD1 at both days 63 and 112 (P<0.05). This study is the first to assess serum protein biomarkers of patients with cutaneous melanoma metastases following topical immunotherapy. Topical DPCP led to an increase in systemic markers of immune activation, particularly the Th1 axis, which has previously been shown in skin and correlates with tumor regression. Additionally, we observed an increase in PD1, which is of great clinical relevance as inhibitors of this receptor are currently standard-of-care treatment for melanoma. Our data suggest potential synergy between DPCP and PD1 checkpoint inhibition as a future cancer therapy regimen for patients with cutaneous melanoma metastases.

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Photodynamic therapy for basal cell carcinoma enhanced by pretreatment with oral vitamin D: interim results of a prospective clinical trial

L. E. Heusinkveld[1], J. Negrey[2], A. Updyke[3], C. B. Warren[4], T. Hasan[5], E. Maytin[4, 3, 1]
[1]Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland Clinic, Cleveland, Ohio, United States, [2]Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, United States, [3]Department of Biomedical Engineering, Cleveland Clinic Lerner Research Institute, Cleveland, Ohio, United States, [4]Department of Dermatology, Cleveland Clinic, Cleveland, Ohio, United States, [5]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts, United States

Photodynamic therapy (PDT) is used in Europe to treat basal cell carcinoma (BCC), but it is not approved in the USA due to uncertainties about efficacy. Vitamin D3 (VD3; cholecalciferol) treatment prior to PDT improves BCC responses in mice. A prospective, double-blind, crossover clinical trial [NCT03467789] was designed to test whether oral VD3 pretreatment enhances BCC response to blue light PDT. Participants received 3 PDT treatments (20% ALA, 4 h; 417 nm, 30 min) 2 months apart. High-dose VD3 or placebo was administered prior to each of the first two PDT sessions. Lesions were recorded with a 3D digital camera to allow software-assisted tumor volume analysis. Treatment-resistant tumors were biopsied at the final visit. To date, 24 patients and 128 BCCs have been analyzed. Two-thirds (70%) of all lesions cleared completely after PDT. Of the 30% of tumors that failed to clear, all except one superficial BCC were either nodular, micronodular, adenoid, or infiltrative subtypes. To assess the ability of neoadjuvant VD3 to potentiate PDT efficacy, we evaluated all available lesions to determine their relative volume reduction after VD3+PDT and placebo+PDT. Tumors that fulfilled a prediction that shrinkage (% volume reduction) would be greater after VD3+PDT compared to placebo+PDT were scored as "Yes". In our analysis, 15 patients scored "Yes" and 6 scored "No". This >2-fold difference provides preliminary evidence that neoadjuvant VD3 augments therapeutic responsiveness to PDT for many BCC tumors. PDT may be an effective treatment for BCC, especially superficial BCC. Oral VD3 given prior to PDT represents a novel and safe combination approach.

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A single-arm, prospective clinical study of blue light phototherapy as a novel treatment for Grover's Disease and psoriasis vulgaris.

M. O. Olagbenro[1], D. Myers[1], S. Ravi[1], S. Xu[1], J. R. Walter[2]
[1]*Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States,* [2]*Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States*

Unlike ultraviolet radiation, blue light (380 nm to 500 nm) is non-ionizing representing a potentially safer and more accessible phototherapeutic modality. We investigated the feasibility, tolerability, and early efficacy of whole-body blue light for the treatment of psoriasis vulgaris and Grover's disease in a single-arm, prospective, open-label clinical study. A total of 11 patients with Grover's disease (mean baseline lesion count 27.4) and 9 patients with psoriasis (mean baseline Psoriasis Area and Severity Index (PASI) 6.1) completed a 2-week washout of all topical and systemic therapies and subsequently underwent whole body blue light phototherapy treatment for 15 sequential treatments over five weeks for 18 minutes using the DUSA BLU-U device. Cumulative dosage of light received was 160 J/cm^2. Clinical status was assessed by lesion count, PASI, and clinical photography before, during (midpoint), and after treatment conclusion. In addition, both the Dermatology Life Quality Index (DLQI) and 12-item pruritus severity score (ISS) were assessed pre- and post-treatment. Wilcoxon signed rank and paired t-tests were used based on normality with a significance level of p<0.05 (Stata v14.2). For Grover's disease patients, lesion count decreased from a mean of 27.4 (95% CI 9.2-45.6) pre-treatment to a mean of 5.6 (95% CI 2.5-8.7) post-treatment (p=0.007) and ISS decreased from a mean of 7.8 pre-treatment to 5.1 (95% CI 3.5-6.8) post-treatment (95% CI 0.4- 4.9, p=0.03). For psoriasis patients, there were no significant changes in ISS or PASI. Neither Grover's disease nor psoriasis patients exhibited a significant change in DLQI. Patients with Grover's disease may benefit from whole blue light therapy as an alternative to topical or systemic treatments as evidenced by a decrease in both lesion count and ISS. Future randomized clinical trials are needed.

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Psoriasis resolution in challenging body areas with ixekizumab: Response trajectories by patient profile clusters over time

A. Egeberg[1], A. Blauvelt[2], G. Gallo[3], K. See[3], Y. Chen[3], R. B. Warren[4]
[1]Department of Dermatology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark, [2]Oregon Medical Research Center, Portland, Oregon, United States, [3]Eli Lilly and Company, Indianapolis, Indiana, United States, [4]Dermatology Centre, Salford Royal NHS Foundation Trust, Manchester NIHR Biomedical Research Centre, University of Manchester, Manchester, United Kingdom

To identify patients that benefit most from ixekizumab (IXE), unsupervised machine learning applied to Psoriasis Area and Severity Index (PASI) improvement was used to define IXE-response clusters in patients with psoriasis. Using these identified clusters based on PASI, responses in challenging body areas (CBA) as well as in Static Physicians Global Assessment (sPGA) were assessed through 52 weeks. These analyses included patients treated with on-label doses of IXE pooled from two Phase 3 clinical trials, UNCOVER-31 and IXORA-S2 .Response trajectories over 52 weeks were summarized for sPGA(0,1)/sPGA(0); CBA areas of Nail Psoriasis Severity Index 0 [NAPSI(0)], Psoriasis Scalp Severity Index 0 [PSSI(0)], and Palmoplantar Psoriasis Area and Severity Index 100 [PPASI(100)]. Two clusters labelled as "Rapid and Stable (RAS)" and "Moderate" were identified. An "Ultimate" sub-group was further distinguished within the RAS cluster, for patients with an even faster and more stable response to IXE. In the RAS and Ultimate clusters, IXE demonstrated marked and sustained skin clearance as per sPGA (including psoriasis resolution) and in all assessed CBA compared with the Moderate cluster. Patients in the RAS and Ultimate clusters showed a higher and earlier response for PSSI(0), PPASI(100), and sPGA(0,1) by Week 8, with responses maintained through Week 52. Rapid and stable complete clearance, including resolution of nail, scalp, and palmoplantar psoriasis, is achievable with IXE for most patients, especially for those identified in the RAS and Ultimate clusters. References: 1. Blauvelt, A., et al. J Am Acad Dermatol, 2021. 85(2):360-368 2. Paul, C., et al. J Am Acad Dermatol, 2019. 80(1):70-79

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Comparison of subcision vs subcision-suction for treatment of acne scars: A split-face randomized controlled trial

N. Anvery, M. A. Dirr, R. Christensen, M. Alam
Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Surgical treatment with subcision is a standard therapy for acne scars. Other resurfacing procedures, including suctioning with microdermabrasion, also offer clinical improvement. This randomized, split-faced study sought to compare the effectiveness of subcision with suctioning to subcision alone for the treatment of rolling acne scars. Patients were randomized to receive subcision alone on one side of the face, and subcision with suction using a microdermabrasion device on the opposite side. Suction treatment continued daily, except weekends, for a total of two weeks. Patient photos were collected at baseline, 1- and 4-months to assess acne scarring via the Acne Severity Scale (AS), Acne Scar Improvement Grading Scale (ASIGS), and the Quantitative Global Scarring Grades (MQGSG) scale. Adverse events and patient preferences were also collected. Of 18 enrolled participants, 14 completed the study. While there was no significant difference in AS scores as assessed by dermatologists or participants between treatment types, AS scores were significantly lower for subcision (1.8, dermatologist: p=0.02; 2.0, patient: p=0.005) and subcision-suction (1.9, dermatologist: p=0.03; 2.0, patient: p=0.002) at 4-months compared to baseline. Participant ASIGS scores at 1-month were higher for subcision-suction (37% improvement) as compared to subcision (23%; p=0.04). Dermatologist MQGSG scores at 1-month were lower for subcision-suction (6.2) as compared to baseline (6.5; p=0.04). Treatment groups did not differ in incidence of adverse events. More patients preferred subcision-suction (43%) over subcision (21%). Subcision-suction appears to provide a clinical advantage in acne scar reduction compared to subcision alone at 1-month, but this is not maintained at 4 months. Further studies may clarify how and if the relative benefit of subcision-suction may be made more durable.

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Effects of ruxolitinib cream on pruritus in black patients with atopic dermatitis

L. F. Eichenfield[7], L. F. Stein Gold[1], K. K. Brar[2], Z. C. Chiesa Fuxench[3], J. I. Silverberg[4], M. E. Venturanza[5], H. Kallender[5], J. Gao[5], J. C. Szepietowski[6]
[1]*Henry Ford Health System, Detroit, Michigan, United States,* [2]*New York University Grossman School of Medicine, New York, New York, United States,* [3]*University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [4]*George Washington University, Washington, District of Columbia, United States,* [5]*Incyte Corporation, Wilmington, Delaware, United States,* [6]*Wroclaw Medical University, Wroclaw, Poland,* [7]*University of California San Diego, San Diego, California, United States*

Atopic dermatitis (AD) is an inflammatory skin disease that has phenotypic differences across race and can be more severe in Black patients. In two phase 3 identical design studies (TRuE-AD1/TRuE-AD2), patients (≥12 years old with AD for ≥2 years, Investigator's Global Assessment [IGA] score 2/3, 3%–20% affected body surface area) were randomized (2:2:1) to twice-daily 0.75% or 1.5% ruxolitinib (Janus kinase [JAK]1/JAK 2 inhibitor) cream or vehicle for 8 weeks. Here we describe the effect of ruxolitinib cream on itch in Black patients using pooled data from the 2 studies (n=292). Mean itch numerical rating scale (NRS) score at baseline was 5.3/5.4 for ruxolitinib cream (0.75%/1.5%) and 5.7 for vehicle. Reductions in mean itch NRS score with ruxolitinib cream (0.75%/1.5%) were evident within approximately 12 hours of first application (–0.6/–0.7 vs –0.2 for vehicle), with statistically significant reductions by Day 4 vs vehicle (–1.4/–1.6 vs –0.6; both P<0.05). For those with baseline itch NRS ≥4 (n=187; 64.0%), more patients achieved ≥4-point itch NRS improvement vs vehicle by Day 2 (6.1%/16.4% vs 0%); this increased to 15.9%/26.6% vs 3.0% on Day 7 and 30.1%/43.2% vs 17.5% at Week 8 (P=0.212/P=0.009). More patients applying 0.75%/1.5% ruxolitinib cream vs vehicle reported no days of itch per question 1 of the Patient-Oriented Eczema Measure (POEM) at Week 2 (19.0%/19.4% vs 5.3%); this increased at Week 8 (34.0%/30.8% vs 12.2%). In summary, ruxolitinib cream monotherapy over 8 weeks was associated with rapid and considerable itch relief in Black patients with AD.

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Ixekizumab citrate-free formulation: Results from 2 clinical trials

S. Chabra[1], B. Gill[2], G. Gallo[3], D. Zhu[3], C. Pitou[3], C. Payne[3], A. Accioly[3], L. Puig[4]
[1]Texas Arthritis Center, El Paso, Texas, United States, [2]Complete Dermatology, Houston, Texas, United States, [3]Eli Lilly and Company, Indianapolis, Indiana, United States, [4]Hospital de la Santa Creu i Sant Pau, Barcelona, Catalunya, Spain

Subcutaneous (SC) injection is a common route of drug administration; however, injection site pain (ISP) might create a negative patient experience. We evaluated ISP, bioequivalence, and overall safety of a new citrate-free (CF) formulation of ixekizumab, a high-affinity monoclonal antibody that selectively targets interleukin-17A. Two phase 1, single-blind studies were conducted in healthy participants. The crossover Study A (NCT03848403) evaluated pain intensity on injection as measured by Visual Analog Scale of Pain (VAS) scores. Subjects (N=70) were randomized 1:1:1 at the beginning to 3 possible treatment sequences and received a 1mL SC injection of the 3 formulations sequentially in the abdomen on Days 1, 8, and 15, respectively. A mixed-effects repeated measures analysis model was used to analyze VAS score by time post-injection. CF formulation 1 was selected for Study B (NCT04259346), where the primary objective evaluated bioequivalence of a single 80mg dose of CF formulation 1 compared to the commercial formulation. Subjects (N=245) were randomized 1:1 to either commercial or CF formulation and received a single SC injection into the abdomen, arm, or thigh. Primary endpoint was achieved in both studies. In Study A, least squares mean (LSM) difference of VAS scores immediately post injection between commercial (n=61) and CF formulation 1 (n=63) was -21.69 ($p<0.0001$), indicating a lower degree of pain associated with CF formulation 1. In Study B, bioequivalence of the CF formulation was established as 90% CIs for the ratio of geometric LSM AUC(0-tlast), AUC(0-∞), and Cmax between treatments were contained within the prespecified limits of 0.8 and 1.25. Except for less ISP in the CF formulation, overall safety profile was comparable. Ixekizumab CF formulation proved to be bioequivalent, was associated with less ISP and no other notable differences in the safety profile compared to the current commercial formulation.

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High-level stabilized retinol serum balances bioactivity and dermal tolerance

E. Zaleski, M. Chang, D. Meza, D. Miller, R. Parsa, V. Rivera, J. Snell, N. Tierney, S. Walsh
Johnson & Johnson Consumer Companies Inc, Skillman, New Jersey, United States

Retinol, an established topical ingredient for improving signs of photo-aging, is often limited in concentration due to the potential for low-level visible and sensory signs of irritation. A new anhydrous, 0.5% stabilized retinol serum was developed, balancing bioactivity and tolerability. This patented formula uses the Relative Polarity Index (RPI) between skin, retinol, and emollient ratios, allowing for controlled bio-delivery of retinol to skin. The product also includes skin-smoothing emollients, and antioxidants to stabilize the retinol. Retinol bioactivity and tolerability were first evaluated through gene and protein expression using human skin explants. Gene expression results indicated significant induction of genes with anti-aging benefits, such as cellular retinoic-acid binding protein-2 (CRABP2), a highly sensitive marker of retinoid bioactivity, Heparin-binding EGF-like growth factor (HBEGF), and hyaluronan synthase-3 (HAS3). Additionally, minimal to non-significant induction of IL-8, an inflammatory irritation marker, was observed compared to untreated controls. Histology results also demonstrated significantly increased collagen production. To further evaluate irritation potential, a clinical modified cumulative patch model was developed to differentiate irritation of retinol products. The new 0.5% retinol serum was found to have significantly less irritation ($p<0.05$) versus a competitive in-market 0.5% retinol formulation. A 4-week clinical in-use test was performed to assess the facial tolerance and self-perceived efficacy of the serum. Study results concluded daily use was well-tolerated, with no statistically significant increases ($p<0.05$) in tolerance parameters. Moreover, self-perception questions yielded positive results for improvements on visible aging attributes, confirming our *ex vivo* results. This new 0.5% retinol serum delivers bioactive retinol to target advanced visible signs of aging, without the increased irritation.

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Risankizumab (RZB) for active psoriatic arthritis (PsA): Integrated subgroup analysis from 2 double-blind, placebo-controlled, phase 3 studies (KEEPsAKE 1 and KEEPsAKE 2)

J. Merola[1], K. Duffin[2], B. Padilla[3], Z. Xue[3], H. Photowala[3], B. Kaplan[3], I. McInnes[4]
[1]Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States, [2]University of Utah Health, Salt Lake City, Utah, United States, [3]AbbVie Inc, North Chicago, Illinois, United States, [4]University of Glasgow, Glasgow, Glasgow, United Kingdom

Objective: Report proportion of patients (pts) with active PsA treated with RZB vs placebo (PBO) who achieved ≥20% improvement in American College of Rheumatology criteria (ACR20) by baseline demographics and by concomitant or prior medication use subgroups. RZB specifically inhibits the p19 subunit of cytokine IL-23. KEEPsAKE 1 (NCT03675308) and KEEPsAKE 2 (NCT03671148) are ongoing, multicenter, randomized, double-blind, PBO-controlled, phase 3 studies. Pts with active PsA with an inadequate response or intolerance to conventional synthetic disease-modifying, anti-rheumatic drug (csDMARD; KEEPsAKE 1 and 2) and/or biologic therapy (KEEPsAKE 2) received RZB 150 mg or PBO (1:1). The primary endpoint was the proportion of pts achieving ≥20% improvement in ACR criteria (ACR20) at week 24. In KEEPsAKE 1 (RZB, n=483; PBO, n=481) and KEEPsAKE 2 (RZB, n=224; PBO, n=219), baseline demographics and characteristics were generally balanced between treatment groups. In this integrated analysis, a greater proportion of pts receiving RZB vs PBO achieved ACR20 at week 24, regardless of age (<65 years, ≥65 years, ≥65 to <75 years, ≥75 years), sex, body mass index (<25 kg/m2, ≥25 to <30 kg/m2, ≥30 kg/m2), race (White, non-White), PsA duration (≤5 years, >5 to ≤10 years, >10 years), baseline hs-CRP (<3 mg/L, ≥3 mg/L), concomitant csDMARD at baseline (any csDMARD, any methotrexate, none), or prior biologics use (yes, no). The proportion of RZB-treated pts who achieved ACR20 was generally similar across most assessed demographic or prior treatment subgroups. No new safety concerns were observed with RZB. RZB demonstrates efficacy vs PBO for active PsA as shown by greater proportions of pts achieving ACR20 at week 24, regardless of baseline demographics, concomitant csDMARD use at baseline, or prior biologic use.

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Improvements in GPPGA score in patients experiencing a generalized pustular psoriasis (GPP) flare: Effisayil 1 study results

M. Anadkat[9], M. Lebwohl[1], B. Elewski[2], U. Mrowietz[3], S. Imafuku[4], J. Xu[5], L. Ling[6], M. Quaresma[7], C. Thoma[7], H. Bachelez[8]
[1]Icahn School of Medicine, Mount Sinai, New York, New York, United States, [2]University of Alabama, Birmingham, Alabama, United States, [3]Psoriasis Center, University Medical Center Schleswig-Holstein, Kiel, Germany, [4]Fukuoka University, Fukuoka, Japan, [5]Huashan Hospital, Shanghai, China, [6]Boehringer Ingelheim Investment Co. Ltd, Shanghai, China, [7]Boehringer Ingelheim International GmbH, Ingelheim, Germany, [8]Hôpital Saint-Louis, Paris, France, [9]Washington University School of Medicine, St. Louis, Missouri, United States

GPP is a rare autoinflammatory disease. In Effisayil 1 (NCT03782792), spesolimab, an anti-IL-36 receptor antibody, rapidly cleared skin pustules in patients with a GPP flare after 1 week. We assessed the proportion of patients with clinical improvements based on GPP Physician Global Assessment (GPPGA) score over 12 weeks (intention-to-treat analysis). Patients (N=53) were randomized 2:1 to receive single IV spesolimab 900 mg or placebo (PBO), could receive open-label (OL) spesolimab on Day (D)8 if symptoms persisted, and another up to Week (W)12 for a new flare. At W1, 79.4% (27/34) and 67.6% (23/34) of patients on spesolimab had ≥1- or ≥2-point improvement from baseline (BL) in GPPGA pustulation subscore vs 50.0% (9/18) and 22.2% (4/18) on PBO. 73.5% (25/34) and 52.9% (18/34) patients on spesolimab vs 38.9% (7/18) and 16.7% (3/18) on PBO had ≥1- or ≥2-point improvement in GPPGA total score, respectively. From D8, 40.0% (14/35) and 88.9% (16/18) of patients in spesolimab and PBO arms received ≥1 OL spesolimab. By W12, GPPGA pustulation subscore improved by ≥1 or ≥2 points from BL in 100% (32/32) and 96.9% (31/32) on spesolimab; all patients randomized to PBO who received ≥1 OL spesolimab had a ≥2-point improvement. All patients had a ≥1-point improvement in GPPGA total score at W12, with a ≥2-point improvement in 87.5% (28/32) and 93.3% (14/15) initially randomized to spesolimab or PBO. Spesolimab provided sustained clinical improvements up to W12 in patients with a GPP flare. All patients initially on PBO saw clinical improvement upon treatment with spesolimab.

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Is it safe to discontinue immunotherapy after a response in merkel cell carcinoma?

L. Tachiki[1], L. Zawacki[1], D. Hippe[2], Y. Moshiri[1], N. Alexander[1], T. Akaike[1], C. Doolittle-Amieva[1], T. Pulliam[1], L. Zaba[3], S. Bhatia1, P. Nghiem[1]
[1]University of Washington Department of Medicine, Seattle, Washington, United States, [2]Fred Hutchinson Cancer Research Center, Seattle, Washington, United States, [3]Stanford Medicine, Stanford, California, United States

Immunotherapy (IMTX) has significantly improved outcomes for patients with advanced Merkel cell carcinoma (MCC). However, data on the durability of response after IMTX discontinuation is limited. We retrospectively assessed 170 persons with advanced MCC treated with a first-line anti-PD-(L)1 agent to determine recurrence rates in patients who discontinue IMTX after complete (CR) or partial (PR) responses to therapy. Reasons for discontinuation were classified as elective or due to toxicity. Of 170 patients, 104 (61%) had objective responses and 66 (39%) did not. Among IMTX responders, 57 patients discontinued IMTX (electively or due to toxicity) and 47 patients were still on. At the 2-year timepoint after initiating immunotherapy, the fraction of patients whose disease progressed was 37% for those who previously discontinued IMTX as compared to 20% who continued IMTX, HR = 2.11 (95% CI: 0.97-4.57), p=0.059. Of the 57 responders who discontinued IMTX, 27 (47%) were due to toxicity and 30 (53%) were elective. Median IMTX duration was shorter in those who discontinued due to toxicities versus electively (median: 273 vs. 561, p = 0.002). The recurrence rate was >2 times higher for patients who discontinued due to toxicity (56%) vs. electively (19%) at 2 years after discontinuation, HR= 2.59, 95% CI: 1.02-6.59, p = 0.046. The recurrence rate was numerically higher for patients with PR (56%) vs CR (27%) at 2 years after IMTX discontinuation, HR=1.88, 95% CI: 0.77-4.6, p = 0.17. Our findings are consistent with an analysis of German MCC outcomes showing there is a significant risk of recurrence after IMTX discontinuation. The risk of disease recurrence is higher in patients who discontinue IMTX for adverse events or if best overall response was less than CR.

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Ixekizumab demonstrates comprehensive psoriasis clearance in patients with moderate-to-severe psoriasis with scalp, nail, and/or palmoplantar involvement: Uncover-1, -2 trials through 5 years

A. B. Gottlieb[1], J. Merola[2], N. Somani[3], B. Konicek[3], K. See[3], M. McKean-Matthews[3], G. Gallo[3], P. Rich[4]
[1]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States, [3]Eli Lilly and Company, Indianapolis, Indiana, United States, [4]Oregon Dermatology & Research Center, Portland, Oregon, United States

Involvement of sensitive or challenging body areas is an important treatment consideration since patients with psoriasis in these areas can have disproportionately greater burden of disease. We assess comprehensive clearance by measuring the ability of ixekizumab to achieve complete skin clearance (PASI 100) up to 5 years when multiple challenging body areas (scalp, nails, palmoplantar [pp] regions) are involved. Data were integrated from UNCOVER-1 (NCT01474512) and UNCOVER-2 (NCT01597245) trials for patients with psoriasis treated with ixekizumab per label dosing. PASI 100 was assessed for the subset of patients who achieved site-specific complete clearance at week 60 among the integrated UNCOVER-1 and -2 dataset: nail (NAPSI 0, N=123); scalp (PSSI 0, N=189); pp (PPASI100, N=57); nail & scalp (NAPSI 0 + PSSI 0, N=115); nail & pp (NAPSI 0 + PPASI100, N=50); scalp & pp (PSSI 0 + PPASI100, N=55); or nail, scalp & pp (NAPSI 0 + PSSI 0 + PPASI100, N=50). Data are observed. PASI 100 at weeks 60 and 264 was achieved among ixekizumab-treated patients who achieved site-specific complete clearance as follows: nail (74% [57/77], 48% [26/54]); scalp (71% [118/167], 71% [70/99]); pp (51% [27/53], 43% [12/28]); nail & scalp (78% [53/68], 59% [23/39]; nail & pp (72% [18/25], 33% [5/15]); scalp & pp (61% [26/43], 61% [11/18]); or nail, scalp & pp (72% [18/25], 63% [5/8]) involvement, respectively. Ixekizumab treats the totality of a patient's psoriasis in a comprehensive manner for up to 5 years, even when multiple challenging body areas are involved concurrently.

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Berdazimer 10.3% gel, a nitric oxide-releasing topical medication for molluscum contagiosum, triggers BOTE (Beginning Of The End) inflammation and accelerates resolution

T. Maeda-Chubachi[1], M. Cartwright[1], A. Paller[2]
[1]Novan, Inc, Durham, North Carolina, United States, [2]Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

The beginning-of-the-end "BOTE" sign describes the inflammation (with intense erythema and variable crust, pustule, scale) that predicts imminent resolution of molluscum contagiosum (MC). Posthoc analyses of 2 phase 3, 12-week, randomized, double-blind vehicle-controlled studies, B-Simple1&2, provided initial evidence that SB206 (berdazimer 10.3% gel) may trigger BOTE and shorten MC duration. We conducted a similar, larger phase 3 study, B-Simple4, and prospectively stratified patients based on baseline (BL) BOTE status (BOTE+ vs BOTE-). A pre-defined analysis of the association between the presence of BL BOTE+ lesion and MC lesion reduction including complete clearance is presented. Treatment assignment was well balanced within BL BOTE status: of 447 patients randomized to vehicle, 50.3% were BOTE+ and 49.7% were BOTE-; of 444 patients randomized to berdazimer (active), 49.3% were BOTE+ and 50.7% were BOTE-. MC lesion counts at Week 12 decreased from BL in both treatment groups at a higher level for BOTE+ vs BOTE- patients (mean+SE); vehicle arm: 44.5+4.5% BOTE+ vs 21.2+4.6% BOTE- (p<0.0001), active arm: 65.6+4.2% BOTE+ vs 59.9+4.2% BOTE- (p=0.2325). At Week 12, a similar pattern was observed for patients achieving complete clearance; vehicle arm: 56 (25.0%) BOTE+ vs 32 (14.3%) BOTE- (p=0.0043), active arm: 85 (38.8%) BOTE+ vs 59 (26.2%) BOTE- (p=0.0043). Of all patients that were BOTE- at BL, a higher proportion of patients in the active arm became BOTE+ by Week 2 vs the vehicle arm: 141 (33.8%) vs 75 (18%). Further, a higher proportion of patients failed to initiate BOTE during the study in the vehicle arm vs the active arm; 73 (16.3%) vs 33 (7.4%). Median Kaplan-Meier estimates of time to complete clearance (days) was 93 (active) vs 100 (vehicle) (p<0.0001). This study suggests that BOTE is an indicator of MC resolution and that berdazimer 10.3% gel promotes earlier BOTE initiation, resulting in faster MC resolution.
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Collagen 7 (C7) protein replacement therapy (PTR-01) durably reduces wound size and symptoms in patients with recessive dystrophic epidermolysis bullosa (RDEB)

A. Bruckner[4], J. Tang[5], W. Chung[1], K. Morel[1], M. Chen[2], D. Woodley[2], D. Keene[6], K. Peoples[4], M. Barriga[5], J. Carroll[1], L. Levin[1], S. Ravindran[3], M. Mangone[3], D. Ramsdell[3], H. Landy[3]
[1]Columbia University Irving Medical Center, New York, New York, United States, [2]University of Southern California Keck School of Medicine, Los Angeles, California, United States, [3]Phoenix Tissue Repair, Boston, Massachusetts, United States, [4]U. Colorado School of Medicine, Aurora, Colorado, United States, [5]Stanford University School of Medicine, Stanford, California, United States, [6]Shriners Hospital for Children, Portland, Oregon, United States

We treated 6 patients (13-30 yrs of age) with confirmed RDEB in a multicenter, open-label Phase 2 study. PTR-01 3.0 mg/kg IV was given weekly x4, then every other week x7 with follow up assessments 1 and 3 months after treatment. Response was defined as improvement of ≥2 points on a 7-point wound impression scale (WIS) in a majority of lesions. Multiple secondary outcomes and safety were assessed. Five patients completed the study, 4 of whom were considered responders on the WIS. Over 75% of lesions (both chronic and recurrent) were closed by 50% or more at the end of treatment and remained so for at least 1 month after treatment. Wound surface area over time indexed to baseline (AUCi) showed a median decline of 46% at Day 43 of treatment in comparison to 25% in a control group and declined further to 70% at the end of treatment. Surveys of EB symptoms, impact, and patient and investigator global impressions, which comprised both cutaneous and systemic manifestations, showed rapid and persistent improvements during treatment. NC2 staining of skin biopsies showed extensive basement membrane incorporation of PTR-01 though no increase in anchoring fibrils by immunoelectron microscopy. Elevated skin fibrosis markers were markedly reduced. There were no serious or unexpected adverse events. One patient withdrew at Day 50 for lack of efficacy in association with high anti-C7 antibodies. Treatment with PTR-01 resulted in durable effects on both cutaneous and systemic manifestations of RDEB and reduced fibrosis markers in a majority of patients.

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Dupilumab treatment restores skin barrier function in adult and adolescent patients with moderate-to-severe atopic dermatitis

E. Goleva[1], R. Bissonnette[2], E. Berdyshev[1], P. Jurvilliers[3], I. Agueusop[4], A. Praestgaard[5], N. A. Levit[6], A. Rossi[5], A. Zhang[5]
[1]National Jewish Health, Denver, Colorado, United States, 2Innovaderm Research, Montreal, Quebec, Canada, [3]Sanofi SA, Chilly-Mazarin, Île-de-France, France, [4]Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany, [5]Sanofi Genzyme, Cambridge, Massachusetts, United States, 6Regeneron Pharmaceuticals Inc, Tarrytown, New York, United States

Atopic dermatitis (AD) is characterized by skin barrier dysfunction, for which the abnormal stratum corneum (sc) ceramide composition serves as one of the driving factors. Here, we assess effect of dupilumab on the regulation of skin barrier function and sc ceramide composition in patients with AD. BALISTAD (NCT04447417) was an open-label trial, in which Trans-epidermal Water Loss (TEWL) was assessed via skin tape strip (STS) samples from lesional skin of 26 patients with AD treated with dupilumab and from normal skin of 26 healthy volunteers over 16 weeks. Quantitative lipidomic analysis of STS samples was conducted by liquid chromatography tandem mass spectrometry. Wilcoxon signed rank tests were performed to assess the change in TEWL from baseline in response to dupilumab treatment. The median TEWL area under curve up to 10 STS in AD lesions was significantly reduced from baseline starting at Week 2 ($P < 0.0001$) and sustained through Week 16 ($P < 0.0001$). Dupilumab treatment significantly increased levels of total C18 esterified omega-hydroxy fatty acid sphingosine ceramides (EOS-CER, long-chain lipids; $P < 0.0001$), and decreased levels of total non-hydroxy fatty acid sphingosine ceramides (NS-CER, short-chain lipids; $P < 0.0001$) in AD lesional skin at Week 16. In conclusion, dupilumab treatment significantly improved skin barrier and normalized composition of skin barrier lipids in adults and adolescents with AD.

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Dupilumab reduces biomarkers indicative of type 2 inflammation in children aged ≥ 6 months to < 6 years with moderate-to-severe atopic dermatitis

A. Paller[1, 2], E. Guttman-Yassky[3, 4], M. Boguniewicz[5, 6], Y. Sun[7], K. Wolfe[8], M. Dillon[7]
[1]Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]Ann and Robert H Lurie Children's Hospital of Chicago, Chicago, Illinois, United States, [3]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [4]The Rockefeller University, New York, New York, United States, [5]National Jewish Health, Denver, Colorado, United States, [6]University of Colorado Denver School of Medicine, Denver, Colorado, United States, [7]Regeneron Pharmaceuticals Inc, Tarrytown, New York, United States, [8]Sanofi, Bridgewater, New Jersey, United States

Dupilumab was previously shown to reduce thymus and activation-regulated chemokine (TARC/CCL17) and total IgE levels in multiple type 2 inflammatory diseases, including atopic dermatitis (AD). Here, we assess the effects of dupilumab treatment on these biomarkers of type 2 inflammation in children aged 6 months – < 6 years with moderate-to-severe AD. In LIBERTY AD INFANT/PRE-SCHOOL (NCT03346434 part B), a phase 3, double-blind trial, children aged ≥ 6 months – < 6 years with moderate-to-severe AD inadequately controlled with topical therapies were randomized 1:1 to subcutaneous dupilumab (200 mg if baseline weight ≥ 5 – < 15 kg, 300 mg if ≥ 15 – < 30 kg) or placebo every 4 weeks for 16 weeks. All patients received standardized low-potency topical corticosteroids. Serum for biomarker analysis was collected at baseline, Week 4, and Week 16. Baseline median serum TARC and total IgE levels for dupilumab/placebo groups (n = 83/79) were 3,295/3,190 pg/mL and 2,190/3,240 kU/L, respectively. After 16 weeks of treatment, median % change from baseline in dupilumab/placebo was –83.1%/–12.8% for TARC and –71.2%/28.1% for total IgE (both $P<0.0001$). Similar reductions were observed in dupilumab- vs placebo-treated patients for all tested serum allergen-specific IgEs (peanut, egg white, soybean, Dermatophagoides farinae and Dermatophagoides pteronyssinus). Dupilumab significantly reduced serum TARC and total and allergen-specific IgEs in children aged 6 months – < 6 years with moderate-to-severe AD vs placebo-treated controls, reflecting reduction of systemic type 2 inflammation.

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Sleep improvement with dupilumab in adults with moderate-to-severe atopic dermatitis: Results of the DUPISTAD study

J. Merola[1], A. Chiou[2], P. Foley[3], M. Ardeleanu[4], J. Wu[5], Z. E. Ozturk[5]
[1]Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Stanford University School of Medicine, Stanford, California, United States, [3]Skin Health Institute, Carlton, Victoria, Australia, [4]Regeneron Pharmaceuticals Inc, Tarrytown, New York, United States, [5]Sanofi Genzyme, Cambridge, Massachusetts, United States

Background: Patients with atopic dermatitis (AD) often report sleep disturbance due to nighttime itching and scratching, leading to daytime fatigue and impaired quality of life. The DUPISTAD phase 4 randomized double-blinded placebo-controlled study (NCT04033367) evaluated the impact of dupilumab treatment on sleep in adults with moderate-to-severe AD. Methods: Adult patients with an Eczema Area and Severity Index (EASI) ≥ 12, Peak Pruritus Numerical Rating Scale (NRS) ≥ 3 and sleep NRS ≤ 5 were randomized 2:1 to dupilumab 300 mg every 2 weeks (q2w) or placebo for 12 weeks (W12); both treatment groups were permitted to apply concomitant topical corticosteroids as needed. The primary endpoint was percentage change from baseline to W12 in sleep quality, assessed on a 0–10 NRS, where 0 was "worst possible sleep" and 10 was "best possible sleep," which was reversed for the analysis to measure sleep disturbance. Results: 127 patients received dupilumab 300 mg q2w, and 61 received placebo. Mean baseline SCORing Atopic Dermatitis (SCORAD) and EASI scores were well balanced between groups: SCORAD 64.7 vs 62.8, and EASI 26.2 vs 26.0). Sleep disturbance NRS significantly improved in dupilumab-treated patients at W12 vs placebo (least squares mean difference [LSMD] –15.1%; $P < 0.001$). Further, SCORAD sleep Visual Analog Scale was also significantly improved at W12 (LSMD –2.3, $P < 0.001$). Treatment-emergent adverse events occurred in 55.9% in the dupilumab group vs 67.2% with placebo. Serious adverse events occurred in 1.6% of patients in both treatment groups. Conclusion: In this study, dupilumab significantly improved sleep in adult patients with moderate-to-severe AD, with an acceptable safety profile.

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Oral difelikefalin improves itch and inflammatory biomarkers in atopic dermatitis subjects with moderate-to-severe pruritus

E. Guttman-Yassky[1], P. Facheris[1, 2], J. Correa Da Rosa[1], E. Del Duca[1], Y. Estrada[1], E. David[1], A. Pavel[3], S. Bose[1], J. Goncalves[4], K. Nograles[4], B. Kim[1], M. Lebwohl[1]
[1]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Italy, [3]The University of Mississippi, University, Mississippi, United States, [4]Cara Therapeutics, Inc., Stamford, Massachusetts, United States

Pruritus is the most frequent and burdensome atopic dermatitis (AD) symptom and can exacerbate disease via the itch-scratch cycle. Difelikefalin (DFK), a kappa-opioid receptor (KOR) agonist is approved for moderate-to-severe pruritus in adults with CKD undergoing hemodialysis. DFK blocks pruritic signaling through peripheral sensory neurons and may have anti-inflammatory activity. We tested the impact of DFK on pruritus-related and inflammatory transcriptomes of AD patients. In a phase 2 clinical study, subjects with AD and moderate-to-severe pruritus were randomized (1:1:1:1) to receive oral twice-daily DFK (3 doses) or placebo for 12 wk. A substudy (n=40) characterized effects of DFK on pruritus- and AD-related gene expression using baseline and wk 12 skin biopsies. Gene expression was measured using RNA-sequencing and TaqMan Low-Density Array qualitative polymerase chain reaction. Data from DFK groups were pooled. DFK altered expression of multiple individual pruritus- and AD-related genes. Gene set variation analysis confirmed downregulation of pruritus-related genes (eg, IL-31, TRPV2) and the Th2 pathway following 12 wk treatment with DFK, but not placebo. Changes in gene expression of pruritus- and immune-related genes significantly correlated with skin improvement for DFK-treated subjects. DFK downregulated expression of key genes implicated in pruritus and AD inflammation. Reports demonstrate an anti-pruritic effect of DFK in AD and other chronic itch conditions. DFK is a promising therapy for AD-related pruritus and may provide anti-inflammatory benefit by impacting the itch-scratch cycle.

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Clinical assessment of low level microcurrent of short duration in enhancing allantoin penetration and improving the appearance of skin cellulite, radiance, texture, and firmness

G. Diwakar[1], D. Kern[1], M. Riggs[1], Z. D. Draelos[2]
[1]Global Product Research and Development, Nu Skin Enterprises, Provo, Utah, United States, [2]Dermatology Consulting Services, PLLC, High Point, North Carolina, United States

Low level DC microcurrent is used to enable electrically conductive drug delivery in skin wounds and ulcerations. However, it is not clear from the literature whether very low level DC current of short-term duration can improve the delivery of active ingredients contributing to the improvement of skin appearance. We conducted a 12-week study in 30 females using a non-invasive negative DC microcurrent device to deliver allantoin from a topical serum followed by a moisturizer. Consistent areas on the upper thigh and upper arm were evaluated visually for improvement in skin cellulite, radiance, texture, and firmness. Subjects treated the upper arm and upper thigh with 375 microamps for 5 min with the topical serum three times weekly followed by post-device treatment with the moisturizer daily. Subjects were evaluated at baseline and weeks 1, 2, 4, 8, and 12. Cellulite was evaluated by measuring the circumference of upper thigh and upper arm and radiance, texture and firmness was assessed by dermatologist investigator grading and subject self-assessment during each visit on a 5 point ordinal scale. Additionally, at the end of study, 20 tape strips were collected from five randomly chosen subjects. Five tape strips from both treated and untreated areas of the upper arm and upper thigh were evaluated for accumulation of allantoin using LC-MS spectroscopy. Our results showed significant improvement in the appearance of cellulite, radiance, texture and firmness at 12 weeks when compared to baseline. Further, the amount of allantoin recovered by the tape stripping method increased over the background skin allantoin level in the early tape strips (1-5) but tended to decrease as the tape strips were removed from lower levels of the epidermis. Low level DC microcurrent of short duration was shown to enhance the skin appearance benefit of the topically applied allantoin.

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The impact of tailored delivery of education on engagement and disease severity: A randomized controlled trial among adults with psoriasis

C. Read[1, 2], J. F. Apperley[1], S. P. Hettiaratchy[1], S. P. Pourali[2], M. E. Jones[2], T. E. Saputera[2], A. W. Armstrong[2]
[1]Medicine, Imperial College London, London, London, United Kingdom, [2]Dermatology, University of Southern California, Los Angeles, California, United States

How the delivery of educational content may impact patient engagement and disease severity is rarely studied in adults with dermatoses. Delivery of education can be tailored such that a patient can choose how they receive information, or non-tailored, such that a patient does not choose how they receive information. Non-tailored delivery of health education may be associated with reduced patient engagement and worse disease severity. We conducted a 3-month randomized controlled study to evaluate the impact of tailored delivery of education on patient engagement and disease severity among U.S. adult patients with psoriasis. 134 patients were randomized 1:1 to receive either tailored or non-tailored delivery of education and associated questionnaires every 1.5 months. Patients in the tailored group could choose to receive their education through e-mail, SMS, WhatsApp, or Facebook. Patients in the non-tailored group received hardcopy. Each patient received weblinks to educational videos and associated questionnaires. The primary outcome was patient response rates as measured by click rates. The secondary outcomes were patient engagement as measured by PAM-13, AVA, and patient drop-out rates. Disease severity was assessed using PGA, BSA, and PASI. The average age was 48.8; 52% male; 63% white. Compared to patients in the non-tailored delivery group, patients in the tailored delivery group had a greater click rate (94% vs 79%; p<0.01), scored a high level of engagement on PAM-13 (95% vs. 75%; p<0.01), and had a lower patient drop-out (15% vs. 54%; p<0.001), but there was no difference in the proportions of patients who reported high patient experience of the videos (90% vs 87%; p=0.32) or the percentage change in PASI (8.86 vs 5.70; p=0.13). In conclusion, tailored delivery of education led to greater engagement but no difference in disease severity compared to non-tailored delivery of education.

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A novel end-to-end deep learning framework for skin lesion segmentation and classification in clinical images

X. He[1], Y. Wang[1], S. Zhao[2], X. Chen[2]
[1]School of Automation, Central South University, Changsha, Hunan, China, [2]Xiangya Hospital, Central South University, Changsha, Hunan, China

Currently, deep learning-based methods have obtained a series of successes in skin lesion analysis. However, most of the existing methods are designed for either lesion segmentation or classification, and ignore the potential benefits between the two tasks. Motivated by the above consideration, this paper proposes a novel end-to-end deep learning framework for skin lesion segmentation and classification. In the proposed framework, in addition to the two basic tasks (i.e., skin lesion segmentation and classification), we add an auxiliary task, i.e., the lesion edge prediction task. First, an edge enhancement module is proposed to utilize the edge prediction results to improve the performance of the segmentation task. Then, a lesion saliency module is proposed to mine useful segmentation information for a better classification. To verify the effectiveness of the proposed method, we have collected a skin lesion dataset at Xiangya Hospital, Central South University, including 1768 high-quality clinical images of five skin diseases. Each image in this dataset contains the label of diagnosis and the segmentation ground-truth. Experimental results on this dataset demonstrate the superiority of the proposed method against the existing segmentation, classification, and multi-task methods.

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A multi-task learning network for skin disease classification

W. Wang[1], Y. Wang[1], S. Zhao[2], X. Chen[2]
[1]School of Automation, Central South University, Changsha, Hunan, China, [2]Xiangya Hospital, Central South University, Changsha, Hunan, China

In recent years, convolutional neural networks (CNNs), due to their powerful end-to-end feature learning capabilities, have been widely used in medical aided diagnosis. In general, a dermatologist's diagnostic process first identifies the type of skin lesions, and then obtains the diagnosis of skin diseases according to the recognition of skin lesions. However, most of existing methods only focus on designing diverse neural networks to directly identify skin diseases, while ignoring the importance of the recognition of skin lesions. In this paper, a multi-task learning network is proposed for skin disease classification. In the proposed network, in addition to the skin disease classification task, multiple skin lesion classification tasks (e.g., cyst, plaque, and macule) are added to determine the types of skin lesions, thereby providing useful skin lesion information for skin disease classification. Specifically, we concatenate the features of skin lesion classification tasks with the original skin disease classification feature at the top of the network, and then use the concatenated feature for the final skin disease classification. To verify the effectiveness of the proposed network, we have collected a skin disease dataset at Xiangya Hospital, Central South University, including 8713 clinical images of five common skin diseases (i.e., basal cell carcinoma, melanoma, nevus, squamous cell carcinoma, and seborrheic keratosis). Preliminary experimental results show that the proposed multi-task learning network performs better than existing skin disease classification methods.

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A residual dense network for skin lesion segmentation in dermoscopy images

Y. Zhao[1], Y. Wang[1], S. Zhao[2], X. Chen[2]
[1]School of Automation, Central South University, Changsha, Hunan, China, [2]Xiangya Hospital, Central South University, Changsha, Hunan, China

Skin lesion segmentation in dermoscopic images is more challenging due to the irregular and blurred skin lesion boundaries, as well as the low visual contrast between the skin lesions and the surrounding normal tissues. This paper proposes a residual dense network for skin lesion segmentation in dermoscopy images. Compared with the existing mainstream image segmentation methods, we propose a novel residual dense module and increase the depth of the convolutional neural network, which not only makes the network easier to converge, but also enables each layer of the network to obtain sufficient information interaction. Furthermore, we design an improved cross-entropy loss to weight each class, thereby alleviating the problem of model background biased caused by the number of foreground pixels being far less than the number of background pixels. To verify the effectiveness of our method, we have constructed a new dermoscopy dataset at Xiangya Hospital, Central South University, containing 4327 dermoscopy images of 5 skin diseases (i.e., basal cell carcinoma, squamous cell carcinoma, seborrheic keratosis, melanoma, and nevus). Experimental results show that our method can obtain more accurate segmentation results compared with classical image segmentation methods. We believe that our method can lay a solid foundation for the establishment of skin lesion auxiliary diagnostic systems.

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New shampoo targeting dry dandruff: Evaluation program and results

M. Froliger
PFDC, Toulouse, France

Introduction: Dry dandruff (DD) is in the form of small, whitish flakes, not adherent to the scalp. Shampoo was developed for DD and formulated according to the requirements of 'conscious care' with 85% natural ingredients. Objective: Clinical studies were designed to assess its tolerance, efficacy and sensoriality with clinical, auto-assessment scoring, instrumental, pharmaco clinical methods and an in-use test. Material and methods: The *in vivo* studies were carried out on a total panel of 121 subjects corresponding to the target of the product. First, in use tolerance under dermatological control was investigated for 3 weeks (W). Efficacy and persistence were evaluated for 8 W to follow anti-dandruff-efficacy, scalp itching and discomfort and to measure hydration (HI), Trans Epidermal Water loss (TEWL) and pH of the scalp. Scalp microflora was analysed by NGS sequencing from swabs sampled after 2 and 4 W. The respect of skin barrier was evaluated on skin explants. Consumer use test of 3W was carried out to assess cosmetic acceptability and efficacy. Results: The results showed a very good tolerance and a decrease of dandruff, itching and discomfort scores. HI increased at W2. PH and TEWL did not change significantly. Regarding scalp microbiome, an increase of mycobiota diversity and significant change from W2 of bacteria population were observed. The respect for the integrity of the skin barrier was also shown. Cosmetic qualities and efficacy were appreciated. Conclusion: The new anti-dandruff shampoo displays a very good tolerance, anti-dandruff and soothing effects that are persistent while respecting skin barrier and a very good acceptability and efficay. The scalp microbiota is rebalanced, reflecting a strong relation between skin disorders and flora. All this evidence provides the consumer with a quality product that is well tolerated, effective and in line with expectations of naturality.

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New anti-dandruff shampoo targeting oily dandruff: Evaluation program and results

V. T Turlier
PF DCPC, Toulouse, France

Introduction/ aims: Before the launch of a dermo-cosmetic product, an evaluation program along 3 major axes (tolerance, efficacy, sensoriality) is conducted to check that the developed product has the expected properties. We present the results of RF0911I shampoo developed for oily dandruff (OD), formulated according to the requirements of 'conscious care', with 85% natural ingredients. Material and methods: The *in vivo* studies were done on a total panel of 121 people corresponding to the target of the product. In use tolerance under dermatological control was investigated for 3 weeks (W). Efficacy and persistence were assessed for 8 W by clinical, instrumental and pharmaco-clinical methods to follow anti-dandruff efficacy, pruritus, discomfort, hydration (HI), lipids (IL), pH of the scalp, and scalp microflora. A consumer use test of 3 W was done to assess cosmetic acceptability and perceived efficacy. The respect of skin barrier was evaluated on skin explants. Results: They showed a very good overall tolerance, a significant improvement in OD with a decrease in clinical signs, pH,an overall increase in HI,no change in IL, a decrease in the ratio Malassezia restricta/Cutibacterium acnes resulting from a rebalancing of the microflora. Except for pH, these changes were globally maintained 4 W after stopping RF0911I shampoo. The respect for the integrity of the skin barrier was also shown. The consumer test showed the good level of sensorial features and the perceived anti-dandruff and soothing effects, and the conscious care aspect was very well received. Conclusion: The new anti-dandruff shampoo RF0911I displays a very good overall tolerance, anti-dandruff and soothing effects that are persistent and a very good acceptability and perceived effectiveness. It rebalances the scalp microbiota and respects the skin barrier. All this evidence provides the consumer with a quality product that is well tolerated, effective and in line with their expectations of naturality.

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High-threshold responses to ruxolitinib cream in adolescents and adults with atopic dermatitis

A. Blauvelt[1], J. C. Szepietowski[2], K. Papp[3], E. Simpson[6], D. Sturm[4], H. Kallender[4], J. Gao[4], L. Kircik[5]
[1]Oregon Medical Research Center, Portland, Oregon, United States, [2]Wroclaw Medical University, Wroclaw, Poland, [3]K. Papp Clinical Research and Probity Medical Research, Waterloo, Ontario, Canada, [4]Incyte Corporation, Wilmington, Delaware, United States, [5]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [6]Oregon Health & Science University, Portland, Oregon, United States

Atopic dermatitis (AD) is a highly pruritic, inflammatory skin disease that can have a significant impact on patient quality of life. Ruxolitinib cream is a topical formulation of ruxolitinib, a Janus kinase (JAK) 1/JAK2 inhibitor. In two phase 3 AD studies of identical design (TRuE-AD1 [NCT03745638]/TRuE-AD2 [NCT03745651]), patients (≥12 years old with AD for ≥2 years, Investigator's Global Assessment [IGA] score 2/3, 3%–20% affected body surface area) were randomized (2:2:1) to twice-daily 0.75% or 1.5% ruxolitinib cream or vehicle for an 8-week, double-blinded, vehicle-controlled period. Here, we describe the effect of ruxolitinib cream on achievement of multiple high-threshold responses in patients with AD (itch numerical rating scale [NRS] score of 0/1 [range 0–10]; IGA score of 0/1 [clear/almost clear skin]; Dermatology Life Quality Index [DLQI] or the children's version [cDLQI] score of 0/1 [no effect of AD on quality of life]) using pooled data from the 2 studies. A total of 1208 patients were evaluated (vehicle/0.75% ruxolitinib cream/1.5% ruxolitinib cream, n=244/483/481). At Week 8, more patients applying 0.75%/1.5% ruxolitinib cream vs vehicle achieved itch NRS 0/1 (35.2%/42.8% vs 16.4%), IGA score 0/1 (55.1%/62.6% vs 20.1%), or DLQI/cDLQI score 0/1 (48.9%/53.8% vs 20.1%). Considerably more patients applying 0.75%/1.5% ruxolitinib cream achieved ≥2 responses at Week 8 vs vehicle (44.9%/54.9% vs 15.6%) or all 3 responses (22.4%/27.0% vs 6.6%). Overall, treatment with ruxolitinib cream over 8 weeks was associated with achievement of multiple high-threshold responses in patients with AD.

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Incremental improvements after switching from dupilumab (DUPI) to upadacitinib (UPA) in the Heads Up open-label extension (OLE) study

V. H. Prajapati[1], B. Glick[2], L. Spelman[3, 4], I. Figueras Nart[7], B. Calimlim[5], B. Ladizinski[5], T. Wu[5], Y. Liu[5], J. Davis[5], H. Aydin[5], B. Ehst[6]

[1]Skin Health & Wellness Centre, Dermatology Research Institute, and Probity Medical Research, University of Calgary, Calgary, Alberta, Canada, [2]Glick Skin Institute, Margate, Florida, United States, [3]Veracity Clinical Research, Brisbane, Queensland, Australia, [4]Probity Medical Research, Waterloo, Ontario, Canada, [5]AbbVie Inc, North Chicago, Illinois, United States, [6]Oregon Medical Research Center, Portland, Oregon, United States, [7]University of Barcelona, Hospital Universitari de Bellvitge, Catalunya, Barcelona, Spain

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by eczematous morphology and intense itch. Heads Up was a phase 3b, randomized, double-blind, double-dummy clinical trial comparing the efficacy and safety of DUPI vs UPA in adults with moderate-to-severe AD over 24 weeks; in the Heads Up OLE, all patients received UPA 30 mg orally once daily for an additional 52 weeks. This analysis characterized the incremental changes in Eczema Area and Severity Index (EASI) and Worst Pruritus Numerical Rating Scale (WP-NRS) scores during the Heads Up OLE in patients who switched from DUPI to UPA after 24 weeks of DUPI treatment. Among patients who did not achieve ≥75% improvement in EASI (EASI 75) with DUPI, 75.0% and 87.5% achieved EASI 75 with UPA after 4 and 16 weeks, respectively, while 0% and 2.0% of patients who achieved EASI 75 with DUPI did not maintain this response with UPA. Similar results were observed for EASI 90. Among patients with WP-NRS ≥4 at baseline who did not achieve WP-NRS improvement ≥4 with DUPI, 56.6% and 57.7% achieved WP-NRS improvement ≥4 with UPA after 4 and 16 weeks, respectively, while 3.7% and 8.8% of patients who achieved WP-NRS improvement ≥4 with DUPI did not maintain this response with UPA. Most patients with a suboptimal response (EASI <75 and WP-NRS improvement <4) to DUPI experienced greater skin clearance and itch reduction after switching to UPA. These are the first observations reported in patients receiving UPA following treatment with DUPI.

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Losartan treatment improves recessive dystrophic Epidermolysis bullosa

M. Pourani[1], H. Vahidnezhad[2], L. Youssefian[2], A. Rakhshan[1], B. Hajimoradi[1], F. Abdollahimajd[1], J. Uitto[2]

[1]Shahid Beheshti University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [2]Thomas Jefferson University, Philadelphia, Pennsylvania, United States

Recessive dystrophic epidermolysis bullosa (RDEB) manifests with blistering and erosions of the skin and mucous membranes due to mutations in COL7A1. The repetitive wounding and healing processes lead to extensive cutaneous scarring. The scarring is driven by inflammatory processes, particularly the TGF-b signaling pathways, resulting in deposition of the extracellular matrix, especially collagen. There is currently no effective or specific treatment for RDEB. Losartan, an angiotensin II type 1 receptor antagonist, is an inhibitor of TGF-b signaling. Previous preclinical studies with hypomorphic Col7A1 mice recapitulating features of RDEB have suggested that losartan may improve the clinical features of RDEB. In this case series, we assessed the effects of losartan on the clinical and histopathologic features in seven patients with RDEB; 3F/4M, age 18.1+9.1 years. The diagnosis was based on characteristic clinical features and the presence of biallelic loss-of-function mutations in COL7A1. Daily oral administration of losartan (0.7 mg/kg) for six weeks resulted in subjective improvement of the clinical features, as judged by the treating physicians and the patients, and the severity of the disease improved based on Birmingham Epidermolysis Bullosa Severity (BEBS) score (30.5+12.8 vs. 23.3+10.4, before and after treatment; p=0.018), accompanied by improvement of quality of life, as determined by the EB-QoL questionnaire (24.0+8.1 vs. 17.7+5.5; p=0.018). Histopathology of the selected lesions revealed increased number of mast cells, and enhanced microvasculature in the mid and lower dermis. The width of collagen bundles in dermis was suggested to be decreased in four samples and changed from dense to loose in appearance. In summary, this case series reports beneficial effects of losartan on RDEB as a potentially novel treatment, along with of histopathological alterations. Larger studies and randomized clinical trials are required to determine the long-term efficacy of losartan in treating RDEB.

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Efficacy and safety of abrocitinib in biologic-exposed versus biologic-naïve patients with moderate-to-severe atopic dermatitis

M. Gooderham[1], B. Strober[2, 3], M. Ardern-Jones[4], E. Guttman-Yassky[5], M. Levenberg[6], G. Chan[7], P. Biswas[8], M. Watkins[8]

[1]SKiN Centre for Dermatology, Peterborough, Ontario, Canada, [2]Yale University, New Haven, Connecticut, United States, [3]Central Connecticut Dermatology Research, Cromwell, Connecticut, United States, [4]University of Southampton, Southampton General Hospital, Southampton, United Kingdom, [5]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [6]Pfizer Inc., Collegeville, Pennsylvania, United States, [7]Pfizer Inc., Groton, Connecticut, United States, [8]Pfizer Inc., New York, New York, United States

Abrocitinib, an oral once-daily Janus kinase 1 selective inhibitor, is being investigated for the treatment of moderate-to-severe atopic dermatitis (AD). This post hoc analysis evaluated if prior treatment with biologic therapy affects response to abrocitinib. Data from patients (pts) with/without previous biologic therapy from each treatment arm (abrocitinib 200 mg or 100 mg, or placebo) of the phase 2b (NCT02780167) and phase 3 JADE MONO-1 (NCT03349060) and MONO-2 (NCT03575871) studies were pooled and evaluated separately from the phase 3 JADE REGIMEN (NCT03627767) open-label induction phase (abrocitinib 200 mg). Investigator's Global Assessment score of 0/1 (IGA 0/1) with ≥2-grade improvement from baseline and ≥75% improvement from baseline in Eczema Area and Severity Index (EASI-75) were assessed. IGA 0/1 response rates among 67/867 pts with/without prior biologic therapy were 43.5%/41.4% (abrocitinib 200 mg), 24.1%/26.7% (abrocitinib 100 mg), and 0%/8.5% (placebo) in the pooled population, and 53.5%/66.9% (abrocitinib 200 mg) among 86/1147 pts in REGIMEN. EASI-75 response rates in pts with/without prior biologic therapy were 65.2%/62.4% (abrocitinib 200 mg), 34.5%/42.7% (abrocitinib 100 mg), and 7.1%/12.7% (placebo) in the pooled population, and 64.0%/76.4% in REGIMEN. Treatment-emergent adverse event rates in pts with/without prior biologic therapy were 71.7%/69.9% (abrocitinib 200 mg + 100 mg arms) in the pooled population, and 66.3%/66.5% (abrocitinib 200 mg) in REGIMEN. Abrocitinib efficacy and safety were consistent in pts with moderate-to-severe AD regardless of prior biologic therapy.

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Treatment of bullous pemphigoid by inhibiting fcrn: Pre-registration report of a phase 2/3 trial with efgartigimod

D. A. Culton[1], H. Ujiie[2], E. Schmidt[3], D. Murrell[4], I. Stoykov[5], P. Verheesen[5], L. Borradori[6], R. Hall[7], P. Joly[8]

[1]University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina, United States, [2]Hokkaido University, Sapporo, Hokkaido, Japan, [3]University of Lübeck, Lübeck, Germany, [4]St George Hospital and University of New South Wales, Sydney, New South Wales, Australia, [5]argenx, Ghent, Belgium, [6]University Hospital Inselspital, Bern, University of Bern, Bern, Switzerland, [7]Duke University School of Medicine, Durham, North Carolina, United States, 8Rouen University Hospital, Rouen, France

Bullous pemphigoid (BP) is an autoimmune blistering disease, characterized by subepidermal blisters and mediated by IgG autoantibodies against BP180 (type XVII collagen) and BP230 structural proteins of the dermal-epidermal junction. Efgartigimod, an engineered Fc fragment, inhibits FcRn activity, thereby decreasing serum IgG and autoantibodies. This pre-registration report describes a Phase 2/3 randomized, double-blind, placebo-controlled trial of efgartigimod in patients with moderate to severe (by PDAI) BP. In Part A (proof of concept, phase 2), 40 participants will be recruited, while in Part B (confirmatory, phase 3) 120 participants will be recruited. Part A and B are identical in schedule, structure, assessments, and conduct. 2000 mg efgartigimod PH20 SC (as two 1000 mg injections) will be given on days 1 (baseline) and 8 (week 1), and 1000 mg efgartigimod PH20 SC will be administered weekly through week 35. The primary endpoint is the proportion of participants who have been in complete remission while receiving minimal oral corticosteroid therapy for ≥8 weeks at week 26.

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Efficacy of infliximab in hidradenitis suppurativa: A meta-analysis

T. Shih[1], K. Lee[6], T. Grogan[3], D. R. De[2], V. Y. Shi[4], J. Hsiao[5]
[1]University of California Los Angeles David Geffen School of Medicine, Los Angeles, California, United States, [2]University at Buffalo Jacobs School of Medicine and Biomedical Sciences, Buffalo, New York, United States, [3]Statistics, University of California Los Angeles Health System, Los Angeles, California, United States, [4]Dermatology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States, [5]Dermatology, University of Southern California, Los Angeles, California, United States, [6]Dermatology, University of California Los Angeles, Los Angeles, California, United States

Introduction: Hidradenitis suppurativa (HS) is a chronic inflammatory dermatosis that is often recalcitrant to treatments. Adalimumab is the only medication for HS that is approved by the FDA/EMA. The tumor necrosis factor inhibitor infliximab is an established treatment for HS, but its evidence is limited. Objective: Systematically conduct a meta-analysis of existing literature on the efficacy of infliximab in HS. Method: MEDLINE and EMBASE databases were systematically searched in September 2021. Inclusion criteria included infliximab as study intervention in at least 5 HS patients. Data was extracted by two independent reviewers. Non-English, non-human, and duplicate articles were excluded. Cochran's Q statistic and I squared index assessed heterogeneity. The random effects meta-analytical model was performed. The primary study outcome was the pooled estimate of the response rate of HS to infliximab. Results: 19 articles between 2003-2021 met inclusion criteria. 314 HS patients were included in this study, with 6 prospective studies (116 patients) and 13 retrospective studies (198 patients). HS response to infliximab treatment was reported as HS Clinical Response (n=4), HS Physician Global Assessment (n=4), HS Severity Index (n=2), International HS Severity Score System (n=1), physician assessment (n=10), patient assessment (n=6), and achievement of stable dosing regimen (n=1). In the meta-analysis, the pooled response rate was 83% (95% CI, 0.71-0.91). Discussion: Overall, infliximab is promising as a highly efficacious treatment for HS. Larger randomized controlled trials are needed to explore the comparative efficacy of infliximab in HS against other biologics.

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Evaluating the effectiveness of topical tofacitinib in reducing non-atopic dermatitis chronic itch

T. Ju, A. Labib, A. Vander Does, G. Yosipovitch
Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery and Miami Itch Center, Miami, Florida, United States

Introduction: Chronic and refractory pruritus is a highly common complaint amongst patients with dermatological conditions and remains difficult to treat with current drug therapies. There is an unmet need for potent topical antipruritics. Janus kinase (JAK) inhibitors have shown significant effect in treating atopic eczema itch. Objective: Topical tofacitinib compounding cream was investigated for treatment of non-AD related chronic pruritic diseases. Methods: A comprehensive chart review was completed where data was collected on numerous variables that help recognize the efficacy of tofacitinib. 19 patients with chronic itch of different types (excluding those with AD) treated with topical tofacitinib 2% compounding cream within the last four years were identified and evaluated regarding their demographic information, diagnosis, location and characteristics of itch, itch intensity scale ratings, adverse effects and reasons for discontinuation (if applicable). Paired-sample t-test was performed to evaluate average 24-hour itch intensity scale ratings prior to and after initiating topical tofacitinib. Results: 13 (68%) out of 19 patients demonstrated a quantifiable reduction in itch. Average 24-hour itch scale ratings decreased significantly ($p<0.001$) after initiation of tofacitinib from 7.82 (± 2.23) to 4.47 (± 3.55) across all patients, with score reduction of 3.34 (± 3.34). Tofacitinib was effective for many causes of non-AD itch, particularly in patients with prurigo nodularis and psoriasiform dermatitis. Overall, the drug was safe with no major adverse effects; the main reason for discontinuation of the drug was no relief or short duration of relief time after application. Conclusion: Topical tofacitinib cream significantly decreased the average itch intensity of patients and is generally well-tolerated, making it a promising therapeutic for non-AD related chronic itch.

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Mass spectrometry-based plasma proteomics analysis reveals IL-31 inhibition modulates cutaneous and systemic inflammation in prurigo nodularis

S. G. Kwatra[1], M. P. Alphonse[1], V. Parthasarathy[1], J. Deng[1], K. K. Lee[1], S. Stander[2], C. Piketty[3], L. Tille[3], H. Kamali[3], J. K. Krishnaswamy[3], V. Julia[3]
[1]Dermatology, Johns Hopkins Medicine, Baltimore, Maryland, United States, [2]Department of Dermatology and Center for Chronic Pruritus, Universitatsklinikum Munster, Munster, Nordrhein-Westfalen, Germany, [3]R&D, Galderma R&D, Entre-deux-villes, Switzerland

Prurigo nodularis (PN) is an intensely pruritic, chronic inflammatory skin disease with diffusely distributed hyperkeratotic nodules featuring cutaneous and systemic inflammation. This study investigated the systemic anti-inflammatory properties of nemolizumab, a humanized anti-IL-31RA antibody, by using an unbiased plasma proteomics with isobaric Tandem Mass Tags® (TMT®) approach to enrich skin-specific proteins detection. The analysis was performed on samples collected from the Phase II study of nemolizumab. Plasma samples were collected from PN patients at three timepoints (baseline, week 4, week 12), from 19 nemolizumab responders (Peak Pruritus NRS reduction at week 12 > 4) and 19 placebo non-responders. PN plasma samples were super-depleted, digested and labelled with TMT® and subjected to LC-MS/MS analysis. To enhance detection of skin proteins, skin biopsies were run in parallel to plasma samples using TMTprocalibrator™, a novel method to amplify the detection of skin-related proteins in plasma. Differentially expressed protein (DEP) analysis was performed from baseline-corrected data and pathway analysis employed GO term enrichment. TMT® coupled to LC-MS/MS analysis of plasma samples from PN patients resulted in the quantification of 3,451 unique proteins and 392 plasma proteins were differentially regulated by Nemolizumab treatment. Nemolizumab-treated patients had dramatic decreases in global inflammatory responses, including decreased STAT3, IL-3, IL-8, IL-15, IL-17 & VEGF signaling, neuronal neuroinflammation, impaired granulocyte activation and myeloid cell activation. IL-31 inhibition appears to be a central regulator in decreasing systemic inflammatory responses in patients with PN.

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Herpes simplex and eczema herpeticum in moderate to severe atopic dermatitis treated with abrocitinib

S. Tyring[1], S. R. Feldman[2], K. Winthrop[3], J. Alderfer[4], W. Romero[5], S. Johnson[6], H. Fan[4], H. Valdez[7]
[1]The University of Texas Health Science Center at Houston, Houston, Texas, United States, [2]Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States, [3]Oregon Health & Science University, Portland, Oregon, United States, [4]Pfizer Inc., Collegeville, Pennsylvania, United States, [5]Pfizer Ltd, Tadworth, United Kingdom, [6]Pfizer Inc., Raleigh-Durham, North Carolina, United States, [7]Pfizer Inc, New York, New York, United States

Patients with atopic dermatitis (AD) have increased risk of herpes simplex (HS) infection. Eczema herpeticum (EH) is a serious complication of HS. In a published safety analysis of abrocitinib, HS was reported in 5.9% (116; IR 11.83) and 6.1% (54; IR 8.73) and EH was reported in 0.4% (8; IR 0.78) and 1.7% (15; IR 2.34) of patients treated with abrocitinib 200 mg (N=1971) and 100 mg (N=885), respectively. Two EH events occurred in patients with IGA score <2 (both 100 mg). This post hoc analysis further characterized HS and EH in the above analysis of pooled data from patients with moderate to severe AD receiving abrocitinib 200 mg or 100 mg in the phase 2b (NCT02780167) and phase 3 JADE MONO-1 (NCT03349060), MONO-2 (NCT03575871), COMPARE (NCT03720470), REGIMEN open-label induction phase (NCT03627767), and EXTEND long-term extension (NCT03422822) trials (data cutoff: April 22, 2020). Most HS/EH infection cases were mild/moderate (HS: 96.3% [200 mg], 100% [100 mg]; EH: 87.5% [200 mg], 86.7% [100 mg]). Serious EH infections were reported in 4 patients (all 100 mg). In patients with HS infection, the first HS event occurred within the first 12 weeks of treatment in 57.8% (200 mg) and 48.1% (100 mg). As of data cutoff, HS resolved in 88.2% of cases; median time to resolution was 9.0 days (200 mg) and 10.0 days (100 mg). In patients with EH infections, the first EH event occurred within the first 12 weeks of treatment in 62.5% (IR 1.13; 200 mg) and 46.7% (IR 3.55; 100 mg). These findings suggest that HS/EH infections tend to occur early after abrocitinib administration and in patients with a history of HS/EH. Most cases were not severe and resolved with therapy.

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Role of platelet and growth factor concentration in platelet rich plasma therapeutic response to alopecia

J. A. Shaik[1], R. Farah[1], G. Bellefeuille[1], B. Paiewonsky[1], O. Raymond[1], N. Sadick[2], S. Arruda[2], M. Hordinsky[1]
[1]Dermatology, Regents of the University of Minnesota, Minneapolis, Minnesota, United States, [2]Sadick Dermatology, New York, New York, United States

The use of platelet rich plasma (PRP) for the treatment of hair loss has increased significantly in recent years. PRP treatment involves obtaining autologous PRP from a patient's blood sample and injecting it into the scalp. Although a promising treatment for alopecia, the role of platelet and growth factor (GF) concentration in PRP on hair growth is poorly understood. Herein, we present a case series of 3 patients (A, B and C) with androgenetic alopecia (AGA) who received PRP therapy containing platelet concentrations that were 15-55% of the baseline in the blood throughout the course of their treatment. Patients received 3 treatments over a 6-month period. Platelets in blood and PRP were counted using an automated hematology analyzer. An aliquot of the PRP was subjected to platelet activation using 20 mM calcium chloride to induce GF secretion and 11 different GFs were measured using commercially available kits. Clinical response was assessed prior to each treatment by a board-certified dermatologist. Additionally, an artificial intelligence photography device, HairMetrix® D200 EVO dermatoscope, was used to quantitate hair fibers in the frontal anterior, midscalp and vertex regions. Data generated 6-12 weeks after 3rd PRP treatment was compared to baseline. Patient A had the best response with a 20-50% increase in hair density and a 20% increase in average hair width across 3 scalp regions. Patient B improved slightly, while patient C's AGA remained stable. Platelet GF secretion profiles showed no fibroblast growth factor 2 (FGF2) in all of patient A samples, whereas the platelets of patient B and C secreted FGF2 upon activation. Secretion levels of brain-derived neurotropic factor was less than 0.5-fold in patient A's PRP samples compared to patients B and C. Our data suggests that high platelet numbers in PRP samples may not be necessary for hair growth and highlights key GFs that may have a negative effect on clinical response.

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Characterization of pharmacokinetics, pharmacodynamics, tolerability and clinical activity in phase I studies of the novel allosteric tyrosine kinase 2 (TYK2) inhibitor NDI-034858.

E. A. Gangolli, S. Carreiro, J. J. McElwee, N. Dave, A. Lombardi, J. Hanna, V. Hosagrahara, B. Srivastava
Nimbus Therapeutics, Cambridge, Massachusetts, United States

Tyrosine kinase 2 (TYK2) is an obligate mediator of signaling via IL-23, IL-12 and the type I interferon receptors, and is a validated therapeutic target in psoriasis and psoriatic arthritis. NDI-034858 is a novel, oral, allosteric, investigational TYK2 inhibitor with high selectivity for TYK2 over JAK1, JAK2, and JAK3 kinases. NDI-034858 was studied in multiple placebo-controlled Phase I studies. These included two trials in healthy volunteers receiving single ascending doses (six dose groups, N = 5-6 per group; versus placebo, N = 12) and multiple ascending doses (four dose groups, N = 6 per group; versus two placebo groups, N = 4 per group) for 2 weeks. NDI-034858 was also studied in patients with moderate to severe psoriasis treated daily for 4 weeks (three dose groups, N = 21 total; versus placebo, N = 5). Across the studies, the pharmacokinetic parameters of NDI-034858 were calculated. Pharmacodynamic effect was tested in a cytokine-induced *ex vivo* assay using whole blood samples, which demonstrated that exposure to NDI-034858 correlated with activity. No serious adverse events or deaths were observed in the studies, and treatment emergent adverse events were as expected for inhibition of TYK2. Exploratory efficacy endpoint analysis in patients with moderate to severe psoriasis using the Psoriasis Area and Severity Index (PASI) score suggested improvement in the disease. These data support further development of NDI-034858. Phase 2b trials in psoriasis and psoriatic arthritis are ongoing.

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Skin rash composition after checkpoint inhibitor therapy varies by therapeutic regimen

P. Ramesh[1], D. Jaishankar[1], C. Cosgrove[1], C. Kosche[1], A. Li[2], S. Hong[3], R. Shivde[1], S. S. Munir[2], H. Zhang[2], J. N. Choi[1], I. Le Poole[1]
[1]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [3]School of Medicine, Yale School of Medicine, New Haven, Connecticut, United States

Cancer patients treated with immune checkpoint inhibitors can experience adverse events affecting different organ systems. Among immune related adverse events, skin rash develops relatively early and might serve as a biomarker of downstream responses to therapy. We questioned whether the cellular composition of skin rash might vary according to the type of treatment applied, to interfere with PD1 signaling alone or combined with CTLA-4 blockade. Skin samples were obtained from consenting adults to perform mono-and multiplex OPAL immunostaining and Vectra imaging of snap frozen or paraffin-embedded rash (n=9) and control (n=8) skin samples. The cellular composition, intercellular interactions and proliferation were evaluated and compared to healthy skin. CD4 and CD8 T cells were prime contributors to skin rash, with T cells and macrophages interacting and proliferating on site. Combination therapy offers greater therapeutic benefit to patients, and here a combination of increased cytotoxic T cell content and decreased macrophage abundance was associated with dual checkpoint inhibition over PD1 inhibition alone. Type 1, 2 and 17, but not type 9 or regulatory T cells were increased among inflammatory skin infiltrates, associated with enhanced epithelial proliferation and -thickening. We conclude that skin rash composition is defined in part by the course of treatment, wherein increased cytotoxic T cell abundance and reduced contact with macrophages are associated with dual checkpoint inhibitor therapy.

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The utilization of 3-dimensional imaging to establish a standardized acne severity scale

A. Schrobilgen[1], A. Lo[1], B. Chandani[1], M. Foss[2, 3], N. Vesely[2, 3], C. Dunn[3, 2], S. Aneja[2, 3], J. A. Solomon[4, 5]
[1]University of Central Florida College of Medicine, Orlando, Florida, United States, [2]ADCS Clinics LLC, Maitland, Florida, United States, [3]Kansas City University, Kansas City, Missouri, United States, [4]University of Central Florida College of Medicine, Orlando, Florida, United States, [5]Florida State University College of Medicine, Tallahassee, Florida, United States

The current methods for classifying acne severity including lesion counting, global acne severity grading, subjective self-assessment and multimodal digital imaging have not demonstrated efficacy in development of a patient-oriented, standardized acne severity scale. Research that integrates the use of 3-D photography to classify and treat acne is limited, however the capability of 3-D photography to accurately assess skin texture, presence/elevation of lesions on darkly pigmented skin, and lesion depth/volume is well-established. The purpose of our study is to develop a standardized, patient-oriented grading system for acne severity through using 3-D photography. We captured 130 images using the Canfield Vectra H2 of patients with all 6 Fitzpatrick skin types and acne severities ranging from clear to extremely severe. Acne patients and board-certified dermatologists will be asked to rank the severity of the images without consideration of the current ranking system to find possible discrepancies between patients and dermatologists regarding interpretations of acne severity. Artificial Intelligence (AI) will be trained to recognize the elements of the images that caused it to be categorized as mild or severe by the survey participants such as the size of the lesions, number of lesions or redness associated with the lesions. With training, AI could accurately and universally categorize the severity of a new patient's acne and track the progress of recurrent patients by comparing each new image to the 3-D image database that we have built. In conclusion, 3-D photography allows for standardized assessment of acne severity for all skin types, taking into account many features that are lost when trying to use 2-D photography or current acne severity ranking methods.

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Regional disparities in the dermatology workforce

J. Tomtschik, E. Sabogal, P. Singh, M. G. Mercurio
University of Rochester Medical Center, Rochester, New York, United States

Socioeconomic disparities are an important barrier to dermatological care in the US. While access to care has grown with telehealth and use of advance practice providers (APPs), gaps in healthcare are still being identified and addressed. Prior studies have shown racial, ethnic, and socioeconomic disparities, but there is little data examining the geographic distribution of dermatologists throughout the US. Our study uses the 2006-2019 Association of American Medical Colleges (AAMC) Data Warehouse data to investigate trends in dermatologist density by US Census Region (Northeast, Midwest, South, or West). Data from the years 2006, 2011, 2013, 2015, 2017, and 2019 were analyzed. We recorded the mean annual dermatologist density, defined as dermatologists per 100,000 people (estimated from the US Census) and the ratio of dermatologists to primary care providers (PCPs), stratifying by US state and region. Significant differences in distribution of dermatologists were tested using Moran's I statistic for spatial autocorrelation. Mean dermatologist density was highest during the study period in the Northeast (4.58/100,000, Moran's I P<0.001), followed by the West (3.86/100,000), South (3.25/100,000), and Midwest (2.98/100,000). PCP/Dermatologist ratios were highest in the Midwest (30.8 PCPs/dermatologists, Moran's I P=0.05), and this trend was stable throughout the study period. Additionally, the number of dermatologists practicing in rural areas grew by an annualized rate of 0.7% per year between 2006 and 2017, compared to 1.8% in urban/suburban areas. Our analyses show that supply of rural dermatologists is growing at less than half the rate of non-rural areas, which implies that geographic disparities worsened over the study period. While PCPs and APPs are helping to address a shortage of dermatology providers in rural areas and the Midwest, there is likely still an unmet need for higher level dermatologic care. Our findings suggest a need for new strategies to attract dermatologists to these areas and further studies to investigate the factors influencing these disparities.

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Regional differences in cardiovascular risk factor screening for patients with psoriasis by dermatology providers

W. B. Song[1], G. M. Peck[2], D. B. Shin[1], A. B. Fleischer[2], S. R. Feldman[3], J. M. Gelfand[1]
[1]University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]University of Cincinnati College of Medicine, Cincinnati, Ohio, United States, [3]Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States

Psoriasis patients have an increased risk of cardiovascular (CV) events and mortality. Psoriasis treatment guidelines recommend counseling patients about CV risk and screening for modifiable CV risk factors. Regional differences in screening psoriasis patients for CV risk factors are not well characterized and could represent unmet screening needs. Using the most recent data from the National Ambulatory Medical Care Survey 2007-2016, a nationally representative sample of office-based ambulatory visits in the United States, we examined screening differences between 4 regions (Northeast, Midwest, South, West) for 4 CV risk factors (blood pressure, body mass index, cholesterol, glucose) for psoriasis patients visiting dermatology providers. For 15 million weighted patient visits, the screening rates in the Northeast, Midwest, South, and West were 12%, 10%, 2.3%, and 7.5% for blood pressure; 11%, 13%, 11%, and 8.7% for body mass index; 2.4%, 5.5%, 3%, and 10% for cholesterol; and 3%, 2%, 0.9%, and 1.3% for glucose, respectively. Using a logistic regression model, we found that regional differences in blood pressure screening persisted after adjusting for sex, age, race, ethnicity, and disease severity. Compared to the rate of blood pressure screening in the South (2.3%), the odds of receiving blood pressure screening were 5.90 (95% CI 1.34–25.92) in the Northeast, 4.87 (95% CI 1.59–14.97) in the Midwest, and 3.42 (95% CI 1.01–11.57) in the West. No statistically significant regional differences were found for body mass index screening. Regression analysis for cholesterol and glucose screening was not performed due to sample size limitations. CV risk factor screening in psoriasis patients by dermatology providers is very low across all regions and lowest in the South. Interventions to improve and standardize CV screening for psoriasis patients across all regions are needed.

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Is social media spreading misinformation on the COVID-19 vaccines within the psoriasis community?

D. Yee[1], C. Zagona-Prizio[2], S. Khan[1], S. Khan[4], N. Maynard[1], M. D. Mehta[1], R. Reddy[3], A. W. Armstrong[1]
[1]University of Southern California Keck School of Medicine, Los Angeles, California, United States, [2]University of Colorado Denver School of Medicine, Aurora, Colorado, United States, [3]The University of Texas Southwestern Medical Center Medical School, Dallas, Texas, United States, [4]The University of Texas Health Science Center at San Antonio Joe R and Teresa Lozano Long School of Medicine, San Antonio, Texas, United States

Many patients turn to social media for support and medical advice. This study analyzes social media interactions among patients with psoriasis and psoriatic arthritis regarding the COVID-19 vaccine to determine the misinformation circulating and the apprehension to receiving the vaccine. Relevant posts uploaded between March 1, 2021 and July 31, 2021 from publicly accessible Facebook and Reddit groups were analyzed. Higher-order and lower-order themes, as well as sentiment and engagement scores, were assigned to each post. 345 posts contained content pertaining to vaccination decision, and 1379 posts contained content pertaining to vaccine reaction. 37.4% of vaccination decision posts reported refusing the vaccine, and 18.3% reported being unsure about getting the vaccine. Common reasons for refusing included fear of psoriasis flare up, fear the vaccine was dangerous, and fear the vaccine was experimental; the first two of which received the most engagement. 41.4% of vaccination decision posts contained positive sentiment, and 38.3% contained negative sentiment. 72.2% of vaccine reaction posts reported no change to psoriasis, 16% reported skin/joint flare up, and 3.7% reported skin/joint improvement. Posts pertaining to skin/joint flare up and skin/joint improvement received the most engagement. 77.8% of vaccine reaction posts contained positive sentiment, and 6.2% contained negative sentiment. Our study identified common COVID-19 vaccine concerns within the psoriasis community which should be used to guide education efforts. It is crucial that clinicians address reasons for vaccine hesitancy and inform patients of evidence-based recommendations to combat the pandemic.

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Getting candid with CAM: Complementary and alternative medicine use among eczema patients

S. Khan[1], S. Khan[2], M. D. Mehta[1], N. Maynard[1], R. Reddy[3], D. Yee[1], C. Zagona-Prizio[4], A. W. Armstrong[1]
[1]University of Southern California Keck School of Medicine, Los Angeles, California, United States, [2]The University of Texas Health Science Center at San Antonio Joe R and Teresa Lozano Long School of Medicine, San Antonio, Texas, United States, [3]The University of Texas Southwestern Medical Center Medical School, Dallas, Texas, United States, [4]University of Colorado Denver School of Medicine, Aurora, Colorado, United States

Complementary and alternative medicine (CAM) use is common among those with skin diseases. However, little is known about the nature and extent of CAM use among adults with eczema in the United States. This study sought to describe CAM use among those with eczema and compare CAM use in adults with and without eczema. We conducted a population-based, cross-sectional analysis using the 2012 National Health Interview Survey. Among 7,513,156 adults (weighted) with eczema, 3,265,350 (43.5%) reported using at least one CAM modality. Compared to non-CAM users with eczema, a greater percentage of CAM users with eczema were younger (47.72±0.58 vs. 49.38±0.58, P=0.031), female (69.9% vs. 58.3%, P<0.001), white (84.1% vs. 79.2%, P<0.001), of non-Hispanic origin (92.4% vs. 88.0%, P<0.001), and higher educated (61.6% vs. 57.6%, P=0.036). Compared to adults without eczema, adults with eczema had 69% higher odds of using CAM when controlling for age, sex, race, Hispanic origin, household income and education attainment (aOR [95% CI], 1.69 [1.50-1.91], P < .001). Among adults with eczema, herbal supplements were the most common modality used for health and for the treatment of eczema. In conclusion, CAM use is common among adults with eczema. It is important for dermatologists to have candid conversations with patients regarding CAM use to optimize care and prevent potential adverse events.

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Biologics gone viral: Perceptions of biologics within online psoriasis and psoriatic arthritis communities

N. Maynard[1], M. D. Mehta[1], R. Reddy[3], D. Yee[1], C. Zagona-Prizio[2], S. Khan[1], S. Khan[4], A. W. Armstrong[1]

[1]University of Southern California Keck School of Medicine, Los Angeles, California, United States, [2]University of Colorado Denver School of Medicine, Aurora, Colorado, United States, [3]The University of Texas Southwestern Medical Center Medical School, Dallas, Texas, United States, [4]The University of Texas Health Science Center at San Antonio Joe R and Teresa Lozano Long School of Medicine, San Antonio, Texas, United States

Limited analyses of social media content among psoriasis (PsO) and psoriatic arthritis (PsA) patients exist. These patients may turn to social media to gain insight on treatments such as biologics. This study aims to analyze the content, sentiment, and engagement of social media posts regarding biologics for PsO and PsA. Posts and comments discussing biologics were extracted from publicly accessible PsO and PsA Reddit groups. Posts were assigned higher and lower order themes (HOTs and LOTs, respectively), sentiments, and engagement scores. Of 1141 posts extracted, 705 posts were classified under the HOT general/efficacy. 12 lower order themes (LOTs) were identified: hoping to start biologics (7.1% of posts), general hesitancy (17%), advice/experience (10.2%), symptoms improved (36.6%), symptoms not improved/worsened (5.7%), switching biologics (10.5%), time to results (13.4%), benefits outweigh risks (0.4%), stubborn areas (2.7%), biologics stopped working (4.9%), biologics mask the problem (1.0%), and other (5.4%). 61.3% of content was of positive sentiment, 24.0% was neutral, and 14.7% was of negative sentiment. The mean sentiment score, defined as the average of all posts' sentiment scores (where negative=-1, neutral=0, and positive=1), was overall positive at 0.47, 95% CI [.41-.52]. Mean sentiment scores between LOTs were significantly different ($p<0.001$). Information regarding biologics on Reddit is mostly positive; however, there remains a significant number of users expressing dissatisfaction with their efficacy or with biologics in general. Many users sought anecdotal advice. These findings can help guide educational efforts to anticipate concerns and appease hesitancy regarding biologic efficacy and use.

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The patient perspective on vaccine uptake in adults with psoriasis and eczema

M. Archila, N. Goldman, L. Perez Chada, M. H. Noe

Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States

Previous studies in the United States and Europe suggest adults with chronic inflammatory skin diseases are not receiving all recommended adult vaccinations. In the general population, age >65 years and having a chronic disease are the most consistent factors associated with receipt of the influenza vaccine, but these factors have not been explored in dermatology patients. The objective of this study was to explore factors associated with vaccine uptake in adults with eczema and psoriasis. A qualitative study using virtual, semi-structured interviews was conducted in a convenience sample of adults who self-identified as having psoriasis or eczema for at least 1 year, recruited from the National Psoriasis Foundation, the National Eczema Association and dermatology clinics at Brigham and Women's Hospital. Each interview was transcribed, edited for clarity, and independently coded by 2 study team members. Thematic analysis was used to analyze the data. Of 34 study participants, 25 participants (74%) were female and 9 (26%) were male, with a mean age of 50.8 years (SD: 16.4). Half of participants (n = 17) had psoriasis and half (n = 17) had eczema. Themes around healthcare decision making, perceived risks/benefits to vaccination, vaccine knowledge, and vaccine administration were explored. Most patients described how their skin disease impacts their lifestyle and personal decision making. Participants recognized both personal and societal benefits to vaccines. The most common barriers to vaccination identified were access to appointments and concerns about side effects. Patients most frequently reported receiving vaccine information from physicians, internet resources and friends, family, or colleagues, but no participant was able to correctly identify all vaccines recommended for adults in the United States. These results summarize the unique dermatology patient perspective around vaccine uptake and can be used to design patient-centered intervention to increase vaccination rates and decrease infectious complications of chronic inflammatory skin diseases.

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High patient acceptability of social needs screening in dermatology clinic

K. Wilkerson[1], J. C. Williams[1], E. De Marchis[2], N. Rudd[1], E. Amerson[1], A. Chang[1]

[1]Dermatology, University of California San Francisco, San Francisco, California, United States, [2]Family & Community Medicine, University of California San Francisco, San Francisco, California, United States

Social risks affect health outcomes and contribute to health inequity. Social needs screening has been evaluated in primary care but not yet in specialty care like dermatology. Our study aimed to: 1) determine patient acceptability of social needs screening and 2) explore patient attitudes & beliefs about social needs screening in dermatology clinic. We conducted a cross-sectional mixed methods study of 140 adult patients from the dermatology clinic at Zuckerberg San Francisco General Hospital. The survey included Accountable Health Communities Health-Related Social Needs Screening questions plus 30 additional questions that explored social factors affecting patient acceptability of screening. The primary outcome measure of acceptability was perceived appropriateness of screening in dermatology clinic. The secondary outcome measure was comfort with including social needs data in the EMR. 28 semi-structured interviews were conducted using convenience sampling to explore patients' attitudes & beliefs regarding social needs screening in dermatology clinic. After coding of transcripts, thematic analysis was used to identify themes. Of 185 patients approached to participate, 140 participants were consented and surveyed (75.7% response rate). Of 140 participants, 85% (116) found it very or somewhat appropriate to conduct social needs screening in clinic and 63% (86) were completely or somewhat comfortable having their social needs documented in the EMR. Four themes emerged: 1) emotional impact of screening, 2) importance of rapport with the healthcare team, 3) expectation of assistance if social needs are disclosed, and 4) views in dermatology clinic vs other healthcare setting. This study—the first to evaluate social needs screening in dermatology clinic— found that dermatology patients at a public city hospital thought screening was acceptable. Future work includes understanding dermatologists' perspectives and developing best practices for implementation.

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Dermatology in the emergency department: Are Hispanics using the ED more than others for inflammatory skin diseases?

C. Zagona-Prizio[1], S. Khan[2], S. Khan[3], N. Maynard[2], M. D. Mehta[2], R. Reddy[2], D. Yee[2], A. W. Armstrong[2]

[1]University of Colorado, Denver, Colorado, United States, [2]University of Southern California, Los Angeles, California, United States, [3]The University of Texas Health Science Center at San Antonio Joe R and Teresa Lozano Long School of Medicine, San Antonio, Texas, United States

Inflammatory skin diseases have high prevalence globally and are associated with significant morbidity. We sought to determine whether differences exist in the usage of emergency department (ED) and outpatient dermatologic offices between Hispanics and non-Hispanic white patients with inflammatory skin disease in the U.S. In this cross-sectional study, we used nationally representative data from the Medical Panel Expenditure Survey (MEPS) between 2016-2018. 151, 753, 634 participants (weighted) were identified with any skin condition. 8, 204, 830 had inflammatory skin conditions, including acne, psoriasis, rosacea, urticaria, and seborrheic dermatitis; among them, 277, 206 (3.4%) attended at least one ED visit, and 494, 171 (56.3%) attended at least one outpatient dermatology visit. Multivariate logistic regression showed that, compared to non-Hispanic white patients, Hispanics with an inflammatory skin disease were more likely to visit the ED (aOR, 2.868; 95% CI, 1.250-6.578) and less likely to attend an outpatient dermatology visit (0.090; 0.009-0.929) after adjusting for insurance status, education level, income, sex, age, and comorbidities. This study reveals a national difference in the use of the ED and outpatient dermatologic offices between Hispanics and non-Hispanic whites with inflammatory skin disease.

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Natural language processing of social media to evaluate patient global impression of change in psoriasis topical steroid treatments

C. M. Infante[1], J. Jueng[1], A. Su[1], I. Brooks[2], S. Shaikh[2], R. Dellavelle[4], O. Burton[5], J. Solomon[3]

[1]*University of Central Florida College of Medicine, Orlando, Florida, United States,* [2]*School of Information Sciences, University of Illinois at Urbana-Champaign, Champaign, Illinois, United States,* [3]*Ameriderm Research, Ormond Beach, Florida, United States,* [4]*University of Colorado Denver School of Medicine, Aurora, Colorado, United States,* [5]*Cyberinstitute, Clemson University, Clemson, South Carolina, United States*

The significant psychosocial burden associated with psoriasis has led patients to seek emotional support, information, and connection through social media. A gap between patient- and provider-perceived treatment efficacy persists and it is crucial to place patient perspective at the forefront. Analyzing social media posts regarding treatment of psoriasis may allow us to enhance adherence and outcomes. 4,236 public posts from May 2008 to January 2020 were extracted from online platforms using high-powered computing and run through Brandwatch, a social media listening program that utilizes natural language processing. Its function was to grade each post based on the standard Patient Global Impression of Change (PGIC) and to pair each with the corresponding emotion. An independent samples t-test was performed for each agent with statistical significance at $p < 0.05$. Each post was related to 1 of 18 topical steroid treatments for psoriasis. For all topical steroids there were more than 63% positive PGIC posts, indicating that most patients using them noticed improvement in their psoriasis. Most positive PGIC posts were associated with underlying negative sentiment for all treatments, possibly reflecting patient dissatisfaction despite relatively good perceived treatment efficacy. Flurandrenolide ($p = 0.045$) had significantly more posts with a positive PGIC and underlying negative sentiment. Topical steroids remain first-line treatment for psoriasis, and it is essential to prescribe an agent that meets patients' individual needs and expectations to establish a foundation of emotional support. A better understanding of patient-perceived efficacy can ultimately be translated into more effective interventions and pharmaceutical responses.

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Financial toxicity and skin cancer care in the US: Population-based survey from 2011-2018

L. Chu[1], K. J. Supapannachart[1], S. C. Chen[2], H. Yeung[1]

[1]*Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States,* [2]*Dermatology, Duke University School of Medicine, Durham, North Carolina, United States*

The cost of skin cancer treatments in the US exceeded $8 billion annually from 2007-2011 and is expected to rise. Financial toxicity, an emerging concept on the financial burden and distress associated with cancer treatment, is not well understood in the context of skin cancer. This cross-sectional study aimed to examine financial toxicity among people with skin cancer using pooled nationally representative National Health Interview Survey data from adults in 2011-2018. Material, behavioral, and psychological markers of financial toxicity were compared by lifetime skin cancer history (any melanoma, any skin cancer beside melanoma, and no skin cancer) using multivariable logistic regression models, which adjusted for survey year, demographics, insurance status, income, education, medical comorbidities, and lifetime history of non-skin cancer. Of 257,652 total participants, 1,874 (0.73%) had known melanoma and 7,073 (2.75%) had any skin cancer beside melanoma. Skin cancer history was not associated with higher odds of material markers (problems paying bills or medical bills over time) or behavioral markers (delaying or forgoing medical care or inability to afford medical supplies and services) of financial toxicity. Skin cancer history was not associated with most psychological markers (worrying about standard of living, retirement, medical costs, housing costs, and monthly bills) of financial toxicity. Participants with any skin cancer beside melanoma were less likely to report worrying about credit card bills as compared with those without skin cancer (adjusted OR, 95% CI; 0.77, 0.66-0.90, P = 0.043 after adjusting for multiple comparisons). Limitations included self-reported data subject to recall bias and no data on skin cancer treatment type. Skin cancer history was not associated with increased markers of financial toxicity in this nationally representative study. Dermatologists should be aware of an increasing chance of financial toxicity as immunotherapy for skin cancer becomes more common.

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Indoor tanning frequency trends: Data from the National Health Interview Survey 2005-2015

N. Trepanowski[1, 2], L. Huang[3], R. Hartman[2, 4]

[1]*Boston University School of Medicine, Boston, Massachusetts, United States,* [2]*Brigham and Women's Hospital Department of Dermatology, Boston, Massachusetts, United States,* [3]*Harvard University T H Chan School of Public Health, Boston, Massachusetts, United States,* [4]*Department of Dermatology, VA Integrated Service Network (VISN-1), Jamaica Plain, Massachusetts, United States*

Indoor tanning is associated with a dose-response increase in melanoma risk. Laws and public health campaigns have resulted in declining indoor tanning rates, but changes in indoor tanning frequency have not been reported. We examined changes in frequency among indoor tanning users using the National Health Interview Survey (NHIS) in 2005, 2010, and 2015. The outcome of interest was past-year frequency of indoor tanning, defined as: never (0), rare (1-2), occasional (3-10), regular (11-24), and frequent (≥25). Associations between indoor tanning frequency and time were investigated with adjusted weighted multinomial logistic regression models. Adjusting covariates included sex, age, race/ethnicity, region, healthcare coverage, alcohol use, smoking status, education, income, personal and family history of skin cancer, and skin phototype. The unweighted and weighted samples included 69,742 and 531,742,536 participants, respectively. In 2015, compared to 2005, a larger proportion of indoor tanners were occasional (41.1% vs. 20.9%), regular (15.9% vs. 10.1%), and frequent (19.8% vs. 10.3%) users, while the proportion of rare users decreased (23.2% vs. 58.8%) (P<.001). The adjusted prevalence of non-indoor tanners increased from 85.9% in 2005 to 96.0% in 2015, with a corresponding decrease in indoor tanners from 14.1% to 4.0%. The adjusted prevalences decreased significantly across all indoor tanning user frequencies, but this was most pronounced among rare users, with a relative decrease of 89.3% vs. occasional (43.0%), regular (52.4%), and frequent (38.9%) users. We observed reductions in indoor tanning from 2005 to 2015, largely driven by a decrease in rare tanners. High frequency tanners should be targeted by public health campaigns, given their associated elevated skin cancer risk and their increasing relative proportion among indoor tanning users.

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Association between oral corticosteroid prescribing patterns and appropriate fracture preventive care in people with atopic eczema in the UK

J. Matthewman[1], M. Tadrous[2], K. Mansfield[1], D. Thiruchelvam[2], D. Redelmeier[2], A. Cheung[2], I. Lega[2], D. Prieto-Alhambra[3], L. Cunliffe[2], S. Langan[1], A. Drucker[2]

[1]*London School of Hygiene & Tropical Medicine, London, London, United Kingdom,* [2]*University of Toronto, Toronto, Ontario, Canada,* [3]*University of Oxford, Oxford, Oxfordshire, United Kingdom*

(Atopic) eczema is commonly treated with oral corticosteroids (OCS), which increase fracture risk. Fracture-preventive medications, including bisphosphonates, are recommended to counter the negative effects of OCS on bone health when individuals are prescribed >=450mg prednisolone equivalent dose (PED) in 6 months. People with eczema are prescribed OCS in different patterns (e.g., continuously or intermittently). We hypothesised people receiving intermittent OCSs were less likely to receive adequate fracture-preventive care than people receiving the same cumulative OCS dose continuously. We conducted a nationwide cohort study using routinely collected UK general practice data (1998-2020). We identified 20,680 individuals aged 66+ with eczema who had received at least 450mg PED in 6 months. Of these, 13,240 were intermittent and 7,440 were continuous OCS users. Hazard ratios from Cox regression suggested those prescribed continuous OCSs had higher rates of fracture preventive care (bisphosphonates, calcium, vitamin D) than those prescribed OCSs intermittently (HR 5.11; 95%CI 4.63-5.63). Effect estimates were attenuated in analyses using different continuous/intermittent OCS definitions, however a strong positive association between continuous use and fracture preventive care prescribing remained. Those prescribed OCS continuously were at higher risk of major osteoporotic fractures (HR 1.46; 95%CI 1.18-1.82). Results were similar when we replicated analyses in a population-based cohort from Ontario, Canada. There may be missed opportunities for appropriate fracture preventative care in people with eczema prescribed intermittent OCSs, suggesting a need for updated treatment guidelines. Implementation research could explore potential solutions such as electronic medical record reminders for individuals receiving threshold cumulative OCS prescriptions.

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Impact of free dermatology clinic on healthcare delivery to uninsured patients – a descriptive analysis study

J. S. Kang, D. C. Moore, F. Nutan
Virginia Commonwealth University School of Medicine, Richmond, Virginia, United States

To evaluate the impact of the only free dermatology clinic in Richmond, VA, a retrospective analysis was conducted in patients seen at Crossover Health Ministry from December 2020 to December 2021. Data was extracted from eClinicalWorks and de-identified patient data was collected. 50 uninsured immigrant patients were seen over 62 visits by dermatologists with study range from 13 to 83 years of age (mean = 40.5 years, median = 39 years), and 30% were pediatric patients. There were more female than male patients (64% vs 36%). Most of these patients were being seen for the first time (mean = 1.26 visits, median = 1 visit), but a few patients needed multiple follow ups. An interpreter was used for 82.3% of the visits – 74% Spanish, 4% Portuguese, 2% Arabic, and 2% Russian. All but 1 patient came in with at least 1 chronic complaint. Common diagnoses included seborrheic keratosis (11.3%), acne vulgaris (8.1%), psoriasis (8.1%), alopecia areata (6.5%), acrochordon (6.5%), and seborrheic dermatitis (4.8%). Patients also presented with less common conditions such as cutaneous leprosy, lichen simplex chronicus, perioral dermatitis, solar elastosis, pityriasis versicolor, vitiligo, pemphigus vulgaris and verruca vulgaris. Biopsies were taken during 6.5% of visits, and prescription medications were written during 46.8% of visits. The results emphasize the benefit of a free clinic that specializes in dermatology and offers interpreters to overcome language barriers to bridge the gap in dermatologic care for skin of color patients. Due to the presence of premedical and medical student volunteers, this is also a great resource for early exposure to dermatology and hence increase diversity in the future dermatological work force.

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A modified delphi consensus exercise: developing a skin-directed Stevens-Johnson Syndrome/toxic epidermal necrolysis scoring system

M. Waters[1], A. Dobry[2], S. Le[3], K. Shinkai[2], E. Maverakis[3], B. H. Kaffenberger[1]
[1]The Ohio State University College of Medicine, Columbus, Ohio, United States, [2]University of California San Francisco, San Francisco, California, United States, [3]University of California Davis, Davis, California, United States

While SCORTEN and ABCD-10 are available to provide a patient prognosis in Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN), they are not intended to assess the severity, progression, or improvement in disease throughout the hospitalization. A skin-directed instrument is needed. A modified Delphi consensus using RAND/UCLA appropriateness criteria was initiated with a core group of members who have published in the topic. A two-part consensus was determined to be needed to first establish agreement on the optimal design for a cutaneous scoring instrument, terminology, morphologies, and locations of involvement. Fifty-four dermatology hospitalists agreed to participate in this study. After one round for part one, all questions and statements reached consensus. Participants consistently agreed on the need of such an instrument and that certain locations including the head and neck, chest, upper back, ocular mucosa, and oral mucosa were almost always affected by SJS/TEN. Participants also agreed on morphologic terminology and that morphologies could be differentiated into blanching erythema, dusky erythema, targetoid erythema, vesicles/bullae, desquamation, and erosions. The second part of the study will establish agreement on time course, progression, and weighting assessments and is in progress. The Delphi consensus has established that there is widespread agreement among expert dermatologists that there is a need for a skin directed scoring system, widespread agreement that specific morphologies can be differentiated consistently on both cutaneous and mucosal surfaces, and that some areas of the body are almost always affected when SJS/TEN occurs. This undertaking has so far revealed best practices as well as areas of uncertainty with SJS/TEN to help develop the framework for an SJS/TEN skin-directed scoring instrument.

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Clinical data registries as a vital tool for academic dermatology

A. Mittal[1], J. Marks[2], M. Butt[2], J. Simmers[2], A. Flamm[2]
[1]Penn State College of Medicine, Hershey, Pennsylvania, United States, [2]Department of Dermatology, Penn State Health Milton S Hershey Medical Center, Hershey, Pennsylvania, United States

Clinical data registries (CDRs) store data pertaining to diseases, patients, treatments, or procedures. We investigated the use of CDRs in academic dermatology departments. A REDCap survey was distributed to departments nationally. Parameters collected include CDR use for research, internal vs. external development, types of data stored, CDR format, and challenges and benefits of use/management. Results were analyzed using descriptive statistics. Responses were obtained from 21 institutions across the United States. 86% (n=18) of departments have used CDRs to aid research, of which 61% (n=11) have published research involving CDR use. Of the 14% (n=3) not having used CDRs, reasons include lack of access (100%) and unawareness of the benefits/utility (100%). Most departments using CDRs indicate that the CDRs store data for a disease/condition (78%, n=14) with 56% (n=10) using internally-developed and 67% (n=12) using externally-developed CDRs. 83% (n=15) indicate that they use CDRs in a parallel electronic format such as REDCap or Excel. Challenges of use/management include time, data quality, regulatory, and personnel, while benefits include scholarship, data consistency, and access to patient populations/data. Our findings reveal that both internally and externally-developed CDRs are widely used in academic dermatology departments. While some barriers do exist in the effective implementation of CDRs, CDRs can be a vital tool for dermatology departments, specifically to inform research, improve patient care, and support academic output.

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Developing a treatment decision aid for patients with moderate to severe atopic dermatitis

W. Baghoomian, R. Dunlap, A. Chang, E. Foster, E. Simpson
Oregon Health & Science University, Portland, Oregon, United States

Shared decision-making (SDM) in healthcare is considered the gold standard of care for supporting the cooperation of physicians and patients. A key component of SDM is the utilization of Patient Decision Aids (PDAs), which are tools used to help patients make informed decisions about treatment options. PDAs are particularly suited for conditions in dermatology, like moderate to severe atopic dermatitis (AD), that have multiple treatment options with varying outcomes. While several PDAs currently exist within the field, none exist for patients with moderate to severe AD. The objective of this study was to develop a PDA in accordance with the International Patient Decision Aid Standards (IPDAS) to assist adult patients with moderate to severe AD in choosing a systemic treatment. Initial focus groups with patients with a history of moderate-to-severe AD (n=10) and clinicians (n=6) were conducted to establish priorities in selecting AD treatment and to collect impressions of existing decision aid models. Thematic analysis was used to evaluate and code the interview responses. Key findings from the initial interviews included: 70% of patients selected efficacy and side-effects as their top priorities when selecting a systemic treatment, 40% of patients preferred a PDA that would be used during a clinical encounter, and 40% of patients preferred both an electronic and paper format. The resulting data were then used alongside a comprehensive literature review to develop a PDA. A second round of focus groups with the same patients (n=8) and clinicians (n=4) was conducted to provide feedback on the prototype PDA. The PDA was revised using the suggested responses. The resulting paper PDA utilizes a table format to list systemic treatment options and their expected efficacies, common side effects, method of delivery, timeline for improvement, and cost. In summary, our study identified several key components of a PDA and developed a prototype that would allow patients to successfully make informed decisions about their treatment options for moderate to severe AD.

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Systemic corticosteroid use among dermatologists for steroid-responsive diagnoses: A cross-sectional survey

A. Snyder[1, 2], Z. Hopkins[3], A. M. Secrest[1, 2]
[1]Dermatology, University of Utah, Salt Lake City, Utah, United States, [2]Population Health Sciences, University of Utah, Salt Lake City, Utah, United States, [3]Dermatology, Broward Healthcare System Inc, Fort Lauderdale, Florida, United States

Use of systemic steroids is common in dermatology. Side effects from systemic steroids are common, well-known, and sometimes severe. This survey study aims to provide a cross-sectional view of systemic corticosteroid use among United States board-certified dermatologists to understand frequency and patterns of use. A random sample of 2000 board-certified dermatologists identified through the American Academy of Dermatology were sent a survey, and 844 (42.2%) completed the survey. Comfort prescribing systemic steroids was assessed with a Likert-scaled question (1 [strongly disagree] – 5 [strongly agree]). Univariate ordinal logistic regression was used to assess association between prescribing comfort and demographic and training differences. Stata 16 was used for calculations. Systemic corticosteroids were most commonly prescribed for contact dermatitis, bullous dermatoses, and eczema (96.7%, 95.0%, and 91.8%, respectively) – 0.8% reported not using any systemic corticosteroids. 90.5% felt comfortable using oral corticosteroids for steroid-responsive dermatoses, whereas 6.2% were neutral and 3.3% did not feel comfortable. Compared to more recent graduates (those practicing <5 years post-residency), participants who had been practicing 15-24 years, 25-34 years, and 35+ years after residency were less comfortable using oral corticosteroids (OR [95% CI]: 0.42 [0.20-0.90]), 0.35 [0.17-0.74], and 0.35 [0.17-0.75], respectively). Compared to participants who typically saw the fewest patients (<25 per week), those who saw the most patients (175+ per week) more strongly felt comfortable using oral corticosteroids (2.32 [1.06-5.09]). This study demonstrates that the majority of dermatologists feel comfortable using systemic corticosteroids. Dermatologists more recently out of training and who saw large numbers of patients were more likely to report higher degrees of comfort.

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Patient perspectives of barriers to accessing care for hidradenitis suppurativa: A proposed model.

L. A. Barnes[1], N. Shukla[2], M. Paul[2], M. C. Halley[1, 3], I. de Vere Hunt[1], E. Linos[1], H. B. Naik[2]
[1]Dermatology, Stanford University School of Medicine, Stanford, California, United States, [2]Dermatology, University of California San Francisco, San Francisco, California, United States, [3]Biomedical Ethics, Stanford University School of Medicine, Stanford, California, United States

Hidradenitis suppurativa (HS) is a painful, disfiguring, chronic inflammatory disease that significantly impacts patient quality of life. We hypothesize that there are unique barriers to care experienced by patients with HS. We conducted an inductive thematic analysis of 45 in-depth 60-90 minute semistructured interviews of patients with HS. Participants were recruited from the UCSF HS patient registry and HS online communities to represent diverse geographical and socioeconomic backgrounds. Three investigators analyzed the data using a grounded theory approach to develop a codebook. Two other investigators performed a thematic analysis of excerpts associated with the following codes: health systems interactions, facilitators to care, barriers to care, income and profession, interpersonal interactions with healthcare. The resultant themes and thematic framework were critiqued by three experienced qualitative researchers to ensure qualitative rigor. Our inductive thematic analysis identified five themes for barriers to HS care: (1) disease activity can influence employment, (2) employment influences health coverage options, (3) health coverage influences costs for care and health care choices, (4) costs for care influence ability to access quality care, and (5) quality care impacts disease activity. These themes generate a theoretical cyclical model for understanding a cycle that HS patients traverse to obtain quality care and improve disease activity. We theorize that HS disease activity can be reduced when cycle elements are optimized. Conversely, should elements of the cycle be perturbed, it will negatively affect all subsequent elements. This qualitative study highlights the barriers to care experienced by patients with HS and provides insights for health care professionals and health care systems caring for this unique patient population.

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Growth in the cost of biologics in Medicare beneficiaries, 2017 to 2019

J. Laborada[1], L. Shin[2], C. Lee[1], S. Shahsavari[3], A. Egeberg[4], J. J. Wu[5]
[1]University of California Riverside School of Medicine, Riverside, California, United States, [2]Loma Linda University School of Medicine, Loma Linda, California, United States, [3]Dartmouth College Geisel School of Medicine, Hanover, New Hampshire, United States, [4]Dermatology, Bispebjerg Hospital, Kobenhavn, Denmark, 5Dermatology Research and Education Foundation, Irvine, California, United States

In 2019, Singh et al. found significant price increases in biologics in Medicare beneficiaries from 2013 to 2016 for adalimumab, etanercept, infliximab, ustekinumab, and abatacept; however, data on IL-12/23, IL-17, and IL-23 inhibitors were not evaluated. We utilized the Centers for Medicare and Medicaid Services' (CMS) Part D Public Use Files from 2017 to 2019 to examine spending and prescription patterns for 11 FDA-approved psoriasis drugs. We found rheumatologists (52.1%) to be the most frequent prescribers of biologics followed by dermatologists (17.9%). The number of prescribers continued to increase each year between 2017 and 2019 with most prescribers from the South. Annualized growth rates for the biologics were as follows in increasing order: ixekizumab (-0.6%), infliximab (-1.3%), brodalumab (-5.7%), guselkumab (-12%), secukinumab (3.1%), etanercept (3.4%), certolizumab pegol (4.1%), adalimumab (4.3%), and ustekinumab (16%). Compared to CMS files from 2013-16, annualized growth rates for adalimumab, etanercept, and infliximab have declined. Guselkumab was initially the most expensive biologic drug for psoriasis in 2017 but was overtaken by ustekinumab in 2018 whereas brodalumab and certolizumab pegol were the least expensive. The rapid price increase of ustekinumab may be due to the drug being double the price for 90 mg versus 45 mg and shortening of the usual interval to 8 weeks for many patients who have recurrence of psoriasis in the last weeks before their next planned injection. In contrast, the cost of guselkumab (approved in July 2017) rapidly declined from 2017 to 2019, likely driven down by approvals of two other IL-23 inhibitors. Future studies in a larger diverse population including non-Part D Medicare enrollees are warranted to better understand and navigate such trends and cost barriers.

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Benzene in sunscreen: Do sensationalizing dermatology reports have a lasting effect on google trends and reddit engagement?

D. Barzallo[1], B. T. Carroll[2]
[1]Dermatology, Case Western Reserve University, Cleveland, Ohio, United States, [2]Dermatology, University Hospitals, Cleveland, Ohio, United States

Skin cancer is the most common form of cancer and has continued to rise in the past decade. Sunscreen has been shown to decrease the risk of developing melanoma by 50%, and squamous cell carcinoma by 40%. Australia has a robust sun-safety program which was implemented in 1988 and has shown to be effective in increasing sun protection behaviors and a decline in melanoma incidence. Behavior change is hard and complex and important for dermatologists to understand to help modify a patient's sun protective behaviors. We explored how anti-sunscreen dermatology reports are consumed by the general public and predict it will not have a lasting effect on behavior modification. On May 25th, 2021, it was reported that benzene was found in sunscreen products on the reddit subforum r/SkincareAddict. Benzene is a known carcinogen, associated with leukemia. On July 14, 2021, on the same subreddit it was posted that the FDA announced that Johnson and Johnson voluntarily recalled specific Neutrogena® and Aveeno® aerosol sunscreen products due to the presence of benzene. Google trend analysis of the year 2020-2021 reported that between May 30th and June 5th there was the highest peak interest in searching "benzene in sunscreen", with 84% peak interest between July 11th to July 17th. Using NVIVO, word frequency was analyzed on the post of May 25th with comments frequently mentioning safety (n=48, 12.33%), Neutrogena (n=41; 10.54%), contaminated (n=26; 6.68%) and carcinogen (n=17; 4.37%). There was only one comment under this post that occurred after June 5th which was the last day of the Google Trends peak, with an overwhelming majority occurring on the same day. No post after July 14th had more than 20 comments in the subreddit r/SkincareAddicts. Our findings suggest that two different internet platforms, Reddit and Google Trends, follow a similar pattern and that while these publications caused a lot of interest it was short-lived. This has an important impact on public education campaigns to create behavior change.

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Hidradenitis suppurativa: Patient perspectives on biologic use

D. R. De[1], T. Shih[2], J. Hsiao[4], V. Y. Shi[3]
[1]University at Buffalo Jacobs School of Medicine and Biomedical Sciences, Buffalo, New York, United States, [2]University of California Los Angeles David Geffen School of Medicine, Los Angeles, California, United States, [3]Dermatology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States, [4]Dermatology, University of Southern California, Los Angeles, California, United States

Hidradenitis suppurativa (HS) was once considered an orphan disease with limited therapeutic options. With the emergence of pipeline biologic treatments for HS, the treatment paradigm for HS is expanding. We aim to identify HS patient use and perspectives of biologic medications. Between 10/2021 to 1/2022 an anonymous survey was disseminated through online HS support groups. Data regarding demographics and perspectives on biologic medications were collected and assessed using Spearman rank correlation. The majority of the 196 respondents were female (92%, 180/196) and white (75%, 147/196) with Hurley stage 3 HS (89.3%, 102/195). A dermatologist was the primary healthcare provider for HS in 65% (128/196) of patients, with only 12% (23/196) seen at an HS specialty clinic. The majority of respondents never tried a biologic medicine (62%, 118/192). The top three barriers to biologic treatment were fear of side effects (61%, 109/179), high cost or lack of insurance coverage (46%, 83/179), frequency of weekly injections (32%, 58/179). Respondents were less likely to report every other week and monthly injections as barriers (23% 40/176, 11%, 19/176). Respondents reporting their main healthcare provider as a non-dermatologist (4.11 vs 3.0, p<0.0001) and not seen at a HS specialty clinic (3.5 vs 2.7, 0.039) were significantly more likely to agree that "I do not know enough about how biologics work to help my HS". Our results highlight the importance of dermatologists and specialty clinic visits in the education and implementation of biologic treatments. Timely initiation of biologics for eligible patients may reduce the irreversible sequelae of HS. Patients may benefit from comprehensive discussion regarding biologics including risks versus benefits, the insurance authorization process, and availability of less frequent dosing.

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Patterns and predictors of NIAMS funding for early career researchers

B. Chiang, K. Abuabara
University of California San Francisco, San Francisco, California, United States

Both basic and clinical research are important for advancing science and public health, and the NIH has been working to increase support for investigators performing clinical research. Our objective was to evaluate the distribution of mentored career development awards in dermatology and to compare it to other specialties funded by the National Institute for Arthritis and Musculoskeletal and Skin Disease (NIAMS). We collected data on K08 and K23 awards from NIAMS through the publicly available NIH Reporter database and classified these awards as dermatology, musculoskeletal, or rheumatology research to compare the K08 and K23 distribution across specialties. We also classified award recipients as men or women according to the gender pronouns used in publicly available institutional biographies. From January 2012 to June 2021, NIAMS granted K23 awards to 104 unique recipients and K08 awards to 108 unique recipients, resulting in an overall ratio of 1.0 for K23 to K08 recipients. This K23:K08 ratio varied by specialty: it was 0.4 for dermatology, 0.8 for musculoskeletal research, and 1.7 for rheumatology. Adjusted multivariate regression models show that the odds of K23 versus K08 awards were over three times higher in rheumatology than dermatology (OR 3.63, 95% CI 1.72-7.90). Women were almost three times more likely to receive K23 awards (OR 2.64, 95% CI 1.47-4.82), and there was evidence of effect modification in the association between specialty and award type by gender (likelihood ratio test, p-value = 0.015). When stratified by gender, the K23:K08 ratio in dermatology was 0.1 for men and 1.1 for women. The imbalance between support for early career researchers in dermatology focusing on basic versus clinical research may have important implications for the state of dermatological science and the academic workforce. Efforts to address these challenges and correct the imbalance in research funding is needed to improve equity in the dermatology workforce and improve patient outcomes.

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Recent trends in healthcare utilization and costs for adults and children with hidradenitis suppurativa

D. Chopra[1], S. Maczuga[2], J. S. Kirby[2], H. Lev-Tov[1]
[1]Dermatology, University of Miami School of Medicine, Miami, Florida, United States, [2]Dermatology, Penn State Health Milton S Hershey Medical Center, Hershey, Pennsylvania, United States

Hidradenitis Suppurativa (HS) is a chronic, inflammatory disease that requires a complex, multidisciplinary approach. Disease awareness and management have significantly changed in recent years in part by the advent of new biologic drugs approval in 2015. It is unclear how these changes affected healthcare utilization. This study aims to characterize how adults and children with HS utilized medical care before and after 2015. We retrospectively analyzed the IBM® MarketScan® Commercial database for outpatient, inpatient, ED, and pharmaceutical claims for 2007 to 2010 and 2015 to 2018. HS cohorts from each period were selected and divided between adult and pediatric subsets and matched to a control population. Overall costs for the HS adult cohort increased from $32,267 in 2007-2010 to $55,236 in 2015-2018, a 71% increase (p<.0001). Healthcare utilization increased or remained consistent amongst all variables over time except the proportion of people hospitalized (30.1% in 2007-2010 to 25.1% in 2015-2018 (p<.0001)). For the pediatric cohort, overall costs increased similarly from $15,559 in 2007-2010 to $28,710 in 2015-2018 an 85% increase (p<.0001). The proportions of members with ED and inpatient claims decreased (54.8% to 51.2% (p=0.047) and 17.4% to 14.3% (p=0.019), respectively). The greatest proportional increase in cost for both cohorts was in drug and pharmacy costs (67% in adults and 60% in pediatrics). Utilization by the HS population was consistently higher compared to controls across all cohorts, settings, and periods. HS patients continue to utilize medical care at a higher rate. Overall costs for HS patients have increased over time, particularly for pharmaceuticals; however, a lower proportion of patients than before are using emergent, high-cost settings such as inpatient or ED services. Although overall cost for pediatric patients is lower than their adult counterparts, pediatric patients are utilizing services at comparative rates.

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Telehealth utilization among patients with skin cancer

A. Munjal[1], R. Tripathi[1, 2]
[1]*The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States,* [2]*Johns Hopkins Medicine, Baltimore, Maryland, United States*

Background: Skin cancers are the most common cancers in the United States. The expansion of teledermatology has been greatly expedited by the COVID-19 pandemic. Our objective was to evaluate trends in the utilization of telehealth among patients with skin cancer. Methods: Patients with skin cancer were identified using the nationally representative National Health Interview Survey (NHIS) 2020, produced by the CDC. Descriptive analyses were initially used to evaluate the relationship of patient characteristics with use of telehealth over the past year. Multivariable logistic regression was used to determine which characteristics were most predictive of using telehealth among patients with skin cancer. Results: 939 patients with skin cancer were included in this study (59.1% keratinocyte carcinoma, 26.3% melanoma, and 14.6% other). 44.7% of patients reported having a virtual appointment with a provider in 2020; 85.5% of these patients reported a virtual visit due to reasons related to COVID-19. In multivariable analyses, the strongest predictors of increased telehealth use were immunosuppression (adjusted odds ratio [aOR] 2.63, p<0.001), living in a larger urban area (aOR 1.48, p=0.005), age over 65 (aOR 1.35, p=0.049), having a college education (aOR 1.58, p=0.002), and having a functional limitation (aOR 2.22, p<0.001). Patients with a regular healthcare provider were more likely to use telehealth than those without one (p=0.02). Telehealth use was associated with increased emergency department use and increased hospitalization rate (p<0.001). Employed patients were less likely to use telehealth (p=0.01). Income, sex, and marital status were not associated with telehealth use. Conclusion: Telehealth improves access to safe and affordable care for patients with skin cancer, particularly among those who may have barriers to attending traditional in-person visits (immunosuppressed and functionally impaired patients). Further expansion of telehealth should target younger patients with skin cancer, those with lower educational status, and patients who live outside large urban centers.

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The burden of atopic dermatitis out-of-pocket healthcare expenses in United States children

R. Chovatiya[1], W. Smith Begolka[2], I. J. Thibau[2], J. I. Silverberg[3]
[1]*Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States,* [2]*National Eczema Association, Novato, California, United States,* [3]*Department of Dermatology, The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, United States*

Atopic dermatitis (AD) is associated with elevated financial costs, including out-of-pocket (OOP) expenses. Yet, the full burden of OOP expenses in children with AD is poorly understood. We characterized categories, impact, and associations of OOP AD healthcare expenses in US children. An online survey was administered to National Eczema Association members (N=113,502). Inclusion criteria (US resident; respondent age ≥18; self or caregiver report of AD diagnosis) was met by 77.3% (1,118/1,447). Children (<18 yrs) vs. adults (≥18 yrs) with AD had similar overall severity but increased healthcare provider (HCP) visits, comorbid food allergy, cutaneous infections, and topical antimicrobial use (P<0.005 for all), and increased OOP expenses for hospitalization (4.6% vs. 1.9%), emergency room visits (17.7% vs. 12.1%), emollients (98.6% vs. 93.1%), hygiene/bathing products (91.1% vs. 83.3%), childcare (14.2% vs. 2.8%), and specialized cleaning products (86.6% vs. 71.6%), and clothing/bedding (62.0% vs. 40.3%) (P<0.05 for all). Children vs. adults with AD had increased median total yearly OOP expenditures ($860 vs. $500, P=0.002) and were more likely to spend ≥$1000 OOP per year (48.9% vs. 40.0%, P=0.03). In children, yearly OOP expenses ≥$1000 were associated with increased AD severity, flares, HCP visits, prescription polypharmacy, and step-up therapy use (P<0.005 for all). Children and adults reported similarly harmful household financial impact from OOP expenses (>60% each). Predictors of harmful impact among children included black race (adjusted OR [95% confidence interval]: 3.86 [1.66-8.98] P=0.002) and ≥$1,000 annual OOP expenditures (6.98 [3.46-14.08], P<0.0001). Children with AD have unique and increased OOP expenses that are associated with significant disease burden. Strategies are needed to reduce OOP costs and improve clinical outcomes in children with AD.

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Health literacy and the ability to understand postoperative instructions in patients undergoing Mohs micrographic surgery

H. Reddy[1], D. DeMeo[1], A. Maytin[2], B. T. Carroll[2]
[1]*Case Western Reserve University, Cleveland, Ohio, United States,* [2]*Dermatology, UH Cleveland Medical Center, Cleveland, Ohio, United States*

Health literacy refers to an individual's ability to navigate the healthcare system and make health-related decisions. Numerous studies link low health literacy with poor outcomes in chronic diseases; however, few studies investigate the impact of health literacy in a surgical setting. Appropriate wound care and pain medication use are imperative for safe recovery after Mohs surgery. Because of this, it is of interest to explore the relationship between health literacy and comprehension of medical instructions in Mohs patients. The primary objective of this study was to determine whether low health literacy was linked to poor comprehension of postoperative wound care instructions in patients undergoing Mohs micrographic surgery. The validated Newest Vital Sign (NVS) survey measured the health literacy level of participants. An additional questionnaire assessed the participants' understanding of pain medication usage as described in their postoperative instructions. A secondary objective of this study was to determine whether a health literacy screening question accurately predicts health literacy in the Mohs population. The widely used screening question employed in this study asks, "How often do you have someone help you read hospital materials?" Of the 24 participants, 58.3% had adequate health literacy as defined by the NVS. Despite this, 79.2% of participants responded "Never" to the health literacy screening question. Furthermore, 45.8% of participants incorrectly identified the timing and dosage of postoperative pain medication. While further investigation is needed to determine the link between health literacy and understanding of postoperative instructions in Mohs patients, preliminary data suggests that some patients are unable to adequately comprehend critical instructions. Identifying methods to screen for and recognize vulnerable patients may allow clinicians to better tailor educational materials to these individuals.

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Patient interest in Mohs surgery telehealth services beyond the COVID-19 pandemic

A. Munjal[1], R. Tripathi[1, 2]
[1]*The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States,* [2]*Johns Hopkins Medicine, Baltimore, Maryland, United States*

Background: Telemedicine in Mohs micrographic surgery (MMS) has expanded significantly throughout the COVID-19 pandemic. Despite this, the role of telemedicine in MMS in the post-pandemic world remains unclear. Our objective was to evaluate trends in patient interest in MMS telehealth services through the COVID-19 pandemic and determine whether heightened interest has persisted following the initial stay-at-home orders. Methods: A Google Trends study was performed to assess interest in MMS telehealth services using the terms "mohs surgery/surgeon + virtual/telehealth" and "skin cancer surgery + virtual/telehealth," which were found to have the greatest inter-term reliability. The results were converted into a combined search volume index (CSIV) with a maximum value of 100. Comparisons were made between the pre-pandemic (1/11/2018-3/1/2020), pre-vaccine (3/1/2020-1/3/2021), and post-vaccine periods (1/3/2021-1/11/2022). Results: The pre-pandemic period mean CSIV was stable at 6.69 (SD 1.19). CSIV peaked at 98.5 on 3/22/2020, decreased to 29.36 (SD 18.95) during the pre-vaccine period, and remained at 18.89 (SD 2.48) during the post-vaccine period (p<0.001). Search interest gradually increased prior to the pandemic (slope 0.016 CSIV/week, r-square 0.189) and plateaued post-vaccine. Prior to the pandemic, search interest for virtual MMS services was highest in metropolitan coastal cities. Pre-vaccine, search interest was highest in rural areas, and post-vaccine, search interest remains highest in smaller metropolitan areas in the Midwest. Conclusion: Public interest in telehealth services for MMS increased drastically during the COVID-19 pandemic and remains significantly elevated when compared to pre-pandemic levels despite reopening of in-person dermatologic care. Given persistent interest among patients, dermatologists should continue to investigate avenues to offer virtual care. Pre-operative evaluations and post-surgical management may be particularly apt for telemedicine when preferred by patients and providers.

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Examining past, present & future shared decision making among eczema patients and caregivers

A. Loiselle[1], I. J. Thibau[1], E. Latour[2], E. Foster[3], W. Smith Begolka[1]
[1]National Eczema Association, Novato, California, United States, [2]Biostatistics Shared Resource, Oregon Health & Science University Knight Cancer Institute, Portland, Oregon, United States, [3]Center for Health & Healing, Oregon Health & Science University, Portland, Oregon, United States

Engaging in shared decision making (SDM) can help patients evaluate and make key healthcare choices for their care. SDM has been shown in several diseases to improve outcomes and is well-suited for conditions like eczema for which several treatment options exist and the clinical and lived experience is varied, yet little is known about the use and experience of SDM in eczema care. To understand SDM in eczema care settings from the patient/caregiver perspective, the National Eczema Association conducted an online survey to assess "past SDM" (SDMQ9, score ranges transformed to 0-100), "present SDM" (Control Preferences Scale), and "future SDM" (self-reported confidence and motivation to engage in SDM) behavior. Inclusion criteria (US resident, patient or caregiver of patient ≤17 yr, respondent age ≥18 yr) was met by 94.7% (1,313/1,387). Respondents were mostly female (80%) adult (82%) patients with mean patient RECAP score of 11.7 (SD 7.2), mean patient age 39.5 yr (SD 22.2), and mean SDMQ9 score of 65.1 (SD 27.4). For present SDM, 50% reported "I prefer to make the final decision after seriously considering my doctor's opinion" and 69% reported being very/extremely confident to engage in SDM in the future. Those "very well informed" about the causes of eczema had a 14.7 point higher (95% CI 9.2-20.2, p<0.001, multiple linear regression) SDMQ9 score than those "not adequately informed" and were 3.4 times more likely (95% CI 2.1-5.7, p<0.001, multiple logistic regression) to be confident to engage in future SDM. Respondents commonly cited healthcare providers (HCPs) initiating SDM conversations and the perception of HCPs valuing their input as motivators to future SDM. This study suggests that a majority of eczema patients/caregivers prefer a large role in decision making for their care and that HCPs have an important opportunity to initiate and facilitate SDM in eczema care settings.

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Development of a digital tool for home-based monitoring of skin disease for older adults

S. van Egmond, Z. Cai, V. Nava, B. Rapaport, J. Ko, A. Chiou, K. Y. Sarin, J. Tang, S. Bousheri, L. Zhang, E. Linos
Dermatology, Stanford University, Stanford, California, United States

The COVID-19 pandemic has accelerated the adoption of telemedicine. However, current tools pose substantial barriers for older adults and those with low digital literacy. By implementing user-centered design, we developed a digital tool, Dermatology for Older Adults (DORA), for home-based monitoring of skin disease, specifically designed for older adults. DORA is a virtual assistant based on REDCap and Twilio APIs that automates image and symptom collection and allows communication between patients and the research team. We evaluated the feasibility, usability, patient compliance, retention, and clinical utility of DORA. Eligibility criteria included patients >70 years with any skin disease, access to a smartphone, and no cognitive impairment. We recruited 62 patients aged 70-94 (mean age 77), 39% female, 81% white from Stanford's Dermatology Clinic from August-December 2021. We asked patients to send weekly photos and answer a questionnaire of a single skin lesion for 4 weeks, then monthly for 4 months. We measured response time, photo quality, and participant satisfaction using mHealth app usability questionnaire (MAUQ). The median response time was 1.4 days (IQR 0.6-3.4). Four participants dropped out. 83% completed photo submission requests (48% at initial request, 19% after 1st reminder and 16% after 2nd reminder). 80% of all questionnaires requested (131 of 163) were completed. Four dermatology clinicians evaluated the quality of the first 88 images and reported good confidence in triaging skin diseases. MAUQ scores were high for ease of use (5.6 SD1.3), interface satisfaction (5.5 SD1.3), and usefulness (5.2 SD1.3). Patients were consistently able to use DORA to submit photos and symptoms and reported high usability and satisfaction. Patient retention was high, and clinicians felt confident making triage recommendations based on DORA images. This approach can be used in other settings where digital literacy barriers and unequal access to dermatologists contribute to healthcare disparities.

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Analysis of fibroblast pen usage on TikTok: A cross-sectional study

L. E. Hernandez[1], N. Mohsin[1], I. Dreyfuss[2], F. S. Frech[1], K. Nouri[1]
[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States, [2]Dr. Kiran C. Patel College of Osteopathic Medicine, Nova Southeastern University, Fort Lauderdale, Florida, United States

Since its inception in 2016, TikTok, a social media platform wherein users can share videos, has served as a tool for the propagation of potentially dangerous cosmetic trends, most recently the fibroblast pen. Potential adverse effects include the development of dyspigmentation, scarring, and mechanical burns. This study assessed social media content to improve our understanding of fibroblast pen, also known as the plasma pen, usage amongst TikTok creators. An initial search of public TikTok posts tagged with "#PlasmaPen," "#PlasmaPenTreatment," "#FibroblastPlasma," or "#FibroblastPlasmaPen" identified 200 posts, of which 78 were eliminated after accounting for overlapping posts, posts that were later deleted, and those in languages other than English. We analyzed posts according to creator type and classified them into 4 main themes. The 122 videos were later re-viewed to provide more detailed subdivisions within the 4 main themes. Analysis showed that 36% of the posts were created by lay-person TikTok users, followed by 25% of posts by self-proclaimed fibroblast skin tightening specialists. Major themes include advertisement of the pen (61%), experience with the pen (26%), education on the pen's uses and benefits (6.5%), and warnings related to its usage (6.5%). TikTok users are more likely to encounter a post regarding pen usage from uncredentialled, non-medical professional accounts. Given that most of the posts were related to advertisement of the procedure and education, respectively, unsubstantiated claims may be disseminated. Only 6.5% of posts were created with the intention of serving as a warning to users, with most being created by medical doctors. Dermatologists should be aware of the misinformation regarding fibroblast pens and consider posting on social media to raise awareness about this potentially dangerous skincare trend.

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Barriers to implementation of a teledermatology virtual clinic within an academic medical center

E. D. Borre, S. C. Chen, M. Nicholas, E. Cooner, T. Ashley, M. Ingrassia, C. Pineo, M. Kheterpal
Duke University, Durham, North Carolina, United States

Background: Teledermatology (TD) may benefit patient access, however detailed barriers to its implementation are unknown. We sought to evaluate barriers to implementation of a pilot virtual clinic TD service at Duke University. Methods: We evaluated the implementation success of TD using the Reach, Efficacy, Adoption, Implementation, and Maintenance framework and the electronic health record. Barriers to implementation were collected through an in-person meeting and survey of site medical directors. Results: Our virtual clinic received referrals and dermatoscopic images from primary care providers (PCPs), with a subsequent video visit with a dermatologist. Implementation at 4 pilot clinics began on 9/10/2021. As of 12/31/2021, there were 128 e-communications and 94 virtual clinic video visits. Compared to in-person wait times of 157 days, the TD virtual clinic occurred on average 2.75 days after e-communication. Most patients returning the patient survey (n=21/22) agreed their clinical goals were met. 92% of PCP images were deemed satisfactory. Participating sites differed in the number of patients referred (range 3-62) and the percent of virtual clinic referrals relative to all dermatology referrals (range 2-24%). Based on the medical director survey (n=4), the primary barriers to placing virtual referrals was lack of fit in the clinic flow, real or perceived time-burden, and little desire to change existing practice. Medical directors also endorsed concerns among PCPs about the complexity and time commitment with dermatoscopic image taking, storage, and attachment. Conclusions: Despite promising access and patient satisfaction results, there is differential volume of referrals to TD by clinic site. Process adjustments to better embed dermatoscopic image taking into clinic flow and increase uptake. Data on the measured PCP time burden required for virtual clinic may indicate where process improvements should be targeted. Lastly, messaging around patient access improvements would highlight the benefits of the virtual clinic to PCPs.

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Trends in office visits for five most common skin diseases in the United States

G. M. Peck[1], S. Muddasani[1], A. Grada[2], A. B. Fleischer[1], S. R. Feldman[3]
[1]University of Cincinnati College of Medicine, Cincinnati, Ohio, United States, [2]Almirall, Malvern, Pennsylvania, United States, [3]Dermatology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States

Introduction: One third of the U.S. is affected by at least one skin condition. Although some obtain care from dermatologists, many patients have skin diseases treated by general practitioners. Methods: We conducted a cross-sectional analysis to determine outpatient visit rates for the five most common skin conditions amongst dermatologists and non-dermatologists in the National Ambulatory Medical Care Survey, 2007 and 2016 (most recent years available). Results: The five most common skin diagnoses amongst all physicians were contact dermatitis, acne vulgaris, actinic keratosis, benign neoplasm, and epidermoid cyst. Over the study interval, visit rates for these five skin conditions remained unchanged (p<0.001). Actinic keratosis, acne vulgaris, and benign neoplasm were the three most common diagnoses amongst dermatologists, whereas contact dermatitis, acne vulgaris, and epidermoid cyst were the most common amongst non-dermatologists. Dermatologist visit rates for contact dermatitis (p=0.0077) and acne vulgaris (p=0.0008) were decreasing over the study interval. Contact dermatitis (p<0.0001) and benign neoplasm (p=0.7) experienced increased visit rates amongst non-dermatologists. Discussion: Contact dermatitis, acne, benign neoplasm of the skin, and actinic keratosis have remained common amongst the U.S. population over the last ten years. Non-dermatologists continue to see almost half of visits for the five most common skin diagnoses. Patients are often referred from the primary care setting for skin lesions; thus, it is not surprising that actinic keratosis has remained the most common dermatologic diagnosis and benign neoplasm the third most common dermatologic diagnosis. Declining dermatology visit rates for contact dermatitis and acne could indicate the condition is readily managed in the primary care setting. While we have identified some changes, the five most common skin diagnoses have remained largely unchanged over the study interval.

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Evaluation of immunization status in psoriasis patients prior to initiation of immunosuppressive therapy- A multidisciplinary approach

D. S. Kim[1, 2], R. S. Gibson[2, 3], M. Her[3, 4], M. Mahoney[4, 5], P. Stering[5], S. Padival[6], D. Taupin[5, 7], M. L. Porter[2, 3, 7]
[1]Tufts University School of Medicine, Boston, Massachusetts, United States, [2]Clinical Laboratory for Epidemiology and Applied Research in Skin, Boston, Massachusetts, United States, [3]Department of Dermatology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [4]Department of Pharmacy, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [5]Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [6]Division of Infectious Diseases, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [7]Harvard Medical School, Boston, Massachusetts, United States

Immunosuppressive medications are a mainstay of psoriasis treatment. As these medications increase the risk of infection, a Pre-Immunosuppression Clinic at Beth Israel Deaconess Medical Center (BIDMC) was created to screen for infectious diseases and evaluate the vaccination status of patients prior to starting immunosuppressive therapies. Of 78 psoriasis patients (mean age= 50, SD= 14, Males= 54% Females= 46%) who were prescribed immunosuppressive medications through the Department of Dermatology at BIDMC from April 2021-Dec 2021, 48 (62%) were referred to the Clinic. Of those who were referred, 39 (81%) patients followed up. Additional vaccination was indicated for 100% of the patients referred, and all referred patients received at least one vaccine. The PPSV23 (n= 33, 85%), PCV13 (n= 31, 80%), and Recombinant, Adjuvanted Zoster Vaccines (n= 21, 54%) were most frequently ordered. 23 (59%) patients had additional screening labs ordered based on history and individual risk. Most frequently prescribed medications that led to referrals include risankizumab (n= 27, 56%), secukinumab and ustekinumab (n= 6, 12%). Mean age of the patients who were ordered vaccinations was 50 years (SD= 14), and mean number of days between the date of referral placed and the clinic visit was 16 days (SD= 11). The high percentage of patients who qualified for vaccinations after evaluation in the Pre-Immunosuppression Clinic demonstrates the need for a systematic, multidisciplinary effort to evaluate and improve the vaccination rates of patients prior to initiating immunosuppressive therapies.

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The paradoxical promotion of UV tanning through sunless tanning

K. Erickson[1], S. J. Eley[2], A. Fan[1], J. Narang[1], J. Bordeaux[1, 3]
[1]School of Medicine, Case Western Reserve University, Cleveland, Ohio, United States, [2]College of Medicine, Northeastern Ohio Medical University, Rootstown, Ohio, United States, [3]Department of Dermatology, University Hospitals, Cleveland, Ohio, United States

Sunless tanning is an alternative to UV tanning with reduced skin safety concerns. We aim to identify retail strategies tanning salons use that influence consumer selection of UV vs. sunless tanning. This cross-sectional secret-shopper study included 155 tanning salons from 31 US cities selected by census data. A Google search identified the five highest consumer-rated salons per city. Three research assistants posing as prospective customers called salons between November 2020-February 2021, using a script to ask about services offered, pricing, and recommendations. Our sample included 36 national, 33 regional, and 86 independent businesses. In total, 81.3%(126/155) of salons offered UV and sunless tanning; 54.8%(86/155) offered combined UV/sunless packages. National chains had higher odds of offering UV tanning (OR=9.93, p=0.03) and combined UV/sunless (OR=31.73, p<0.001) compared to independent businesses. National chains also had higher odds of offering combined UV/sunless (OR=9.71, p=0.005) than regional chains. Regarding salon recommendations, 22.6% endorsed UV, 25.8% combined UV/sunless, 23.9% either, 20% did not answer, and only 7.7% recommended sunless. UV cost less than sunless for monthly packages ($38.73 vs. $66.05, p<0.001) and single sessions ($12.02 vs. $29.93, p<0.001). The majority of salons offered both UV and sunless tanning and most frequently recommended UV tanning. Young adults are the main tanning industry consumers, so the lower cost of UV may influence purchasing. National chains, who often market to young adults, have higher odds of offering combined UV/sunless tanning. Thus, customers who engage in sunless tanning in a salon may be implicitly encouraged to UV tan. Dermatologists should maintain a high index of suspicion with patients who sunless tan, as this study demonstrates sunless tanning is heavily promoted with UV tanning. Further studies are needed to assess if sunless tanning is a predictor for future UV usage.

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Responding to a call of action: An analysis of ethnic diversity within dermatology residency programs

B. Cooper[1], K. Pulsipher[1], C. Presley[2], R. Dellavalle[3, 4, 5]
[1]Rocky Vista University College of Osteopathic Medicine, Parker, Colorado, United States, [2]Division of Dermatology, Lehigh Valley Health Network, Allentown, Pennsylvania, United States, [3]Department of Dermatology, University of Colorado - Anschutz Medical Campus, Aurora, Colorado, United States, [4]Department of Epidemiology, Colorado School of Public Health, Aurora, Colorado, United States, [5]Dermatology Service, VA Eastern Colorado Health Care System, Aurora, Colorado, United States

Recent efforts have been made to increase diversity in healthcare within the United States (US). Among physician specialties, dermatology was identified as one of the least ethnically diverse among residents and practicing dermatologists. Numerous calls of actions to increase diversity among dermatologists have been published in the literature. To evaluate recent trends and the current state of ethnic diversity within dermatology, this study provides a ten-year analysis of ethnicity data from dermatology residents, compared to total graduate medical education (GME) residents. For the academic years of 2011-2021, the total number of GME and dermatology residents were recorded, as well as the residents' self-reported ethnicity data. Data was obtained through analysis of the Accreditation Council for Graduate Medical Education (ACGME) Data Resource Book. Ethnic groups were defined as White, Asian or Pacific Islander, Hispanic, Black, and Native American or Alaskan. Our results showed that from 2011-2021, 56.38% of dermatology residents were white, compared to 44.17% of GME residents overall. The representation of all other individual ethnicities was lower among dermatology residents when compared to GME residents. The gap between minority ethnicities in dermatology and other medical specialties continues to persist, with little improvement over the last ten years. Our study shows only a 1.4% improvement of the gap between ethnic minority dermatology residents and ethnic minority GME residents. Diversity among physicians has been shown to improve health outcomes, particularly for ethnic minority patients. Future studies and efforts should be directed towards increasing ethnic minority representation within the dermatology physician workforce.

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Infantile hemangioma referral delays persist despite 2019 American Academy of Pediatrics Clinical Practice Guideline: Experience at a single quaternary pediatric institution

L. Montoya, K. Johnson, J. A. O'Haver, H. N. Price
Dermatology, Phoenix Children's Hospital, Phoenix, Arizona, United States

Infantile hemangiomas (IH) are vascular tumors that often require timely treatment to reduce morbidity.1,2 The 2019 American Academy of Pediatrics (AAP) Clinical Practice Guidelines (CPG) for the Management of IH recommend referral to dermatology prior to 4 weeks of age, enabling timely treatment initiation.1 This study examines adherence to national guidelines and aims to identify barriers to appropriate referral timing & treatment. This retrospective cohort study examined IH patients, ages 0 to 24 months, referred to Phoenix Children's Hospital (PCH) Dermatology from 1/1/2019 to 12/31/2020, following release of AAP CPG. Patients were categorized into age appropriate (≤4wks) or late (>4wks) referral groups. Associations of referral age w/ demographics/treatments were examined. Among 791 patients identified, 46 (6%) were appropriately referred at ≤4 weeks of age, 680 (86%) were referred late at >4 weeks of age, and 65 (8%) had missing referral dates. For the group of 343 patients who were referred and treated w/ propranolol, mean age at referral, initial dermatology visit, and propranolol initiation was 3.2, 3.8, & 4.2 months, respectively. No statistical differences (p≤0.05) were detected in gender, race, insurance, language, or rates of propranolol/timolol treatment between referral groups. Despite AAP recommendations, the vast majority of infants with IH are referred to PCH Dermatology after 4 weeks of age. Late referral has led to treatment initiation after the rapid growth phase in most patients, which is problematic for those w/ high-risk hemangiomas. Patient demographics were not correlated w/ referral category suggesting that other factors, such as primary care provider referral practices and the COVID-19 pandemic, may have contributed to delayed referrals. Based on mean age at referral and treatment initiation, patients may have already experienced complications from their hemangiomas, which could result in increased healthcare utilization, costs, & morbidity. References: 1) Krowchuk DP et al. Clinical Practice Guideline for the Management of Infantile Hemangiomas: American Academy of Pediatrics. Pediatrics, Jan 2019; 143(1). 2) Tollefson MM and IJ Frieden. Early growth of infantile hemangiomas: what parents; photographs tell us. Pediatrics, Aug 2012; 130(2): e314-20.

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Skin cancer treatment delays during the COVID-19 pandemic

C. B. Lau, K. Yang, C. X. Pan, W. C. Lau, B. Kassamali, V. Nambudiri
Department of Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States

The COVID-19 pandemic has considerably disrupted health care delivery, presenting challenges for dermatologists. We investigated the scope of delayed treatment for melanoma and non-melanoma skin cancers in relation to other cancer types during the COVID-19 pandemic. Respondent data from the National Health Interview Survey (NHIS) was analyzed for the year 2020. Data included demographic information as well as delays and cancellations of cancer treatments due to the coronavirus pandemic. Multivariate odds ratios were calculated using the statistical software, IBM SPSS. P-values <.05 were considered statistically significant. The study cohort comprised a weighted frequency of 5,120,506 respondents with non-melanoma skin cancer (NMSC) and 2,171,953 respondents with melanoma. The average age for patients with NMSC and melanoma was 67 and 65.9, respectively. Patients with NMSC were found to have more delays/cancellations with cancer treatment during the pandemic compared to patients with breast cancer, brain cancer, lung cancer, colon cancer, colorectal cancer, and bladder cancer. Patients with melanoma had more delays and cancellations of treatment compared to patients with breast cancer, leukemia, brain cancer, lung cancer, colon cancer, pancreatic cancer, colorectal cancer, and bladder cancer. Notably, patients with melanoma were found to have more delays in treatment than patients with NMSC (OR 1.23, P<.001). Treatments for NMSC and melanoma were delayed more often compared to most other cancers during the pandemic. These observations suggest that skin cancer care may be particularly vulnerable to care disparities during the pandemic as delays in skin cancer treatments may potentially lead to adverse outcomes. Our finding that melanoma treatment was delayed significantly more often compared to NMSC treatment is surprising given the higher likelihood of metastasis and mortality associated with melanoma. Our study provides valuable insight into the delays of skin cancer treatments experienced by patients in relation to other cancer treatments.

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Qualitative study of medical decision making among patients with hidradenitis suppurativa

Y. Sow[2], N. Salame[1], M. Siira[3], N. Flowers[1], A. Garg[4], D. Kavalieratos[3], R. Patzer[3], S. C. Chen[5], L. Orenstein[1]
[1]Emory University School of Medicine, Atlanta, Georgia, United States, [2]Morehouse School of Medicine, Atlanta, Georgia, United States, [3]Emory University School of Public Health, Atlanta, Georgia, United States, [4]Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Hempstead, New York, United States, 5Duke University School of Medicine, Durham, North Carolina, United States

Hidradenitis suppurativa (HS) is a chronic inflammatory disease characterized by recurrent, painful tunnels and abscesses predominantly in intertriginous sites. Despite the Food and Drug Administration's approval of adalimumab for HS, prescription rates remain low. Low biologic prescription rates may result from patient preferences, provider comfort, inadequacy of real-world outcomes, or health systems factors. Poor disease control may increase need for analgesics and alternative pain-relief seeking strategies. This qualitative study aims to elucidate HS patient attitudes and knowledge about opioid and biologic therapies, preferences for provider communication, and factors influencing treatment decisions. Interviews were conducted with English-speaking patients ≥18 years of age with average Numeric Rating Scale (NRS) pain score in the past 7 days of ≥1. Data collection continued until thematic saturation was reached at 21 interviews. Mean age was 38.5 years (IQR 27.9-43.4); 76% of participants were female and 71% were African American. Almost all (96%) participants had Hurley Stage II or III disease. Mean NRS score for pain over the preceding week was 6 (IQR 3-7) and 62% of patients had Dermatology Life Quality Index scores ≥11. Factors that influenced patients' medical decision making include perceived therapeutic risks, social influences, and access barriers. Personal factors such as disease severity, effectiveness of current regimen, and attitudes towards healthcare also influenced the decision to pursue therapies. Elucidating patient medical decision-making may uncover opportunities for improved patient-provider communication, adherence to treatment plans, and, potentially, outcomes in HS.

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Public sunscreen dispenser distribution in the United States: Continued COVID-19 trends during 2021

M. D. Szeto[1], R. Kokoska[2], J. Maghfour[3], C. Rundle[4], C. Presley[5], T. Harp[6], A. Hamp[7], V. Wegener[8], J. Hugh[9], R. Dellavalle[1, 10]
[1]Department of Dermatology, University of Colorado, Aurora, Colorado, United States, [2]Indiana University School of Medicine, Indianapolis, Indiana, United States, [3]Dermatology, Henry Ford Hospital, Detroit, Michigan, United States, [4]Dermatology, Duke University Hospital, Durham, North Carolina, United States, [5]Division of Dermatology, Lehigh Valley Health Network, Allentown, Pennsylvania, United States, [6]College of Osteopathic Medicine, Rocky Vista University, Parker, Colorado, United States, [7]College of Osteopathic Medicine, Midwestern University, Glendale, Arizona, United States, [8]Pre-Medical Postbaccalaureate Program, University of California Berkeley, Berkeley, California, United States, [9]Dermatology, Colorado Kaiser Permanente Medical Group, Centennial, Colorado, United States, [10]Dermatology, VA Eastern Colorado Health Care System, Aurora, Colorado, United States

The COVID-19 pandemic may have significantly affected consumer preferences and societal behavior regarding sun protection and skin cancer. IMPACT Melanoma is a United States nonprofit organization for skin cancer prevention/education, and a prominent nationwide sunscreen distributor. Substantial decreases in the distribution of public dispensers and sunscreen were noted at the onset of the pandemic in 2020, especially to public health departments and parks/recreation facilities. Analysis of 2021 data has revealed that total distribution remained at similar levels relative to 2020. However, private business (-77%), public health department (-71%), and healthcare facility (-41%) orders decreased the most, while nonprofits (+612%) and educational institutions (+86%) greatly increased orders. 2021 orders continued to demand only hybrid (physical combined with chemical formulation) sunscreens. Maine, Massachusetts, and Wyoming received the greatest total numbers of dispensers and sunscreen in 2021. Despite organizational and regional fluctuations, these persistent overall reductions in public access to sunscreen are concerning, and corroborate broader pandemic patterns of falling retail consumer sunscreen sales. Dermatologists should be made aware of this pandemic-era erosion of consumer attitudes towards sun protection and sun damage risk, and encouraged to continue advocating for sunscreen use during the pandemic.

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Trends in teledermatology utilization in the United States

A. Patel[2], C. Rundle[1], B. Liu[3], C. Green[3], M. Kheterpal[1]
[1]Department of Dermatology, Duke University School of Medicine, Durham, North Carolina, United States, [2]Duke University School of Medicine, Durham, North Carolina, United States, [3]Department of Biostatistics & Bioinformatics, Duke University School of Medicine, Durham, North Carolina, United States

Background: Teledermatology is an effective healthcare delivery model that has seen tremendous expansion over the last decade, which has been particularly pronounced during the Coronavirus Disease 2019 (COVID-19) pandemic. Objective: To better understand teledermatology utilization and patient demographic trends throughout the COVID-19 pandemic. Methods: National-level data were curated for all practices enrolled in the American Academy of Dermatology's DataDerm registry from April 1, 2020, through June 30, 2021. Encounter utilization rates were collected for visit type (i.e., teledermatology versus in-person), sex, race, age, insurance provider, and location. Results: Data from up to 13,964,816 encounters across the United States were analyzed. Sex, race, age, insurance provider, and location were each found to have a significant association with telemedicine utilization (adjusted p<0.001). The proportion of women who utilized services via teledermatology (n=65,023, 66.0%) was greater than those who utilized in-person services (n=2,940,122, 58.3%). Non-white patients made up a higher percentage of teledermatology utilizers (n=8,920, 14.3%) when compared to in-person utilizers (n=394,680, 11.2%). Younger patients (age<40) contributed more to teledermatology service utilization (n=62,695, 83.2%) when compared to in-person services (n=1,329,218, 40.3%). Medicare and Private were larger payor contributors for in-person services (n=1,089,777, 25.2%; n=2712594, 62.6%) than for teledermatology services (n=8232, 5.4%; n=73940, 48.2%). Utilization by out-of-state patients was proportionally higher for teledermatology services (n=19,422, 14.6%) compared to in-person services (n=580,358, 4.2%). Conclusions: Teledermatology services may reach and benefit certain populations (females, younger patients, non-White races, out-of-state patients) more so than others.

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Utilization of resources for cellulitis in hospitalized patients: Predictors of cutaneous abscess diagnosed on ultrasound

B. Cucka, B. Biglione, S. Chand, R. Rrapi, C. Gabel, S. Song, D. Kroshinsky
Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Cellulitis and cutaneous abscess are the most common skin and soft tissue infections (SSTI), accounting for 4 million emergency department (ED) visits and 1.5 million incision and drainage (I&D) procedures. Ultrasound (US) may differentiate cellulitis from abscess more accurately than clinical evaluation and reduces inappropriate I&D procedures, unnecessary antibiotics, and failure to resolve post-drainage. However, there are currently no standardized guidelines for imaging patients. This study assesses the use of imaging in patients diagnosed with cellulitis and the association with clinical risk factors to predict the likelihood of cutaneous abscess diagnosed on US. A retrospective chart review of adult inpatients treated for cellulitis from 2013 through 2018 was conducted. Of the 788 patients who met inclusion criteria, 300 (38.1%) obtained an ultrasound for evaluation of cellulitis, of which 34(11.3%) received a diagnosis of cutaneous abscess. Infection over permanent hardware(8.8%, p=0.018, OR 10.5), infection of groin and buttocks(17.6%, p=0.008, OR 6.86), and active intravenous drug use (IVDU)(44.1%, p=0.003, OR 2.44) were identified as positive predictors of cutaneous abscess on ultrasound. A history of heart disease(5.9%, p=0.014, OR 0.26), tachycardia at presentation(41.2%, p=0.029, OR 0.32) and hemoglobin less than 11.5 g/dl(p=0.003, OR 0.32) were identified as negative predictors of cutaneous abscess on ultrasound. This study is retrospective at a single academic medical center, which may limit its generalizability on a national level. These findings may guide the clinical decision to obtain imaging preferentially in patients with cellulitis overlying permanent hardware, cellulitis of groin and buttocks, and a history of active IVDU and limit use of imaging in cases of cellulitis that lack specific clinical features concerning for abscess.

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Recommendations from cross-sectional, Chinese-language survey of knowledge and prevention of skin cancer among chinese populations internationally

A. Lo[1], L. Y. Chen[1], W. Niu[1, 2], K. Lim[3], J. A. Solomon[1, 4, 5]
[1]University of Central Florida College of Medicine, Orlando, Florida, United States, [2]LSU Health New Orleans, New Orleans, Louisiana, United States, [3]Lehigh Valley Health Network, Allentown, Pennsylvania, United States, [4]Florida State University College of Medicine, Tallahassee, Florida, United States, 5Kansas City University, Kansas City, Missouri, United States

Although there is evidence that skin cancer rates are increasing among Chinese populations, sociobehavioral understanding of perceptions and behaviors among the demographic globally relating to skin cancer risks and protection are poorly understood. We report the current knowledge and beliefs of Chinese populations in North America and Asia regarding skin cancer and propose recommendations for closing the gap. Participants recruited via social media answered a 74-question, internet-based survey in Chinese. Comparisons with chi-squared and Fisher's exact tests were made between responses by Chinese participants in Asia versus in North America and by those with modified Fitzpatrick scores ≤ 14 versus ≥ 15. Of the 113 completed responses (participation rate of 65.7%), 84.1% were Han Chinese, 96.9% were born in China, and 71.4% resided in China. Few but more North American Chinese than Chinese Asians received annual skin checks (4.2% vs 0%, p=0.0086) and believe that their clinician provided adequate sun safety education (43.3% vs 23.1%, p=0.0441). Participants with higher Fitzpatrick scores less frequently received sun safety education from a clinician (11.8% vs 36.1%, p=0.0154). More participants with lower Fitzpatrick scores use sunscreen (67.2% vs 47.1%, p=0.0546), but alternative sun protection usage rates are similar across groups. In conclusion, cultural differences and Fitzpatrick scores can affect knowledge and practices with respect to sun protection and skin cancer among Asian and North American Chinese communities. Through a collective and adaptive effort across all levels of healthcare, knowledge and practices with respect to sun protection and skin cancer can be improved to reduce morbidity and mortality among Chinese populations globally.

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Analysis of dermatology consultation follow-up after emergency department evaluation: An assessment of disparities and potential interventions to increase post-discharge care among vulnerable populations

B. Biglione[1], B. Cucka[1], S. Chand[1], G. P. Smith[1], B. J. Yun[2], D. Kroshinsky[1]
[1]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Emergency Medicine, Massachusetts General Hospital, Boston, Massachusetts, United States

The emergency department(ED) is a frequent source of care for patients with dermatologic disease likely owing to limited access to routine and urgent outpatient dermatologic care. Patients without adequate follow-up planning are at risk for re-presentation to the ED. A retrospective chart review of 152 adult patients who received a dermatology consultation while in the ED for diagnosis and outcomes. 112(73.7%) patients were referred for outpatient dermatology follow-up. An electronic referral resulted in most appointments being scheduled(74.6%) and ultimately attended(90.1%). Expecting the patient to call independently resulted in the lowest rates of scheduled appointments(30.0%). Risk factors for not attending a scheduled appointment include being widowed(OR 11.38,p=0.01), unemployed(OR 8.30, p=0.03), and having unstable housing(OR 19.91, p<0.05). 10.5% re-presented to the ED within 30 days. Patients who re-presented were more likely to have a history of substance use disorder(p=0.04), had received a psychiatry/addiction medicine consult(p=0.01), and/or were recommended follow-up within 3-4 weeks(p=<0.01). Significant predictors of re-presentation included frequent ED history(OR 4.66,p=0.03), initial refusal of treatment(OR 19.24,p=0.01), and Black or African American race(OR 5.16,p=0.02). We identify risk factors for patients who may benefit from additional attention during care planning due to re-presentation risk.

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Improving hairdressers' knowledge and identification of hair loss disorders with use of an educational video

S. Ali[1], M. Collins[1], L. Burns[1], I. Pupo Wiss[1], D. Hagigeorges[1], L. Burns[1], M. Senna[1, 2]

[1]*Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States,* [2]*Harvard Medical School Department of Dermatology, Boston, Massachusetts, United States*

Hair loss, regardless of cause, can have a significant impact on self-esteem, mental health, and quality of life. Early detection and treatment help to improve outcomes and lessen the emotional burden on patients. Lichen planopilaris (LPP), frontal fibrosing alopecia (FFA), and central centrifugal cicatricial alopecia (CCCA) are forms of scarring alopecia that primarily affect women. Haircare professionals are often the first to note signs and symptoms of hair loss in their clients. However, the signs of hair loss, especially cicatricial alopecia (CA), for which early intervention is paramount, are not well known by hair stylists. We conducted a single-group, pretest-posttest intervention study to assess the use of an educational video in training hairdressers to identify signs of CA. Subjects included 40 hairdressers with a mean age of 44.1 and a mean of 20.3 years' experience in haircare. Subjects completed a pre- and post-video questionnaire that assessed the subjects' ability to identify clinical signs of scarring alopecia. Subjects showed increased knowledge about signs of CA after watching the video, with significantly more hair stylists correctly identifying perifollicular scale and redness as a sign of CA after watching the educational video compared to before (90% vs 50%, p<.001). Subjects also had increased ability to correctly identify photographs of persons with CA after watching the video (82.5% vs 57.5%, p=.003). Our data demonstrate that the education of hair stylists using a video can be effective in improving hairdressers' ability to identify signs of CA. These results echo previous studies that have shown the effectiveness of videos in training hairdressers to detect melanoma. This intervention could lead to recommendations to seek dermatologic care and earlier treatment, improving patient outcomes.

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Transition of care in patients with epidermolysis bullosa: A survey study

M. Dykman[1], J. Han[2], S. Lunos[3], A. Nguyen[1], C. Boull[2]

[1]University of Minnesota Medical School Twin Cities, Minneapolis, Minnesota, United States, [2]Dermatology, University of Minnesota Twin Cities, Minneapolis, Minnesota, United States, [3]Clinical and Translational Science Institute/Biostatistical Design and Analysis Center, University of Minnesota Twin Cities, Minneapolis, Minnesota, United States

Epidermolysis bullosa (EB) is a rare hereditary blistering condition with a wide spectrum of disease severity. Children with severe forms of EB have multi-disciplinary medical needs including wound treatments, infection management, nutritional maximization, and psychosocial support. These needs are initially addressed early on in the pediatric setting, but patients eventually age out of the pediatric sphere, transitioning to adult specialists. Furthermore, transition of care is fraught with emotional stress and logistical difficulties for patients and their families. There is little published data on transition of care in EB. We aimed to identify at what rate EB patients successfully transition to adult care and outline the barriers they face along the way. We conducted a survey study recruiting EB patients from the Dystrophic EB Research Association (Debra) website and centers caring for high numbers of EB patients in the United States and internationally from Sept 17, 2019 to Nov 3, 2021. Among adult patients (≥18 years) nine percent of adults identified a pediatrician as their primary care provider. The majority of participants have not discussed transition of care with their healthcare providers nor the healthcare needs required as an adult. Ongoing pediatric subspecialty care was reported by 12% of adults, most commonly in pediatric dermatology and pediatric cardiology. Identified barriers to transition included the perceived lack of adult providers' knowledge about EB patient healthcare needs including challenges with physical activity, work, foot health, hot climate, oral health, and cost of care. Our study suggests the need for transition guidelines, early discussions with families about transition, and practical information for the adult providers accepting care.

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Comparing patient perspectives towards treatment for alopecia areata before and during COVID-19 using social media data mining

J. Jueng[1], V. Bhupalam[1], A. Su[1], C. M. Infante[1], L. Dupuis[1], S. Shaikh[2], R. Dellavalle[3], I. Brooks[2], O. Burton[4], J. Solomon[1, 5, 6]

[1]*UCF College of Medicine, Orlando, Florida, United States,* [2]*Center for Health Informatics, University of Illinois, Champaign, Illinois, United States,* [3]*University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States,* [4]*Clemson Cyber Institute, Clemson, South Carolina, United States,* [5]*Carle-Illinois College of Medicine, Champaign, Illinois, United States,* [6]*USF Morsani College of Medicine, Tampa, Florida, United States*

Alopecia Areata (AA) is often associated with psychosocial distress due to its chronic nature and clinical presentation. Though treatment modalities are available, little is known regarding the emotional responses to these treatments. Thus, treatment response may not always match patient perceived efficacy of treatment or underlying emotional response. The restrictions and lockdowns associated with COVID-19 may change patient perspective towards treatments for AA. Brandwatch, an artificial intelligence-powered social media database was used to identify publicly available social media posts regarding AA treatment modalities before COVID-19 (May 2008- February 2020) and during COVID-19 (February 2020 – April 2021). Natural language processing was used to grade posts based on the Patient Global Impression of Change (PGIC) scale to assess patient-perceived clinical efficacy towards treatment. Emolex was used to determine underlying patient emotions which were compared to PGIC value for each post. 43,969 full-text posts were identified for minoxidil, dexamethasone, prednisone, and JAK inhibitors before COVID-19 and 5701 were identified during COVID-19. Minoxidil, prednisone, and dexamethasone were associated with more positive than negative sentiment during COVID-19 compared to pre-COVID-19 in posts with positive patient perceived efficacy of treatment. In the future, full text analysis will be done to identify and quantify specific reasons for these differences, such as increased rates of telemedicine, fewer aesthetic procedures, and reduced patient load in clinics. Understanding patient perspective towards treatment of AA may improve patient-centered care.

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Googling acne: Analyzing ingredients and price of over the counter acne products

K. Elhage[2], J. Yousif[2], M. Kwa[1], L. F. Stein Gold[1]

[1]*Dermatology, Henry Ford Medical Group, Henry Ford Health System, Detroit, MI, US, health/system, Detroit, Michigan, United States,* [2]*Wayne State University, Detroit, Michigan, United States*

Introduction: Given the convenience of the over-the-counter (OTC) market, many individuals trial OTC products as a means to combat their acne. Within the OTC acne market, there is great heterogeneity in ingredients and price. Herein, we analyze the distribution of ingredients and price among OTC acne products in top Google searches, which the public may encounter when performing an online search. Methods: Google searches for key terms "acne", "acne treatment", "top acne treatment", and "best acne regimen" were performed. Unique acne products for the first 100 websites for each term were collected. Summary statistics for median, range, mean, and standard deviation for price per topical therapy were analyzed. A factorial ANOVA was performed assessing effect of ingredient on price. Results: A total of 272 unique products were collected out of the 400 websites analyzed. The mean price per ounce of all products was $24.79 (standard deviation of $31.84) and median[range] was $10.40 [$0.28-$166]. Retinol (p<0.001), resorcinol (p=0.013), and tea tree oil (p=0.001) were associated with higher product prices. Notably, 12% of products (10% benzoyl peroxidel(BPO), 2% adapalene) contained an active ingredient that carries a grade A strength of recommendation based on AAD clinical guidelines. BPO products were the most affordable with average price per ounce (median [range]) of $8.15 [0.91-138.16]. Adapalene products had an average price per ounce of $18.74 [$12.26-$29.37]. Conclusion: Providers play an important role in educating and helping patients to navigate the OTC market. Based on efficacy and affordability, benzoyl peroxide and adapalene should remain the active ingredient of choice when turning to the OTC market. Given the heterogeneity of the OTC market, patients should carefully evaluate OTC products and be aware that not all products will have ingredients containing a grade A strength of recommendation and know that products with the same topical therapy can vary dramatically in price.

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An analysis of telangiectasia-related social media videos

A. Quinn[1], K. Pulsipher[1], C. Presley[2], J. Anderson[3], M. Laughter[4], C. Rundle[5], R. Dellavalle[6,7]

[1]Rocky Vista University College of Osteopathic Medicine, Parker, Colorado, United States, [2]Division of Dermatology, Lehigh Valley Health Network, Allentown, Pennsylvania, United States, [3]Department of Pathology, Stanford University, Stanford, California, United States, [4]Transitional Year Residency, The University of Texas at Austin Dell Medical School, Austin, Texas, United States, [5]Department of Dermatology, Duke University Hospital, Durham, North Carolina, United States, [6]Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States, [7]Dermatology Service, US Department of Veterans Affairs Rocky Mountain Regional Medical Center, Aurora, Colorado, United States

Recent studies have reported a notable rise in social media use (i.e., TikTok) by dermatologists to educate users about dermatological conditions. Similarly, patients have reported utilizing social media to obtain dermatological information. Due to the nature of Tik-Tok, specifically, any user is permitted to create and post content with very few regulations that might ensure content accuracy. Thus, arises the likelihood that inaccurate medical information is propagated on social media, potentially misleading users in dermatological disease management. We have analyzed the top 10 posts from each of the 13 most searched hashtags on TikTok relating to either disease state or treatment options for lower extremity telangiectasias/spider veins. Information regarding content creator, post type, and user engagement was gathered for each post individually. Of the analyzed posts, 59.2% were published by medical providers while 40.8% were by influencers and businesses (i.e., med spas, equipment suppliers). 81.5% of total posts were classified as educational, 13.1% as advertisement, and 5.4% as promotional. The proportion of creators producing educational content who are not licensed or trained healthcare providers emphasizes the opportunity for inaccurate medical information to be spread via social media. This study highlights the need for dermatologists to engage with social media apps to correct unreliable information as well as prioritize correct patient education during clinical encounters regarding vein pathology treatment.

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Utility of price-estimator tools within dermatology

L. E. Drake[1,2], K. Yang[1,2], S. Ghatnekar[1], V. Nambudiri[2]

[1]Tufts University School of Medicine, Boston, Massachusetts, United States, [2]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States

In 2019, the Centers for Medicare & Medicaid Services (CMS) introduced a requirement for hospitals to publish prices online allowing patients to price shop. This study examined the utility of CMS-mandated price estimators for dermatology procedures. Chargemasters and price-estimator tools from the five largest hospitals in each state (n=250) were reviewed. They were considered accessible if there were publicly available, written in English, and searchable. Each was searched for the presence of dermatologic procedures and their estimated price. Of the 250 hospitals, 89.6% of chargemasters and 92.8% of price estimators were accessible. Of the accessible chargemasters and price estimators, 98.7% and 63.8% contained pricing data on dermatologic procedures, respectively. Of the 36.2% hospitals without dermatologic procedures on their price-estimators, 100% had dermatologic procedures present on their chargemasters. Formatting of price-estimators included searchable databases (93.1%, n =216) and spreadsheets (6.9%, n = 16). The most common dermatology procedures listed were debridement of subcutaneous tissue (CPT 11042) with a median price of $1,135.50 (IQR $577-$3533.67; n= 80), followed by simple incision and drainage of skin abscess (CPT 10060) with a median price of $956.00 (IQR $465.50-$6506.00, n=79), and single punch biopsy of skin (CPT 11104) with a median price of $506.06 (IQR $356.50-$922.08, n=29). Although price estimator tools are widely available in patient-friendly and searchable formats, they are not optimized for shoppable dermatologic services. Dermatologic procedures were present in nearly all available chargemasters, indicating that these services are offered by these hospitals, yet over a third of hospitals omitted dermatologic procedures from their price-estimator tool. Given the wide range of prices, adding more dermatologic procedures to patient friendly price-estimator tools may increase their utility and improve cost savings for dermatology patients.

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An analysis of androgenetic alopecia treatment content on Instagram and TikTok

J. Hatch[8], J. Albrecht[1], C. Presley[2], J. Anderson[3], A. Hamp[3], J. Anderson[4], C. Rundle[5], M. Laughter[6], R. Dellavalle[7]

[1]School of Medicine, University of Utah Health, Salt Lake City, Utah, United States, [2]Division of Dermatology, Lehigh Valley Health Network, Allentown, Pennsylvania, United States, 3Midwestern University Arizona College of Osteopathic Medicine, Glendale, Arizona, United States, [4]Department of Pathology, Stanford University, Stanford, California, United States, [5]Department of Dermatology, Duke University, Durham, North Carolina, United States, [6]Department of Medicine, University of Texas, Austin, Texas, United States, [7]Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States, [8]Texas College of Osteopathic Medicine, University of North Texas Health Science Center, Fort Worth, Texas, United States

Androgenetic Alopecia (AGA) plagues up to 75% of men and 50% of women aged 50. Currently, only two FDA-approved forms of treatment exist: topical minoxidil (Rogaine) and finasteride (Propecia). TikTok and Instagram, prominent social media platforms, allow patients to view content related to these therapies. Unfortunately, social media platforms are unable to monitor those who teach, endorse, or criticize alopecia treatments, often creating a juxtaposition among what is shared on social media and evidence-based guidelines. We collected 78 posts on TikTok and Instagram (40 on Instagram, 38 on TikTok) using hashtags #finasteride, #propecia, #minoxidil, and #rogaine to evaluate dermatologist contributions to social media content. Overall, dermatologists only created 23% of social media posts regarding AGA therapy. User engagement analysis reveals that dermatologists have the highest average number of followers across both platforms (305,695.35) in comparison to others posting about hair loss treatments. This finding indicates dermatologists who choose to utilize these platforms will likely garner a large audience to engage and educate. The high prevalence of AGA and the increasing number of individuals relying on social media for medical advice would allow dermatologists to use Instagram and TikTok to increase awareness and improve education of the pharmacologic treatment of AGA.

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Fear of negative evaluation in hidradenitis suppurativa patients is correlated with worse quality of life

R. Singh[1], A. Senthilnathan[1], I. M. Richardson[1], S. G. Kaplan[4], S. R. Feldman[1,2,3], R. O. Pichardo[1]

[1]Dermatology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States, [2]Pathology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States, [3]Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States, [4]Psychiatry and Behavioral Medicine, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States

Introduction: Patients with hidradenitis suppurativa (HS) may be differentially impacted by social anxiety. We evaluated the tendency for HS patients to have social anxiety and assessed its impact on patient quality of life (QOL). Methods: A total of 63 respondents completed Brief Fear of Negative Evaluation (BFNE) survey to assesses social anxiety, Dermatology Life Quality Index (DLQI) survey to assess QOL and Patient Health Questionnaire (PHQ-9) to assess depression. Respondents were stratified on the median BFNE score 31. Respondents with a score of 31 and greater were classified as high BFNE and respondents with 30 and less were classified as low BFNE. Differences in DLQI scores were compared between the two groups. Results: Respondents mean BFNE score was 31.7 (SD: 9.6). There was a moderate correlation between social anxiety (per BFNE) and worse QOL (per DLQI) ($r=0.418$, $P<0.001$) and depression (per PHQ-9) ($r=0.4007$, $P<0.01$). Respondents in the high BFNE group had worse QOL per sum DLQI and individual questions pertaining to leisure, sports, social, and sexual activities, compared to respondents in the low BFNE group ($P<0.05$). Discussion: In our cohort, HS patients experienced social anxiety (per mean BFNE), and greater social anxiety was associated with worse QOL and depression. HS currently has no definitive treatment, and patients are predisposed to depression and anxiety. According to our cohort, HS patients with high social anxiety experienced worse QOL. Therapeutic interventions to decrease psychological burden may help improve patient QOL. Conclusion: In our cohort, HS patients had a high fear of negative evaluation and high social anxiety may be associated with poor QOL and depression.

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Factors associated with adherence to skin self-examination recommendations for melanoma patients: A prospective cohort study

D. J. Lewis[1], S. Nugent[2], D. B. Shin[1], M. E. Ming[1]
[1]University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]Thomas Jefferson University, Philadelphia, Pennsylvania, United States

Skin self-examination (SSE) by melanoma patients is an important tool in our armamentarium against this deadly cancer, having been found to be associated with decreased melanoma thickness and improved survival. However, patient adherence to SSE recommendations is imperfect, and little is understood about factors associated with improved adherence. We sought to identify factors associated with adherence to SSE recommendations through a prospective cohort study of newly diagnosed melanoma patients seen at the Pigmented Lesion Clinic(PLC) of the University of Pennsylvania from 2012 to 2018. Eligible subjects had their 1st melanoma within 6 months of original PLC visit and had PLC follow up for at least 2.5 years. Patients with metastases were excluded. At each visit, patients were advised to perform monthly SSE and asked about home SSE practices. A total of 369 melanoma patients fulfilled eligibility criteria. The mean age was 56.4 years (SD=13.9), and 202 (54.7%) were male. At their most recent visit during the study period, 260(60.5%) reported doing home SSE since their prior PLC visit. Using a multivariable logistic model, factors significantly associated with SSE included younger age (OR=0.98 for each year increase in age, p=0.005), female sex (OR=1.60, p=0.049), increased disease severity as assessed by calculating expected 8-year survival (OR=2.17, p=0.009), and having a significant other (a "helper") assist with the examination (OR=2.51, p=0.001). Having had total body photography, having an increased number of nevi, and having had more than one melanoma were not associated with adherence either on univariate analysis or when included in a multivariable model with the other variables described herein. We have identified characteristics associated with improved adherence to SSE recommendations. Understanding which patients may be non-adherent allows us to improve targeting of efforts aimed towards increasing adherence to patient populations less likely to comply.

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Retrospective algorithmic application of a dermatological complexity tool

A. Patel[1], M. M. Sarver[1], B. Liu[2], C. Green[2], M. Nicholas[3], S. C. Chen[3, 4]
[1]Duke University School of Medicine, Durham, North Carolina, United States, [2]Department of Biostatistics & Bioinformatics, Duke University School of Medicine, Durham, North Carolina, United States, [3]Department of Dermatology, Duke University School of Medicine, Durham, North Carolina, United States, [4]Division of Dermatology, Durham VA Medical Center, Durham, North Carolina, United States

Background: Current tools to assess patient dermatological complexity are labor intensive and not easily applicable on a large scale. Objective: Create an automated algorithm from a previously implemented manual tool to assess dermatological patient complexity using electronic health record data. Methods: Dermatology outpatient encounters from 1/1/2019 through 4/30/2021 were retrieved and refined to include top 20 primary and associated top 10 secondary ICD-10 coded visits. Provider reported stability scores were also retrieved. Complexity scores were calculated from the following Components: 1) number of diagnoses or treatment options, 2) amount and/or complexity of data to be reviewed, and 3) risk of complications, morbidity, and mortality. Forty encounters ("Test Cohort") were randomly selected from the refined cohort (n=5,863) for manual complexity tool scoring (gold-standard) and comparison to the automated score. Results: McNemar's symmetry test revealed similar scores between automated and manual methods (p=0.906) from the Test Cohort. There was statistically significant and fair agreement between automated and manual methods when including (weighted kappa= 0.36, 95% CI 0.15-0.57, p<0.001) or excluding (weighted kappa=0.30, 95% CI 0.09-0.52, p=0.004) stability scores. Sensitivity analysis of the components revealed the following: moderate agreement for Component 1 (weighted kappa = 0.53, 95% CI 0.37-0.70, p<0.001), fair agreement for Component 2 (weighted kappa = 0.35, 95% CI 0.13-0.56, p=0.005), and moderate agreement for Component 3 (weighted kappa = 0.47, 95% CI 0.25-0.69, p<0.001). Conclusion: Compared to the gold standard, our automated tool was relatively precise in capturing patient encounter complexity.

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529 dermatologists' perspectives on active surveillance for low-risk basal cell carcinoma

S. van Egmond[1], I. de Vere Hunt[1], Z. Cai[1], N. Rizk[1], M. Wakkee[2], M. Chren[3], N. Goldfarb[4], J. Simard[1], E. Linos[1]
[1]Stanford University, Stanford, California, United States, [2]Erasmus MC, Rotterdam, Netherlands, [3]Vanderbilt University Medical Center, Nashville, Tennessee, United States, [4]Minneapolis VA Health Care System, Minneapolis, Minnesota, United States

Active surveillance (AS) might be an appropriate management option for frail older adults with low-risk basal cell carcinoma (BCC) when risks of treatment outweigh benefits. However, AS is not yet incorporated in US clinical guidelines for BCC. The goal of this study was to investigate dermatologists' perspectives on AS, by identifying clinical scenarios in which this option would be recommended. An online survey including demographic questions and clinical scenarios was emailed to 11,476 US dermatologists in December 2021 with 2 reminders. Each clinical scenario was identical except for characteristics that randomly varied including patient age (91 or 71 years), life expectancy (healthy, 3-year- or, 1-year life expectancy), and tumor location (arm or shin). Dermatologists were asked "Which BCC management option would you recommend to this patient?". We compared responses using logistic regression to define odds ratios. 529 dermatologists responded (4.6% response rate); 43% women, 60% general dermatologists, 12% dermatologic surgery, 63% >10 years of experience, 56% group or private practice, 20% academic. AS was the preferred choice (58% of responses) for patients with 1-year life expectancy, 38% for patients with 3-year life expectancy and 6% for healthy patients. The odds ratio comparing AS in 91- to 71-year-old patient was 1.64 (95%CI 1.27-2.12), 9.33 (95%CI 5.71-15.25) comparing 3-year life expectancy to a healthy patient and 20.44 (95%CI 12.52-33.35) comparing 1-year life expectancy to a healthy patient. No significant differences were noted when comparing tumor location on the arm to the shin. In this survey study of over 500 dermatologists, most recommended active surveillance in situations of limited life expectancy and advanced age. This may help guide clinical guidelines and future trials of active surveillance.

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Lived experiences of acne and acne treatment in transgender persons: A qualitative study

C. Alcid[2], S. Gold[1], S. Willner[2], R. Radi[1], J. Barron[1], H. Yeung[1]
[1]Department of Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States, [2]Emory University Rollins School of Public Health, Atlanta, Georgia, United States

Transgender persons receiving hormonal therapy face health disparities related to acne, but the effect of acne on psychosocial health and their access to dermatologic care are not well understood. In this study, we explored the lived experiences of acne and acne treatment 26 transgender persons recruited from private tertiary endocrinology & dermatology clinics and public safety-set multidisciplinary gender centers in Atlanta. Semi-structured 60-minute interviews were conducted, transcribed verbatim, coded, and analyzed deductively using the social ecological model and minority stress theory frameworks and inductively. Participants often noted that acne negatively affected their daily life and acne was viewed negatively by others. Specifically, transmasculine participants more often reported negative self-image due to the acne presence to transfeminine and nonbinary participants. Some transgender participants perceived differences in acne-related stigma from other transgender persons as compared with other cisgender persons. Factors that affected acne care included healthcare coverage, treatment cost, access to acne care knowledge, social support, and internet access. Most participants relied on over-the-counter products and cleansing tools, especially those without dermatologic care access. Participants recommended systems-level changes such as increased access to dermatologists, gender-inclusive language within healthcare setting, multidisciplinary communication between specialists, and more tailored healthcare solutions for transgender persons to facilitate acne care and self-management. Small study sample size limits saturation of some themes. Multi-level healthcare changes, including culturally competent and responsive healthcare environments, are needed to improve acne care and psychosocial health for transgender persons.

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Trends in oral antibiotic use for acne treatment: A population-based study in the United States, 2014-2016

A. Grada[3], J. S. Barbieri[1], A. W. Armstrong[2], R. Salem[3], S. R. Feldman[4]
[1]*Brigham and Women's Hospital, Boston, Massachusetts, United States,* [2]*University of Southern California, Los Angeles, California, United States,* [3]*Almirall, LLC, Malvern, Pennsylvania, United States,* [4]*Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States*

Oral antibiotics are a mainstay in the treatment of moderate to severe inflammatory acne vulgaris. Widespread and prolonged use of oral antibiotics contributes to antibiotic resistance, which has negative public health implications. This retrospective cohort study, conducted from January 2014 through September 2016 using the IBM® MarketScan® claims database, evaluated trends in oral antibiotic use for acne treatment. Patients were ≥9 years of age, prescribed an oral antibiotic, and had ≥2 diagnoses of acne ≥7 days apart. The primary outcome was duration of oral antibiotic treatment over 12 months, with continuous use defined as no longer than a 30-day gap between prescriptions. Among all patients (N=46,267), the most commonly prescribed oral antibiotics were doxycycline (36.7%, n=16,972) and minocycline (36.5%, n=16,903); 73.4%, 11.4%, 4.1%, and 10.9% of patients were prescribed tetracycline-class antibiotics, penicillins, macrolides, and another antibiotic class, respectively. Overall, 36.3%, 17.6%, 10.3%, and 5.4% of patients continuously used any oral antibiotic at 3, 6, 9, and 12 months, respectively. Among patients who continuously used tetracyclines, a similar percentage continuously used minocycline (40.2%, 18.6%, 10.5%, and 5.1%) and doxycycline (34.7%, 14.6%, 7.7%, and 3.9%) at 3, 6, 9, and 12 months. Over 12 months, a greater percentage of patients continued use of tetracycline-class antibiotics than other antibiotic classes. In this retrospective study, nearly 20% of patients continuously used oral antibiotics for ≥6 months, exceeding guideline recommendations of 3-4 months. Considering alternatives such as narrow-spectrum oral antibiotics, hormonal therapy, earlier initiation of isotretinoin, and laser and light-based modalities, is a potential opportunity for reducing the emergence of antibiotic resistance and antibiotic-associated complications.

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Trends in racial diversity of dermatology residency applicants from 2016-2020

S. Ghanian[2], F. S. Frech[1], L. E. Hernandez[1], I. Dreyfuss[3], K. Nouri[1]
[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States, [2]Dermatology, Brown University Warren Alpert Medical School, Providence, Rhode Island, United States, [3]Nova Southeastern University Dr. Kiran C. Patel College of Osteopathic Medicine, Miami, Florida, United States

Disparities in racial diversity in the field of dermatology continue to persist given that dermatology has the second lowest percentage of underrepresented minorities (URM), only second to orthopedic surgery. This study aims to investigate any trends in racial representation of dermatology residency applicants over a five-year period. Dermatology residency applicant race data was extracted from the Electronic Residency Application Service (ERAS) of the Association of American Medical Colleges (AAMC) for application seasons 2016-2020 for a retrospective review study. There was an overall increase in the number of dermatology residency applicants during the five-year study period. Prior to 2018 (midpoint of the study) 14.1% of applicants identified as URM compared to 16.6% after 2018, although this difference was not statistically significant (p=1.42). Our findings suggest that in the study period analyzed racial representation remained relatively similar, with a non-statistically significant increase in URM applicants. Outlining the current trends in dermatology residency applicants may be helpful in identifying factors affecting the disparity in racial representation within the field. Factors that may explain this phenomenon include the discordance between the number of URM medical school applicants accepted and those who pursue dermatology, the intrinsic competitive nature of the specialty that may deter URM students from applying to dermatology residency, residency application fees and travel costs for interviews, inadequate mentorship/recruitment efforts, and emphasis on United States Medical Licensing Examination scores. There is hope that dermatology residency applicants are becoming more racially diverse with improved representation of URMs. Dermatologists should continue to be mindful of efforts to promote cultural diversity in the field in order to be able to address dermatologic concerns among increasingly diverse patient populations.

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Real-world utilization of Delphi consensus diagnostic criteria for pyoderma gangrenosum referrals

W. Liakos, A. Ji-Xu, K. Artounian, L. Downing, J. Nava, A. Toussi, S. Le, E. Maverakis
Department of Dermatology, University of California Davis, Sacramento, California, United States

Pyoderma gangrenosum (PG) is a rare neutrophilic dermatosis characterized by chronic, painful ulcerations. PG is challenging to diagnose due to its variable presentation, clinical overlap with other conditions, and the absence of defining markers. Consensus diagnostic criteria have recently been proposed for the clinical diagnosis of PG but remain underutilized. Our objective was to assess the use of published Delphi Consensus (DC) diagnostic criteria in patients with suspected PG, and to evaluate differences in criteria met between true PG and ulcers due to alternate diagnoses. We prospectively identified patients with suspected PG referred to one dermatologist over a four-year period. PG was diagnosed based on published DC diagnostic criteria. Overall, 27 referrals for PG were identified. Most patients were >55 years (70.4%, 19/27) and female (59.3%, 16/27). Median follow-up was 23 months. Patients were most commonly referred by a dermatologist (51.9%, 14/27) after a median of 6.5 months of care. Twenty-four (88.9%) patients were found to have alternate diagnoses, namely exogenous injury-induced ulcer (37.0%, 10/27), venous/vascular ulcer (22.2%, 6/27), vasculitis (11.1%, 3/27), and infection (11.1%, 3/27). Three patients (11.1%) were diagnosed with PG. The major criterion in the DC criteria was found in 13 (48.1%) referrals. Of the minor criteria, morphology (55.6%, 15/27) was the most commonly met, while cribriform scarring (7.4%, 2/27) was the least commonly met. Our findings show that consensus diagnostic criteria can aid in the objective and systematic evaluation of patients with suspected PG, which can lead to optimization of provider heuristics and benefit patients by reducing misdiagnosis rates, delays in care, and iatrogenic exacerbation of alternate conditions.

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I'm sensitive! Understanding the patient experience of self-reported sensitive skin

S. Neadle
Johnson & Johnson Consumer Companies Inc, Skillman, New Jersey, United States

Consumer self-reported sensitive skin has become more prevalent yet there is limited knowledge on exactly what sensitive skin is and the skin attributes associated with sensitive skin. An online survey was conducted with 1,000 participants in the US with two key objectives: 1) understand who sensitive skin sufferers are and 2) understand their skin concerns and experiences with skin care products. Consumer insights on the most commonly perceived sensitivity triggers, symptoms, and associated skin conditions were evaluated. Almost 65% of those who self-identify as having sensitive skin discover their sensitivity before they are 30 years old. In addition, those who self-identify as extremely sensitive are about 200% more likely to have been diagnosed with eczema, rosacea, acne vulgaris, or skin allergies than those who identify as slightly sensitive. Moreover, itchiness (74%), dryness (61%), and redness (64%) are the most prevalent skin reactions among self-perceived sensitive skin consumers. This study contributes to the overall understanding of consumers who self-report as having sensitive skin and provides important context to healthcare professionals on the sensitive skin experience.

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Quality of life impact of acne by region in transgender and gender diverse persons: A cross-sectional study

S. Willner[1], C. Alcid[1], S. Gold[2], R. Radi[2], J. Barron[2], H. Yeung[2]
[1]Department of Behavioral, Social, & Health Education Sciences, Emory University Rollins School of Public Health, Atlanta, Georgia, United States, [2]Department of Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States

Transgender and gender diverse persons face specific skin health disparities. Little is known about the severity, distribution, and impact of acne in the setting of gender-affirming hormone therapy. Our aim is to examine the severity and distribution of acne in transgender and gender diverse persons and its association with quality of life impact and depression symptoms. Participants were surveyed in tertiary academic endocrinology and dermatology clinics and a public safety-net multidisciplinary gender center in Atlanta, Georgia. Acne severity in the past week was self-reported using the Patient Global Assessment overall and separately for the face, chest and back. Quality of life impact and depression symptoms, measured using Skindex-16, COMPAQ, and CES-D, were compared by gender identity and overall acne severity (mild vs. moderate-to-severe acne) using linear regression. 13 transgender men, 10 transgender women, and 4 non-binary persons completed the survey. Mean acne severity differed by gender identity (3.5, SD (0.9) in transgender men; 2.4 (1.0) in transgender women; 2.5 (1.7) in non-binary persons, p=0.03). Mean acne severity differed by gender identity on the back (3.3 (1.0) vs 2.5 (0.7) vs 1.7 (0.6), p=0.02), but not the face or chest. After adjusting for acne severity, mean Skindex-16 Symptoms score differed by gender identity (55.8 (27.2) vs 28.8 (24.7) vs 17.7 (20.0), p=0.01), while Skindex-16 Emotions, Functioning, COMPAQ, and CES-D scores did not. Small sample size of participants recruited from healthcare settings may limit generalizability. Acne severity and quality of life impact differed by gender identity, particularly due to back acne in transgender men. Culturally responsive care will be required to facilitate clinical examination and treatment of truncal acne and to ameliorate its impact in transgender and gender diverse persons.

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Assessing the ease of use and adherence of topical corticosteroid drug delivery devices in caregivers of pediatric patients with atopic dermatitis

W. Baghoomian, E. Simpson
Oregon Health & Science University, Portland, Oregon, United States

Topical corticosteroids are the mainstay of treatment for atopic dermatitis (AD) and when used as directed, are highly effective. Upwards of 50% to 88% of children and adolescents are non-adherent with their prescribed regimen. In addition, inaccurate dosing of topical steroids is a large source of treatment failure. Existing studies exploring the barriers and interventions in adherence place the blame and responsibility on patients and clinicians to improve outcomes. Little is known about topical drug delivery devices as a source of non-adherence, specifically issues concerning inaccurate dosing. The objectives of this study were to identify specific barriers in dosing and topical treatment application processes that may impair treatment adherence. Caregivers (n=46) of pediatric patients with a history of AD and topical corticosteroid use were identified in outpatient dermatology clinics at Oregon Health & Science University and given a 28 question Likert scale survey to complete. Questions ranged from the caregiver's user experience, issues regarding dosing, adherence, identification, and preferences for alternative drug delivery devices. 74% of responses "strongly agree" or "agree" with the statement "I often must guess how much I am applying on my child" and "knowing how much medication to apply on my child would make me more likely to use it on my child." 83% of responses "strongly agree" or "agree" with the statement "if my topical medication came out of the tube in the right amount, I would be more likely to use it on my child" and "I would prefer a topical medication tube that gives out the consistent amount of medication each time." Other statements caregivers "agree" 45% of the time include: their current topical drug delivery device often leaks, is difficult to open and close, is messy to use, and is often wasteful. In summary, our survey data reveals that addressing inaccurate dosing and improving the ease of use of topical drug delivery devices may improve adherence for caregivers of children with AD.

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Skin cancer-related behaviors and perceptions in cyclists: A cross-sectional survey

L. Chu, H. A. Braun, H. Yeung
Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States

Exercise was the most common activity reported during recent sunburns in US adults. Cycling, an increasingly popular sport, includes long periods of sun exposure. This cross-sectional survey aimed to examine sun-protective behaviors and beliefs related to tan skin within the context of cycling tans in a population of road cyclists in Atlanta. 57 recreational to competitive cyclists in the Atlanta Peloton "ATLPLN" group participated in a pilot survey distributed via email and Instagram. Sun-protective behaviors including applying sunscreen before rides, reapplying after two hours, and carrying sunscreen on rides. We also explored beliefs related to tan lines obtained while cycling. A subset of 43 participants (75%) who used a popular exercise tracking app estimated the time spent riding outdoors and the number of sunburns and sunburns from cycling within the past 12 months. Respondents were 79% male. 81% at least "sometimes" applied sunscreen before rides, 100% "never" or "rarely" reapplied sunscreen on rides, and 91% "never" or "rarely" brought sunscreen on rides. 56% believed tan lines reflect the number of miles ridden, and 46% believed tan lines reflected how hard they have trained. Among Strava users who reported at least 1 sunburn, 61% of sunburns were attributed to cycling. Study limitations included small sample size, missing data, and potential for selection bias. Sunburns due to cycling are common. All respondents reported low use of sun-protective behaviors during cycling, which likely contributed to cycling-related sunburns. Positive attitudes towards cycling tan lines represented a previously underrecognized risk factor for skin cancer risk behaviors. Future tailored interventions aimed to improve cycling-related sun-protective behavior and reducing cycling-related sunburns may be warranted.

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Hidradenitis suppurativa patient perspectives on clinic visits

T. Shih[1], D. R. De[2], V. Y. Shi[3], J. Hsiao[4]
[1]University of California Los Angeles David Geffen School of Medicine, Los Angeles, California, United States, [2]University at Buffalo Jacobs School of Medicine and Biomedical Sciences, Buffalo, New York, United States, [3]Dermatology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States, [4]Dermatology, University of Southern California, Los Angeles, California, United States

Introduction: Given the complexity of hidradenitis suppurativa (HS) management, it may be challenging for healthcare providers(HCPs) to meet patient needs in time-constrained clinic visits. Objective: Characterize patient priorities during HS clinic visits. Method: An anonymous survey was distributed to 3 online HS support groups from 10/2021-1/2022. Patients responded to questions on demographics and perspectives on HS clinic visits. Associations between demographics and Likert scale (0-5) questions were assessed using Spearman rank correlation. Statistical tests were conducted in R(v1.2.1335). Results: Of 158 participants, 92.4% were female and 77.8% White. Mean age was 40.8 years(SD 11.13, range 18-64). Participants self-reported Hurley stages as I (8.2%), II (41.1%), or III (49.4%). Over 70% of HS patients are interested in discussing treatment plans (80.4%), treatment options (79.1%), current/past treatments (73.4%), symptoms (72.8%), and self-managing flares (71.5%) during first clinic visits. At subsequent visits, patients prioritized discussing emotional well-being (64.6%), treatment plans (61.4%), and HS symptoms (58.2%). Only about 1/4 of patients expressed interest in discussing weight loss or sexual health with their health care provider (HCP). Patients reported their HCP is managing their HS well when they spend an adequate amount of time during clinic (83.7%), suggest new treatment options when needed (77.8%) and help their HS improve (76.5%). About 1/2 (52.0%) would recommend their main HS HCP to other patients; patients who were seen in HS specialty clinics were more likely to recommend their HCP (4.6 v 3.4, p<0.0001). Discussion: Understanding HS patient preferences during clinic visits will help HCPs meet expectations and improve patient satisfaction. Topics such as weight loss and sexual health should be approached with sensitivity.

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Impact of the COVID-19 pandemic on dermatology visits among older adults and racial and ethnic minorities

N. Rizk[1], M. B. Mathur[2], J. Ko[1], A. Chiou[1], I. de Vere Hunt[1], S. van Egmond[1], E. Linos[1]
[1]*Stanford University School of Medicine, Stanford, California, United States,* [2]*Quantitative Sciences Unit, Stanford University, Stanford, California, United States*

Little is known about how the COVID-19 pandemic affected the demographic make-up of in-person and telemedicine visits during the pandemic and the trend in the proportion of older adults and of racial and ethnic minorities seen at dermatology visits over time. We evaluated differences in demographic and diagnostic characteristics comparing in-person versus telemedicine visits during the pandemic and conducted two interrupted time series (ITS) analyses modeling older adult patient visits and racial and ethnic minority patient visits. Our sample included 351,253 visits from 91,290 patients at Stanford Dermatology from 2016-2021. Overall, we found that compared to in-person visits, a greater proportion of telemedicine visits were with patients aged 18-30, female patients, Hispanic patients, as well as Asian and Black patients ($p<0.001$ for each analysis). In regard to diagnostic characteristics, compared to in-person visits, a lower proportion of telemedicine visits were conducted with patients with malignant neoplasms ($p<0.001$). In our ITS analysis focused on older adults, the proportion of patient visits with adults >65 years was stable in the pre- and post-pandemic announcement period. Similarly, the ITS analysis modeling the proportion of racial and ethnic minority visits also remained stable in the pre- and post- pandemic announcement period. The number of telemedicine visits increased from an average of 1,296 visits (approximately 2% of all visits) per year from 2016 to 2019 pre-pandemic to 21,975 visits (24% of all visits) from March 2020 to July 2021. Our findings suggest that the introduction of telemedicine may have helped stabilize access to dermatologic care for older adults and minorities throughout the pandemic and improved access to dermatologic care for younger patients, female patients, and racial and ethnic minority patients.

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Determinants of antibiotic stewardship for acne: A pilot survey of key stakeholders

K. B. Case, E. C. Thompson, J. Barron, R. Radi, L. Chu, H. Yeung
Department of Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States

Oral antibiotics remain one of the most frequently prescribed systemic treatments for acne, despite growing concerns for widespread antibiotic resistance. The American Academy of Dermatology recommends limiting oral antibiotics to less than three months for acne treatment, yet adherence to these guidelines remains inconsistent. An important first step in addressing guideline adherence is to examine determinants of antibiotic stewardship in acne clinical practice. We surveyed 30 key stakeholders: 22 dermatologists, 4 infectious diseases specialists, 2 dermatology advanced practice providers, and 2 dermatology residents. Influences on antibiotic prescribing practices were quantified using the validated 23-item "Influences on Patient Safety Behaviors Questionnaire" (IPSBQ). IPSBQ scores were summarized descriptively. Participants were 53% female. 73.3% identified as non-Hispanic White, 20% non-Hispanic Asian, 3.3% non-Hispanic Black, and 3.3 % Hispanic. Participants had practiced for an average of 15.1 (SD, 13.4) years. Factors reflecting highest barriers to antibiotic stewardship included "social influence", "beliefs about capabilities", "memory, attention, and decision processes", and "environmental context and resources" with mean IPSBQ domain scores (SD) of 3.28 (0.79), 3.21 (1.09), 2.9 (0.93) and 2.67 (1.01), respectively. Lowest barrier was "knowledge", with mean score of 1.8 (0.677). IPSBQ domain that varied the most was "skills" (mean, 2.13, SD, 1.09, coefficient of variation, 51.1%). Key stakeholders identified important barriers to translating existing guidelines into real-world clinical practice, which extend beyond knowledge deficits. These barriers can be targeted in future interventions that address growing concerns for antibiotic stewardship in acne treatment. Designing and implementing evidence-based interventions in a systematic fashion may improve acne antibiotic guideline adherence in routine clinical practice.

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Impact of telehealth appointments on pharmaceutical management of dermatological conditions

A. Munjal[1], R. Tripathi[2], P. Kinn[3], K. Percival[4], D. Ince[5], J. G. Powers[6]
[1]*The University of Iowa Roy J and Lucille A Carver College of Medicine, Iowa City, Iowa, United States,* [2]*Internal Medicine, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States,* [3]*Pharmacology, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States,* [4]*Pharmacology, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States,* [5]*Internal Medicine, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States,* [6]*Dermatology, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States*

The impact of the COVID-19 pandemic caused dermatology providers to use telemedicine to safely arrange clinic appointments during lockdowns. This study aimed to evaluate the impact of telehealth on antibiotic prescription length. Specifically, we sought to compare antibiotic length prescription for virtual vs. in-person visits before, during, and after COVID-19 shutdowns. A retrospective cohort study was performed using all documented pharmaceutical prescriptions of tetracycline in 2019-2021 prescribed by dermatology providers at a large academic tertiary referral center. Results show an increase in telemedicine visits from 0.75% (2019) to 18.51% (2020), with a decrease to 3.98% in 2021 ($p<0.0001$). Analysis demonstrates that a tetracycline prescription of over 91 days was given in 37.90% vs. 28.83% of visits for virtual vs. in-person visits respectively ($p<0.0001$). Interestingly, 52.64% of antibiotic prescriptions written by staff physician dermatologists exceeded 91 days vs. 18.18% for dermatology fellows, 25.74% for resident physicians, and 21.35% for physician-assistants ($p<0.001$). The demonstrated increase in duration of tetracycline prescription during virtual visits is perhaps indicative of less data available for clinical decision-making, longer wait times between provider appointments during this era of lockdowns, and providers desire to make the visit worthwhile. Future studies should explore factors related to provider decision-making in virtual compared to in-person visits. This research is important in laying a foundation for how virtual visits may play a greater role in dermatologic care as we move towards a post-COVID world.

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Consumer attitudes, knowledge, and behavior towards aging skin during the COVID-19 pandemic

L. Yang, J. Knoll, R. V. Kundu
Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Background: Among the many changes brought about by the COVID-19 pandemic, makeup sales decreased while skin, nail, and hair care sales increased1, suggesting a shift in focus amongst consumers. There has been little research examining how consumer knowledge, attitudes, and behavior towards how aging skin have changed during the COVID-19 pandemic. Methods: A cross-sectional, online survey was administered through REDCap. Eligible participants (English-speaking participants 18 years or older) were recruited and consented online through ResearchMatch. Results: A total of 1434 participants were recruited. 15% (216/1434) were between ages 18-30, 28% (408/1434) between ages 31-50, and 56% (810/1434) were ages 51 or above. 19% (272/1434) identified as men, 78% (1125/1434) were women, and 3% (37/1434) were genderqueer. Overall, the COVID-19 pandemic reduced the amount of time people spent per day covering up their age-related skin changes; 13% (181/1434) spent over 10 minutes prior to the pandemic, compared to 9% (128/1434) during the pandemic. The most influential factor that affected how people felt about their skin during the pandemic was spending more time on video conferencing platforms (28% (401/1434) were affected). Other factors included wearing masks and pandemic-induced stress, anxiety, or self-isolation. These factors tended to make people care more about their skin. Conclusion: Many consumers are affected both physically and emotionally by age-related skin changes. The pandemic has played an important role in how consumers feel about their skin.

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Impact of crisaborole & tacrolimus 0.03% on patient-reported outcomes and caregiver burden in children with atopic dermatitis

J. Wieser[1], A. Chen[2], G. Lee[1], L. Baughman[1], E. M. Pope[1], A. Franco[1], B. Verhave[1], B. Johnson[1], T. Love[2], L. A. Beck[1], J. Ryan Wolf[1]
[1]*Dermatology, University of Rochester Medical Center, Rochester, New York, United States,* [2]*Biostatistics, University of Rochester Medical Center, Rochester, New York, United States*

Poor quality of life (QoL) is common in children with atopic dermatitis (AD) and their caregivers. This study used patient-reported outcomes (PROs) to monitor the impact of crisaborole (CRIS) and tacrolimus 0.03% (TAC) on children (2-15 years) with mild/moderate AD and on caregiver burden. This open-label study randomized 47 child-caregiver dyads to CRIS or TAC for 12 weeks. Disease severity (Eczema Area & Severity Index [EASI], % body surface area, Investigator Global Assessment [IGA]), QoL (Children's Dermatology Life Quality Index, Family Dermatology Life Quality Index), itch (PROMIS Peds Itch-Short Form), anxiety (PROMIS Anxiety-Peds), pain interference (PROMIS Pain Interference-Peds), depression (PROMIS Depression-Peds), sleep (Children's Sleep Habits Questionnaire), and caregiver burden (Caregiver Burden Inventory) were assessed at baseline, 6 and 12 weeks. A total of 36 dyads completed the study. Children (mean age=8.0±3.9 yrs) had mild baseline AD (EASI=4.9±3.7) and were diverse by race (39% white; 36% Black) and gender (53% males). Caregivers were mostly female (78%; 37±7.6 yrs). Both arms improved disease severity over 12 weeks (EASI: CRIS=-2.4 vs. TAC=-1.9, p=0.577). At 6 weeks, TAC had worse caregiver burden on emotional health (-0.1 vs. 1.8, p=0.017) and social relationships (-0.4 vs. 2.2, p=0.006) than CRIS. Within arm analysis at 12 weeks revealed TAC significantly improved caregiver burden, QoL (caregiver, child), and all child PROs except sleep (all p<0.05). In contrast, CRIS only improved QoL (caregiver, child), depression, and pain interference (all p≤0.05). Our results confirm the utility of PROs to monitor treatment response. Both CRIS and TAC reduced disease severity, but TAC improved more child PROs. Future trials should implement PROs to fully understand the impact of treatment.

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Impact of the COVID19 pandemic on the execution of real world, pragmatic trials: The LITE study experience

B. Hefele[1], K. Duffin[2], L. Howard[3], D. B. Shin[1], J. M. Gelfand[1]
[1]*University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [2]*University of Utah Health, Salt Lake City, Utah, United States,* [3]*National Psoriasis Foundation, Portland, Oregon, United States*

The LITE study (NCT03726489) is the largest US-based academic pragmatic trial in dermatology to date. It compares home v office phototherapy for psoriasis at 38 sites with goals of 1050 enrolled and equal skin type representation embedded in routine clinical care. The COVID19 pandemic resulted in profound impacts to routine dermatological care and execution of clinical research. To determine the impact of the pandemic on enrollment, we defined time periods as pre-pandemic (March 2019-March 2020) and post-pandemic (April 2020-January 1, 2022). Enrollment rate was calculated over each time period as per patient per active site to account for the closures and additions of sites throughout the study. To determine barriers and facilitators we collected qualitative responses monthly throughout the post-pandemic timeframe and analyzed. The overall enrollment rate dropped from 1.2 patients per site per month to 0.5 patients per site per month as of April 2020. Of the 29 active sites pre-pandemic (11 academic/18 private), 87% closed to enrollment at one time post-pandemic; all that remained open (n=4) were private. Post-pandemic, 12 academic and 2 private sites were newly activated, and 5 private practices were withdrawn. Commonly reported barriers included institutional holds, staffing shortages, worsening patient financial situations, reduced capacity for patient visits and office phototherapy, uncertain childcare and at-home situations, and increased loss of insurance coverage. LITE developed and transitioned to a remote consenting model, including assessments via teledermatology reflective of the shifting standard of care. Site-specific support for engagement, recruitment, and remote coordinating efforts were employed by the centralized team. Though creative solutions were implemented to address many reported barriers, the residual impact of the pandemic on the economy, healthcare, and family life continues to challenge the execution of the LITE study.

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Real world prescribing patterns of dupilumab for atopic dermatitis

T. E. Sivesind[1], G. Bosma[2], C. Hochheimer[2], L. M. Schilling[3], R. Dellavalle[1]
[1]Dermatology, University of Colorado, Denver, Colorado, United States, [2]CIDA, Colorado School of Public Health, Aurora, Colorado, United States, [3]Medicine, University of Colorado, Denver, Colorado, United States

The prevalence of atopic dermatitis (AD) in the USA is ~12% in children and ~7% in adults. We conducted a retrospective, observational, cohort study examining prescribing patterns of the systemic biologic dupilumab for AD using University of Colorado Hospital and Children's Hospital Colorado electronic medical record databases. We sought to test the hypothesis that this expensive medicine (~$3.5K/dose https://www.drugs.com/price-guide/dupixent, accessed 1/19/22) might be less commonly prescribed for Black and Hispanic patients. Study subjects were between the ages of 4 and 85 years of age as of 3/28/2017 (the date dupilumab was FDA approved to treat moderate to severe AD in those age 12 and older). AD diagnosis inclusion criteria included having at least two diagnoses of AD (defined as having at least two ICD-10 codes L20, L20.0, L20.8, L20.81, L20.82, L20.84, L20.89, L20.9). Any prescription of dupilumab was included. Dupilumab start date was required to be on or after 3/28/2017, and had to occur on or after a diagnosis of AD. 267 persons out of 7723 persons meeting AD diagnosis criteria received at least one dupilumab prescription. Mean (standard deviation) age among those prescribed dupilumab for AD was 47 (22) years and 57% were female. Analysis by patient characteristics revealed the following associations (odds ratio [confidence interval] p value): age per ten year increment (1.06 [1-1.12] 0.051), female (0.93 [0.73-1.20] 0.59), Black (2.3 [1.58-3.33] <0.001), and Hispanic (0.79 [0.50-1.19] 0.28). Our preliminary data revealed surprisingly that Blacks were more likely to be prescribed dupilumab than other racial and ethnic groups in our Colorado academic health care system. Our analysis will further explore associations with severity of disease, insurance type, and inclusion of patients who had multiple diagnoses of AD plus another diagnosis that may also be treated with dupilumab.(e.g. certain cases of chronic rhinosinusitis with nasal polyps and asthma).

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Factors affecting electronic dermatology consultations for patients with uncertain cutaneous neoplasms

S. Himed[1], B. H. Kaffenberger[2]
[1]*University of Cincinnati College of Medicine, Cincinnati, Ohio, United States,* [2]*Internal Medicine, Division of Dermatology, The Ohio State University, Columbus, Ohio, United States*

With the growing incidence of skin cancer globally, electronic consultations (e-consults) can be a useful tool for dermatologists in the assessment of cutaneous lesions. In this study, we sought to characterize the social and institutional factors affecting completion of initial e-consults as well as in-office follow-ups. Patients with an ICD 10 code of neoplasm with uncertain behavior at The Ohio State University Medical Center that received an e-consult order from May 2017 to May 2021 were queried. Additional information collected included patient demographics, zip code affluence, follow-up in-office appointment and referral status, as well as number of completed visits. In-office follow-up appointments were defined as completed or as no-contact. These factors were then used to assess differences in the completion of e-consults, and the status of in-office follow-up appointments. A total of 667 patients were identified as having received an order for an e-consult, of which 427 (64%) had a completed e-consultation while 240 (36%) did not. Year of encounter (p<0.0001) and number of completed visits (p<0.004) were found to be significantly associated with completion of initial e-consult and remained significant in the multivariate model. For in-office follow-up appointments, 429 patients presented for an in-office appointment, while 82 had no contact. The status of follow-up appointments was significantly associated with patient's race (p<0.0001), language spoken (p<0.0028), and referral status (p<0.018). In the multivariate model, patient race remained as the only significant association (p<0.003). Electronic consultations to assess possible cutaneous neoplasms is an important tool in the growing incidence of skin cancer. Our study demonstrated a successful increased utilization of e-consults, though the follow-up model was affected by social factors. Additional systems need to be implemented to ensure minorities and non-native English speakers are obtaining adequate dermatologic care.

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Dietary factors differentiate vitiligo patients with stable or active disease.

M. Daniel[1], K. Cedercreutz[1], S. M. Rangel[1], Y. Ali1, K. M. Daftary[1], E. Dellacecca[1], L. van Horn[2], S. Green[3], I. Le Poole[1], R. V. Kundu[1]

[1]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [3]Internal Medicine, Rush University, Chicago, Illinois, United States

Vitiligo is mediated by T cells targeting melanocyte antigens. Depigmentation in vitiligo-prone mice can be accelerated or delayed by oral antibiotics in vitiligo-prone, but not in healthy mice. We questioned whether lifestyle factors influence microbiome composition and the development of human vitiligo. Patients and controls matched for age, sex and skin type were consented before administering dietary and lifestyle questionnaires, collecting skin and stool samples and evaluating clinical parameters related to vitiligo. Five-20 individuals per group were included per parameter. Patient VASI, BSA and VitiQol were measured. The NHANES validated dietary questionnaire (DHQII) provided nutritional data that were analyzed using R. DNA isolated from skin and stool samples was subjected to 16SrRNA and shotgun sequencing. No significant differences between patient and control groups were found for skin care parameters, household composition, occupation or diet. However, significant reductions in the % Parabacteroides and a trend towards reduced Bacteroides were observed in patient stool. Correlation analysis revealed biologically and statistically significant differences in dietary composition between patients with active or stable disease. Notable differences include elevated carbohydrate, total fat and cholesterol consumption and caffeine intake among patients with stable disease. These patients also consumed more fruit. Importantly, stable disease was associated with 80% increased dietary intake of vitamin B2 and Ca2+, each associated with a healthy gut. Meanwhile a strong trend towards increased energy derived from dietary alcohol was found among patients with active disease. We tentatively propose that reduced stress combined with increased dietary calcium and riboflavin can support a healthy gut and disease stabilization in vitiligo.

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An analysis of dermatology resident population's medical trainings and degrees

B. Cooper[1], K. Pulsipher[1], C. Presley[2], R. Dellavalle[3, 4]

[1]Rocky Vista University College of Osteopathic Medicine, Parker, Colorado, United States, [2]Division of Dermatology, Lehigh Valley Health Network, Allentown, Pennsylvania, United States, [3]Department of Epidemiology, Colorado School of Public Health, Aurora, Colorado, United States, [4]Dermatology Service, VA Eastern Colorado Health Care System, Aurora, Colorado, United States

Thousands of medical students apply for residency each year in the United States (US). The majority have graduated medical school and received a degree from a US based allopathic program (US MD), a US based osteopathic program (US DO), or are international medical graduates (IMG MD). The underrepresentation of US DO and IMG MD residents, compared with US MD residents, within the field of dermatology has been well documented in the literature. Numerous calls to action have both highlighted this disparity and advocated for an increase in representation of US DO and IMG MD residents within dermatology. To assess recent trends, we provide a ten year analysis of medical training background data of dermatology residents, compared to total Graduate Medical Education (GME) residents. For the academic years of 2011-2021, the total number of GME and dermatology residents were recorded. The residents' background medical training was determined through analysis of the Accreditation Council for Graduate Medical Education (ACGME) Data Resource Book. Our results demonstrate that from 2011-2021, 90.41% of dermatology residents were US MD, 6.16% US DO, and 3.41% IMG MD compared to 63.16% US MD, 11.94% US DO, and 24.84% IMG MD GME residents overall. Improvements have been made from 2011-2021, as the representation of US DO and IMG MD residents in dermatology increased from 2.0% to 10.7% for US DO's and from 3.0% to 3.5% for IMG MD's. While the percentage of US DO and IMG MD residents within dermatology has been increasing, attention should be directed towards making the representation of these two groups in dermatology similar to what is seen in other specialties. Future studies and endeavors should attempt to increase the representation of US DO and IMG MD physicians within the field of dermatology.

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Characteristics and reasons for litigations involving dermatitis: An exploratory analysis

R. Raiker[1], K. Jenkins[2], H. Pakhchanian[3], L. Shen[2]

[1]West Virginia University School of Medicine, Morgantown, West Virginia, United States, [2]Boston University School of Medicine, Boston, Massachusetts, United States, [3]The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, United States

Previous research has examined the causes and characteristics for litigation among patients who listed psoriasis and cutaneous malignancies as reasons for lawsuits. However, reasons for litigation among patients with any form of dermatitis have not been assessed. Therefore, the goal was to assess this by an exploratory analysis using Westlaw, one of the largest online legal databases. Search terms "dermatitis or eczema" were used. Cases with judges and settlements were manually reviewed and excluded if dermatitis was unrelated to the overall lawsuit. Demographics, prosecution reason, verdict, dermatitis type, and case payouts were examined. 98 out of 155 cases met the inclusion criteria, ranging from years 1983-2021. 59% of plaintiffs were female and most cases were in the West (30%). Juries returning plaintiff and defendant verdicts were 37% and 43% respectively with others being settlements. Dermatitis was the primary cause of litigation in 72% of cases and the most prevalent form involved was contact dermatitis (62%) with minimal atopic dermatitis cases (5%). The median and mean payout among cases was $25,000 and $224,781 respectively (range from $325-$5075000). Top reasons for litigation included toxic exposure (41%), medical malpractice (32%), and negligent business practices (16%). Subgroup analysis of toxic exposure cases revealed 42% were from an adverse reaction to a product, 30% from an occupational hazard, and 27% were from improper cosmetic services. Among medical malpractice cases: 81% were won by defendants, 7% of overall defendants were dermatologists, and only 4% mentioned adverse effects of topical steroids. The findings here demonstrate majority of plaintiff-won cases came from lawsuits where patients suffered contact dermatitis and sued businesses. Additionally, minimal cases involved atopic dermatitis, topical steroids side effects, or lawsuits against physicians. Further research is warranted.

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Utilization of teledermatology services for dermatological diagnoses during the COVID-19 pandemic

A. He[1], T. T. Kim[2], K. Nguyen[1]

[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Naveen Jindal School of Management, The University of Texas at Dallas, Richardson, Texas, United States

Little is known about trends in teledermatology adoption and use for managing dermatologic patients, especially changes in use influenced by the COVID-19 pandemic. In this retrospective cohort study, we analyzed encounter data from the Healthjump dataset (containing electronic health record data from throughout the US) for visits from November 2019 to July 2021 with a primary dermatology-related diagnosis. There was a striking rise in teledermatology use with the onset of the pandemic in February 2020, peaking in April 2021 with 2,178 teledermatology encounters (32.8% of all encounters). Subsequently, teledermatology use waned. When compared to those with neoplastic skin diseases, patients with inflammatory skin diseases were more likely to be seen via teledermatology (OR 3.30, 95% CI 3.12-3.49). Certain demographic groups were less likely to receive care via teledermatology, such as men (compared with females, OR 0.76, 95% CI 0.74-0.78) and patients 65 and older (compared with those below 65, OR 0.59, 95% CI 0.57-0.62). Our work shows increased adoption of teledermatology at the onset of the COVID-19 pandemic with decreasing use over time. Future efforts are needed to ensure continued and expanded use of a valuable care modality to reach vulnerable populations.

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Characterizing inpatient hospitalizations for hidradenitis suppurativa and assessing the impact of outpatient dermatology care on hospitalizations

J. Maghfour, V. Liu, R. Huggins, I. H. Hamzavi
Dermatology, Henry Ford Health System, Detroit, Michigan, United States

Introduction: Hidradenitis suppurativa (HS) is associated with a significant disease burden. The use of high-cost settings care are common among HS patients. Objective: To explore factors that may influence hospital admissions and readmissions among HS patients. Methods: Using ICD-9/10 codes (705.83 and L73.2), we extracted the medical records of adult HS patients who visited the Henry Ford Health System (HFHS) ED between 2010 and 2020. Results: Of the 100 HS patients, 52 (52%) were admitted to an inpatient service. Hypertension (OR:2.55,95% CI:1.11-5.83, p value=0.027), diabetes mellitus (OR:2.42, 95%CI:1.05-5.61, p value =0.039), cellulitis (OR: 19.28, 95%CI:4.23-87.96 p<0.001), sepsis (OR:10.25, 95%CI:1.34-89.24, p value=0.025), and depression (OR:3.32, 95%CI:1.10-10.04, p value =0.002) were significant predictors of admission. Chronic kidney disease (OR:3.05, 95% CI:1.00-9.23,p value=0.049), congestive heart failure (OR:4.06, 95%CI:1.19-13.80, p value =0.025), coronary artery disease (OR:15.20, 95%CI:2.80-82.65, p value=0.002), chronic obstructive pulmonary disease (OR:8.94, 95%: 1.51-52.86, p value =0.003), cellulitis (OR:4.62, 95%CI:1.66-12.88, p=0.003), sepsis (OR:3.75, 95%CI:1.02-13.82,p value =0.047), and depression (OR:4.50, 95%CI:1.54-13.18, p value=0.006) were positively associated with readmission. Those who received outpatient dermatology care had a lower risk of being admitted (n=87, 28.7% vs n=13,100%, p <0.001) and readmitted (n=10, 11.5% vs n=5, 38.5%, p value =0.0108). Discussion: In this study, we demonstrate that certain comorbidities, that are common among HS patients, are significant determinants of admission to an inpatient service. Furthermore, the increase access to outpatient dermatology care significantly reduces the likelihood of HS patients being admitted and readmitted. Conclusion: The findings of this study illuminate the pivotal role of dermatologists in improving patients' health outcomes while minimizing the avoidable use of high-cost settings care.

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Characterization of ABCA12 gene variant by electron microscopy in an infant with an ichthyosiform dermatitis and MALT1 deficiency

L. Gardner, A. K. Dewan, J. Meyer
Department of Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States

The ABCA12 gene encodes an ATP-binding cassette transporter vital to skin barrier function. In keratinocytes, ABCA12 transports ceramides into the lumen of lamellar bodies as part of the widely conserved water barrier system. Alterations in the ABCA12 gene are associated with the autosomal recessive congenital ichthyoses: harlequin ichthyosis, non-bullous congenital ichthyosiform erythroderma, and lamellar ichthyosis. We report a 4-month-old female who presented with erythroderma, diffuse fine scale, and recurrent infections. Hematoxylin & eosin skin biopsy demonstrated parakeratosis, spongiosis, and a T-cell lymphocytic infiltrate at the dermoepidermal junction. Congenital ichthyosis and severe combined immunodeficiency genetic panels revealed the patient to be heterozygous for p.Ala833Val on ABCA12. This variant is present in population databases but has not been reported in the literature in individuals with ABCA12 related ichthyoses; its associated phenotype is unknown. The patient was also found to have a known pathogenic variant of MALT1, which is associated with autosomal recessive combined immunodeficiency as well as an "Omenn or Netherton syndrome-like" exfoliative dermatitis. We sought to further characterize her ABCA12 mutation with electron microscopy. Electron microscopy revealed foci of inflammatory cells, dermal edema, and intact lamellar bodies present within the granular and spinous layers of the epidermis. We concluded that this ABCA12 variant is not pathogenic, and that skin findings in this patient were best explained by the MALT1 variant. This case provides an example of how in-depth molecular understanding of protein function can inform the clinical management of genetic disease, especially in cases where the pathogenicity of a gene variant is in question.

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Comparison of epidermal gene expression profiles in mice aged 1 to 20 months

S. Wen[1], L. Ye[2], X. Wang[1], D. Liu[1], B. Yang[1], M. Man[2]
[1]*Guangdong Provincial Dermatology Hospital, Guangzhou, Guangdong, China,* [2]*Guangdong Provincial Dermatology Hospital, Guangzhou, Guangdong, China*

Although it is well known that epidermal function changes with aging, the transcriptomic basis and possible signaling pathways for aging-associated functional changes remain largely unknown. Here we employed RNA sequencing technique to assess epidermal gene expression profiles in the epidermis of mice aged 1, 2, 6, 12 and 20 months old. Our results showed that a total of 132 genes displayed reductions in expression levels with aging while expression levels of 406 genes increased with aging. Epidermal gene expression was prominently upregulated in 2 months vs. 1 months old mice, while more genes were downregulated after 12 months old. Upregulation of genes associated with immune/inflammatory responses were observed in the epidermis of aged mice in comparison to that of young mice, whereas down-regulated signaling pathways in the epidermis of aged mice were primarily involved in metabolism such as fatty acid elongation, glutathione metabolism, and biosynthesis of antibiotics. Some signaling pathways such as chemokine signaling, cytokine/cytokine receptor interaction signaling and IL-17 signaling pathways were remarkably upregulated in 12 months old mice. Steroid synthesis, metabolic pathway, thermogenesis, and proteasome pathways were steadily downregulated, starting at 2 months old. These results indicate that the epidermis of aged mice displays a upregulation of genes associated with inflammation signaling pathways, and downregulation of genes related to metabolic signaling pathways.

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Tralokinumab treatment modifies stratum corneum lipid composition in skin of adolescents with atopic dermatitis

E. Berdyshev[1], E. Simpson[8], I. Bronova[1], A. Pavel[2, 7], W. Soong[3], R. Antaya[6], S. Imafuku[4], M. A. Røpke[5], L. Jiang[5], E. Guttman-Yassky[2], D. Y. Leung[1]
[1]*National Jewish Health, Denver, Colorado, United States,* [2]*Icahn School of Medicine at Mount Sinai, New York, New York, United States,* [3]*AllerVie Health-Alabama Allergy and Asthma Center, Birmingham, Alabama, United States,* [4]*Fukuoka University, Fukuoka, Japan,* [5]*LEO Pharma A/S, Ballerup, Hovedstaden, Denmark,* [6]*Yale School of Medicine, New Haven, Connecticut, United States,* [7]*University of Mississippi, University Park, Mississippi, United States,* [8]*Oregon Health & Science University, Portland, Oregon, United States*

In atopic dermatitis (AD), Th2 cytokines, including interleukin (IL)-13, alter skin lipid metabolism. Tralokinumab is a high-affinity, monoclonal antibody that neutralizes IL-13. We evaluated effects of tralokinumab on stratum corneum (SC) lipid composition in adolescents with moderate-to-severe AD (ECZTRA 6, NCT03526861). Adolescents (age 12-17) were randomized to subcutaneous tralokinumab 150mg, 300mg every 2 weeks, or placebo. Primary endpoints were IGA 0/1 and EASI-75 at Week 16. Tape strip samples were collected for SC lipid and transcriptomics analyses in lesional and non-lesional skin while ceramides, sphingomyelins and natural moisturizing factor (NMF) components were quantified using targeted LC-ESI-MS/MS. At Week 16, greater proportions of patients receiving tralokinumab achieved IGA 0/1 (150mg/300mg 21.4%/17.5% vs placebo 4.3%; P<0.001/P=0.002), and EASI-75 (28.6%/27.8% vs 6.4%; P<0.001/P=0.001). At baseline, key NMF components, urocanic acid (UCA) and 2-pyrrolidone-5-carboxylic acid (PCA), and barrier lipid omega-acyl ceramide (EOS-CER) were significantly downregulated in lesional vs non-lesional skin while short-chain NS-ceramide (NS-CER) and sphingomyelin levels were upregulated. Tralokinumab substantially increased NMF content and improved lipid composition by increasing EOS-CER content and proportion of NS-CER with long-chain fatty acids while decreasing sphingomyelins. Tralokinumab improved AD severity and shifted skin NMF and lipid parameters from a lesional to non-lesional skin profile, demonstrating the effectiveness of neutralizing IL-13 in improving the skin barrier, evidenced by shifts in lipids of importance for maintaining intact SC structure.

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Microfluidic culture insert device for fabrication of full-thickness human skin equivalent with perfusable vascular-like channels

D. Myasnikova[1], M. Mori[2], A. Toyoda[2], K. Yo[2], S. Takeuchi[1]
[1]*Mechano-Informatics, Tokyo Daigaku, Tokyo, Tokyo, Japan,* [2]*POLA Chemical Industries, Inc., Yokohama, Japan*

Full-thickness skin equivalent(SE) is widely used to assess the safety of chemicals. However, a lack of important components, such as vascular and neuro-immune systems, prevents the use of such models in acute toxicity, repeated dose toxicity, and reproductive developmental toxicity tests. Several approaches to fabricate vascularized SE have been published. However, each of them had its limitations. Some models did not contain vascular-like channels but instead had an underlying channel, which does not allow differentiation between percutaneous penetration and absorption by vessels. The models containing vascular-like channels used collagen as a matrix, which led to uncontrollable shrinkage and made it impossible to do TEER measurement or permeation test directly in the device used for SE fabrication. We present an improved version of the microfluidic device for the fabrication of SE with vascular-like channels, previously designed in our laboratory. We designed a smaller one that fits into a 6-well plate like a culture insert. An anchor's unique design allows controlling collagen shrinkage, which results in fabricating SE with a reproducible size of 1 cm in diameter. The SE is tightly attached to the device, allowing TEER measurement and permeation test directly in the device. Cultured SE consisted of ~100 μm epidermis, ~2 mm dermis and ~200 μm diameter vascular-like channels covered with endothelial cells. Immunohistological and immunofluorescence staining confirmed the formation of stratum corneum and living layers. We successfully measured TEER and performed a permeation test for caffeine to characterize barrier function. We collected samples both from underneath a SE and from vascular-like channels, representing percutaneous penetration and absorption by vessels. We believe that our device will be helpful in the establishment of an in vitro system for chemical toxicity testing, which requires the assessment of systemic exposure. This study was funded by POLA Chemical Industries, Inc.

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PP2A-B55α dephosphorylates desmoplakin's C-terminus to regulate cell adhesion

A. Perl[1], J. Koetsier[1], S. Roselli[3, 4], N. Panicker[3, 4], N. Verrills[3, 4], K. Green[1, 2]
[1]Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [3]The University of Newcastle School of Biomedical Sciences and Pharmacy, Callaghan, New South Wales, Australia, [4]The University of Newcastle Hunter Medical Research Institute, New Lambton, New South Wales, Australia

Critical for the maintenance of epidermal stability and function are attachments between intermediate filaments (IF) and intercellular junctions called desmosomes. The desmosomal cytoplasmic plaque protein desmoplakin (DP) is essential for anchoring IF to the junction. DP-IF interactions are regulated by a phospho-regulatory motif within the DP C-terminus. Previously we showed that hypo-phosphorylated DP increased DP-IF interaction strength, impairing desmosome formation due to DP retention on IF, but generated stronger, more stable desmosomes in mature cell sheets. Thus, DP's phospho-regulation provides a mechanism to finely tune junction assembly dynamics and intercellular adhesion strength. Our group identified GSK3b as capable of phosphorylating DP's C-terminus; however, the phosphatase responsible for DP's dephosphorylation was unknown. Protein phosphatase 2A (PP2A) comprises a scaffolding subunit (A), a catalytic subunit (C), and one of 23 different regulatory subunits (B) responsible for binding PP2A substrates. Here, we identify the PP2A-B55a holoenzyme as capable of dephosphorylating DP's C-terminus. Using co-immunoprecipitation and colocalization analysis we demonstrate B55a as a new DP binding partner in keratinocytes and uncover a novel DP-dependent membrane-bound fraction of B55a in both 2D- and 3D-epidermal models. Furthermore, we show that PP2A-B55a activity increases tissue integrity in a manner similar to expression of a hypo-phosphorylated DP variant, S2849G DP. Finally, PP2A signaling is required for proper DP recruitment during desmosome assembly in vitro and *in vivo* in embryonic epidermis of B55a-deficient mice. Together, our data uncovers a potential mechanism for PP2A-B55a's regulation of the DP-IF association and intercellular adhesion in the epidermis.

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Effect of myrciaria fubia on in vitro tattoo model

L. Restellini, C. Meyrignac, C. Capallere, I. Imbert
Ashland Global Specialty Chemicals Inc, Covington, New Jersey, United States

Insertion of pigments under the skin to create tattoos is around since about 6 000 years old (Neolithic period). Nowadays, United States have more than 20 000 tattoo parlors with one more opening every day. 36% of Americans between the age of 18 and 29 have at least one tattoo. Among the population with tattoos, 70% have more than one and 20% have more than five. 32% of people with tattoos claim that they are addicted to ink. The increasing number of tattooed persons urges the development of reliable test systems to assess tattoo associated risks (phototoxicity, inflammation, sensitization, etc.). In this study, 3 main color inks (carbon black, pigment orange and titanium oxide) were tested to determine their ability to initiate inflammation. Several biological models (fibroblasts and macrophages in 2D, reconstructed tissues in 3D) have been used to test these inks. First, we tested the phototoxicity potential of ink compounds on validated 3T3NRU phototoxicity test (OECD TG432) and phototoxicity was observed according to the ink tested. Then, these inks were tested in dermal fibroblasts and macrophages derived PBMC. H&E staining observations demonstrated that the inks were ingested inside the macrophages 24h after the application, whereas the inks were staying at the surface of the fibroblasts. Inks application induced an anti-inflammatory response by an increase of IL8 cytokine expression and also an increase of MMP1 (involved in extracellular matrix destruction). A treatment with a Myrciaria dubia fruit extract was investigated to help reduce the inflammatory response. These innovative in vitro models have been developed to evaluate biofunctionals and natural ingredients to prevent tattoo-induced skin reactions focusing particularly on fibroblasts and immune specialized cells.

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Glyoxal induces senescence in in vitro skin models

C. Nizard[2], R. Halkoum[2, 3], C. Plaza[1], V. Salnot[4], K. Pays[2], A. L'Honoré[3], B. Friget[3], C. Capallere[1], I. Petropoulos[3], I. Imbert[1]
[1]Ashland Global Specialty Chemicals Inc, Covington, New Jersey, United States, [2]LVMH Recherche, Saint-Jean-de-Braye, Centre, France, [3]Sorbonne Universite, Paris, Île-de-France, France, [4]Université Paris Descartes 3P5, Paris, France

Senescence is a well-known cellular event characterized by specific markers like a permanent cell proliferation arrest and the secretion of messenger molecules forming the Senescence-Associated Secretory Phenotype (SASP). The SASP composition depends on many factors such as the cell type and the nature of the stress that induced senescence. Since the skin constitutes a barrier with the external environment, it is particularly subjected to different types of stresses and thus to premature cellular aging. In this study, we have demonstrated that glyoxal provokes senescence in our skin in vitro models. Indeed, application of glyoxal on keratinocytes during the reconstruction induced a senescent phenotype of epidermis with a thinning of epidermal thickness (H&E), an increase of senescent markers (p16), an increase of inflammatory cytokine (IL1a), and a decrease of terminal differentiation epidermal protein expression (filaggrin, loricrin). Our study provides evidence that glyoxal can affect keratinocyte function and act as a driver of human skin aging. Hence, senotherapeutics aimed at modulating glyoxal-associated senescent phenotype, could be relevant to prevent the aging process in skin. These innovative in vitro models have been used to develop and screen ingredients. Results allowed to select ingredients with high potential to fight against consequences of glyoxal stress.

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Novel glycerin compounds improve skin health.

G. Shiota, R. Tsuruoka, K. Hanada
R&D, Mediplus Pharma,.Inc, Tokyo, N/A, Japan

Novel glycerin compounds (NGCs) were developed which generated new reaction products, endoperoxide derivatives. NGCs have been reported to exhibit various biological effects. In addition to their deodorizing, disinfecting and antiviral effects, NGCs were found to promote wound healing in the skin by stimulating epithelial growth and regeneration with the synthesis of extracellular matrix such as hyaluronic acid. In this study, in order to clarify the effects of NGCs on normal human skin, we quantified the mRNA gene expression of heme oxygenase-1 (HO-1) and DAD(P)H quinone dehydrogenase 1 (NQO-1) as antioxidant factors by PR-PCR and the protein content of intracellular glutathione (GSH) using a culture system of normal human epidermal keratinocyte. Moreover, gene expression of factors related to skin barrier functions (involucrin (INV), filaggrin (FLG), and serine palmitoyl transferase (SPT)) was examined to clarify the effects of NGCs on epidermal cell differentiation. Our results showed that NGCs enhanced expression of HO-1, NQO-1 mRNA and GSH protein in a concentration-dependent manner from 20 ppm after 24 h of stimulation. These results suggest that the oxidative property of NGCs activates Nrf2 (NF-E2-related factor 2) transcription factor, which is a biological defense sensor, and induces the expression of antioxidant factors. Since their expression was caused by oxidative stimulation at low concentrations of NGCs, it was strongly suggested that their hormesis effect would be working protectively for the skin. Consistent with this, the expression of INV, FLG, and SPT genes also increased at low concentrations of NGCs: FLG showed a threefold increase compared to the control group. These results suggest that NGCs promote epidermal cell differentiation and have positive effects on skin barrier functions. Mild oxidative stimulation of NGC may lead to healthy skin through hormesis effect. We are currently conducting detailed analysis of the effects of NGCs using three-dimensional cultures of human epidermis.

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Atopic dermatitis patients have decreased epidermal innervation but increased neuronal substance p expression

G. N. Calco[1], W. Baghoomian[1], D. B. Jacoby[2], E. Simpson[3]
[1]School of Medicine, Oregon Health & Science University, Portland, Oregon, United States, [2]Pulmonary and Critical Care Medicine, Oregon Health & Science University, Portland, Oregon, United States, [3]Department of Dermatology, Oregon Health & Science University, Portland, Oregon, United States

Severe pruritus in atopic dermatitis (AD) significantly impairs patients' quality of life, primarily due to relentless itching. Sensory nerves in the epidermis are important in mediating sensation of itch, but there is conflicting evidence whether epidermal nerve density is increased or decreased in lesional skin of AD. Here we measured nerve length, branching, and neurotransmitter expression in epidermis of healthy controls and lesional and non-lesional skin of AD patients. Four millimeter punch biopsies were taken from skin of 8 healthy adult control patients and 8 lesional and non-lesional skin of patients with moderate-to-severe AD. Biopsies were fixed and immunostained. Epidermal sensory nerves were identified by staining with pan-neuronal marker, PGP9.5, and neuroinflammatory marker, substance P. Sensory nerve length and branching in epidermis were measured in optically cleared, whole-mount tissue biopsies using confocal microscopy and Imaris 3D digital modeling software. Average epidermal sensory nerve length was 667 (±352) nm in control skin and decreased to 445 (±270) nm in AD non-lesional skin and 273 (±177) nm in AD lesional skin (p = 0.04). Average number of branch points also decreased from 7 (±6) in control patient skin to 3 (±3) in AD non-lesional skin and 2 (±1) in AD lesional skin. Colocalized neuronal substance P volume increased from 13 (±17) μm3 in skin of control patients to 21 (±27) μm3 in non-lesional AD skin and 27 (±18) μm3 in lesional AD skin. The ratio of substance P to PGP increased in epidermal skin from 0.03 (±0.03) in control skin to 0.26 (±0.4) in non-lesional skin and 0.28 (±0.3) in lesional AD skin (p = 0.02). In conclusion, patients with AD have decreased epidermal innervation in lesional skin. Despite this decrease in overall sensory nerve density, increased neuronal substance P expression in the epidermis may contribute to pruritus in AD.

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Modulation of calcium channel activity in Darier's disease keratinocytes improves disease phenotypes

L. M. Godsel, J. Koetsier, R. M. Harmon, M. Hegazy, A. L. Huffine, C. T. McCullough, S. A. Svoboda, L. Luass, M. Prakriya, B. E. Perez White, K. Green
Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Darier's disease (DD) is an autosomal dominant skin disorder characterized by acantholysis and dyskeratosis associated with mutations in ATP2A2 encoding for the sarco/endoplasmic reticulum Ca2+-ATPase pump type 2 (SERCA2), resulting in patients being functionally haploinsufficient for SERCA2. DD keratinocytes displayed a 1.5-fold increase in Ca2+ levels above that observed in control keratinocytes. This Ca2+ imbalance negatively influenced localization of protein constituents of the cell-cell adhesive junction, the desmosome, resulting in decreased desmosome function and loss of epidermal integrity. Toward resolving the Ca2+ imbalance, DD keratinocytes and controls were treated with a pharmacological SERCA2 activator, CDN1163. CDN1163 treatment of DD cells increased border localization of desmosome components plakophilin 3 and desmoplakin 2-fold above DMSO treatment, restoring desmosome formation to the level of control keratinocytes. Desmosome uniformity along cell borders was enhanced 2.5-fold above DMSO treated DD cells and 1.5-fold above DMSO treated control keratinocytes. Correspondingly, cell-cell adhesive strength was restored, evaluated by measurement of monolayer integrity upon challenge with mechanical stress. As a second approach, we utilized the compound dantrolene sodium, administered clinically as a muscle relaxant for patients with spasticity associated with CNS disorders such as multiple sclerosis, cerebral palsy and stroke. Dantrolene acts to dampen the activity of Ryanodine receptors, Ca2+ SR/ER pumps responsible for regulating Ca2+ release from ER stores. Dantrolene treatment resulted in a 2-fold increase in desmosome protein localization at DD cell borders and the restoration of monolayer integrity upon challenge with mechanical stress. These results highlight the potential for drugs designed to balance Ca2+ levels in the epidermis of DD patients as promising avenues for therapeutic intervention to reduce disease severity.

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***In vivo* evaluation of skin volatile organic compounds and squalene peroxidation on stressed and aged skin**

Y. Ferreira, L. Mouret, A. Veitch, A. Perrin, A. Le Mestr, J. Botto, I. Imbert
Global Skin Research Centre, Ashland Global Specialty Chemicals Inc, Sophia Antipolis, France

Volatile organic compounds (VOCs) are emitted from various natural and chemical sources. Some VOCs may have therapeutic benefits (e.g., the VOCs emitted by forests, usually odorant and pleasant). Others may have harmful effect, like the ones due to solvents, adhesives, fuels, or industrial wastes. The human body also emits various VOCs (via breath, skin...) which represent the footprint of the cellular activity and can thus reveal certain dysregulation of the metabolism. These emanations depend on the physiological status as they may vary with the donor, the gender, with aging, the diet and the microbiota. Skin VOCs arise from eccrine, sebaceous and apocrine gland secretions and their interactions with microbiota determine our body odors. Specific skin VOCs such as the aldehydes 2-nonenal (Asian skin) and nonanal (Caucasian skin) increase with age. These VOCs derive from lipid peroxidation which increases with age and oxidative stress. A clinical study was designed to evaluate the variations of nonanal, on subjects applying on the face, a formulation containing a botanical extract vs placebo. The study enrolled 25 stressed Caucasian volunteers, aged 36 to 66 yo. After one month of application twice a day, the use of the formulation was associated with a decrease of squalene peroxidation and nonanal emanation compared to placebo. In parallel, the skin luminosity (known to be influenced by lipid peroxidation) and fine lines parameters were evaluated. An improvement of skin luminosity as well as an attenuation of fine lines parameters were noticed on the formula-treated side, compared to placebo. This study pointed out the concomitant decrease of both skin lipid peroxidation level and nonanal emanation level, associated with the application of the formula in aged or stressed population.

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Efficacy of cosmeceuticals on skin structure-function utilizing preclinical models

S. Iglesia[1], A. Zahr[2], T. Kononov[1], M. Palm[3]
[1]Research and Development, Revision Skincare, Irving, Texas, United States, [2]Research and Development, Revision Skincare, Irving, Texas, United States, [3]Art of Skin MD, Solana Beach, California, United States

Objective: To investigate the efficacy of three cosmeceutical products; vitamin C serum (VCS) containing Tetrahexyldecyl (THD) Ascorbate, a multi-ingredient anti-aging facial and eye moisturizer (MFM and MEM), in improving epidermal and dermal structure and function utilizing preclinical models. Background: Dermatologists confidently recommend cosmeceuticals that demonstrate efficacy and tolerability across a broad patient population. Robust clinical studies are needed to substantiate efficacy, as ingredient lists provide limited insight. Companies focused on physician selected cosmeceuticals are tasked with finding innovative and credible methods to demonstrate product efficacy. Execution of preclinical models can provide valuable results that can be helpful to physicians, especially when supported with *in vivo* data. Methods: MatTek tissues (EpiDermFT™) were treated with the MFM and MEM. Controls were daily rinsed paralleling treated conditions. On Day 7, tissues were fixed, and thin histological sections were performed and stained with hematoxylin and eosin (H&E) or processed with antibodies for immunohistochemical analysis. Facial skin explant from a 60-year-old Asian female with Fitzpatrick Skin Type III were incubated with the VCS and MFM for five days, fixed and stained with H&E and Masson's Trichrome Stain (MCS). Results: MFM and MEM significantly increased epidermis thickness and upregulated collagen IV and elastin by 2-fold in the EpiDermFT™. The morphology of the MFM and VCS treated skin explants demonstrated a stronger network of extracellular matrix proteins compared to control. The MFM generated a denser papillary dermis. The VCS produced dense protein fibers and upregulated collagen by 145% (*p < 0.05) versus control visualized by MCS. Conclusion: The preclinical results strongly suggest that the VCS, MFM, and MEM are strongly effective in improving skin structure and function and may offer physicians a solution for patients looking for highly effective cosmeceuticals.

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Corneoptosis, functional keratinocyte cell death, is tightly associated with spaciotemporal dynamics of epidermal tight junctions

T. Matsui[1, 2], M. Urabe[2], K. Fukuda[2, 3], M. Amagai[2, 3]
[1]Laboratory for Evolutionary Cell Biology of the Skin, School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo, Japan, [2]Laboratory for Skin Homeostasis, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan, [3]Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

Epidermis has two physical barrier components, stratum corneum (SC) and tight junction (TJ). Formation of SC or cornification is initiated by the cell death of the top layer (SG1) of stratum granulosum, termed 'corneoptosis' after prolonged intracellular Ca2+([Ca2+]i) elevation (~60min), followed by rapid intracellular acidification. However, the exact timing of the onset of corneoptorsis remains to be clarified. In this study, we aimed to determine the spatiotemporal relationship between the turnover of TJs and corneoptosis. We performed intravital confocal microscopic imaging of mouse ear co-expressing ZO-1-Venus (TJ strand marker) and GCaMP3 (intracellular Ca2+ marker). Hoechst 33342 was intradermally injected to monitor the change of nuclear pH. Consistent with our previous observation, ZO-1-Venus signals showed two TJ strand-pattens in upper SG. According to the TJ turnover, three phases are observed; TJ-phase 1 which shows single old TJ strand formed in SG2 cells; TJ-phase 2 which shows double TJ strands; TJ-phase 3 which shows new single TJ strand. Time lapse imaging analysis revealed that the elevation of GCaMP3 signal was observed only in TJ-phase 2 which shows double TJs, but not in other TJ-phases. The elevation of [Ca2+]i lasted for approximately 60min. Subsequently, intracellular acidification was induced in these SG1 cells as detected by both the decrease of GCaMP3 signal and the increase of Hoechst 33342 signals. Simultaneously, 'upper larger TJ strand' of double-TJ strand in SG1 cells disappeared, resulting in change from TJ-phase 2 to TJ-phase 3. These findings indicated that the onset of corneoptosis is tightly associated with the spaciotemporal dynamics of TJs.

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Three stepwise pH zones to form functional stratum corneum

K. Fukuda[1, 2], Y. Furuichi[1, 2], T. Miyano[3], R. J. Tanaka[3], T. Matsui[1, 4], M. Amagai[1, 2]
[1]Skin Homeostasis, RIKEN-IMS, Yokohama, Japan, [2]Dermatology, Keio University School of Medicine, Shinanomachi, Japan, [3]Bioengineering, Imperial College London, London, United Kingdom, [4]Evolutionary Cell Biology of the Skin, Tokyo University of Technology, Hachioji, Japan

Stratum corneum (SC) is the outermost layer of the epidermis, which consists of nonviable anuclear keratinocytes (corneocytes) and function as a protective skin barrier. The pH distribution of SC has long been considered to impact its homeostasis. However, pH distribution and its biological significance in SC homeostasis remain unclear due to challenges in measuring and visualizing pH at a single-corneocyte resolution and elucidating the biological significance of pH profiles in SC. To tackle these challenges, we generated mice expressing Venus–mCherry, the fusion protein of pH-sensitive probe Venus and pH-insensitive probe mCherry from the uppermost stratum granulosum cells (SASP-Venus-mCherry mice), which enables the observation of relative pH changes and calculation of pH values via simultaneous ratiometric imaging. Confocal microscopic analysis of SASP-Venus-mCherry mice revealed that the SC has three stepwise pH zones, lower-moderately acidic (pH 6.0), middle-acidic (pH 5.4), and upper-nearly neutral (pH 6.7) zones, rather than gradual change over SC layers, in various body parts. Topical application of phosphate buffers with pH 4.1, 5.3, 6.4 and 7.2 did not affect pH of lower and middle SC-pH zones, while it changed pH of upper SC-pH zone according to the applied buffer. Additionally, the lower and middle SC-pH zones were dependent on claudin-1, a tight junction protein, which diminished in claudin-1-deficient mice; thus, the entire SC exhibits neutral pH. Finally, mathematical modeling demonstrated that the stepwise SC-pH profile pH6.0–pH5.4–pH6.7, but not the gradient SC-pH profile pH7–pH5 reproduced the increase of effective catalytic activity of kallikrein-related peptidases toward the SC surface. Collectively, these findings indicate that the three stepwise SC-pH zones are tightly regulated and are necessary to mediate adequate desquamation, which leads to functional SC differentiation.

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Redefining "normal" TEWL: a systematic review

M. Green[1], A. Feschuk[2], N. Kashetsky[2], H. Maibach[3]
[1]Tulane University School of Medicine, New Orleans, Louisiana, United States, [2]Memorial University of Newfoundland, St. John's, Newfoundland, Canada, [3]University of California San Francisco, San Francisco, California, United States

Background: Transepidermal water loss (TEWL) has historically been used as an objective measure of skin water barrier function and integrity. Variation in TEWL has been associated with variables such as age and anatomic location, yet regulatory agencies provide minimal guidance on controlling for variation in settings such as in vitro permeation testing (IVPT). Suggestions by the FDA broadly advise skin samples with <15 g m-2h-1 TEWL for IVPT studies. However, many variables have been described in the literature that influence TEWL. Thus, this review summarizes variables that may cause TEWL variation. Methods: A literature search was performed using Embase, PubMed, and Web of Science to find human studies with quantitative data on variables affecting TEWL. Results: 31 studies that examined 22 variables were identified. Ten variables consistently caused increases in TEWL across studies: mask-use (n=1), Dry Eye Disease (n=1), Chronic Venous Disease (n=1), Coronary Artery Disease (n=1), age (infants vs. adults) (n=4), nourishment in infants (n=1), stress within individuals (n=2), Body Mass Index (n=2), bathing versus showering (n=2), and scratching/friction (n=1). Two variables caused decreases in TEWL: genetic variability with SNPs on chromosome 9q34.3 (n=1) and cancer-cachexia (n=1). The remaining ten variables showed no significant association or contradictory results across studies. Conclusion: We summarized 12 variables that cause variation in TEWL that are currently not accounted for in regulations. This suggests the problematic nature of FDA regulations trying to define normal TEWL without controlling for these variables. As a result, researchers should continue identifying factors that cause TEWL variation. Additionally, regulatory agencies should provide detailed guidance on controlling for TEWL variation and proper TEWL measurement to minimize human error.

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An emollient attenuates the progression of cognitive impairment in the elderly

L. Ye[1], Z. Wang[2], Y. Kim[3], P. M. Elias[4], T. Li[2], S. Wen[1], J. Song[5], B. Yang[1], C. Lv[5], M. Man[1]
[1]Dermatology Hospital, Southern Medical University, Guangzhou, China, [2]The 7th People's Hospital of Shenyang, Shenyang, China, [3]CRID Center, NeoPharm, Seoul, Korea (the Republic of), [4]Dept of Dermatology, UCSF and VAMC, San Francisco, California, United States, 5Dept of Dermatology, Dalian Skin Disease Hospital, Dalian, China

Cognitive impairment (CI) is common in the elderly. Prior studies suggest a link between chronic inflammation and CI. Additionally, an age-associated increase in cutaneous cytokines has been connected to elevated circulating cytokines. We therefore assessed here whether topical emollient can mitigate the progression of CI. Methods: This randomized, open-label pilot trial was carried out in Dalian and Shenyang, in northern China. Eligible participants were ≥65 years old and were randomly assigned to the treated and untreated groups at 1:1 ratio. The treated group were treated topically with Atopalm cream® twice-daily from November to the following May each year for three consecutive years, while the untreated subjects served as controls. The Global Deterioration Scale (GDS) was used to assess the severity of CI in November and the following May of each year, while epidermal biophysical properties were measured on the forearms and the shins. Results: A total of 200 eligible participants, including 63 males and 137 females, aged 75.83 ± 0.62 years were enrolled. Over the 3-year trial, GDS increased significantly in the controls, while in the treated group, GDS remained stable. While stratum corneum hydration (SCH) on the forearms did not change significantly in the controls, transepidermal water loss rates (TEWL), significantly increased by the end of the trial compared to baselines in the controls. Skin surface pH declined slightly in the controls. On the forearms of the treated group, SCH increased significantly, while surface pH markedly decreased. TEWL was comparable before and after the trial. These results demonstrate that improvements in epidermal function can mitigate the progression of CI. However, the sample size was relatively small, and trials in a larger cohort are needed to validate the present results.

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Quantitative morphometric analysis of intrinsic and extrinsic ageing in Caucasian individuals

L. Costello[1], K. Goncalves[1], P. De Los Santos Gomez[1], A. Simpson[1], V. Maltman[1], E. Markiewicz[2], C. Bascom[3], S. Przyborski[1,4]

[1]Department of Biosciences, Durham University, Durham, Durham, United Kingdom, [2]Helix Lab, Newcastle upon Tyne, United Kingdom, [3]The Procter & Gamble Company Mason Business Center, Mason, Ohio, United States, [4]Reprocell Europe Ltd, Glasgow, United Kingdom

Introduction: The field of skin ageing is important due to the world ageing population. Due to its interface with the external environment, skin ageing is attributed to both intrinsic and extrinsic factors. The aim of this study was to define histomorphic disparities between intrinsically and extrinsically aged skin, and increase understanding of how skin structures change with age and photoexposure. Methods: Full-thickness skin biopsies were obtained from the photoexposed dorsal forearm and photoprotected buttock of young (21-24 years, n=10 females) and aged Caucasian volunteers (61-65 years, n=10 females). Biometrics analysis of epidermal thickness (Emin), epidermal proliferation, basal keratinocyte morphology, interdigitation index and morphology of rête ridges and dermal papilla was performed on blinded histological and immunofluorescence images (n=40 biopsies; n=160-240 images). Results: Our study found that Emin and epidermal proliferation do not change with age or photoexposure (P>0.05). The size and height of basal keratinocytes is increased by photoexposure in young and ageing individuals, but no differences were identified with age (P<0.0001). The dermal-epidermal junction is affected by both age and photoexposure with regards to decreased interdigitation and decreased height of rête ridges and dermal papilla (P<0.0001). Discussion: Our data suggest that basal keratinocyte morphology is affected by photoexposure, and dermal-epidermal junction characteristics are altered by age and photoexposure. Interestingly, flattening of the dermal-epidermal junction was most apparent in photoexposed areas, and we propose that there is an accelerated ageing phenotype due to the contribution of extrinsic factors. These data are important for both academic and industrial scientists to validate in vitro findings, and identify targets for cosmetic interventions.

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Exogenous ceramide serves as a precursor to endogenous ceramide synthesis and as a modulator of keratinocyte differentiation

K. Shin[1,2], H. Mihara[3], K. Ishida[3], Y. Uchida[1,4], K. Park[1]

[1]Hallym University, Chuncheon, Gangwon-do, Korea (the Republic of), [2]LaSS Lipid Institute (LLI), LaSS Inc, Chuncheon, Korea (the Republic of), [3]Takasago Koryo Kogyo Kabushiki Kaisha Hiratsuka Kenkyujo, Hiratsuka, Kanagawa, Japan, [4]University of California San Francisco, San Francisco, California, United States

Since ceramide (Cer) is a key epidermal barrier constituent and its deficiency causes barrier-compromised skin, several molecular types of Cer are formulated in commercial topical agents to improve barrier function. Topical Cer localizes on the skin surface and in the stratum corneum, but certain amounts of Cer penetrate into the stratum granulosum, becoming precursors to endogenous Cer synthesis following molecular modification. Moreover, exogenous Cer as a lipid mediator could modulate keratinocyte proliferation/differentiation. We here investigated the biological roles of exogenous NP (non-hydroxy Cer containing 4-hydroxy dihydrosphingosine) and NDS (non-hydroxy Cer containing dihydrosphingosine), both widely used as topical Cer agents, in differentiated-cultured human keratinocytes (KC). NDS, but not NP, becomes a precursor for diverse Cer species required for a vital permeability barrier. Loricrin (late differentiation marker) production is increased in KC treated with both NDS and NP vs. control, while bigger increases in involucrin (early differentiation marker) synthesis were observed in KC treated with NDS vs. NP and control. NDS increases a key antimicrobial peptide (innate immune component), cathelicidin antimicrobial peptide (CAMP/LL-37), that is upregulated by a Cer metabolite, sphingosine-1-phosphate. Our current studies demonstrate that NDS could be a multi-potent Cer species, forming heterogenous Cer molecules, and a lipid mediator to enhance differentiation and innate immunity.

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Using a cosmetic blend to produce a contraction response in human keratinocytes and deliver tightening of skin around the eye

G. Huang[1], S. Strikarsky[1], J. Weinstein[1], M. Ellahi[2], A. Gonzalez[1], J. Idkowiak-Baldys[1], J. Glynn[1]

[1]Avon Products Inc Innovation Center, Suffern, New York, United States, [2]Avon Products Inc, London, United Kingdom

Human keratinocytes have been known to shrink in the presence of certain compounds such as small amines. This cell shrinkage suggests a tension exerted by the cells that could lead to a perception of skin firmness and changes in skin mechanical properties that together with reinforcing dermal matrix would provide long-term benefit. Cosmetic ingredients were investigated for their ability to elicit a contraction response from keratinocytes. Keratinocyte shrinking due to a treatment can be observed in real time by measuring the impedance on a cell culture surface. Human keratinocytes were treated with a combination of cosmetic ingredients: an amino alcohol and a non-proteinogenic amino acid previously shown to increase both epidermal turnover as well as dermal collagen production. After 5 hours of treatment, this blend of ingredients resulted in a synergistic effect of keratinocyte contraction that was significantly greater than the vehicle ($p<0.05$). This combination of ingredients could elicit a tension in skin cells that may suggest a perception of firmness in the skin. A formulation containing this blend of ingredients was tested with consumers (n=127, double blind monadic study) for use on skin around the eye. After 2 days, consumers indicated that the eyes looked (69%) and felt (76%) firmer. After one week, 81% of consumers agreed that the formulation left skin feeling dramatically firmer. This suggests the formulation contributes to the consumer perceived changes in skin firmness by stimulating keratinocyte contraction in the epidermis.

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Pyroptosis of keratinocyte in Stevens-Johnson syndrome / toxic epidermal necrolysis

Y. Saito, A. Hasegawa, T. Nishiguchi, N. Hama, R. Abe

Dermatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Niigata, Japan

Stevens Johnson syndrome (SJS)/ toxic epidermal necrolysis (TEN) are characterized by the remarkable cell death of keratinocytes. Our previous studies have revealed that keratinocyte death pathway involve apoptosis and necroptosis. However, in recent years, it has been found that there is a crosstalk between apoptosis, necroptosis and pyroptosis. Pyroptosis is an inflammatory programmed cell death characterized by caspase 1-mediated cleavage of GSDMD and the release of IL-1b and IL-18. The aim of our study is to reveal whether pyroptosis is associated with the pathogenesis of SJS/TEN and develop specific therapeutic agents that can suppress these cell death programs in SJS/TEN. Expression of cleaved GSDMD and cleaved caspase1, which were key players of pyroptosis, were observed in the keratinocytes of SJS/TEN patients by immunofluorescent staining. To prove that pyroptosis can occur in keratinocytes, we stimulated HaCaT cells with pyroptosis inducers, LPS and nigertin, and evaluated pyroptosis reaction by live/dead staining assay, observation of morphological changes and cleaved GSDMD expression. HaCaT cells after LPS and nigertin stimulation formed large bubbles and a punctate positive reaction of cleaved GSDMD on the cell membrane, occurring as a result of membrane pore-forming activity of the gasdermin-N domain. Pyroptosis occurred even after stimulating HaCaT cells with SJS serum. Our study showed that pyroptosis occurred in keratinocytes of SJS/TEN patients and substances that induce pyroptosis to keratinocyte were present in the serum of the patients with SJS/TEN. We have investigated SJS/TEN-specifically elevated proteins by selected reaction monitoring. We are currently exploring the substances that induce pyroptosis. It is expected that understanding the mechanisms of keratinocyte death in SJS/TEN lead us to develop a specific therapeutic drug that can suppress cell death in SJS/TEN.

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Evaluation of a novel skin emollient on skin lipidome and lipids organization

C. Jacques[1], C. Dejean[1], C. Klose[3], E. Leccia[4], S. Bessou-Touya[1], A. Delarue[2], H. Duplan[1]

[1]Centre R&D Pierre Fabre, Innovation et Développement Pharmacologie, Pierre Fabre Dermo-Cosmétique, Toulouse, France, [2]Pierre Fabre SA, Lavaur, Les Cauquillous, France, [3]Lipotype GmbH, Dresden, Germany, [4]Novitom SAS, Les Ulis, France

The stratum corneum (SC) matrix is composed of free fatty acids, cholesterol and ceramides (CERs), which play a key role in the skin barrier function. The effect of a glycerol/petrolatum-based emollient (G/P-emollient) cream on the lipid profile of the SC in a reconstructed human epithelial (RHE) model was measured. The spatial organization of the cream and the SC intercellular matrix was studied using X-ray diffraction. The inter-bilayer distance in the multi-lamellar lipid structures and lattice type were analyzed using small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS), respectively. The crystallized components of the G/P-emollient cream are lipids, which were mainly packed in hexagonal and orthorhombic lattices, similar to the SC structure. The G/P-emollient cream penetrated the SC but did not alter the WAXS profile. It increased long chain length CERs, all chain length acyl-CERs, acyl-CER classes (CER EOS, EOH, EOP, EOdS) and total CER classes, and decreased short chain CER C34 for hydroxylated and non-hydroxylated CERs. The cream had a positive impact on the [S] and [P] CER forms (increased the [NP]/[NS] and [AP]/[AS] ratios), indicating it could help to reduce the relative feedback mechanism observed in inflammatory pathologies e.g., atopic dermatitis. The cream increased CER[NP], which is decreased in dry skin. In conclusion, G/P-emollient cream can help to balance the negative impact of pathologies by modifying SC lipids, balancing CER levels and ratios, and improving the barrier function. Importantly, the cream structure mimics that of the SC and penetrated the lower SC layers without compromising its lamellar structure

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Commensal C. acnes promote epidermal keratinocyte lipid synthesis via PPAR

S. Almoughrabie[1, 2], L. Cau[2], C. Mainzer[2], B. Closs[2], K. J. Williams[3], S. J. Bensinger[3, 4], R. Gallo[1]

[1]Dermatology, UC San Diego, La Jolla, California, United States, [2]R&D Department, SILAB, Brive, France, [3]Microbiology, Immunology and Molecular Genetics, Los Angeles, California, United States, [4]Molecular and Medical Pharmacology, UCLA, Los Angeles, California, United States

We observed in human and mouse skin that exposure to C. acnes resulted in greatly increased epidermal lipids as indicated by bodily staining and hypothesized that this microbe may regulate keratinocyte lipid metabolism. To test this, human keratinocytes (NHEKs) were exposed to sterile C. acnes supernatant and showed a dose dependent increase in Oil Red O staining (p-value <0.001), and increases of ceramides, cholesterol and free fatty acids detected by differential mobility spectrometry-based shotgun lipidomic analysis. Triglycerides increased to the greatest degree; specially those which contain the following fatty acids: (16:0), (16:1), (18:1) and (18:2). Whole mouse epidermis exposed to C. acnes showed a similar response. Transcriptional analysis further defined the pathway for these induced lipids as RNA-seq analysis and subsequent validation by qPCR showed that C. acnes promoted upregulation of genes involved in ceramide, free fatty acid and triglyceride metabolism. This induction was induced in part by short chain fatty acids (SCFAs) made by C. acnes during its growth (propionic acid and isovaleric acid) as exposure to SCFAs also induced keratinocyte lipid accumulation and transcription of the lipid synthesis genes. Activation was mediated by peroxisome proliferator-activated receptors (PPARs) since siRNA silencing of PPARa and g receptors in NHEKs blocked increases in total lipid or induction of lipid synthesis genes (AGPAT4, GPAT3 and DGAT1) when exposed to C. acnes supernatant. This effect did not occur upon silencing of PPARb. Induction of lipids in NHEKs delayed C. acnes growth. Taken together, these results suggest that metabolites from C. acnes trigger upregulation of lipid synthesis in the skin that may decrease its survival while also influencing the epidermal barrier and host defense.

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Glucose controls protein-protein interactions and epidermal differentiation

V. Lopez-Pajares[1], A. Bhaduri[2], Y. Zhao[1], G. Gowrishankar[1], L. Donohue[1], M. Guo[1], A. Guerrero[3], A. Ji1, O. Garcia[1], S. Gambir[1], P. Khavari1, [4]

[1]Stanford University, Stanford, California, United States, [2]University of California Los Angeles David Geffen School of Medicine, Los Angeles, California, United States, [3]UPMC, Pittsburgh, Pennsylvania, United States, [4]VA Palo Alto Health Care System, Palo Alto, California, United States

Glucose serves as a universal energy currency in living organisms, however, its potential non-energetic biomolecular functions are less well defined. Glucose was among the most increased analytes among >14,000 assessed across epidermal differentiation, an elevation verified in tissue engineered with fluorescent glucose sensors. Free glucose accumulation, but not its increased metabolism, was essential for epidermal differentiation and required GLUT1, GLUT3, and SGLT1 transporters. Consistent with this, decreasing cellular glucose levels, by restricting available glucose or by increasing intracellular glucose catabolizing enzymes, HK1/2 and G6PD, blocked differentiation and differentiation was also rescued with a non-metabolizable glucose analog. Furthermore, RNA-seq analysis of glucose-depleted epidermal tissue revealed 34% of the genes downregulated by glucose are part of the epidermal differentiation gene signature. ATAC-seq identified candidate transcription factors (TFs) that may act on glucose-regulated genes, including ZNF750, NFE2L2, and IRF6. Glucose affinity chromatography and azido-glucose click chemistry revealed direct glucose binding to a variety of regulatory proteins, including the IRF6 TF whose epidermal knockout confirmed its requirement in glucose-dependent differentiation. Small molecule docking models suggest glucose binds within the DNA binding domain of IRF6. Glucose binding mediated IRF6 dimerization, DNA affinity, and genomic targeting. The IRF6R84C mutant found in poorly differentiated cancers and ectodermal dysplasias was unable to bind glucose. These data demonstrate a non-energetic role for glucose in modulating protein multimerization to control epidermal differentiation.

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In silico prediction of the skin biological activity for botanical active ingredients based on their composition in phytocomponents

J. Botto, L. Mur, I. Imbert

Global Skin Research Centre, Ashland Global Specialty Chemicals Inc, Covington, Kentucky, United States

Network pharmacology links phytocomponents to target genes and proteins and helps predict biological activity. Thereby, this type of study relies on the analytical determination of the major components of a botanical extract. Major plant secondary metabolites are more likely to belong to terpenoids, phenylpropanoids and polyketides class of compounds. The determination of validated relationships (scientific literature, databases, and experimental data) associated with the prediction of undescribed but potential connections (e.g., via structural biology approaches) leads to a list of targets that will serve as a base for gene enrichment studies, to point out specific associated biological processes and pathways of interest. The output can be represented as a tripartite “active phytocompound / target proteins & genes / biological activity & signaling pathway” set of data that can be used as a predictive ground to further perform experimental validation on dedicated skin models. We present in this study the application of network pharmacology to a particular botanical extract.

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Involvement of olfactory receptor OR2AT4 in skin aging and the response to environmental pollution

L. Mur, A. Perrin, A. Lebleu, L. Labourasse, C. Plaza, C. Meyrignac, C. Capallere, J. Botto, I. Imbert
Global Skin Research Centre, Ashland Global Specialty Chemicals Inc, Covington, Kentucky, United States

Olfactory receptors (ORs) are 7-transmembrane G protein-coupled receptors expressed in the olfactory epithelium and mediate the odor perception. Around 400 ORs are known in human, and their expression has been evidenced in various peripheral tissues such as intestinal epithelium, prostate, spermatozoa, liver, and skin. These ectopic receptors might regulate physiological functions beyond olfaction. The OR called OR2AT4 is expressed in the skin, especially the epidermal keratinocytes, melanocytes, dendritic cells, and hair follicles. Its activation increases the proliferation and migration of keratinocytes, allowing faster re-epithelialization during wound healing. OR2AT4 downregulation induces the decrease of IGF-1 expression in hair follicles suggesting its link with hair growth. However, the roles of ORs in the skin are still incompletely described, especially, the possible link with intrinsic and extrinsic aging. In this study, OR2AT4 expression was investigated in various senescent skin models in vitro, such as human cultured keratinocytes, and reconstructed epidermises. Besides, the consequences of ultrafine particle-induced skin damage were studied via the evaluation of OR2AT4 expression level and on markers involved in skin senescence and differentiation. In parallel, botanical extracts were screened for their ability to modulate the expression of OR2AT4 and related markers. Our results showed that the expression of OR2AT4 was inversely correlated with aging and UFP-induced skin damage, suggesting that its modulation could be beneficial to limit the consequences of intrinsic and extrinsic aging into the skin.

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Keratinocyte media differences uncovered during COVID-19 supply shortages

E. M. Pope, M. C. Moran, M. G. Brewer, L. A. Beck
University of Rochester, Rochester, New York, United States

Keratinocyte (KC) culture medias are used interchangeably with the expectation of comparable results. The COVID-19 pandemic resulted in supply chain shortages necessitating substitutions to standard protocols. We screened available medias on the KC cell line N/TERT2G and found biological responses varied considerably across three culture medias: KSFM (Gibco, #17005042), KGM2 (PromoCell, #C-20211), and Defined (Gibco, #10744019). We observed qualitative and quantitative differences in proliferation among KC grown in the different medias; KC cultured in Defined had a significantly lower proliferative capacity. KC differentiation was assessed by Western blot for claudin-1 (CLDN1), occludin (OCLN), cytokeratin-10 (CK10), and loricrin (LOR). CLDN1, OCLN, and CK10 were below the limit of detection in undifferentiated KC cultured in KSFM whereas KC cultured in KGM2 and Defined showed robust expression. Even after differentiation in the same media, expression of CLDN1, OCLN, and LOR was detected earlier and at higher levels in KC cultured in KGM2 and Defined compared to KSFM. KC cultured in KGM2 and Defined developed significantly higher transepithelial electrical resistance (TEER) than cells cultured in KSFM. When treated with IL-4&13, TEER was initially lower and then significantly increased compared to untreated KC cultured in KSFM. TEER significantly decreased in KGM2, and no changes were observed in Defined. Lastly, we observed similar kinetics in susceptibility to infection with vaccinia virus over the course of differentiation; undifferentiated KC showed resistance to infection across all medias. In summary, the use of different culture medias impacts biological responses (i.e., proliferation, protein expression, barrier function) of KC in a manner that persists even through differentiation in the same media. Our results highlight the misconception that these medias can be used interchangeably for propagating KC in vitro and emphasize the importance of providing methodologic details in epidermal biology publications.

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Keratin diversity modulates cytoskeletal dynamics and force generation during epidermal remodeling

B. A. Nanes[1, 2, 3], E. Azarova[3], K. Bhatt[2, 4, 3], J. Chi[2, 4, 3], T. Isogai[2, 4, 3], K. M. Dean[2, 4, 3], G. Danuser[2, 4, 3]
[1]Department of Dermatology, UT Southwestern Medical Center, Dallas, Texas, United States, [2]Lyda Hill Department of Bioinformatics, UT Southwestern Medical Center, Dallas, Texas, United States, [3]Department of Cell Biology, UT Southwestern Medical Center, Dallas, Texas, United States, [4]Cecil H. and Ida Green Center for Systems Biology, UT Southwestern Medical Center, Dallas, Texas, United States

Keratin intermediate filaments (KFs) allow the epidermis to resist external force, but their interplay with the force-generating actomyosin cytoskeleton is poorly understood. This is particularly relevant in situations such as wound healing, where actomyosin drives keratinocyte migration and morphology changes. Notably, KF composition changes during epidermal remodeling, with increased expression of five wound-associated keratins relative to steady-state keratins. To explore the potential link between KF composition and actomyosin-driven remodeling behavior, we expressed different levels of steady-state keratin 5 (K5) and wound-associated K6A in cultured keratinocytes. Live cell imaging revealed that increasing K6A expression decreased KF network dynamics and altered actin flow. Furthermore, as measured by traction force microscopy, K6Ahigh cells generated increased contractility compared to K5high cells. These changes in cytoskeletal behavior and force generation were associated with a migration advantage for K6Ahigh cells compared to K5high cells, a difference accentuated in three-dimensional stratified epidermal cultures compared to monolayers. Together, these results establish a novel functional link between the KF and actomyosin cytoskeletons, where changing KF composition modulates cytoskeletal dynamics and force generation to support epidermal remodeling.

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C10orf99 governs keratinocyte inflammatory response and barrier formation of the skin

T. Dainichi[1, 2], Y. Nakano[2], H. Doi[2], S. Nakamizo[2, 4], S. Nakajima[2], R. Matsumoto[2], T. Farkas[3], P. Wong[4], V. Narang[4], E. Kawakami[5], E. Guttman-Yassky[6], O. Dreesen[4], T. Litman[3], B. Reversade[4], K. Kabashima[2, 4]
*[1]Kagawa University, Miki-cho, Kagawa, Japan, [2]Kyoto University, Kyoto, Japan, [3]University of Copenhagen, Copenhagen, Denmark, [4]A*STAR, Biopolis, Singapore, [5]RIKEN, Yokohama, Japan, [6]Icahn School of Medicine at Mount Sinai, New York, New York, United States*

The defense system of the skin has three layers: barrier, innate immunity, and acquired immunity. Epidermal keratinocytes participate in all these three processes and play central roles in the inflammatory responses. However, a shared molecule in keratinocytes that responds to diverse dangers and governs the protective machinery of the skin remains obscure. Here we report that the orphan gene C10orf99 plays a primary role in the innate response of keratinocytes and barrier formation in the skin. C10orf99 peptide expression was highly induced in the suprabasal layer of the epidermis in the lesional skin of patients with atopic dermatitis or psoriasis. In addition, strong expression of the mouse honolog 2610528A11Rik in the lesional skin was common to several types of murine dermatitis models. C10orf99 transfection into normal human epidermal keratinocytes induced the expression of inflammatory mediators and reduced the expression of barrier-related genes. Gene ontology analyses of the RNA-Seq showed its association with lipid synthesis, cadherin, and mitochondria, and transcription factor (TF) enrichment analysis found 4 TF groups: stress-inducible TFs; repressors IRF2/STAT2; polycomb/SMAD-related TFs; and SREBP1 regulating sterol synthesis. Treatment with C10orf99 peptide reduced the filaggrin and loricrin expression in human keratinocyte 3D cultures. Furthermore, LPS-induced expression of Il1b and Il6 was less in 2610528A11Rik deficient mouse keratinocytes, and imiquimod-induced psoriatic dermatitis was blunted in the deficient mice. Moreover, topical injection of 2610528A11Rik peptide in mice induced skin inflammation in a dose-dependent manner. These results suggest that C10orf99 is a master regulator that induces the inflammatory response of keratinocytes and reduces the barrier formation.

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Acute inflammatory cytokines differentially effect epidermal barrier expression and function

B. Shi, A. Klopot, T. Mahi, S. Buiter, E. Khan, I. Budunova, B. E. Perez White
Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Inflammatory cytokines are released in skin by immune cells and keratinocytes in response to a number of stimuli. This inflammation influences epidermal function, including disruption of the barrier. Barrier disturbances are known to contribute to and promote the inflammatory process. While the cytokine milieu in skin can vary between individuals, acute innate immune responses cause increases in tumor necrosis factor α (TNFα), interleukin (IL)-1β, IL-6, and interferon γ (IFNγ). We asked what the single or combined effects of these pro-inflammatory cytokines on epidermal barrier function and the expression of key elements of tight junctions and the cornified envelope in single donor-derived 3D human epidermal equivalents (N = 8 individuals). Transcript analysis by qPCR shows a significant negative impact of TNFα and a combination of all four cytokines on the expression of cornified envelope elements filaggrin and loricrin. Although IL-1β, IL-6, or IFNγ alone did not affect filaggrin or loricrin, the combination of the four cytokines reduced the expression of these cornified envelope transcripts by more than 50-fold. Conversely, TNFα or the combination of four did not affect the expression of tight junction components claudin 1, occludin, or zona occludens-1, while claudin 4 was significantly upregulated by both treatments ($p < 0.01$). Using transepithelial electrical resistance (TEER) assays, we found that concurrent treatment with TNFα, IL-1β, IL-6, and IFNγ significantly decreased TEER ($p < 0.01$). As a single treatment, TNFα alone significantly reduced TEER ($p < 0.05$). Only the dual combination of IL-6 and TNFα did not adversely affect epidermal barrier function. These results suggest that TNFα is a potent barrier disruption factor in acute inflammation but that IL-6 may have protective effects. Further, TNFα specifically disturbs gene expression of the cornified envelope, not the tight junction barrier.

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Effect of L-4-thiazolylalanine on skin barrier strength

C. Haxaire, F. Liebel, L. DiNatale, J. Idkowiak-Baldys, J. Glynn
Avon Research and Development, Suffern, New York, United States

Skin barrier properties are critical for maintaining epidermal water content, protecting from environmental factors, and providing first line of defense against pathogens. In this study, the effect of a non-proteinogenic amino acid, L-4-Thiazolylalanine (L4), on this important aspect of skin health was evaluated by in vitro and *in vivo* biomarkers. 3D human skin models were treated with 0.3% L4. After 48h, RNA samples were collected and gene expression analyzed. Significant increase in the expression of ALOXE3 (protein eLOX-3) vs. vehicle (VEH) (+27%) was measured. eLOX-3 is a member of the lipoxygenase family required in the epidermis for the covalent linkage of ceramides to proteins of the cornified envelope, an important step in establishing skin barrier function. To further evaluate skin barrier integrity, trans-epithelial electrical resistance (TEER) was measured in the 3D tissues at basal level as well as upon barrier disruption. TEER is a quantitative method that allows barrier strength evaluation. TEER values are highly related to the tight junction barrier function and decrease upon disruption induced by calcium depletion. L4 treatment induced a significant increase in TEER vs. vehicle and protected from low calcium-induced barrier decrease. Clinically, skin treated with L4 for 4 weeks had better barrier integrity vs. vehicle control. This was shown via instrumentation and evaluation of biomarkers in skin samples collected via tape stripping. A fluorescence-based assay was used to quantify the enzymatic activity of 12R-LOX, another lipoxygenase critical for maturation of skin barrier ceramides. Significant increase in 12R-LOX activity was measured after L4 treatment vs. vehicle (+31%) with a stronger effect in subjects 40 years-old and older (+45%). In conclusion, L4 led to modulation of in vitro and *in vivo* biomarkers critical for skin barrier strength, making it desirable ingredient for topical treatments.

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The autophagy receptor TEX264 initiates endoplasmic reticulum degradation in differentiating epidermal keratinocytes

C. L. Simpson, C. Johnson
Dermatology, University of Washington, Seattle, Washington, United States

During late stages of differentiation, epidermal keratinocytes break down organelles and nuclei to become compact corneocytes that form a barrier for the body. Our prior work uncovered a novel pathway driving degradation of mitochondria in granular layer keratinocytes, which engage the selective autophagy machinery to route mitochondrial fragments into lysosomes as they begin to cornify. These findings led us to ask if the epidermis uses distinct autophagy receptors to orchestrate breakdown of other organelles as keratinocytes transition into the stratum corneum. Applying confocal microscopy to live organotypic human epidermis, we found the endoplasmic reticulum (ER) undergoes fragmentation in the outermost cell layers and hypothesized that keratinocytes initiate selective autophagy of the ER (reticulophagy) to degrade this organelle compartment. Treatment of organotypic epidermis with tunicamycin, which causes ER stress and upregulates reticulophagy, resulted in premature cornification and reduced tissue thickness, suggesting precocious ER breakdown accelerates keratinocyte maturation. To discern the mechanism by which cornifying keratinocytes normally degrade the ER, we queried RNA sequencing and protein expression data from murine and human epidermis and found the outermost cell layers upregulate TEX264, a protein recently shown to mediate reticulophagy. While TEX264 was not expressed in undifferentiated human keratinocytes, ectopic expression of the receptor was sufficient to induce ER fragmentation. Further examination of TEX264-positive ER fragments revealed colocalization with the autophagosome marker LC3, establishing that TEX264 serves as a reticulophagy receptor in keratinocytes. Finally, mutation of its LC3-interacting region abolished the ability of TEX264 to fragment the ER, confirming that interaction with Atg8 family proteins is essential to its function. In sum, our findings indicate an essential role for ER homeostasis in epidermal cornification and identify TEX264 as an autophagy receptor promoting reticulophagy during keratinocyte differentiation.

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RORα promotes keratinocyte differentiation by reducing SOX9 stability.

Y. H. Bryner, H. Li, J. Dai
Division of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin-Madison, Madison, Wisconsin, United States

Retinoic acid receptor-related orphan receptors α (RORα) is a transcription factor essential for keratinocyte differentiation. The transcriptional network of RORα and how it regulates various keratinocyte functions remain understudied. We have identified Sex Determining Region Y-Box9 (SOX9) as a critical downstream target gene of RORα in human keratinocytes. SOX9 is an epithelial-to-mesenchymal transition (EMT)-inducing transcription factor involved in embryonic development and cancer metastasis. In skins, SOX9 promotes the proliferation of human keratinocytes but inhibits their differentiation, and the SOX9 expression level is upregulated in keratinocyte-derived basal cell carcinomas (BCCs). We found that the RORα knockdown using its shRNAs caused a substantial increase of the SOX9 protein expression in KerCT human keratinocytes. The simultaneous SOX9 gene depletion could partially restore the downregulation of differentiation markers caused by RORα deficiency. Despite a marginal effect on SOX9 mRNA expression, RORα gene silencing reduced the speed of SOX9 protein degradation. In addition, SOX9 elevation in RORα deficient cells was resistant to the stabilizing effect of MG132, an inhibitor of proteasome degradation, thereby suggesting that RORα regulates SOX9 by altering its protein stability. Also consistent with the role of SOX9 in promoting EMT, KerCT cells with RORα depletion displayed accelerated migration and increased expression of the EMT markers, including N-cadherin. These results suggest that RORα promotes keratinocyte differentiation partially through downregulating SOX9 protein stability and that it prevents skin cancer development through a novel mechanism also likely involving SOX9.

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Case report: Pemphigus vulgaris mimicking dermatitis herpetiformis

I. Olugbade, Y. Farid, L. Robinson-Bostom, A. Qureshi
Brown University Warren Alpert Medical School, Providence, Rhode Island, United States

A 23-year-old woman with a history of interstitial lung disease, positive ANA, and weakly positive anti-Scl-70 presented with periumbilical blisters. Biopsy revealed a subepidermal split with eosinophils and neutrophils and suprabasilar acantholysis. Intercellular C3 on direct immunofluorescence (DIF) was suggestive of pemphigus vulgaris (PV). She subsequently developed a tense vesicle on her right shin. Repeat biopsy only demonstrated intraepidermal blister with re-epithelialization of blister floor. Her bullae were pauci-inflammatory and not histologically classic. Two months later, the patient returned with intensely pruritic and painful grouped vesicles and scattered pink papules on her trunk and extremities, along with a blister on her back. This eruption had the appearance of dermatitis herpetiform (DH) however tissue transglutaminase, deamidated gliadin peptide, and endomysial antibodies were negative. A third biopsy showed suprabasilar acantholysis with intercellular IgG on DIF and she later developed ulceration of the lower lip all supporting the diagnosis of PV. Mycophenolate mofetil and prolonged oral prednisone taper resulted in significant improvement of cutaneous and mucosal lesions. PV classically presents with flaccid blisters and DIF showing intercellular IgG and C3 deposits. DH typically presents as a pruritic erythematous papulovesicular rash over extensor surfaces, buttocks, and knees. Previously described pemphigus herpetiformis combines clinical DH and histologic pemphigus. Unlike our patient, this entity usually has urticarial lesions and eosinophilic spongiosis. This case highlights an example of PV that clinically mimicked DH. It also serves as a reminder to repeat biopsies when there is conflicting clinical-pathologic correlation in order to optimize patient care and management.

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An inflamed and infected reconstructed human epidermis to study the efficacy of a plant extract on atopic dermatitis

A. Garlet[3], S. Cadau[1], M. Gault[1], N. Berthelemy[1], D. Gauché[1], C. Pons[2], C. Leprince[2], M. Simon[2], S. Pain[1], V. Andre[1]
[1]BASF Beauty Care Solutions France SAS, Lyon, Rhône-Alpes, France, [2]Toulouse Institute for Infectious and Inflammatory Disease, Toulouse, France, [3]BASF Corp Tarrytown, Tarrytown, New York, United States

We previously developed an innovative reconstructed human epidermis (RHE) combining two main characteristics of Atopic Dermatitis (AD): an inflammatory medium (a cocktail of TNFa and interleukins IL-4, IL-13 and IL-31) and Staphylococcus aureus (S. aureus) surface colonization. We identified an extract of Castanea sativa leaves with potential protective properties against epidermal barrier defects. This was confirmed in vitro using the AD model and *in vivo* on a cohort of 22 volunteers with mild to moderate AD (EASI grade 3-11). The expression of two skin barrier proteins (filaggrin and claudin-1) in the AD-mimicking RHEs was analyzed by immunohistology and confocal microscopy. *In vivo*, the action of the daily topical application of the plant extract versus a base cream was evaluated by measuring the transepidermal water loss (TEWL) after 14 days, one month and two months. Immunostainings showed that filaggrin and claudin-1 expression was impaired when RHEs were treated with the cytokine cocktail. The reduction was worsened by the addition of S. aureus. Castanea sativa extract application reduced the decrease expression of filaggrin (-18% in the presence vs -49% in the absence of the plant extract (control) and claudin-1 (-22% vs -36% in control). *In vivo*, after 14 days of use of a formula containing 2% of Castanea sativa extract, we observed an improvement of the water loss by 10% vs baseline. An increasing improvement of the barrier function was observed from -21% after one month ($p<0.001$ vs baseline) to -27% after two months ($p<0.001$ vs baseline). As expected from its in vitro properties to counteract mechanisms involved in skin barrier impairments, the effects of the Castanea sativa extract observed on AD patients evidenced that it may help to improve the skin barrier function in atopic-prone skin.

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AMP-IBP5 improves tight junction barrier function in human keratinocytes and suppresses dermatitis-like lesions in a mouse model of atopic dermatitis

H. L. Nguyen[1, 3], J. Trujillo[1], G. Peng[1, 3], H. Yue[1, 3], R. Ikutama[1, 3], M. Takahashi[1, 3], Y. Umehara[1], K. Okumura[1], H. Ogawa[1], S. Ikeda[1, 3], F. Niyonsaba[1, 2]
[1]Atopy (Allergy) Research Center, Juntendo Daigaku, Bunkyo-ku, Tokyo, Japan, [2]Faculty of International Liberal Arts, Juntendo Daigaku, Bunkyo-ku, Tokyo, Japan, [3]Dermatology and Allergology, Juntendo Daigaku, Bunkyo-ku, Tokyo, Japan

Background: A novel antimicrobial peptide derived from insulin-like growth factor-binding protein 5 (AMP-IBP5) displays both antimicrobial and immunomodulatory properties. However, the role of AMP-IBP5 in the regulation of skin barrier remains unclear. Objective: To investigate the effects of AMP-IBP5 on skin barrier function. Methods: Normal human epidermal keratinocytes were stimulated with AMP-IBP5 and Western blot was used to analyze the expression of tight junction (TJ)-related proteins. Immunofluorescence staining was used to examine the intercellular distribution of TJ-related proteins. Activation of aPKC z and Rac1 was evaluated by Western blot and the TJ barrier function was investigated by measurement of transepithelial electrical resistance (TER). BALB/c mice treated with 2,4-dinitrochlorobenzene were used as a mouse model of AD. The TJ permeability assay and immunofluorescence staining were used to investigate the TJ barrier function in mice. The production of inflammatory cytokines was examined by real-time PCR, and total serum IgE level in AD mice was examined by ELISA. Results: AMP-IBP5 increased the expression of various TJ-related proteins and enhanced their distribution along the cell-cell borders. AMP-IBP5 also improved the TJ barrier function as shown by increasing TER values. Furthermore, AMP-IBP5 enhanced the phosphorylation of aPKC z and Rac1 and supppressed the effect of their respective inhibitors on TER. . In AD mice, AMP-IBP5 improved dermatitis-like symptoms, recovered the expression of TJ-related proteins, suppressed the production of inflammatory cytokines and decreased total serum IgE levels. Conclusions: AMP-IBP5 improves AD symptoms and enhances the skin barrier function, suggesting a possible implication of AMP-IBP5 in the treatment of AD.

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IL-33 is a negative regulator of filaggrin expression in the skin but not in barrier homeostasis

M. Hossain, T. Ansary, M. Komine, M. Ohtsuki
Dermatology, Jichi Ika Daigaku, Shimotsuke, Tochigi, Japan

The skin protects the human body by providing barrier function. Interleukin (IL)-33 is a tissue-derived cytokine, mainly expressed by epithelial and endothelial cells, and is known to activate Th2 lymphocytes, mast cells, ILC2, and eosinophils. We aimed to study the role of IL-33 in skin barrier function. Wild-type (WT), and IL-33 knockout (IL-33KO) mice were tape stripped, and trans-epidermal water loss (TEWL) was monitored. Skin samples were corrected, and hematoxylin and eosin staining and other immunohistochemical analyses were performed. Total RNA was extracted from the skin samples and real-time PCR was performed. Tape stripping increased TEWL both in IL-33KO and in WT mice ($p<0.0001$), but there was no statistical difference between WT and IL-33KO mice (n.s.). Tape stripping increased the thickness of the epidermis and granular layer in IL-33KO mice compared to WT mice. We observed an increased level of filaggrin (FLG) in immunohistochemical analyses at days 1, 2, and 3 after tape-stripping in IL-33KO, but real-time PCR showed increased expression of filaggrin before tape-stripping at day1 in IL-33KO ($p<0.0001$) mice, and reduced by tape-stripping. Increased mRNA expression of TSLP without tape-stripping was observed at day2 IL-33KO ($p<0.0001$) mice, which was reduced by tape-stripping. Tape-stripping induced the expression of acid ceramidase (ASAH1 and ASAH1.2) both in immunohistochemical and real-time PCR analysis in WT ($p<0.0001$) mice, but not in IL-33KO mice. These results imply that filaggrin protein existence did not contribute to barrier disruption inhibition by tape-stripping in IL-33KO mice. Expression of acid ceramidase was increased with tape-stripping in WT mice but not in IL-33KO mice, of which absence neither contributed to the barrier disruption inhibition in IL-33KO mice by tape stripping. There would be some other molecules involved in barrier disruption in IL-33KO mice by tape-stripping.

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Loricrin imprints adaptive immunity

Y. Ishitsuka[1], T. Ogawa[2], D. Roop[3], M. Fujimoto[1]
[1]*Dermatology, Osaka Daigaku Daigakuin Igakukei Kenkyuka Igakubu, Suita, Osaka, Japan,* [2]*Dermatology, Tsukuba Daigaku Igaku Iryokei, Tsukuba, Ibaraki, Japan,* [3]*Department of Dermatology and Charles C. Gates Center for Regenerative Medicine, University of Colorado Denver, Denver, Colorado, United States*

Langerhans cells (LCs) are skin dendritic cells (DCs) that communicate with epidermal keratinocytes (KCs) and activate endogenous transforming growth factor-beta (TGF-β) signaling. Loricrin provides the stratum corneum (SC) with force resistance through promoting disulfide-mediated cross-linkages of keratins and desmosomes, and structural maturation of the desmosome-keratin scaffold has previously been shown to be hampered in the SC of loricrin-knockout (LKO) mice. We suspected that LOR might promote the maturation of the actin-anchored apical junctional complex (adherence/tight junctions) and integrins, the latter of which is a prerequisite for the retention of epidermal LCs. Steady-state LKO LC expressed higher surface expression levels of CD207, MHC-II, and CD86 than wild type (WT) mice. DCs in LKO mice delivered topically applied fluorescein isothiocyanate to draining lymph nodes more efficiently than WT mice. Percutaneous inoculation with the innocuous hapten 2,4-dinitrothiocyanobenzene, but not with the immunogenic hapten 1-Fluoro-2,4-dinitrobenzene (DNFB), induced enhanced lymphoproliferative responses in LKO mice. Topical application of the nonantigenic irritant 12-O-Tetradecanoylphorbol 13-acetate in LKO mice upregulated E-cadherin ($p < 0.001$) on the LC surface and delayed LC migration more than two-fold ($p < 0.005$) compared with WT mice. Although LC-deficiency in LKO mice reversed the attenuation of DNFB-induced contact hypersensitivity comparable to WT mice, a series of passive transfer experiments revealed that LCs were dispensable for the immunosuppression at the effector phase. These preliminary results indicate that LOR may imprint adaptive immune responses, possibly by regulating the bioavailability of TGF-β in the epidermis.

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Molecular events in the epidermis upon aryl hydrocarbon receptor targeting: AHR-TFAP2A axis drives epidermal keratinocyte differentiation

J. P. Smits[1], J. Qu[2], F. Pardow[1, 2], D. Rodijk-Olthuis[1], I. van Vlijmen-Willems[1], S. van Heeringen[2], P. Zeeuwen[1], J. Schalkwijk[1], H. Zhou[2, 3], E. van den Bogaard[1]
[1]*Dermatology, Radboudumc, Nijmegen, Gelderland, Netherlands,* [2]*Molecular Developmental Biology, Radboud Universiteit, Nijmegen, Gelderland, Netherlands,* [3]*Human Genetics, Radboud Universiteit, Nijmegen, Gelderland, Netherlands*

The aryl hydrocarbon receptor (AHR) is an environmental sensor and ligand-activated transcription factor that is involved in epithelial homeostasis and barrier development of the skin. Through genome-wide transcriptomic and epigenomic analyses of human primary keratinocytes upon AHR activation using TCDD we identified early and late AHR responsive genes. The early responsive genes were enriched for canonical transcription factors known to promote keratinocyte differentiation, barrier development, and host defense, such as Transcription Factor AP-2α (TFAP2A). Genes related to epidermal differentiation and structure (e.g. filaggrin, keratins, and transglutaminases), and host defense (e.g. PI3 and S100 genes) were identified as late AHR responsive genes. DNA binding motif analysis and siRNA-based knockdown of TFAP2A expression in primary keratinocytes indicated that TFAP2A regulates late AHR responsive genes involved in epidermal differentiation. CRISPR-Cas9 mediated knockout of TFAP2A in keratinocytes resulted the severely impeded epidermal stratification, terminal differentiation, and additional functional barrier formation in 3D human epidermal equivalent cultures. The herein identified AHR-TFAP2A axis provides important insights into epidermal differentiation in response to environmental cues, which opens new avenues for the targeting of this axis to treat barrier dysfunction related diseases.

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Identification of the EGF receptor extracellular domain as a cell-surface sensor for ubiquitous organic pollutants

N. C. Sondermann[1], C. Vogeley[1], S. Woeste[1], A. A. Momin[2], A. Rossi[1], S. T. Arold[2], T. Haarmann-Stemmann[1]
[1]IUF – Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany, [2]King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

Chloracne, a persistent acne-like skin eruption, is a result of acute intoxication with dioxins and related halogenated aromatic hydrocarbons. The underlying pathomechanisms are not well understood, but may involve alterations in proliferation and differentiation of sebocytes and epidermal keratinocytes. The fact that in contrast to dioxin-like compounds (DLCs) some chloracnegenic agents do not bind to the aryl hydrocarbon receptor (AHR) implies the involvement of other cellular effector molecules. Preliminary data of our group point to an involvement of the epidermal growth factor receptor (EGFR), an important regulator of keratinocyte biology. Therefore, it was investigated whether the EGFR serves as a cell-surface sensor for DL and non-DL halogenated organic pollutants. Western blot analyses and EGFR internalization assays showed that DLCs like TCDD and PCB126 interfere with EGF-induced activation of the receptor. In silico docking analyses predicted that several halogenated organic compounds interact with the EGFR extracellular domain (ECD) closely to the EGF-binding site. A mutational exchange of amino acid residues predicted to be essential for DLC-binding (Q8A, Q408A) and subsequent western blot analyses confirmed that EGFR ECD serves as cell-surface receptor for DLCs. Further experiments with AHR knockout HaCaT keratinocytes revealed that a variety of halogenated organic compounds (brominated flame retardants, pesticides, DL and non-DL PCBs) interfered with EGFR-ligand-induced DNA synthesis independently of AHR. Thus, halogenated DL and non-DLCs seem capable of interfering with EGFR signaling via binding of its ECD and mitigating growth factor-induced signal transduction and DNA synthesis in human keratinocytes. These data may enhance our understanding of the pathogenesis of chloracne and other diseases associated with an exposure to ubiquitous organic pollutants.

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Modelling darier disease using human epidermal organoids

R. Agarwal[1, 2], T. Dittmar[1, 2], E. Contassot[1, 2], A. Navarini[2]
[1]*Department of Biomedicine, Universitat Basel, Basel, Basel-Stadt, Switzerland,* [2]*Dermatology, Universitatsspital Basel, Basel, BS, Switzerland*

Darier disease (DD) is a dominantly inherited skin disorder. It is characterized by painful and malodourous plaques and papules on the skin of patients. The typical features of DD include: acantholysis and abnormal keratinization. These histological features are due to mutations in the gene, ATP2A2, which encodes the sarco/endoplasmic reticulum (ER) Ca2+-ATPase isoform 2 (SERCA2), a Ca2+-ATPase pump of the ER. This leads to malformation of the desmosomes and adherent junctions. Currently, there are no good in vitro models to study DD. An organoid is a collection of organ-specific cells that develops from stem cells or progenitors and is capable of recapitulating specific functions of the organ. Human epidermal organoids (HEOs) are obtained from the skin epidermis and have several advantages over 2D keratinocyte cultures. They have an *in vivo*-like complexity and architecture and have the ability to recapitulate the physiology of the epidermis. Exposure of normal keratinocytes to the SERCA2 inhibitor thapsigargin (TG) recapitulated the abnormalities observed in DD. HEOs exposed to TG showed acantholysis thus, recapitulating DD. In addition to this artificial, chemical-induced model, we also developed an HEO model derived from keratinocytes isolated from Darier patients' lesional skin biopsies as well as an HEO model generated with CRISPR-Cas9-modified primary keratinocytes. These models can be used to screen for potential drugs to treat DD as well as study the pathomechanisms of the disease.

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Postbiotics power in supporting skin

D. Collins[1], Y. Qu[2], N. Huang[2], R. Cao[2], N. Pernodet[1, 3]
[1]Research & Development, Estee Lauder Companies, New York, New York, United States, [2]Asia Innovation Center, Estee Lauder Companies, Shanghai, China, [3]Estee Lauder Research Laboratories, Melville, New York, United States

Prebiotics and probiotics are known for their use for gut health, and now they are increasingly seen as an ingredient source in topical skin treatments. In particular, postbiotics show numerous advantages for the skin. Postbiotics are non-viable molecules/metabolites produced by the bacteria during fermentation, and they are functional bioactive compounds. Research has shown that postbiotic metabolites have health benefits on the guts by helping against inflammation and oxidative stress. Postbiotics use does not require having live bacteria in the product. Only the molecules that the bacteria have produced are introduced in the product. Lactobacillus strains have been shown to have positive effects on skin for decades now. Here, we investigated the benefits of postbiotic metabolites, like pinitol and amino acids, produced during the lactobacillus fermentation process. After treatment with these postbiotics, we were able to measure an increase of cell viability and energy and an increase of cell differentiation markers such as involucrin and filaggrin, which resulted in an increased thickness of viable epidermal layers. Over time, such activity can help the skin reinforce its epidermal layer and build a very strong barrier to defend itself better against environmental factors.

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An efficacious cosmetic formulation for managing barrier damage following mild-moderate skin disruption

U. Santhanam[1], J. Emmetsberger[1, 2], C. Saliou[1]
[1]Research Laboratories, Estee Lauder Companies, Melville, New York, United States, [2]Max Huber Research Laboratories, Melville, New York, United States

Stratum Corneum is a compacted layer of corneocytes embedded in a lipid matrix, that forms a semipermeable barrier on the surface of the skin. Despite its seemingly delicate thinness of 10-30 µm, the Stratum Corneum [SC] plays a vital role in serving as a first line of defense to protect against external environmental stressors. Even minor disruption to this layer can lead to a loss of barrier integrity that manifest as an increase in Transepidermal Water Loss [TEWL]. Many common cosmetic dermatological treatments can inflict varying degrees of short-term damage to the SC. In order to help the SC maintain and restore barrier function, a topical serum was developed containing a combination of Macrocystis pyrifera ferment and other ingredients. This preparation is shown in clinical studies to strengthen the barrier of intact skin, making the barrier not only more robust against mild skin disruption but also to enhance barrier recovery even after the intense effects of cosmetic dermatological treatments such as peels and laser treatment. Moreover, this barrier repair serum is shown to be more effective at helping the barrier recover than a conventional moisturizing lotion during post-treatment recovery. Through a series of *in vivo* evaluations, this serum is shown to reduce TEWL of intact skin, to help build back SC quality, to reduce the immediate barrier disruption caused by cosmetic glycolic peel or laser procedures and to help SC barrier recovery post-procedure. To build upon the *in vivo* findings, immunohistochemical analysis of *ex vivo* skin demonstrated that 70 % glycolic acid acutely disrupts the tight junction protein, claudin-1 and filaggrin. *Ex vivo* skin pretreated with the serum exhibited intact claudin-1 morphology and showed a greater signal intensity of both claudin-1 and filaggrin compared to *ex vivo* skin treated with glycolic acid alone, suggesting that the serum may contribute to accelerated barrier recovery.

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Macrocystis pyrifera kelp ferment affects several factors for skin barrier function

D. Collins[1, 2], J. McCarthy[2], T. Mammone[2], N. Pernodet[2], N. Karaman-Jurukovska[2]
[1]Max Huber Research Laboratories, Melville, New York, United States, [2]Research Laboratories, Estee Lauder Companies, Melville, New York, United States

The skin's primary function is to act as a protective barrier between the organism and its external environment. Physically, it protects from external threats such as chemicals, allergens, and irritants. Internally, the skin helps to maintain homeostasis and protects from enhanced loss of water. The lipid matrix in the stratum corneum of the skin is crucial for skin barrier function. The proper formation and function of the lipid matrix can be adversely affected by a deficiency in lipids. Also, the presence and appropriate processing of filaggrin are crucial for skin barrier function. Evidence from several scientific reports has shown that damage to the skin barrier can not only result in enhanced water loss but can also induce inflammatory mediators which can accelerate irritation, skin aging, prematurely diminishing skin's youthfulness. Our *ex vivo* studies showed the Macrocystis pyrifera ferment that we have developed was found to increase the level of neutral lipids in a human skin model. The ferment was also shown to increase filaggrin in Normal Human Epidermal Keratinocytes. Lastly, the strength and integrity of skin barrier function were measured by TEER (trans-epidermal electrical resistance), the measurement for physiological state generated by the ion concentration gradient. The treatment of wounded skin models with the ferment increased TEER, indicating a decrease in skin permeability and an increase in barrier function, followed by a measured increase in water content of these same wounded skin models. Together, these results indicate that the Macrocystis pyrifera ferment acts to enhance overall barrier integrity and function.

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Topical phosphodiesterase 4 (PDE4) inhibitor crisaborole (crisa) improves skin transcriptomic and proteomic biomarkers of mild-to-moderate AD towards normal skin

A. Pavel[1, 2], E. Del Duca[1], J. Cheng[1], P. Facheris[1], Y. Estrada[1], A. Cha[3], J. Werth[3], R. Bissonette[4], K. Nocka[5], C. Zang[3], E. Guttman-Yassky[1]
[1]Icahn School of Medicine at Mount Sinai, New York City, New York, United States, [2]University of Mississippi, University, Mississippi, United States, [3]Pfizer Inc., Collegeville, Pennsylvania, United States, [4]Innovaderm, Montreal, Quebec, Canada, [5]Pfizer Inc., Cambridge, Massachusetts, United States

This study was conducted to identify changes in genomic and proteomic skin profiles of 40 patients with AD lesions treated with crisa, a nonsteroidal PDE4 inhibitor, and vehicle and compare them to those of nonlesional (NL) and normal (N) skin. 2 randomly assigned (1:1 intrapatient manner) target lesions were treated with crisa or vehicle BID for 14 days. Punch biopsies were collected from lesional and nonlesional skin from patients with AD and 20 healthy controls at baseline and days 8/15 of treatment. Gene expression (microarray), OLINK proteomic (644 proteins), and qRT-PCR analyses were performed. Differentially expressed genes (DEGs) and proteins (DEPs) were determined by fold change >2 and false discovery rate <0.05. Transcriptomic and proteomic skin profiles of crisa-treated lesions at day 15 showed significant improvement towards NL and N skin compared to those of vehicle-treated lesions ($p<0.05$), especially lesional gene expression (478 DEGs; 92%NL/82%N) and baseline proteomics (315 DEPs; 59%NL/52%N) signatures and Th1 (48%NL/44%N), Th2 (58%NL/51%N), and Th17/Th22 (67%NL/38%N) pathways. These results were corroborated by PCR analysis showing improvement towards both NL and N skin in key AD-related biomarkers (innate immunity [IL6: 63%NL/52%N, IL8: 70%NL/59%N], Th1 [CXCL9: 39%NL/39%N], Th2 [IL31: 64%NL/51%N; CCL17: 48%NL/73%N; CCL22: 64%NL/68%N], Th17/22 [IL17A: 48%NL/44%N; IL19: 74%NL/66%N; PI3: 85%NL/62%N; IL23A: 76%NL/47%N] and epidermal hyperplasia [KRT16: 68%NL/60%N; S100A9: 70%N/43%N]). Crisa treatment improves the transcriptomic and proteomic immune/barrier signatures of AD lesions such that they are comparable to those of NL and N skin, highlighting the therapeutic utility of targeting PDE4 in AD patients.

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Super-resolution imaging for nuclear pore quantification in human keratinocyte differentiation

A. Neely[1], Y. Zhang[3], H. Zhang[3], X. Bao[1, 2]
[1]*Molecular Biosciences, Northwestern University, Evanston, Illinois, United States,* [2]*Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States,* [3]*Biomedical Engineering, Northwestern University, Evanston, Illinois, United States*

Nuclear Pore Complexes (NPCs) serve as essential gateways between the nucleus and cytoplasm. Dysregulation of NPC function is associated with aging and cancer, yet how NPC is implicated in epidermal homeostasis and skin disorders remains largely unexplored. Here, we show that the 74% of genes encoding nucleoporins (NUPs), the building blocks constituting the NPC, are significantly downregulated ($p<0.05$) in primary human keratinocyte differentiation. NUP93, an essential NUP for determining the protein diffusion size limit across the nuclear pores, is among the most drastically downregulated NUPs. To investigate potential changes of NPC number and composition in keratinocyte differentiation, we applied Stochastic Optical Reconstruction Microscopy (STORM), which enabled super-resolution optical imaging with resolution down to ~20 nm to visualize individual nuclear pores and quantify pore compositions. Furthermore, we developed a "multiplex nuclear isolation staining" method, which allowed efficient antibody labeling for NUPs especially for the differentiated keratinocytes and enabled multiplexed imaging of four conditions on a single coverslip. Using the MAB414 antibody that recognizes a shared region among multiple NUPs, or an antibody that binds the essential scaffolding NUP133, we consistently observe no significant changes of cluster number. These data suggest that keratinocyte early differentiation is not associated with changes of nuclear pore numbers. Interestingly, with the NUP93 antibody, we identified a reproducible reduction in cluster density ($p<0.05$) but not the intensity per cluster, suggesting decreased incorporation of NUP93 into a subset of the pores in differentiation. Taken together these data indicate that keratinocyte early differentiation does not involve changes of NPC number per cell but involves altered NPC composition that can impact the diffusion limit of protein sizes through the nuclear pore.

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WITHDRAWN

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Inducible IL-2Rγ/IL-4Rα receptor in keratinocytes is involved in epidermal barrier alterations

A. Progneaux[1], C. Evrard1, V. Deglas[1], A. Fontaine[1], E. De Vuyst[1], C. Lambert de Rouvroit[1], V. García-González[2], Y. Poumay[1]
[1]URPHYM-narilis, Universite de Namur, Namur, Belgium, [2]Almirall SA, Barcelona, Spain

Atopic dermatitis (AD) is an inflammatory skin disease triggered by a vicious circle between epidermal barrier alteration and immune system dysregulation. This circle can notably be initiated by exposure of epidermis to interleukins (IL) 4 and 13 that alter keratinocyte phenotype through activation of their IL-4Rα/IL-13Rα1 (type II) receptor. Expression of IL-2Rγ/IL-4Rα (type I) receptor for IL-4 is normally limited to hematopoietic cells, however, gene expression analysis in reconstructed human epidermis (RHE) exposed to IL-4, IL-13 and IL-25 revealed that IL-2Rγ is being expressed by keratinocytes. To investigate if the induced type I receptor could alter AD keratinocytes, expression of IL-2Rγ-encoding mRNA and protein was firstly confirmed in RHE exposed to IL-4 and IL-13, as well as in RHE made of N/TERT keratinocytes (N/TERT-RHE). Keratinocyte expression of IL-2Rγ is partially inhibited by incubation of RHE with JAK inhibitors, and totally in IL13RA1 KO N/TERT-RHE. Consequently, when IL-2Rγ is expressed by keratinocytes, activated STAT6 and MAPK signaling pathways can result from IL-4 activation of both receptors. To determine if type I receptor can activate cell signaling in RHE, type II receptors can be blocked by anti-IL-13Rα1 antibodies. Alternatively, N/TERT keratinocytes edited to knock-out IL2RG through CRISPR/Cas-9 were prepared. Interestingly, the barrier analyzed in IL2RG KO RHE exposed to IL-4 and IL-13 appears unaltered, conversely to alterations observed in unedited RHE. Interestingly, IL2RG KO RHE exhibit stronger expression of IL-13Rα2 decoy receptor when compared to unedited RHE, suggesting limited expression of IL-13Rα2 when IL-2Rγ is expressed and functional. Altogether, the results collected with RHE edited for type I or type II IL-4 receptors highlight the inducible involvement of type I receptor in keratinocytes and consequences for epidermal barrier alteration, as is the case in the context of AD.

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***In vitro* and clinical improvement of sensitive skin functions by ADE-G3 formulations**

C. Viode, A. Rouquier, C. Mias, E. Questel, S. Bessou-Touya, H. Duplan
R&D, Pierre Fabre Dermo-Cosmetique SAS, Toulouse, Occitanie, France

Sensitive skin is a common condition that can severely impact quality of life. Several mechanisms are thought to be involved, including those affecting the skin barrier function, hydration and skin innervation. Our Goal was to investigate clinically and on in vitro models, the benefit of formulations dedicated to sensitive skin and containing Aquaphilus dolomiae extract-G3 (ADE-G3) on skin barrier function and hydration, as well as protective responses to dry and pollution stresses. In vitro sensitized reconstructed human epidermis (RHE) were subjected to dehydration and pollution stress with or without the formulations. Endpoint measurements included transepithelial electric resistance (TEER), and protein expression. Clinical measurements included transepithelial water loss (TEWL), skin pH, the lipid index, and hydration scores. When applied topically in formulations, ADE-G3, increased the TEER in sensitized RHEs. In dehydrated RHEs, formulations increased recovery of skin barrier integrity, evident as a return of the ratios of filaggrin/pro-filaggrin and Caspase-14/pro-Caspase-14 to values measured in control RHEs, as well as the "natural moisturizing factors". In clinical studies, performed on dry human skin, the formulations helped to improve the skin barrier function and the level of hydration as maintained over 24 h after application. This was evident as an intense and sustained moisturization, with total lipids and lipid esters increase, an increase in pH, and a decrease in the TEWL. Moreover, when exposed to pollution stress by treating the RHE with benzo[a]pyrene and airborne particulate matter (PM10), application of formulations prior to exposure attenuated the induction of CYP1A1, CYP1B1 and UGT1A7 expression, indicating a protective effect. ADE-G3 formulations increased the hydration of the skin but also protected and improved the skin barrier integrity of sensitive skin exposed to dry and cold and airborne pollutant induced stress environments.

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Oat components provide skin protection against exposomal-factor-induced damages, increase ceramide production and balance skin's pH *in vitro*

W. Li, T. Futterer, K. J. Spisak, J. Thakrar, H. Li, A. Schladebeck, F. Zhang, R. Parsa
Johnson & Johnson Consumer Companies Inc, Skillman, New Jersey, United States

Our skin is under continuous assault from damaging exposomal factors ultraviolet irradiation, heat and cold temperatures, air pollutants smoke and ozone which alter the skin barrier, affect skin sensitivity, and can increase chronic low-level inflammation accelerating the skin aging process (inflammaging) and exacerbate symptoms of compromised skin. Studies have shown anti-inflammatory properties of oat (Avena sativa). Furthermore, oat-containing topical treatments showed significant clinical improvements in a variety of skin concerns, including erythema, pruritus, and atopic dermatitis. The objective of the present study was to evaluate the effects of oat components colloidal oatmeal, oat extract and oat oil on protection against exposome-induced damages and underlying mechanisms such as ceramide production and pH buffering capacity in a controlled way difficult to achieve clinically. Epidermal equivalent tissues were topically pre-treated with oat-containing formulations and then exposed to UV-radiation, heat/humidity, cold, cigarette smoke, and ozone. Skin barrier properties were evaluated by transepidermal electrical resistance and dye penetration; inflammatory response by ELISA. Further, human keratinocytes were treated with 400 ppm oat oil for 7 days and ceramide production was evaluated by UPLC-MS. pH buffering capacity of oat flour solution was evaluated using 0.01N hydrochloric acid solution to reduce the pH of oat flour solution by two units. Oat-containing formulations showed statistically significant reductions in exposomal-factor-induced skin barrier damage (~50%) and proinflammatory cytokine levels (49%-121%) vs untreated tissues. Moreover, 400 ppm oat oil induced a 50% increase of total ceramide production. 1-2% oat flour provided active pH control in a dose-dependent manner. This study provides additional insights supporting the ability of colloidal oatmeal, oat extract and oat oil to help protect skin against damaging exposomal factors.

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ALOX12B and PNPLA1 have distinct roles in lamellar lipid organization

P. M. Elias[3, 4], K. Vavrova[5], T. Mauro[3, 4], J. Meyer[1, 2]
[1]Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States, [2]Dermatology, VA Tennessee Valley Healthcare System, Nashville, Tennessee, United States, [3]Dermatology, University of California San Francisco, San Francisco, California, United States, [4]Dermatology, San Francisco VA Health Care System, San Francisco, California, United States, [5]Department of Organic and Bioorganic Chemistry, Univerzita Karlova Farmaceuticka fakulta v Hradci Kralove, Hradec Kralove, Hradec Králové, Czechia

Changes in the abundance and organization of lipid lamellae (LL) in the stratum corneum (SC) are proposed to underly barrier dysfunction in conditions such as psoriasis, atopic dermatitis and ichthyosis. However, there are few reports on LL organization in diseased SC. The current investigation tested the hypothesis that LL organization is altered in omega-O-acylceramide metabolism defects associated with congenital ichthyosis. Pnpla1 and Alox12b knockout mice were used as experimental models with defective omega-O-acylceramide synthesis and oxidation, respectively, and LL organization was quantitatively evaluated by electron microscopy. Pnpla1-/- and Alox12b-/- SC were thicker than control SC and contained a greater quantity of mature LL. Pnpla1 deletion, but not Alox12b deletion, was associated with LL disorganization, with loss of the normal pairing and triplet-grouping of immature and mature LL, respectively. Pnpla1-/- and Alox12b-/- mature LL were 19 and 21% thinner than their corresponding control LL. Contrary to previously thought, there was no evidence that barrier dysfunction resulted from failure to form LL or from LL-poor extracellular spaces. Together, these results indicate that LL organization is differentially altered by loss of Alox12b or Pnpla1 expression, while both defects are associated with thinner mature LL. These changes could contribute to increased skin permeability in ichthyosis and other skin diseases and provide insights into the functions of Alox12b and Pnpla1.

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HERC6 negatively regulates type I interferon activity in keratinocytes through modulation of STING-IRF3 signaling

R. Uppala[1], M. K. Sarkar[4], W. R. Swindell[5], L. C. Tsoi[3], J. M. Kahlenberg[2], A. Billi[4], J. E. Gudjonsson[4]
[1]Immunology Graduate Program, University of Michigan, Ann Arbor, Michigan, United States, [2]Internal Medicine, University of Michigan, Ann Arbor, Michigan, United States, [3]Computational medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan, United States, [4]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [5]Internal Medicine, University of Cincinnati, Cincinnati, Ohio, United States

Canonical STING-DNA sensing pathway activation leads to type I interferon (IFN) production in keratinocytes (KCs). Aberrant STING and IFN signaling is a hallmark of lupus KCs, where amplified type I IFN responses by epidermal KCs lead to increased expression of IFN stimulated genes (ISGs) like MX1 and OASL. While a pathogenic role for type I IFNs and STING signaling in autoimmune skin diseases is well established, the key regulators of IFN signaling and the crosstalk between type I IFN and STING signaling in KCs remain unidentified. To identify regulators of the IFN response, we analyzed MX1-correlated genes from 118 human primary KCs microarray dataset. This revealed a striking enrichment of ISGs associated with ubiquitination activity, with HERC6 exhibiting the highest score (r=0.93). HERC6 is an E3 ubiquitin ligase, constitutively expressed by basal and differentiated KCs of the epidermis with an unidentified role in the skin. We found that human primary KCs stimulated with a type I IFN (IFNα) or STING agonist (cGAMP) have increased HERC6 expression. KCs lacking HERC6 show enhanced induction of ISGs ($p<0.05$) upon treatment with IFNα or cGAMP but not RNA-sensing TLR3 agonist (Poly(IC)) suggesting that HERC6 is a negative regulator of ISG expression and specific to dsDNA but not exogenous RNA sensing. HERC6 KO KCs exhibited increased STING activation and STING-IRF3 signaling, resulting in feedback amplification of ISG expression upon cGAMP stimulation. Collectively, these data suggest a role for HERC6 in response to cytosolic DNA and activation of downstream type I IFN responses.

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Physiological function of krox20 (Egr2) in epithelial stem cells

E. J. Tchegnon[1, 2], C. Liao[1, 3], E. Ghotbi[1], L. Q. Le[1, 4, 5]
[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Genetics, Development and Disease Graduate Program, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [3]Graduate Institute of Medical Sciences, Taipei Medical University, Taipei, Taiwan, [4]Hamon Center for Regenerative Science and Medicine, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [5]Simmons Comprehensive Cancer Center, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

Krox20, a zinc finger-containing transcription factor, is well known for mediating stem and progenitor cell activation and differentiation in a variety of tissues. In a recent study, we reported for the first time a population of epithelial-derived Krox20-expressing keratinocytes in the hair follicle that ultimately terminally differentiate to form the structural component of the hair shaft. These Krox20-lineage cells in the hair follicle also mediate melanocyte differentiation via Stem Cell Factor production for hair pigmentation. In light of the importance of Krox20 in other cells types, the role of Krox20 cells in epidermal and hair development warrants the elucidation of Krox20 function in epithelial cells. We report here that ablation of Krox20 in skin epithelial cells caused spontaneous hair loss, correlated with increased epidermal differentiation. On the other hand, overexpression of Krox20 in epithelial cell lines resulted in the upregulation of various epidermal SC markers, suggesting maintenance of stemness as a potential role of Krox20 in epithelial cells. Moreover, we also observed a reduction of apoptosis in Krox20-overexpressing cells, pointing to an additional Krox20 function in regulating cell survival. Analysis of the molecular mechanisms underlying these observations showed that they occur through the modulation of Notch and Wnt/βcatenin pathways. These results highlight the importance of Krox20 in regulating epidermal homeostasis, through the maintenance of a resident SC population.

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Investigating the role of ferroptosis in epidermal differentiation

N. Kuprasertkul[1, 2], S. Egolf[1, 2], J. Zou[1], A. Anderson[1], C. L. Simpson[1], K. Ge[3], J. T. Seykora[1, 2], B. C. Capell[1, 2, 4]

[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Epigenetics Institute, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [3]NIDDK, National Institutes of Health, Bethesda, Maryland, United States, [4]Genetics, University of Pennsylvania, Philadelphia, Pennsylvania, United States

Ferroptosis is a recently described form of regulated cell death gaining significant interest for its involvement in a variety of disease states. It is broadly defined by iron-dependent, lethal accumulation of lipid peroxides and dysregulation of cellular redox homeostasis. However, both a physiologic role for ferroptosis and how it functions in skin homeostasis remains elusive. Recent evidence from our lab identified a critical link between ferroptosis, the epigenetic tumor suppressor MLL4, and epidermal differentiation (Egolf et al., Science Advances, 2021). We demonstrated that loss of Mll4 in mouse epidermis impairs epidermal differentiation and promotes hyperplasia tied to lost expression of key lipoxygenase enzymes (Alox12, Alox12b, Aloxe3) that mediate ferroptosis. This provoked the hypothesis that ferroptosis may play a broader role in terminal differentiation and tumor suppression in the skin. Staining for ferroptotic markers in human skin and inhibiting ferroptosis in 3D human organotypic skin models reinforced this idea. Here, we present further evidence for a functional link between ferroptosis and epidermal differentiation using global transcriptomic and lipidomic analyses as well as specific assays measuring critical signaling metabolites. We show that triggering ferroptosis in keratinocytes upregulates NRF2 antioxidant defense, ER stress signaling, and terminal differentiation genes. Taken together, our data supports the idea that ferroptosis alters key metabolic pathways in the skin to orchestrate epidermal differentiation. Overall, this opens exciting therapeutic avenues for regulating ferroptosis in skin ichthyoses and cancers, as well as other skin diseases characterized by altered differentiation dynamics.

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Ephrin-A ligand engagement of EPHA2 receptor tyrosine kinase reverses Th2 cytokine-induced epidermal barrier disruption

B. Ansbro, B. Shi, B. E. Perez White

Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Atopic dermatitis (AD) is a chronic inflammatory skin disorder affecting up to 20% of children and adults. Underlying the pathogenesis of AD are Th2 cytokines interleukin (IL)-4 and IL-13, secreted by immune cells. In addition, epidermal barrier disturbances add to the exacerbation of AD. Recent studies identify an important role for epidermal tight junction (TJ) barrier disruption in the pathogenesis of AD. Our laboratory previously demonstrated that loss of EPHA2, a receptor tyrosine kinase (RTK), led to the abrogation of epidermal TJ barrier formation and function. Our current data further supports a role for EPHA2 signaling in TJ function as engagement of this RTK with its cognate ephrin-A ligand strengthens the barrier as measured by transepithelial electrical resistance (TEER) in 3D human epidermal equivalents derived from individual donors (N = 5). We further found that ephrin-A ligands prevented the collapse of TJs in response to IL-4+IL-13 treatment. Analysis of Gene Expression Omnibus (GEO) datasets revealed a significant loss of ephrin-A in AD lesional skin ($p < 0.05$), suggesting an imbalance of ephrin-A-mediated signaling in this disease. IL-4 and IL-13 can induce keratinocytes to release other cytokines, including thymic stromal lymphopoietin (TSLP), which is known to activate dendritic cells and promote CD4+ Th2 differentiation. We wanted to ask if TSLP itself can influence keratinocytes and epidermal barrier function, and if ephrin-A ligand stimulation of EPHA2 can reverse any negative effects. We find that TSLP alone significantly reduces barrier function by TEER in 3D human epidermal equivalents from unique donors ($N = 3$; $p < 0.05$). However, concurrent treatment of ephrin-A ligand with TSLP prevented epidermal barrier disturbance and brought function back to baseline. Taken together, these results suggest that restoring ephrin-A in the setting of AD may reinforce TJs and bolster epidermal barrier function.

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Modeling darier disease using gene-edited human keratinocytes and organotypic epidermis to identify therapeutic targets

C. L. Simpson[1], M. Sarkar[2], S. Egolf[3], J. Zou[3], B. C. Capell[3], J. E. Gudjonsson[2]
[1]*Dermatology, University of Washington, Seattle, Washington, United States,* [2]*Dermatology, University of Michigan, Ann Arbor, Michigan, United States,* [3]*Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States*

Darier disease(DD) is a genetic disorder characterized by impaired intercellular adhesion and aberrant differentiation of keratinocytes, leading to hyperkeratotic papules and epidermal erosions. While mutations underlying DD are known to target SERCA2, an endoplasmic reticulum(ER) calcium pump, current therapies for this chronic, painful skin disorder are limited and some, such as oral retinoids, may pose significant long-term risks. Unfortunately, mice lacking SERCA2 do not replicate the cutaneous findings of DD seen in human patients. Thus, we aimed to establish an in vitro model of DD to improve our understanding of its pathogenesis downstream of SERCA2 deficiency and to reveal new potential therapeutic strategies. TERT-immortalized human keratinocytes were subjected to CRISPR/Cas9 gene editing to generate cells deficient in SERCA2. Transduction of keratinocytes with GCaMP, a cytosolic calcium sensor, allowed us to demonstrate augmented spikes in intracellular calcium in SERCA2-depleted cells upon exposure to elevated extracellular calcium. Culturing of SERCA2-deficient keratinocytes in an organotypic model of epidermis revealed features of DD histopathology including dyskeratosis and reduced intercellular adhesion. To elucidate potential pathogenic drivers of DD resulting from SERCA2 deficiency, we performed RNA sequencing of keratinocytes heterozygous or homozygous for loss of SERCA2. While dysregulated calcium homeostasis due to SERCA2 depletion is expected to disrupt numerous cellular pathways, preliminary analysis of gene expression profiles revealed alterations in potentially druggable targets, including regulators of cornification, intercellular adhesion, calcium homeostasis, inflammatory responses, and cell death. Further studies are ongoing to assess whether dampening downstream effects of SERCA2 loss using existing pharmacologic compounds can effectively reverse DD pathology in organotypic epidermis.

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Molecular and histopathologic profiling of melanocytic nevi in patients with RASopathies

E. Simmons[1], I. Rybak[1], J. Urban[1], J. D. McPherson[2], K. A. Rauen[1], M. Kiuru[1]
[1]Dermatology, University of California System, Davis, California, United States, [2]Biochemistry & Molecular Medicine, University of California System, Sacramento, California, United States

Melanocytic nevi are among the most common benign human neoplasms and are mimics, risk factors, and potential precursors for melanoma. Many drivers of melanoma and tumor progression have been identified, such as somatic mutations in the RAS pathway genes. It is also known that germline genetic variants influence nevus count in RASopathy patients. However, why nevi develop and why certain nevi undergo melanomagenesis remains largely unknown. This project aims to evaluate the role of RAS pathway germline mutations in nevogenesis and melanomagenesis by studying individuals with RASopathies, specifically cardio-facio-cutaneous (CFC) syndrome and Costello syndrome (CS) patients. Melanocytic nevi and corresponding normal tissue were collected or already collected biopsy slides were retrieved from RASopathy patients. The samples underwent histopathological analysis, and if material was available, immunohistochemistry screening for BRAF V600E and whole-genome sequencing. Eight melanocytic nevi from 5 patients were included (ages 5 to 25, 80% female, 20% male). Two patients had CFC and 3 patients had CS. Histopathological analysis of the nevi revealed 4 compound nevi, 3 intradermal nevi, and 1 junctional nevus. Dysplastic features were noted in one nevus. Immunohistochemistry screening for BRAF V600E performed on two nevi was positive in one CFC patient. Sequencing in this patient revealed a BRAF p.V600E somatic driver mutation and a germline BRAF p.E501G mutation. In one CS nevus, a germline HRAS p.G12C mutation was identified, but no somatic BRAF mutations were detected. This project utilizes the combination of histopathology and whole-exome sequencing to investigate melanocytic nevi in individuals with germline mutations in the RAS pathway. This study will expand our knowledge on melanocytic tumor risk in RASopathies and germline regulation of the development of nevi. Analyses of additional RASopathy nevi are required and forthcoming.

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H2AZ1 and H2AZ2 regulate divergent programs in epidermal progenitor maintenance

S. Droll[1], B. Zhang[1], D. Leon[1], X. Bao[1, 2, 3]
[1]*Molecular Biosciences, Northwestern University, Evanston, Illinois, United States,* [2]*Dermatology, Northwestern University, Chicago, Illinois, United States,* [3]*Robert H Lurie Comprehensive Cancer Center, Chicago, Illinois, United States*

Collectively known as H2A.Z, the H2AZ1 and H2AZ2 isoforms differ by only three amino acids. H2AZ2 emerged in vertebrates, and both histone variants play key roles in modulating mammalian gene expression. However, the regulatory role of H2A.Z in epidermal homeostasis remains unclear. We demonstrate that progenitor keratinocytes express the highest amount of H2A.Z at both the mRNA and protein levels, and that H2A.Z expression decreases during differentiation ($p < 0.0001$). Knocking down either H2AZ1 or H2AZ2 with four shRNAs per target suppresses keratinocyte proliferation in the holoclone assay ($p < 0.0001$) and impairs progenitor regeneration in organotypic culture ($p < 0.0001$). Seeking to understand the mechanism behind the proliferation defect, we performed RNA sequencing on progenitor keratinocytes with H2AZ1 or H2AZ2 knockdown. GO term analysis reveals that H2AZ1 depletion primarily downregulates genes related to “cell cycle” and multiple DNA repair pathways while also upregulating genes related to “cell adhesion,” “collagen catabolism,” and “extracellular matrix organization.” In contrast, H2AZ2 knockdown downregulates oxidation-reduction and protein folding processes and upregulates epidermal differentiation and collagen catabolism. Thus far, this study demonstrates H2A.Z is required for epidermal progenitor maintenance, and that the isoforms regulate distinct genetic networks.

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Genotype-phenotype associations in recessive dystrophic epidermolysis bullosa (RDEB)

J. So[1], N. Harris[1], S. Fulchand[1], E. Gorell[2], J. Nazaroff[1], V. Yenamandra[1], M. Marinkovich[1, 3], J. Tang[1]
[1]*Department of Dermatology, Stanford Medicine, Stanford, California, United States,* [2]*Department of Dermatology, University of Cincinnati, Cincinnati, Ohio, United States,* [3]*Dermatology Service, Veterans Affairs Palo Alto Medical Center, Palo Alto, California, United States*

RDEB is a rare bullous genodermatosis caused by mutations in COL7A1. Clinical features range from severe wounds to esophageal strictures and anemia. Prior work has identified associations of biallelic COL7A1 premature termination codon (PTC) mutations with more severe disease, likely from absent or severely truncated type VII collagen (C7), but genotype-phenotype associations for other mutations including splice site (SP) or missense (MS) mutations remain unexplored. We analyzed data from 83 RDEB patients for clinical characteristics and functional genotypes as defined by a mutation’s impact on C7 function using available literature and in silico predictions, classifying genotypes by degree of deleterious effect on C7 function as “severe” (PTC/PTC, n=40; PTC/SP, n=12), “moderate” (PTC/MS, n=20; SP/SP, n=2) and “mild” (SP/MS, n=4; MS/MS, n=5). Mean age was 24 years. Genotype severity is as follows: 63% severe, 27% moderate and 11% mild. 89% of subjects with mild genotypes had 2 COL7A1 mutations in the C7 collagenous domain (vs 46% with severe or 46% with moderate genotypes, p=0.05). Greater mutation severity correlated with more clinical disease burden including higher frequency of generalized blistering (vs limited; severe=90% vs moderate=73% vs mild=56%, p<0.01), complete hand pseudosyndactyly (37% vs 25% vs 22%, p=0.02) and loss of fingernails (79% vs 59% vs 33%, p<0.01). Mutation severity also correlated with presence of extracutaneous findings including anemia (79% vs 55% vs 44%, p<0.01), ocular disease (79% vs 46% vs 33%, p<0.01) and use of gastrostomy tube (48% vs 23% vs 22%, p=0.02). Our work suggest that like PTC/PTC mutations, PTC/SP mutations correlate with more severe phenotypes, while SP/MS and MS/MS correlate with less severe disease. Further study of genotype-phenotype associations in larger samples is needed to improve genotype-related prognostics for RDEB patients.

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Non-viral gene therapy for recessive dystrophic epidermolysis bullosa: Hyper branched aminated polyesters mediated minicircle DNA delivery

X. Wang, Y. Li, D. Manzanares, Z. He, S. A, I. Lara-Saez, W. Wang
Charles Institute of Dermatology, University College Dublin, Dublin, Dublin 4, Ireland

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare autosomal inherited skin blistering disorder caused by mutations in the type VII collagen gene (COL7A1). The large size of COL7A1 is a big delivery challenge for both viral and non-viral vectors and seriously hindered the development of therapeutic gene replacement therapy for RDEB. Our group has developed a non-viral minicircle DNA mediated gene replacement strategy for RDEB, which achieved high transfection efficiency while safety is improved by avoiding the usage of viruses and the removal of bacterial backbone from the plasmid DNA. Here we further synthesized a new hyper branched aminated polyesters (HAPE) with greater DNA delivery ability, higher gene expression efficiency and better biocompatibility, which performed better than commercial reagents like lipofectamine 3000 and JetPEI. The HAPE delivered minicircle DNA coding full COL7A1 gene successfully restored type VII collagen expression in RDEB keratinocytes. This improved system developed here has a high potential for use as an efficient and safe non-viral topical treatment for RDEB patients in the clinic and can be adapted to other genetic diseases.

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Independent causal effect of psoriasis on multiple sclerosis identified by Mendelian randomization

M. Patrick[1], R. Nair[1], K. He[2], P. Stuart[1], A. Billi[1], J. E. Gudjonsson[1], J. Oksenberg[3], J. T. Elder[1, 4], L. C. Tsoi[1, 2, 5]
[1]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [2]Biostatistics, University of Michigan, Ann Arbor, Michigan, United States, [3]Neurology, University of California San Francisco, San Francisco, California, United States, [4]Ann Arbor Veterans Affairs Hospital, Ann Arbor, Michigan, United States, [5]DCMB, University of Michigan, Ann Arbor, Michigan, United States

Psoriasis and multiple sclerosis (MS) are physiologically distinct yet share multiple genetic signals and have overlapping pathogenesis. Previous studies suggest psoriasis patients are at greater risk of MS than the general population, and the two diseases also have overlapping comorbidities and traits (for example, they are both more prevalent in northern latitudes). To evaluate whether there is a direct causal relationship, we conducted Mendelian randomization (MR) analysis with six different techniques, using existing GWAS for psoriasis (11,024 cases and 16,336 controls) and MS (14,802 cases and 26,703 controls); we also addressed 10 potential confounding exposures, previously suggested to be associated with both diseases, by including GWAS data from body mass index, coronary artery disease, inflammatory bowel disease (IBD), type 1 diabetes (T1D), type 2 diabetes (T2D), asthma, rheumatoid arthritis, drinks per week, cigarettes per day, and vitamin D levels. Four of the MR techniques indicated a significant effect of psoriasis on MS (FDR<0.05) and two showed nominal significance, whereas the effect of MS on psoriasis was not significant in any of the MR analyses. When including all covariates nominally significant from at least one technique in a multivariable MR analysis, psoriasis still has a significant effect on MS (p=5.8×10-3, OR=1.04), independent of T1D (p=4.3×10-7, OR=1.05), T2D (p=2.3×10-3, OR=1.08), IBD (p=1.6×10-11, OR=1.11) and vitamin D (p=9.4×10-3, OR=0.75) in the multivariable analysis, while BMI and drinks/per week were not significant. By applying multiple different MR techniques to multiple comorbidities and traits, we have been able to reveal the most important modifiable risk factors and determine there is indeed an independent causal relationship between psoriasis and MS.

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Filaggrin gene P478S and R501X polymorphisms in atopic dermatitis in Dakahlia, Egypt

O. Atef[1], M. Zohdy[1], S. Metwally[2], H. Salem[1]
[1]Dermatology, Mansoura University Faculty of Medicine, Mansoura, Dakahlia, Egypt, [2]Clinical Pathology, Mansoura University Faculty of Medicine, Mansoura, Dakahlia, Egypt

Background: Atopic dermatitis (AD) is a common skin disease characterized by chronic relapsing pruritus affecting patients of all ages. Several studies focused on epidermal barrier dysfunction as an etiology. Filaggrin (FLG) is a protein essential for maintaining skin barrier function. Filaggrin gene mutations have been linked to the development and the increased severity of AD. The aim of this study was to investigate the possible association between R501X and P478S polymorphisms of FLG gene and AD patients in Dakahlia, Egypt. Patients and methods: A prospective case-control study, was done on 100 subjects (7 months -34 years), grouped into 50 patients with AD and 50 healthy volunteers. Clinical evaluation of all patients including SCORAD assessment of the severity of AD was done. Genotyping was established using (RFLP-PCR) technique with determination of both alleles and genotypes after DNA extraction. Results: FLG P478S CT genotype, C allele, FLG R501X RX genotype, X allele, and P478S–R501X combined genotypes CC-RX, CT-RR, CT-RX haplotypes showed significant high frequency in patients with AD when compared to controls (p=0.032, 0.045, 0.016, 0.035, 0.001, 0.001, and 0.011 respectively), with significant high risk to acquire AD within healthy subjects in univariate and multivariate analyses. Significant increased amounts of IgE, eosinophils in the sera of the FLG P478S TC carriers were observed compared to non-carriers in AD patients (median=315.5, 17 and 505, 254; p<0.001). P478S CT genotype was significantly associated with positive family history for allergy (p=0.019), especially bronchial asthma (p=0.001). P478S CT genotype was significantly associated with more severe cases, higher IgE concentration, eosinophilic count, SCORAD S when compared to CC genotype (p<0.001, 0.024, 0.001, <0.001 respectively). Conclusion: The contribution of FLG mutations to the susceptibility to AD could be useful for developing a predictive test and informing diagnostics as well as therapeutic advice.

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A focused analysis of genome-wide associations of dermatologic conditions in the UK biobank

J. C. Klein, R. Mahapatra, R. C. Wang
Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

Genome-wide association studies (GWAS) link common variants to a trait of interest. Decreasing sequencing costs enable large-scale efforts such as the UK Biobank to genotype and phenotype ~500,000 individuals for over 7,000 traits. However, due to multiple testing corrections for approximately 200 billion tests, many clinically and statistically significant associations are unappreciated. By re-analyzing the data for 13 dermatologic conditions, we hypothesized we would identify well-established and new genetic associations that can shed insight into disease prediction and novel therapeutic targets. After filtering over 7,000 phenotypes for dermatologic conditions with at least 500 cases, we identified 13 traits for this analysis: malignant melanoma, melanoma in situ, squamous cell carcinoma, basal cell carcinoma, actinic keratosis, seborrheic keratosis, psoriasis, lichen planus, systemic lupus erythematosus, hyperhidrosis, pilonidal cyst, sebaceous cyst, and lipoma. We filtered 166,373,136 associations with a Benjamini-Hochberg false discovery rate of 5% to yield 447 sentinel variants (the most significant variant within a haplotype block (250Kb and R2>0.1)). These were enriched for protein-coding variants (p<1e-5). The sentinel variants validated previously identified associations including genes affecting skin pigmentation—Tyrosinase (TYR), Melanoma Antigen AIM1 (SLC45A2/AIM1), and Melanocortin 1 Receptor (MC1R)—with actinic keratosis, basal cell carcinoma, and malignant melanoma. Novel associations such as LDL Related Receptor 3 (LRP3) with lipomas and Phospholipase C Delta 1 (PLCD1) with sebaceous cysts were also identified. When analyzing all sentinel variants, we noted a higher CADD score (predictor for deleteriousness) compared to random variants (p=0.0037). The availability of databases such as the UK Biobank are redefining the modern GWAS. Our focused analysis has validated numerous dermatologic genetic associations and identified hundreds of novel associations for a variety of dermatologic conditions.

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Regulation of the keratinocyte progenitor to differentiation switch by alternative mRNA splicing

S. Takashima[1], P. Cai[2], W. Sun[2], J. Bui[1], A. Otten[1], K. Qu[2], B. Sun[1]
[1]Department of Dermatology, University of California San Diego, La Jolla, California, United States, [2]Division of Molecular Medicine, University of Science and Technology of China, Hefei, Anhui, China

Alternative splicing (AS) of messenger RNAs diversifies gene function by generating protein isoforms with different biological properties. Over 95% of human genes are alternatively spliced, but the functional consequences of most AS events are still not known. Here, using long-read RNA sequencing, we identified 5,826 AS events associated with human epidermal differentiation (percent spliced index >10%, FDR <0.01). AS events were enriched in genes associated with histone modification and phosphorylation ($p < 0.001$). The most common type of AS was cassette exon splicing, in which an intervening exon between two other exons is included or skipped in the mRNA sequence. We functionally characterized a cassette exon in mitogen-activated protein kinase (MAP3K7), which had not been previously studied in human epidermis. Basal epidermal progenitors preferentially express a shorter MAP3K7 isoform but switch to the longer isoform in suprabasal, differentiated epidermis with inclusion of an additional 81 bp exon. Using isoform-specific knockdown and overexpression, we demonstrate that inclusion of a single exon in MAP3K7 is necessary and sufficient to transition human epidermal keratinocytes from a replicating progenitor state with high clonogenic capacity into a post-mitotic differentiated state. We also performed binding motif analysis of sequences adjacent to epidermal AS events which identified 26 RNA binding proteins predicted to mediate mRNA splicing in epidermal keratinocytes, including ten with previously unstudied roles in the skin. In summary, our study defines the repertoire of alternative mRNA splicing events in the human epidermis and demonstrates the potential of this resource to identify functional AS events and splicing RBPs that regulate epidermal differentiation.

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Rapid activation of epidermal progenitor differentiation via CDK9 activity modulated by AFF1 and HEXIM1

S. Lloyd[1], M. Brady[1], D. Rodriguez[1], D. Leon[1], M. McReynolds[1], J. Kweon[1], A. Neely[1], X. Bao[1, 2]
[1]Molecular Biosciences, Northwestern University, Evanston, Illinois, United States, [2]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Epidermal barrier function requires differentiation initiation from a sustainable pool of progenitors. The mechanisms underlying the earliest events initiating differentiation remain unclear. Here we show that RNA Polymerase II (Pol II) pause release, controlled by the CDK9 kinase activity, plays a crucial role in the rapid induction of differentiation. Using RNA-seq in combination with Pol II ChIP-seq, we identified a cluster of upregulated genes featuring robust Pol II pause release in keratinocyte differentiation. A subset of these genes showed rapid induction within 3 hours by Peptidomimetic inhibitors targeting the super elongation complex (SEC), in keratinocytes cultured in the undifferentiation condition. A longer-term (24 hour) treatment led to significant changes (fold change >2, $p<0.05$) of 1286 keratinocyte-differentiation-signature genes. Mechanistically, we found that the SEC scaffold protein AFF1, but not AFF4, is essential for repressing differentiation in the progenitor state. AFF1 knockdown induced epidermal hypoplasia and impaired progenitor regenerative capacity. We further identified that AFF1 represses differentiation in progenitors through sequestering CDK9 in the inactive state via its interaction with HEXIM1. AFF1 and HEXIM1 directly bind near the transcription start sites of 92 rapid-response genes, sustaining Pol II pausing in the progenitor state. These rapid response genes include ATF3, whose overexpression is sufficient to drive the activation of other differentiation-activating transcription factors, including ZNF750, PRDM1, OVOL1, and GRHL3. In addition, we found that the AFF1 and CDK9 activity are both involved in the immediate of response of PKC-signaling activation. Taken together, our findings suggest a model that AFF1 and HEXIM1 mediated CDK9 activity switch underlies the initial steps of epidermal progenitor differentiation, in response to cellular signaling such as PKC activation.

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Identification of Z-DNA, G4-DNA and B-DNA in epidermis: Spatial genomic organization of different DNA structures (Genomesorganizomics)

C. Gagna[1], L. Elkoulily[2, 3], A. Zaman[1], W. Lambert[3]
[1]Biological and Chemical Sciences, New York Institute of Technology, Old Westbury, New York, United States, [2]Biological and Chemical Sciences, New York Institute of Technology, Old Westbury, New York, United States, [3]Pathology, Rutgers - New Jersey Medical School, Newark, New Jersey, United States

Human epidermis is an exceptional tissue to examine biological processes, such as mitosis, basal stem cells, cellular differentiation, and two different types of cell death. We wanted to establish, for the first time, the presence and distribution of three totally different types of DNAs in the epidermis of human skin. Nucleic acids are dynamic molecules that can adopt different structural conformations, such as left-handed double-stranded (ds-) Z-DNA, four-stranded G4-quadruplex DNA, and canonical right-handed ds-B-DNA. These nucleic acids have different biological functions in human cells. Human epidermis (thin skin) of 15 individuals (ages: 21 to 55) was histotechnologically processed to obtain paraffin-embedded tissue sections. Employing anti-Z-DNA MAb, anti-quadruplex DNA MAb, and anti-B-DNA MAb, DNAs were quantified in the epidermis. Cell death was characterized: terminal differentiation, i.e., denucleation, and apoptosis. Immunofluorescence (IF) was performed using a confocal microscope and computer analysis software. Tissues were counterstained with wheat germ agglutinin. AR enhanced IF of certain DNAs. Both positive and negative controls were used. Data shows that all three types of DNA can be identified within the nucleated cells of the epidermis. IF was highest in the stratum basale and lowest in the stratum granulosum. IF gradually decreased at different rates within the epidermis, depending on type of DNA structure. No IF was observed in the stratum corneum. These three DNAs are regulating genes and telomeres in the epidermis which control cell death and synthesis of biomolecules. A novel “omics” approach allowed us to characterize DNA structures found within the architecture of the nucleus, i.e., “Spatial Genomic Organization of Different DNA Structures: Genomesorganizomics”.

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Mitochondrial genetics in skin aging

A. R. Vandiver[4], A. Hoang[2], N. Kokikian[1], T. Soriano[4], J. Kim[4], M. Teitell[3], J. Wanagat[2, 5]
[1]University of California Los Angeles David Geffen School of Medicine, Los Angeles, California, United States, [2]Division of Geriatrics, Department of Medicine, University of California Los Angeles, Los Angeles, California, United States, [3]Department of Pathology and Laboratory Medicine, University of California Los Angeles, Los Angeles, California, United States, [4]Division of Dermatology, Department of Medicine, University of California Los Angeles, Los Angeles, California, United States, [5]Veterans Administration of Greater Los Angeles Healthcare System, Los Angeles, California, United States

Deletions within the mitochondrial genome (mtDNA) have been repeatedly observed in photoaged skin and purported as a marker of photoaging. The mitochondrial genome is implicated in many important cellular functions through regulation of the metabolome; thus mtDNA mutations are an appealing target to prevent or reverse aging-induced loss of function. However, methodological limitations have prevented a complete understanding of mtDNA deletions, limiting their use as a metric and target for interventional therapies. Here, we have applied two novel methods to begin understanding the trajectory through which deletions arise and to map these changes on the genome. Using droplet digital PCR to quantify large mtDNA deletions in 25 remnant skin samples, we demonstrated an exponential increase in deletion frequency in both facial and truncal skin with increasing age. Examining dispase-separated tissue layers, we demonstrated that these mutations predominate in dermal tissue. Next, using Cas9 based enrichment with nanopore sequencing, we obtained single read full length sequencing of the mitochondrial genome for two aged skin samples, mapping the location of mtDNA deletions in a manner not previously available. In this data, we identified both the previously reported sun-exposure associated mtDNA “common deletion” and novel large deletions not previously reported in aged skin. These findings emphasize the magnitude and breadth of mtDNA mutations in aging skin. The newfound ability to quantitate and map these mutations increases the potential for understanding the role of mtDNA deletions as both of metric of skin aging and a possible therapeutic target.

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Guttate leukoderma as the predominant feature of epidermolysis bullosa simplex caused by a novel EXPH5 mutation

T. Koren[1], F. Zagairy[1], J. Krausz[1], E. Cohen Barak[1, 2]
[1]Emek Medical Center, Afula, Northern, Israel, [2]Technion Israel Institute of Technology, Haifa, Haifa, Israel

Inherited epidermolysis bullosa (EB) are a heterogeneous group of skin fragility disorders, due to mutations in genes, encoding structural proteins involved in cell-cell or cell-matrix adhesion. One of the rare and recent subtypes of EB simplex, is due to bi-allelic mutations in EXPH5 gene, which encodes exophilin5/ synaptotagmin-like protein lacking c2 domains b (Slac2-b). To date, only 10 patients with EB simplex resulting from EXPH5 mutations have been described, characterized by early onset skin fragility, mainly on the extremities, with a variable degree of pigmentary alteration such as mottled pigmentation and hypopigmentation. Here in we present a 35-year-old female, who suffered of diffuse guttate leukoderma on the trunk and extremities since early childhood. In addition, she mentioned a "feeling" of easy skin fragility predominantly following mechanical trauma, with no blisters or scars. To identify the underlying genetic cause, we employed a whole exome sequencing to germline DNA extracted from patient's leukocytes. A novel homozygous variant in EXPH5, c.1153C>T, was identified, causing premature stop codon at amino acid Glutamine 385 (p.Gln385).This case expands the clinical spectrum of inherited EB simplex, especially regarding mild phenotype with dyschromatosis findings. The novel homozygous variant in EXPH5, c.1153C>T, should be included in the gene mutations causing inherited EB.

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Nanopore Cas9-targeted sequencing as a tool for identifying revertant mosaicism in the skin

K. Natsuga[1], Y. Furuta[2], S. Takashima[1], T. Nohara[1], H. Kosumi[1], Y. Mai[1], H. Higashi[2], H. Ujiie[1]
[1]Department of Dermatology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan, [2]Division of Infection and Immunity, International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan

Revertant mosaicism (RM) is a phenomenon in which germline mutations are spontaneously corrected in tissues. RM skin is often observed in genodermatoses, including epidermolysis bullosa (EB). However, the rigorous validation of RM in the skin has been technically challenging, especially when homologous recombination (HR) causes RM. Recently developed nanopore Cas9-targeted sequencing (nCATS) enables the enrichment and sequencing of specific regions (up to a few tens of kb) on gDNA without PCR amplification or the reverse transcription of mRNA. Here, we explore the feasibility of using nCATS to identify HR-induced RM in the skin. We used skin samples from a recessive dystrophic EB patient who was compound heterozygous for missense and nonsense COL7A1 mutations. Immunofluorescent labeling of type VII collagen, encoded by COL7A1, did not clearly distinguish between revertant and non-revertant skin due to the patient's mutational profile. The Cas9-mediated enrichment of gDNA covering the two mutation sites (>8 kb) and subsequent MinION sequencing succeeded in detecting intragenic crossover in the epidermis of the clinically revertant skin. This method is advantageous because it does not involve PCR amplification or reverse transcription, which can technically induce recombination. We propose that nCATS is a practical tool for validating RM in the skin.

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GEM-3: phase 3 safety and immunogenicity results of beremagene geperpavec (B-VEC), an investigational, topical gene therapy for dystrophic epidermolysis bullosa (DEB)

M. Marinkovich[1], M. Gonzalez[2], S. Guide[3], I. S. Bagci[1], S. Chitra[4], B. Agostini[5], H. Chen[5], T. Parry[5], S. Krishnan[5]
[1]Stanford University, Stanford, California, United States, [2]University of Miami, Coral Gables, Florida, United States, [3]Mission Dermatology Center, Rancho Santa Margarita, California, United States, [4]Savio Group Analytics & Statistics, Hockessin, Delaware, United States, [5]Krystal Biotech, Pittsburgh, Pennsylvania, United States

DEB is rare, severe, genetic disorder caused by mutations in the COL7A1 gene. B-VEC is an HSV-1-based topical, redosable gene therapy designed to restore functional COL7 protein in DEB patients. In a Phase 3, multi-center, intra-patient, randomized, double-blind, placebo-controlled study (NCT04491604), patients with confirmed COL7A1 mutations were enrolled. Each patient contributed 1 primary wound pair, randomized 1:1 to weekly treatment with B-VEC or placebo for 26 weeks. B-VEC dose ranged from 4e8-1.2e9 PFU/wound, determined by wound size at baseline. The primary endpoint was complete wound healing at month 6 (M6). To evaluate safety and tolerability of repeat B-VEC use, adverse events (AEs), and changes in vitals, physical exam, and laboratory results, including anti-COL7 and anti-HSV-1 antibodies were assessed. 31 patients enrolled (ITT=31). At M6, B-VEC resulted in 67.4% wound closure compared to 21.6% for placebo (absolute difference (95% CI): 45.8% (23.6%-68.0%); p<0.005). Due to the difficulty of blood draws for DEB patients owing to skin fragility, 22/31 subjects (71.0%) were able to provide a serum sample at baseline, and matched serum samples were obtained at M6 for 19 of these subjects. 14/22 (63.6%) tested patients had anti-HSV-1 antibodies at baseline; 6 patients seroconverted by M6. 1/22 (4.5%) patients were positive for anti-COL7 antibodies at baseline; 13 patients seroconverted by M6. 1 mild drug-related AE was reported. B-VEC was well-tolerated with no drug-related serious AEs or discontinuations due to treatment. Despite antibodies overall efficacy of B-VEC was preserved.

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KIR allelic variation is associated with atopic dermatitis

D. Margolis[1], N. Mitra[2], E. Philips[3]
[1]Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]Biostatistics, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [3]Vanderbilt University School of Medicine, Nashville, Tennessee, United States

Atopic dermatitis (AD) is a common, pruritic, inflammatory skin disease characterized by life-long periods of acute disease flares and remissions. Predisposition to AD is associated with genetic variation. Killer cell Ig-like receptor family (KIR) receptors interact with HLA ligands and are thought to be a primary regulator of NK cell function. NK cells have also been implicated in the pathophysiology of AD and other skin inflammatory diseases. In this study, we examined allelic variation in KIR genes KIR2DL5, KIR2DS5, and KIR2DS1 with respect to AD. We used a classic case-control design to evaluate individuals with (n=313) and without (n=176) AD. The three KIR genes listed above were sequenced using allelic typing and then associations with AD were evaluated using logistic regression. Interactions between KIR and known HLA ligand pairs were also assessed. Overall, the prevalence of KIR2DL5 was 52.5% (95% CI: 48.0,57.0), KIR2DS5 was 33.0% (28.8,37.3), and KIR2DS1 was 33.6% (29.4,38.0). For KIR2DL5, homozygote individuals for KIR2DL5*001:01 were more likely to have AD (OR: 2.16 (95% CI:1.31,3.53) p=0.0023) as compared to those without the KIR2DL5 gene as was only the KIR2DL5A location (1.85(1.22,2.79) p=0.004 (telomeric). The effect of KIR2DL5*001:01 did not vary by race. AD was not associated with alleles in the other two KIR genes. KIR2DL5 is not known to be associated with HLA ligands; however, the effect of KIR2DL5*001:01 increased in the presence of HLA-B *-21TT leader sequence (2.46(1.37,4.41) p=0.0025) as well as HLA-C*C2 ligand (2.07 (1.37,4.41), which is known to interact with "KIR2DL" genes. This is the first study to explore KIR allelic variation in AD. KIR2DL5*001:01 allele is independently associated with an increased risk of AD. Our study adds to the growing literature on the genetic basis of the immune dysregulation of AD and, specifically, that KIR2DL5*001:01 is associated with an increased risk of AD.

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Crucial epigenetic modules in skin differentiation

S. Nayak, K. Jiang, M. Cross, E. Hope, D. Bajpai, S. Worrell, K. Hasneen, F. Naz, S. Brooks, S. Dell'Orso, M. Morasso

National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, United States

Tissue homeostasis is directed by temporal-spatial regulation of gene expression, where lineage specification is a critical step, requiring precise expression profiles. Epigenetic regulation and chromatin accessibility play a major role in ensuring adequate regulation of transcriptional machinery. We amalgamated data from RNA-Seq, ATAC-Seq, ChIP-Seq and HiC to gain transcriptomic/chromatin profile of distinct skin epidermal layers. The data was generated from basal (proliferating) and suprabasal (differentiated) cells of mouse neonate skin epidermis. We observed a repressive gene signature in the suprabasal layer, where chromatin processes were enriched in the repressed genes. This observation was supported by the ATAC-seq analysis. Systematic integration of ATAC-Seq and RNA-Seq data is currently being investigated. Given the significance of Dlx3 in skin differentiation established by our lab, we profiled chromatin occupancy of histones (H3k4me1, H3k4me3, H3k27ac and K3k27me3) and Dlx3 in the suprabasal compartment to decipher the differentiation driving modules. We used this data to delineate super-enhancers in the suparabsal layer and overlay with the chromosome conformation data from HiC-seq. We validated a subset of these modules using Dlx3-knock-out models, marked by impaired differentiation programs leading to significant skin phenotype. This systemic approach of data integration will lead to comprehensive understanding of the dynamics and crosstalk underlying the two epidermal strata in the context of Dlx3-mediated higher order chromatin regulation, paving the way for better understanding and management of skin differentiation disorders.

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Transcriptional profiling of the rare acantholytic disorders Darier disease, Hailey-Hailey disease, and Grover's disease suggests common mechanisms of pathogenesis

H. Burks[2], Q. Roth-Carter[2], L. M. Godsel[2], X. Xing[1], L. C. Tsoi[1], J. Kirma[1], J. E. Gudjonsson[1], K. Green[2]

[1]*University of Michigan, Ann Arbor, Michigan, United States,* [2]*Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States*

Darier, Hailey-Hailey, and Grover's diseases are rare non-autoimmune, acantholytic, inflammatory skin disorders. While these diseases share some features, they have varying clinical presentations and etiologies. Darier disease and Hailey-Hailey disease are caused by mutations in the ATP2A2 or the ATP2C1 gene, respectively, while the etiology for Grover's disease is unknown. To better characterize the shared and divergent molecular and cellular processes driving these acantholytic disorders we performed RNAseq on lesional skin samples (10 Darier disease, 7 Hailey-Hailey disease, and 8 Grover's disease samples). Spearman correlation analysis showed marked correlation between the three diseases, with rho values between 0.81 and 0.86 when comparing any two of these disorders. Functional enrichment analysis of significantly upregulated genes revealed epidermal differentiation pathways as the most changed pathways in all three diseases, suggesting that defects in keratinocyte differentiation are common across these disorders. We also observed enrichment in genes associated with inflammatory pathways in all disorders, in particular upregulation of IL-17 and IL-36 response pathways. Corresponding with this, TNF was identified as the most enriched upstream regulator in all three disorders and p38 signaling is predicted as a commonly activated pathway, indicating a shared inflammatory signature across all three diseases. These observations show that while Darier disease, Hailey-Hailey disease and Grover's disease have different etiologies and clinical presentations they are remarkably similar at the transcriptional level suggesting that patients may benefit from similar targeted therapeutic approaches.

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Altered expression of genes regulating mTOR and other signaling pathways in skin tumors from patients with tuberous sclerosis complex

A. Afrin[1, 2], X. Zhang[3], C. L. Dalgard[3], M. D. Wilkerson[3], J. Moss[2], T. Darling[1]

[1]*Dermatology, Uniformed Services University of the Health Sciences, Bethesda, Maryland, United States,* [2]*Pulmonary Branch, National Heart Lung and Blood Institute, Bethesda, Maryland, United States,* [3]*Anatomy, Physiology, and Genetics, Uniformed Services University of the Health Sciences, Bethesda, Maryland, United States*

Individuals with tuberous sclerosis complex (TSC) develop tumors in multiple organs due to pathogenic variants in TSC1 or TSC2, with skin tumors showing mTOR activation and aberrant cell growth. To identify transcriptional signatures and dysregulated signaling pathways that are shared in different types of TSC skin tumors, we performed RNAseq on 58 samples of fibroblast-like cells grown from TSC skin tumors (27 angiofibromas, 11 fibrous cephalic plaques, 10 ungual fibromas, 8 shagreen patches, 2 oral fibromas) and 28 samples of fibroblasts from patient normal appearing skin, in patients whose blood DNA showed germline variants in TSC2 in 14, TSC1 in 1, mosaicism in 3, and no mutation identified in 3. mRNA libraries were sequenced using the Illumina NovaSeq platform. Sequenced reads were aligned to the human reference genome (hg38) using Mapsplice. Transcript expression quantification was performed using HTSeq. Differential expression analyses were performed using DESeq and g:Profiler was used for functional enrichment analysis. The levels of TSC2 expression were lower in tumor samples than patient normal fibroblasts (p-value = 0.0011). 1357 differentially expressed genes were identified, including 945 upregulated and 412 downregulated in skin tumor samples. KEGG pathway enrichment for three tumor types showed upregulation of genes involved in lysosomes, cancer pathways, cytokine-cytokine receptor interactions, and PI3K-AKT signaling, and downregulation of genes involved in basal cell carcinoma, breast cancer, Wnt signaling, and Hippo signaling. Two genes associated with the mTOR pathway were upregulated in TSC skin tumors, PIK3R3 and RPS6KA1, whereas DEPTOR was downregulated. These studies provide insights into the complex interplay of mTOR and other signaling networks in TSC leading to tumor formation.

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Investigating modifiers of cutaneous neurofibroma development in adults with neurofibromatosis type 1

E. Patel[1], J. Ramos[1], X. Hu[1], J. Roberts[3], F. McCormick[2], J. Blakeley[3], C. Romo[3], K. Y. Sarin[1]

[1]*Dermatology, Stanford University School of Medicine, Redwood City, California, United States,* [2]*Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, California, United States,* [3]*Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States*

Neurofibromatosis type 1 (NF1) is a multi-system genetic disorder involving the cutaneous, nervous, hematopoietic, ocular, and skeletal systems. Almost all individuals with NF1 (>95%) develop benign dermal tumors called cutaneous neurofibromas (cNFs) which frequently lead to significant physical and social morbidity. cNFs exhibit high variability in number, subtype, growth rate, and anatomic location. Despite substantial research into the molecular drivers of cNFs, the genotypic-phenotypic correlation and the factors which modulate cNF development are not well understood. To address this, we have created a biobank of surveys, skin photographs and genetic data from individuals with NF1. 1150 participants aged 40 or older are being recruited from the US, Canada, and Europe to provide quality of life data using validated tools, photographs, and saliva samples for whole genome sequencing. Thus far, 430 participants have provided survey data, 265 participants have provided photographs, and 217 participants have submitted saliva samples. Preliminary analysis suggests a positive correlation between age and self-reported number of cNFs. There is no significant correlation between gender and number of cNF, age of cNF development, or age of NF1 diagnosis. Ongoing analyses include correlating participant self-report of their cNF burden with investigator assessment using clinical photography to assess accuracy in self-reporting and the quality of participant photographs for diagnostic and monitoring purposes. We aim to conduct a large-scale GWAS analysis to elucidate clinical and genetic factors that might explain the phenotypic variability of cNFs in people with NF1. An understanding of these factors will inform the pathogenesis of these tumors, serve as biomarkers for individual risk stratification, and guide future clinical trial design.

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Integrated psoriasis GWAS and eQTL analysis reveals risk-associated genetic control of TRAF3IP2-AS1 expression in activated CD8 T-cells

L. C. Tsoi[1], Z. Zhang[1], P. Stuart[1], N. Dand[2], M. Patrick[1], M. A. Simpson[2], J. Voorhees[1], J. Barker[2], R. Nair[1], J. T. Elder[1]

[1]University of Michigan, Ann Arbor, Michigan, United States, [2]Kings College, London, United Kingdom

We performed an international meta-GWAS of psoriasis, increasing effective sample size by 3.4-fold. Using COJO, we identified 191 independent psoriasis loci at genome-wide (GW) significance (179 outside the MHC). We conducted eQTL analysis of 1,195 strand-specific RNA-seq libraries passing QC (MQ30, ~25M reads/sample) from 9 FACS-purified immune cell types (CD4/CD8, CLA-/CLA+, and 0h/24 h CD3-CD28-activated T-cells; also resting mDC) from 153 genotyped individuals, yielding 9,091 significant (p<5x10-3) cis-eQTLs mapping to the 179 non-MHC 95% Bayesian credible sets. Here we focus on TRAF3IP2 encoding Act1, an adaptor that transduces signals through IL-17R. COJO found 14 independent risk signals for this region (2 potentially protein-altering and 12 potentially regulatory). The lead protein-coding variant, rs33980500 C/T encoding Act1 D10N, is the top GWAS hit with a posterior probability of 1.00 on unconditional analysis. We have shown that rs33980500-T increases psoriasis risk and promotes Th17 expansion. We identified cis-eQTLs for the lncRNA TRAF3IP2-AS1 in activated CD8 T-cells, but not in activated CD4 or resting T-cells. In contrast, cis-eQTL signals for TRAF3IP2 itself were markedly reduced after CD8 T-cell activation. Moreover, the best cis-eQTL signal for TRAF3IP2-AS1 in activated CD8 T-cells (rs11153301, p=2.9x10-6) is a GW-significant psoriasis hit (p=3.7x10-8) after conditioning on Act1 D10N. The risk allele rs11153301-T results in reduced expression of TRAF3IP2-AS1 in activated CD8 T-cells, and a recent study (J Immunol 206:2353) describes an inhibitory effect of TRAF3IP2-AS1 on Act1 protein level and IL-17 signaling. Notably, the expanded GWAS also implicates a cluster of noncoding variants near IL17RA (p=3.9x10-9), which encodes a key subunit of IL-17R targeted by brodalumab, a highly effective psoriasis treatment. These results suggest that TRAF3IP2-AS1 acts as a brake on psoriasis via its effects on IL-17 signaling in activated CD8 T-cells.

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Distribution of hidradenitis suppurativa monogenic etiologies in a racially diverse specialty clinic cohort

A. Colvin[1], E. Baugh[1], K. Babbush[2], T. Adriano[2], G. Benesh[2], M. E. Torpey[2], A. Nosrati[2], A. T. DeWan[3], S. M. Leal[1], D. Goldstein[1], S. Cohen[2], L. Petukhova[1]

[1]Columbia University, NY, New York, United States, [2]Albert Einstein College of Medicine, Bronx, New York, United States, [3]Yale University, New Haven, Connecticut, United States

Hidradenitis suppurativa (HS) is an inflammatory skin disease that disproportionally affects African American women and has many unmet needs. Disease mutations have been reported in three γ-secretase genes (NSCTN, PSENEN, and PSEN1) and PSTPIP1. The population relevance of these monogenic etiologies is incompletely characterized. Previous studies, limited in size and ancestral diversity, utilized small cohorts of either European or Asian ancestry, yielding unstable prevalence estimates (0%-6%) that may not be generalizable to other populations. We aimed to determine the prevalence of HS monogenic etiologies in a large multiracial HS cohort. We performed exome sequencing of 219 HS research participants using standard sample and variant quality thresholds. We used a validated ancestry classification model to determine genetic ancestry of participants. Mutations, defined as having a maximum allele frequency less than .001 in large diverse databases, were extracted for NSCTN, PSENEN, PSEN1 and PSTPIP1. Genetically-defined ancestry indicates 79% of participants have African admixture, 7% have South Asian ancestry, and 14% are genetically-defined Caucasian. We identified seven mutations, notably, 6/7 were in those with genetic ancestries hitherto unrepresented in HS genetic studies. We identified two loss-of-function mutations in NCSTN (p.D443fs; splice site c.148-1G>T), and five missense mutations in PSEN1 (p.P355S; p.G206A) and PSTPIP1 (p.R320Q; p.P283L, p.R44C). PSEN1 mutation p.G206A is annotated as pathogenic in ClinVar. Our preliminary estimate for the prevalence of HS monogenic etiologies in a racially diverse cohort is 3%. Pathogenicity remains to be determined for these mutations, like many others reported in prior HS genetic studies. A lack of in vitro systems to efficiently determine the biological effects of mutations in these genes is a major barrier to advancing precision medicine in HS.

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The role of lanosterol synthase in patients with congenital hypotrichosis

S. Ali[1], M. Collins[1], I. Pupo Wiss[1], M. Senna[1, 2]

[1]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Harvard Medical School Department of Dermatology, Boston, Massachusetts, United States

Hypotrichosis Simplex (HS) is a monogenic hair loss disorder that presents with childhood onset of diffuse and progressive scalp/body hair loss. Literature has identified mutations that cause autosomal-dominant and autosomal-recessive forms of this disorder. We present a rare case of hypotrichosis simplex in a patient with a mutation in lanosterol synthase (LSS). In our case, a 13-year-old Caucasian female presented to the clinic with pronounced scalp alopecia and thinning of the eyebrows since birth. She had no rash, visual deficits, nail or teeth abnormalities, and no other history of illness. She also had no family history of similar hair loss. Upon exam, >90% scalp hair loss was noted, with short coarse hairs minimally scattered. Eyebrows also showed a 50% decrease in hair density. Genetic testing revealed variants of unknown significance in the LSS gene, specifically, in exons 6 and 9 out of a total 23 exons. Microscopy of hair follicles revealed tapering and hypopigmentation of the distal hair shaft. Mutations in this gene can result in ocular abnormalities, such as congenital cataracts, or, as in this case, hypotrichosis.The clinical phenotypes seem to manifest as a result of aberrant and/or reduced expression of LSS. Mutations nearer the N-terminus of the LSS gene seem to manifest as hypotrichosis, while those closer to the C-terminus are primarily associated with eye lens defect. Hypotrichosis simplex in the setting of LSS mutations has been described previously in few patients and this case is an additional example of this unusual condition.

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Ratio-dependent adenine base editing engenders highly efficient correction of RDEB mutations with minimal bystander mutations

A. Sheriff[1], I. Guri[1], P. Zebrowska[2], V. Llopis Hernandez[1], I. Brooks[1], G. Newby[3], D. Liu[3], L. Laczmanski[2], J. McGrath[1], J. Jackow[1]

[1]St John's Institute of Dermatology, King's College London, London, London, United Kingdom, [2]Instytut Immunologii i Terapii Doswiadczalnej im Ludwika Hirszfelda Polskiej Akademii Nauk, Wroclaw, Poland, [3]Merkin Institute of Transformative Technologies in Healthcare, Broad Institute, Cambridge, Massachusetts, United States

Recessive dystrophic epidermolysis bullosa (RDEB) is a currently incurable blistering genodermatosis caused by mutations in COL7A1 encoding type VII collagen (C7), the major component of anchoring fibrils. Adenine base editors (ABEs) are a class of gene editor which can install A-T to G-C base pair changes and therefore hold promise for reversing pathogenic COL7A1 variants. Patient-derived fibroblasts harboring c.5047 C>T (p.Arg1683*) in exon 54 of COL7A1 were electroporated with 2ug of mRNA of the 'ABE8e' ABE variant and 5ug of single guide (sg)RNA. Post-editing analysis uncovered 100% correction of the mutation in edited cells, however 98% of alleles contained an aberrant mutation of a bystander nucleotide at position c.5052. To reduce this bystander mutation we tested a range of ratios and concentrations of sgRNA and ABE8e and found 1:2, 0.5 ug sgRNA: 1 ug ABE8e was the best, maintaining 64% correction efficiency of c.5047 C>T and diminishing bystander mutations to just 2%. Western blot analysis found 35% of normal C7 expression post-correction, which is likely to be enough for functional correction. Furthermore, analysis of the top 10 predicted off-target sites with the higher concentration of ABE8e confirmed no detectable off-targeting activity and screening of the entire COL7A1 gene found no other changes post editing, demonstrating a promising safety profile. Ongoing work will use 3D skin equivalents to evaluate functional restoration of normal skin architecture and also focus on *in vivo* delivery strategies for base editing tools directly to patient skin. Optimized concentrations and ratios of ABE8e and sgRNA appear to be both safe and effective and may hold clinical utility in RDEB.

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High resolution chromatin loops associate with gene targets for psoriasis susceptibility regions

H. Zhang[1], Z. Zhang[2], P. Stuart[2], M. Patrick[2], J. Liu[3], R. Nair[2], L. C. Tsoi[2, 1], J. T. Elder[2]

[1]Biostatistics, University of Michigan, Ann Arbor, Michigan, United States, [2]Dermatology, University of Michigan Michigan Medicine, Ann Arbor, Michigan, United States, [3]Computational Medicine and Bioinformatics, University of Michigan Medical School, Ann Arbor, Michigan, United States

There are over 80 psoriasis-associated loci identified, with a majority playing regulatory roles; however, their gene targets are yet to be identified. Chromatin loops play an important role in gene regulation, and can be detected by chromosome conformation capture techniques like Hi-C. In this study, we deep sequenced Hi-C libraries (~1 billion reads/reaction) to generate nine PBMC-derived subsets from 2 individuals (~50,000 cells each): 4 unstimulated CD3+CD45RO+ memory T cells (CD4+CLA-, CD4+CLA+, CD8+CLA-, CD8+CLA+); the same 4 subsets after 24 hours of CD3/CD28 stimulation of CD1c- cells (primarily T cells); and mDC. Using a 5k-10k contact map resolution, we identified 13,186 ± 2,857 Hi-C loops per library, ranging from 30 to 1,980 kb long, using Mustache. Notably, we found that ~50% of the identified loops have at least one end overlapping a transcription start site, providing a unique resource for mapping long-range promoter interactions in immunocytes. We identified 27 ± 6 loops per library that linked to promoters and whose loop ends could also be mapped to known psoriasis signals, defined by the 95% credible intervals generated from a recent transethnic GWAS (HGG Adv 2022). Of 47 target genes involved in such loops, we found 8 previously suggested gene targets for psoriasis: ANXA6, ELMO1, ETS1, FASLG, MBD2, IFI44, PTGER4 and STARD6. Among them, ANXA6, ETS1, FASLG and IFI44 were present only in T cells, while the rest presented in both T cells and mDC. We further revealed other psoriasis gene candidates including KAT5, NHLRC3/PROSER1, and SUCO, which were linked to known psoriasis signals through loops in multiple Hi-C libraries for both individuals and in both T cells and mDC. This study provides complementary support for known psoriasis-related genes at the level of 3D genome structure, and nominates other genes of interest for future studies.

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Non-viral gene delivery platform for topically treating rare genodermatoses.

I. Lara-Saez, X. Wang, Y. Li, Z. He, D. Manzanares, M. Negru, Q. Xu, S. A, W. Wang

Charles Institute of Dermatology, University College Dublin, Dublin, Dublin Co., Ireland

Gene therapy is the most promising treatment for recessive dystrophic epidermolysis bullosa (RDEB), however genetic cargo delivery efficiency is still a technical limitation. Viruses are the traditional vector of preference for gene therapy, as virus trophism increase tissue specificity. However, drawbacks related with safety and high manufacturing costs have facilitated the expansion of non-viral vectors, such as liposomes and cationic polymers. Our group is focused on the development of highly branched cationic polymers for gene therapy to treat RDEB. Our polymers have demonstrated encapsulation and delivery of a full COL7A1 cDNA with no toxicity, performing better transfection efficiencies than commercial counterparts in RDEB keratinocytes. In this work, we show the research progress expanding the polymer technology for mRNA and ribonucleoprotein complex delivery for developing CRISPR/Cas9 based gene editing therapies for RDEB. Gene edition *in vivo* has been achieved by a single topical application, obtaining efficiencies comparable with viral vectors (Ad5). Endosomal escape, by peptide polymer decoration is being investigated to improve efficiency *in vivo* by avoiding endosomal retention. Storage of the nanoparticles, formed by polymers and the genetic cargo, at -20C ensures no reduction in efficiency for more than 6 months. However, in order to avoid cold chain challenges, nanoparticles have been lyophilised, increasing dose concentration and facilitating formulation with skin absorption enhancers for topical application. Proven ability to transfect stem cells combined with high efficiencies transfecting with multiple plasmids at the same time, should contribute to the success of prime editing strategies to pursue permanent correction of potential >89% of all described EB disease-associated mutations. The developed polymer platform shows high potential to be adapted to a wide range of genetic approaches for RDEB, including the most novel ones.

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Efficient ex-vivo COL7A1 correction of patient's primary keratinocytes and fibroblasts using RNP-based CRISPR/Cas9 and homology-directed repair to treat recessive dystrophic epidermolysis bullosa

A. Izmiryan[1], C. Berthault[1], O. Gouin[1], M. Chen[2], D. Woodley[2], S. Gaucher[1], A. Hovnanian[1]

[1]INSERM 1163-Imagine Institute for Genetic Diseases, Paris, France, [2]Norris Comprehensive Cancer Center, the Keck School of Medicine, Los Angeles, California, United States

RDEB is a severe and life-threatening genetic skin disease responsible for blistering of the skin and mucosa after minor trauma. RDEB is caused by a wide variety of mutations in COL7A1 encoding type VII collagen (C7), the major component of anchoring fibrils (AF) which are critical attachment structures that adhere the epidermis to the dermis. We aimed to achieve highly efficient correction of a null mutation (c.6508C>T, p.Gln2170*) in exon 80 of COL7A1 in a patient's primary RDEB keratinocytes (KC), fibroblasts (FB) and 3D-skin equivalents (SE) through CRISPR/Cas9-mediated HDR. We designed three gRNAs specifically targeting the mutation or adjacent sequences. Chemically modified gRNAs were delivered together with hfCas9 as a ribonucleoprotein complex (RNP) by nucleofection. One gRNA targeting intron 79 achieved up to 73% cleavage activity in primary RDEB-KC and RDEB-FB. The search for off-target activity in RDEB cells found no evidence for non-specific cleavage activity at in-silico predicted sites. For the correction purpose, RDEB-KC and FB were treated with specific RNPs containing the most active gRNA and hfCas9 together with Donor ssODN. Gene correction and C7 rescue were estimated to be up to 58% by allele-specific TaqMan-ddPCR and Western blotting. Grafting of genetically corrected 3D SE onto nude mice induced re-expression and normal localization of C7 as well as AF formation at the dermal-epidermal junction at 5 and 10 weeks post-grafting. Keratin 5, keratin 10 and loricrin showed similar epidermal differentiation patterns in corrected, uncorrected and normal SE. With this approach, we achieved highly efficient and specific gene editing which could be applicable to all mutations in exon 80 of COL7A1 in primary RDEB-KC and FC. This approach has a potential for future *ex vivo* clinical applications.

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CRISPER/Cas 9 mediated introduction of the keratin 1 mutation in mice that are identified in patients with epidermolytic ichthyosis

M. Sashikawa[1], M. Hossain[1], M. Komine[1], C. Sarai[2], Y. Nagao[2], M. Ohtsuki[1]

[1]Dermatology, Jichi Ika Daigaku, Shimotsuke, Tochigi, Japan, [2]Animal center, Jichi Ika Daigaku, Shimotsuke, Tochigi, Japan

Mutations in the KRT1 cause epidermolytic ichthyosis (EI). There is a strong demand for the development of novel treatment measures. A mutation of c. 1436 T >C; p. Ile 479 Thr of K1 was identified in our case with severe palmoplantar keratoderma, and exaggerated skin lesion on joint skin. Our in silico survey revealed that this amino acid change caused a severe conformational alteration in the K1 protein, strongly suggesting that it interfered with the formation of the K1 / K10 coiled- coil complex. We introduced this K1 mutation into C57/BL6 mice using CRISPR / Cas9 method to investigate the effect of mutant K1 on epidermal biology, and to find out the novel therapeutic measures. We also applied mechanical stretch stimuli on cultured keratinocytes with mutant K1, and assessed the mRNA expression. These mice developed erosions on the skin at birth. Many baby mice died within the first 3 days of life with further deletion of DNA occurred in the experimental process. Mortality had declined from the third day of life. After 2 weeks of age, the footpad became thickened and hyperkeratosis occurred with pigmentation. The mice showed hyperkeratotic and erosive skin lesions similar to those in EI patients, especially on the neck and perioral areas, where mechanical stimuli always apply. Hematoxylin and eosin staining showed accumulation of keratohyalin granules, and granular degeneration. Aggregation of keratin filament was also confirmed with the electron microscopic technique. Preliminary study showed that mechanical stretch of mutant K1-introduced keratinocytes showed reduced expression of autophagy-promoting genes, such as EGFR and enzymes that degrade FLG. Since EGFR is involved not only in proliferation but also in downstream autophagy, it is speculated that applying mechanical stimulation to keratinocytes with mutant K1 caused inhibition of autophagy, which may result in phenotypic changes in mice.

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Distinct patterns of gene expression in skin biopsies differentiate generalized pustular psoriasis (GPP) from psoriasis vulgaris (PV)

S. Garcet[1], H. Bachelez[2], P. Baum[3], S. Visvanathan[4], J. G. Krueger[1]
[1]Laboratory for Investigative Dermatology, Rockefeller University, New York, New York, United States, [2]Service de Dermatologie, Assistance Publique–Hôpitaux de Paris Hôpital Saint-Louis and INSERM Unité 1163, Imagine Institute of Genetic Diseases, Université de Paris, Paris, France, [3]Boehringer Ingelheim GmbH & Co. KG, Biberach, Germany, [4]Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut, United States

GPP is a rare, severe, clinically heterogeneous disease characterized by acute, life-threatening flares, and widespread systemic effects. Historically, GPP has been considered a variant of PV; however, evidence indicates that these are distinct diseases, potentially requiring different treatment. We aimed to better understand the differences between GPP and PV by comparing molecular profiles of lesional skin (LS) and nonlesional skin (NLS) from patients with GPP (N=7) or PV (N=17) with skin from healthy volunteers (HV, N=10). Global transcriptome-wide RNA sequences from skin biopsies were analyzed to identify differentially expressed genes (DEGs; absolute fold change >1.5 and Benjamini–Hochberg false discovery rate <0.05) relative to HV skin. NLS from patients with PV showed 541 DEGs. NLS from patients with GPP showed 3683 DEGs (6% shared with PV NLS), demonstrating considerable nonfocal widespread skin involvement in GPP but not PV. There were more DEGs in GPP LS (6874: 3510 upregulated, 3364 downregulated) than in PV LS (3643: 1709 upregulated, 1934 downregulated). In GPP LS, 4115 DEGs (60%) did not overlap with DEGs found in PV LS; among DEGs that did overlap (40%), 1379 were upregulated in both diseases, but 789 (57%) of these showed higher dysregulation in GPP ($p<0.05$). The largest differences were seen in genes involved in neutrophil-associated inflammation (CXCL1 [566 vs 31], CXCL8 [1965 vs 67], CD177 [85 vs 12], CCL20 [476 vs 32]) or connected with the Th1 axis (IL1B [298 vs 9], IL36A [2339 vs 199]). These results add to a growing body of data supporting classification of GPP as a disease separate to PV, based on genetics, transcription, and clinical features.

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The spectrum of oligogenic variants in the RAS pathway in a PHACE cohort

D. Siegel[1], E. Partan[3], O. Davies[9], S. Chamlin[2], B. Drolet[4], A. Mancini[2], L. Sundaram[1], M. Tutaj[8], I. Frieden[5], D. Metry[6], F. Blei[7], C. Lin[8], K. Wang[1], I. Karakikes[1], A. Urban[1], A. Oro[1], N. Sobreira[3]
[1]Stanford University School of Medicine, Stanford, California, United States, [2]Ann and Robert H Lurie Children's Hospital of Chicago, Chicago, Illinois, United States, [3]Johns Hopkins Medicine, Baltimore, Maryland, United States, [4]University of Wisconsin-Madison, Madison, Wisconsin, United States, [5]University of California San Francisco School of Medicine, San Francisco, California, United States, [6]Baylor College of Medicine, Houston, Texas, United States, [7]NYU Langone Health, New York, New York, United States, [8]Medical College of Wisconsin, Milwaukee, Wisconsin, United States, [9]Harvard Medical School, Boston, Massachusetts, United States

The acronym PHACE (posterior fossa anomalies, infantile hemangiomas, arterial anomalies, cardiac defects and eye anomalies) was coined to describe the features of an uncommon sporadic condition with a vascular tumor (infantile hemangioma), developmental and progressive vascular abnormalities. Here, we report the findings of our analysis of whole genome sequencing of germline samples from 98 unrelated trios in which the probands had PHACE. Two coding variants, RASA3–p.Val85Met and THBS2–p.Asp859Asn, were predicted to be pathogenic by numerous algorithms. This analysis was negative for a shared gene across multiple probands; however, a g:Profiler pathway analysis of the genes with rare, de novo variants demonstrated combinatorial variants in the RAS/MAPK pathway. Coding and noncoding variants in six RAS pathway genes were prioritized based on the vascular abnormalities reported in knockout mouse models. To identify lineages in which the genes may be acting, we explored the expression of the prioritized candidate genes RASA3, AFF2, DLC1, EPHA3, PIK3CA, and THBS2 across diverse cell types in the human developing heart by incorporating chromatin accessibility (scATAC-seq) and gene expression (scRNA-seq) datasets. We observed that AFF2, EPHA3, PIK3CA, and THBS2 are co-expressed in the vascular smooth muscle cells in the fetal heart, whilst AFF2, EPHA3, and PIK3CA are co-expressed in the vascular endothelium. These findings suggest oligogenic variants in RAS pathway genes may contribute to the developmental vascular abnormalities in PHACE.

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Pityriasis rubra pilaris transcriptomics further implicate IL-17 signaling and correlate with response to IL-17A inhibitor therapy

R. C. Velasco, D. Haynes, T. Reitner, M. Chang, R. P. Kulkarni, G. Kent, P. Cassidy, T. Greiling
Dermatology, Oregon Health & Science University, Portland, Oregon, United States

Pityriasis rubra pilaris (PRP) is a rare inflammatory skin disorder associated with significant patient morbidity. The pathophysiology of PRP has been under ongoing investigation with studies implicating alterations in Th17 signaling, phospholipase processing, and novel germline CARD14 gene variations. In this study, 11 patients with moderate-to-severe PRP (as defined by a Psoriasis Area and Severity Index [PASI] ≥ 10) had lesional skin biopsies taken prior to and following 24 weeks of Il-17A inhibitor therapy for RNA sequencing analysis. Differential expression analysis was conducted using the R package "DESeq2" comparing age- and sex-matched controls to pre- and post-treatment patients, with delimitations by depth (epidermis vs dermis) and response to treatment (≥50% improvement in PASI [responder] vs <50% [non-responder]). Results were filtered to an adjusted false discovery rate of < 0.05 and absolute log2 fold change (log2FC) > 2. Prior to treatment, 2012 dermal and 4532 epidermal genes were found to be differentially expressed in PRP patients, with mean ± SD absolute log2FC of 3.00 ± 1.26 and 3.23 ± 1.41, respectively. Following treatment, mean absolute log2FC improved to 1.73 ± 1.24 and 1.69 ± 1.43 in dermis and epidermis, respectively ($p<0.001$ by paired t-test). When stratifying pre-treatment IL-17A expression by therapeutic response, both pre-treatment dermis (log2FC 6.02) and epidermis (log2FC 7.60) had elevated expression of IL-17A, whereas neither epidermis nor dermis of non-responders were significantly different. Pretreatment epidermal IL-17C and CCL20, two cytokines in the IL-17 pathway previously implicated during proteomic analysis of PRP, were more expressed in responders (log2FC 10.80 and 7.68, respectively) than non-responders (log2FC 9.03 and 6.07, respectively). Together, these findings demonstrate that IL-17A inhibition improved the cutaneous transcriptomic profile of patients with PRP and identified potential screening targets for treatment success.

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Differences in chromatin accessibility in male vs female keratinocytes using ATAC-seq

Y. Chung[1], L. C. Tsoi[1], B. E. Perez White[2], C. Zeng[1], A. Billi[1], J. E. Gudjonsson[1]
[1]Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, United States, [2]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Sexual dimorphism contributes to autoimmune diseases, such as systemic lupus erythematosus (SLE), where the female-to-male ratio is 9:1. Although hormonal and sex chromosome variation contribute to women being more prone to such diseases, the predominant driving mechanism(s) remain unknown. We hypothesize that male and female keratinocytes display distinct differences in chromatin landscape and nucleosome accessibility that enforce differences in inflammatory responses. We have assessed chromatin landscape differences by performing assay for transposase accessibility sequencing (ATAC-seq) on primary keratinocytes (KCs) isolated from healthy male and female donors (n=3, each). In addition, we have used ATAC-seq to determine changes in chromatin landscape in response to pro-inflammatory cytokines (i.e., IL-17A, type I IFN, and TNF) in KCs and compared differences between male and female. We demonstrate specific sexual differences in chromatin accessibility in female vs. male KCs. Specifically, our data identifies differences in chromatin regions from female KCs adjacent to genes related to epidermal differentiation responses and inflammatory responses, particularly to type I IFNs. These data corroborate our previous ChIP-seq data, where female KCs showed higher level of H3K4me3 within inflammatory gene regions. In addition, our preliminary ATAC-seq data demonstrates that treatment with pro-inflammatory cytokines, including TNF, leads to modification of chromosomal accessible regions in KC. Collectively, our findings reveal that male and female KCs contain distinct open chromosome regions, and these differences may contribute to differential response to inflammatory stimuli between men and women.

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Epigenetic age acceleration in a pediatric cohort of atopic dermatitis

R. Jeremian[1, 2], C. Bouchard[2], V. Hladky[3, 2], D. M. Lebo[3, 2], C. Jack[2]

[1]McGill University Faculty of Medicine and Health Sciences, Montreal, Quebec, Canada, [2]Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada, [3]Universite de Montreal Faculte de Medecine, Montreal, Quebec, Canada

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that varies tremendously in its clinical presentation. It is a manifestation of the poorly understood interplay between genetic, physiologic, and environmental factors. Such heterogeneity adds substantial barriers to predicting disease course and providing targeted, timely treatment, necessitating the discovery of dynamic biomarkers that reflect the nuances of this disease. The epigenetic clock is a marker that has garnered significant interest in complex disease studies due to its ability to reliably predict biological age in healthy human tissues (Horvath, 2018). Consequently, a discrepancy between epigenetic (molecular) and chronological age is thought to be a mark of "epigenetic age de/acceleration," and has been observed in human tissues from cancer, cardiovascular disease, and schizophrenia. To this end, we leveraged a publicly-available dataset of DNA methylation (DNAm) from whole blood, interrogated using Illumina HumanMethylation450 microarray from a pediatric AD population (n = 24, mean age 2.56 [SD 2.02] y) and healthy controls (n = 24, mean age 2.09 [SD 1.01] y). Employing the DNA Methylation Age Calculator, we observed significantly increased mean DNAm age in AD versus control across three clock algorithms (Horvath: +1.47 y, p = 0.042; Hannum: +6.13 y, 4.97 x 10-5; PhenoAge: +8.22 y, p = 0.0011). To our knowledge, this is the first study to investigate epigenetic aging in AD. Despite the relatively small sample size, we observed significant evidence of epigenetic age acceleration in this disease cohort. The implications of our findings, pending validation in independent samples (including skin), have the potential to identify a new disease-associated marker that could serve as a proxy for disease, provide clues to better understanding AD pathophysiology and potentially enable prediction of disease course in the clinical setting.

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New insight of itch mediators and proinflammatory cytokines in epidermolysis bullosa

H. Nguyen[1], S. Shinkuma[1, 2], R. Hayashi[1], T. Katsumi[1], T. Nishiguchi[1], K. Natsuga[3], Y. Fujita[3, 4], R. Abe[1]

[1]Dermatology, Niigata Daigaku Igakubu Igakuka Daigakuin Ishigaku Sogo Kenkyuka, Niigata, Niigata, Japan, [2]Dermatology, Nara Kenritsu Ika Daigaku Igakubu Igakuka Daigakuin Igaku Kenkyuka, Kashihara, Nara, Japan, [3]Dermatology, Hokkaido Daigaku Daigakuin Igaku Kenkyuin, Sapporo, Hokkaido, Japan, [4]Dermatology, Sapporo City General Hospital, Sapporo, Hokkaido, Japan

Epidermolysis bullosa (EB) is a hereditary disorder characterized by mechanical stress-induced blistering. The presence of extracutaneous complications such as cardiomyopathy and renal disease observed in severe EB subtypes and the fact that pruritus is a common symptom across all EB subtypes indicate that EB is not only a skin fragility disease but also a systemic inflammatory disorder. Our study aims to elucidate the basis of the systemic inflammation seen in EB patients. We analyzed serum samples of 20 EB patients by Luminex bead-based cytokine assays and enzyme-linked immunosorbent assays. In the group of itch mediators, the levels of Th2 cytokines including IL-4, IL-5, and IL-13 were not elevated in EB patients ($p > 0.05$). In contrast, TSLP levels significantly increased ($p = 0.01$), and IL-31 and levels of oncostatin M were not statistically significant but tended to be higher in the patients than in the healthy controls. Among proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, IL-6 was elevated in EB patients ($p < 0.01$). In addition, the serum levels of sIL-2R, HGF, M-CSF, SCGF-β, IL-8, IL-16, IFN-γ, MIF, MIP-1α ($p < 0.01$, $p < 0.01$, $p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.05$, $p < 0.05$, $p < 0.05$, $p < 0.05$, respectively) were found to be significantly elevated in EB patients, whereas TNF-β ($p < 0.01$) decreased. In conclusion, the imbalance of several itch mediators and proinflammatory cytokines was identified. Biologics targeting the cytokines, which were found to be elevated in the sera of patients, is considered as a beneficial treatment option for EB.

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The association between juvenile xanthogranulomas in neurofibromatosis type 1 patients and the development of leukemia: A systematic review

S. N. Meyer[1], A. Vaughn[1], Y. Li[2], K. A. Rauen[3], M. Kiuru[1, 4]

[1]Dermatology, University of California Davis, Davis, California, United States, [2]Public Health Sciences, University of California Davis, Davis, California, United States, [3]Pediatrics, University of California Davis, Davis, California, United States, [4]Pathology and Laboratory Medicine, University of California Davis, Davis, California, United States

Importance: A subset of patients with neurofibromatosis type 1 (NF1) develop juvenile xanthogranulomas (JXGs), a non-Langerhans cell histiocytosis, and some of these patients also develop juvenile myelomonocytic leukemia (JMML). Yet, these associations are poorly delineated. Objective: Our objectives in this systematic review was to: (1) clarify the relationship between NF1, JXGs and JMML and identify patients that may benefit from additional screening, (2) describe the clinical characteristics of JXGs arising in NF1. Evidence Review: A literature search was performed within the PubMed database on July 7th, 2021. Articles were included if they were peer-reviewed human studies, in English, and discussed any association between NF1, JXG, and/or leukemia with individual patient data. Findings: Sixty-five articles met eligibility criteria, which included 181 individual patients. Fifty-six patients had NF1 and JXG without leukemia (Group 1), 98 patients had NF1 and leukemia (Group 2), 18 patients had NF1, JXG, and leukemia (Group3), and 9 patients had JXG and leukemia (Group 4). Among the 78 patients with NF1 and JXG, 23% (18/78) developed leukemia, and more specifically, 19% (15/78) developed JMML, which is higher than what has been reported in NF1 patients alone. Majority of patients with the triple association were male (93%, 14/15) and had a family history of NF1 (67%, 8/12). Conclusions: Our findings suggest that the NF1 patients with JXGs have an increased risk of developing JMML and leukemia, especially in males with a family history of NF1. Although the triple association remains rare, closer surveillance and screening of male patients with familial NF1 and JXGs lesions may be reasonable, particularly at early ages.

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Glibenclamide ameliorates skin inflammation in a TRPM4 gain-of-function murine model of imiquimod-mediated psoriasiform dermatitis

D. Yamada[1], S. Vu[2], X. Wu1, Z. Shi[1], M. Huynh[1], J. Zheng[2], S. T. Hwang[1]

[1]Dermatology, University of California Davis, Sacramento, California, United States, [2]Physiology and Membrane Biology, University of California Davis, Davis, California, United States

A heterozygous (het) gain-of-function (GoF) mutation (I1033M or I1040T) of TRPM4, a calcium-activated nonselective monovalent cation channel, has been reported as the cause of progressive symmetric erythrokeratodermia. This genetic disease is characterized by red, scaly plaques in periorificial and acral regions that are reminiscent of human psoriasis. To determine whether the TRPM4 mutation alone is sufficient to lead to skin disease, we created a mouse (TRPM4I1029M, genetically equivalent to the human I1033M mutation) using CRISPR-Cas9 methods. Unperturbed homo and het TRPM4I1029M mice showed no apparent skin changes compared to wild type (WT) littermates. We compared the response of TRPM4I1029M and WT littermates to topical imiquimod (IMQ) application, a model of psoriasiform dermatitis (PsD). Het TRPM4I1029M showed enhanced skin inflammation with greater clinical severity scores (200% vs WT), thicker epidermis, more infiltration of CCR6+ γδ low T cells, higher expression of p-STAT3 protein, and higher mRNA levels of Il17a (1.5-fold) compared to WT mice. *In vivo* dendritic cell (DC) migration assay showed enhanced DC migration in het TRPM4I1029M. Cell cycle analysis showed that keratinocytes (KCs) from het TRPM4I1029M were in proliferative cell cycle status compared to KCs from WT mice. However, TRPM4I1029M did not show exaggerated responses in other *in vivo* models such as croton oil-induced irritant dermatitis and DNFB-induced contact dermatitis models. Glibenclamide, a classic diabetes drug, is a known TRPM4 inhibitor and ameliorated IMQ-PsD both in TRPM4I1029M and WT mice with milder clinical scores (50% vs vehicle), thinner epidermis, less infiltration of CCR6+ γδ low T cells, and lower mRNA levels of Il17a (30% vs vehicle). Our results suggest a possible therapeutic application of TRPM4 inhibitors in psoriasis.

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Lipidomic and proteomic analyses identify increased degradation of sphingolipids in prematurely senescent human dermal fibroblasts

M. Sochorova[1,2], M. Narzt[1], C. Kremslehner[1,2], I. Nagelreiter[1,2], G. Gendronneau[3,2], S. Forestier[3,2], F. Gruber[1,2]

[1]Department of Dermatology, Medizinische Universitat Wien, Wien, Wien, Austria, [2]Christian Doppler Laboratory for Skin Multimodal Analytical Imaging of Aging and Senescence, Vienna, Austria, [3]Chanel PB, Pantin, France

A wide range of environmental factors (e.g. UV radiation, pollution) challenges skin homeostasis and its prolonged imbalance accelerates skin aging. Aged skin is characterized by presence of senescent cells, which have a changed appearance and phenotype, accumulate damaged biomolecules and simultaneously produce a mixture of danger signaling molecules which negatively affect their microenvironment and activate the immune response. We previously described increased levels of lysophosphatidylcholines, in both replicative senescent and in in vitro stress-induced premature senescent (SIPS) fibroblasts. Global lipidomics with an UHPLC triple quadrupole MS/MS method confirmed the increased lysophospholipid content in SIPS fibroblasts. Moreover, a broader spectrum of analyzed lipid classes revealed that phospholipids are not the only senescence regulated lipids. In combination with the proteomic data analysis, upregulation of sphingolipid degradation pathway`s enzymes, e.g. β-galactosidase or acid ceramidase, were recognized, supporting the decreased amounts of sphingolipids found in SIPS cells` extracts. Predominant sphingolipids within the identified classes were lipids with long-chain (C16) and very-long chain (C24) acyl, which together represent 70-80% of sphingolipids in cultured fibroblasts. In the SIPS cells` sphingolipidome we can see a shift to long-chain acyls at the expense of very-long acyls, suggesting the elongation process is regulated. Recognition of structure and function of senescence regulated (epi-) lipidome contributes to a better understanding of the biological base of a senescent phenotype. It also will allow us targeting the consequences of lipid-mediated modulation of the immune system and therefore to help find approaches how to mitigate development of associated skin pathologies.

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Ground reaction forces(GRF) in patients with epidermolysis bullosa simplex(EBS) during walking

M. Hunjan[1], V. Devecchi[2], A. Bardhan[1], N. Harper[1], D. Falla[2], A. Hegearty[1]

[1]University Hospitals Birmingham NHS Foundation Trust, Birmingham, Birmingham, United Kingdom, [2]University of Birmingham School of Sport Exercise and Rehabilitation Sciences, Birmingham, Birmingham, United Kingdom

In EBS mutations in proteins encoding KRT5 & KRT14 result in abnormalities affecting the structural stability of basal keratinocytes. This causes blisters and keratoderma on the hands and feet which cause significant pain and difficulty walking. However, the impact on gait has not yet been studied. We aimed to study how people with EBS walk, and if they adopt any strategy to modify the distribution of forces under their feet. Adults with a diagnosis of EBS were asked to walk barefoot at a spontaneous speed. GRF produced by the contact of feet with the ground were collected by 2 force plates, and were decomposed into the anteroposterior (AP),mediolateral(ML) and vertical(V) components. Patients with EBS were matched by sex and age with a control group obtained from a published dataset of gait in asymptomatic adults1. Waveforms of the force components were compared between groups using statistical parametric mapping, allowing identification of which phases of gait differed between groups. 21 adults were present in each group(12 female,9 male). 14 EBS patients had KRT14 mutations and 7 had KRT5 mutations. Of these,11 patients had blisters during assessment. Mean age of EBS and control groups were 45.8 years & 45.0 years,respectively. A significant reduction of GRF were observed for the EBS group, specifically for the ML component during heel contact($p<0.01$) and AP component during both heel contact($p<0.001$)and push phase($p<0.001$). Patients with blisters showed lower AP force during the push phase compared with patients without blisters($p<0.05$).
These novel results show that people with EBS reduce shear forces under their feet whilst walking; likely a purposeful strategy that might be adopted to protect feet from blistering. This gait pattern could lead to negative consequences on gait stability and biomechanical loading of foot structures. Future research is needed to develop rehabilitation treatments and prevention strategies. 1.SchreiberC DOI:10.1038/s41597-019-0124-4

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Whole-transcriptome sequencing-based concomitant detection of viral and human genetic determinants of cutaneous lesions

A. Saeidian[1], L. Youssefian[1], C. Huang[1], F. Palizban[2], M. Naji[1], Z. Saffarian[3], H. Mahmoudi[3], A. Goodarzi[4], S. Sotoudeh[3], F. Vahidnezhad[5], M. Amani[6], A. Ajami[7], S. Mozafarpoor[7], M. Teimoorian[8], S. Dorgalaleh[8], S. Shokri[4], M. Shenagari[9], N. Abedi[2], S. Zeinali[10], P. Fortina[1], V. Béziat[11], E. Jouanguy[11], J. Casanova[11], J. Uitto[1], H. Vahidnezhad[1]

[1]Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [2]University of Tehran, Tehran, Tehran, Iran (the Islamic Republic of), [3]Tehran University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [4]Iran University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [5]University of California, Santa Cruz, California, United States, [6]Gonabad University of Medical Sciences, Gonabad, Iran (the Islamic Republic of), [7]Isfahan University of Medical Sciences, Isfahan, Isfahan, Iran (the Islamic Republic of), [8]Golestan University of Medical Sciences and Health Services, Gorgan, Golestan, Iran (the Islamic Republic of), [9]Guilan University of Medical Sciences, Rasht, Gilan, Iran (the Islamic Republic of), [10]Kawsar Human Genetics Research Center, Tehran, Iran (the Islamic Republic of), [11]The Rockefeller University, New York, New York, United States

Severe viral infections of the skin can occur in patients with inborn errors of immunity (IEI). We report an all-in-one whole-transcriptome sequencing-based method by RNA-Seq on a single skin biopsy for concomitant identification of the cutaneous virome and underlying IEI. Skin biopsies were obtained from normal and lesional skin from patients with cutaneous infections suspected to be of viral origin. RNA-Seq was utilized as the first-tier strategy for unbiased human genome-wide rare variant detection. Reads unaligned to the human genome were utilized for the exploration of 926 different viruses in a viral genome catalog. In nine families studied, the patients carried pathogenic variants in six human IEI genes, including IL2RG, WAS, CIB1, STK4, GATA2, and DOCK8. Gene expression profiling also confirmed pathogenicity of the human variants and permitted genome-wide homozygosity mapping which assisted in identification of candidate genes in consanguineous families. This automated, all-in-one computational pipeline, called VirPy, enables simultaneous detection of the viral triggers and the human genetic variants underlying skin lesions in patients with suspicion of IEI and viral dermatosis.

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Splice modulation as a therapeutic strategy applied to deep intronic mutations in COL7A1 causing recessive dystrophic epidermolysis bullosa

M. Titeux[1,2], N. Pironon[1,2], C. Prost[3,4,5], M. Chen[6], D. Woodley[6], E. Bourrat[4,7], A. Hovnanian[1,2,8]

[1]Laboratory of genetic skin diseases, Institut Imagine Institut des Maladies Genetiques, Paris, Île-de-France, France, [2]Universite de Paris, Paris, Île-de-France, France, [3]Department of Dermatology, Hopital Avicenne, Bobigny, France, [4]Assistance Publique - Hopitaux de Paris, Paris, Île-de-France, France, [5]Universite Sorbonne Paris Nord, Villetaneuse, Île-de-France, France, [6]Department of Dermatology, University of Southern California Keck School of Medicine, Los Angeles, California, United States, [7]Department of Dermatology, Hopital Saint-Louis, Paris, Île-de-France, France, [8]Department of Genetics, Hopital universitaire Necker-Enfants malades, Paris, Île-de-France, France

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare autosomal inherited skin disorder caused by mutations in COL7A1 encoding type VII collagen (C7), the major component of anchoring fibrils. In this study, we report two cases of RDEB patients with new pathogenic deep-intronic point mutations in COL7A1. Patient 1 is a 45-year-old man with RDEB inversa from unrelated healthy parents, for whom sequencing of COL7A1 from genomic DNA and cDNA extracted from skin biopsies revealed two recessive loss-of-function mutations. A maternal nonsense mutation and a deep-intronic paternal mutation which disrupts COL7A1 pre-mRNA splicing and leads to the inclusion of an out-of-frame 96bp pseudo-exon resulting in a PTC. This mutation is the deepest COL7A1 intronic mutation reported so far and the first leading to the inclusion of a pseudo-exon in RDEB. Patient 2 is a 32-year-old woman with RDEB pruriginosa from consanguineous healthy parents, for whom NGS revealed that the proband was a compound heterozygote for a paternal nonsense mutation and a maternal deep-intronic mutation causing partial or complete intron retention. Splice modulation using antisense oligonucleotides targeting intronic mutations identified in patients 1 and 2 restored the synthesis of wild-type COL7A1 mRNA and C7 protein up to 50% of the normal level. Splice modulation applied to counteract deep intronic mutations in COL7A1 represents a promising therapeutic strategy for personalized medicine directed at individual patients with private mutation.

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Recalcitrant cutaneous warts in a family with inherited ICOS deficiency

L. Youssefian[1], A. Saeidian[1], A. Tavasoli[1], E. Kalamati[1], K. Naghipoor[2], A. Hojabrpour[3], M. Mesdaghi[4], Z. Saffarian[5], H. Mahmoudi[5], M. Nabavi[3], S. Shokri[3], S. Zeinali[6], V. Béziat[7], J. Casanova[7], E. Jouanguy[7], J. Uitto[1], H. Vahidnezhad[1]

[1]Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [2]Golestan University of Medical Sciences and Health Services, Gorgan, Golestan, Iran (the Islamic Republic of), [3]Iran University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [4]Shahid Beheshti University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [5]Tehran University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [6]Pasteur Institute of Iran, Tehran, Tehran, Iran (the Islamic Republic of), [7]The Rockefeller University, New York, New York, United States

Recalcitrant warts (RW), caused by human papillomaviruses (HPVs), can be a cutaneous manifestation of inborn error of immunity. This study investigated the clinical manifestations, immunodeficiency, single-gene susceptibility, and HPV repertoire in a consanguineous family with severe sinopulmonary infections and RWs. Clinical and immunological evaluations, including fluorescence-activated cell-sorting and lymphocyte-transformation test, provided evidence for immunodeficiency. Combined whole-exome sequencing and genome-wide homozygosity mapping was utilized to disclose candidate sequence variants. Whole-transcriptome sequencing (RNA-Seq) was used to concomitantly investigate the HPV genotypes and the consequences of detected sequence variants in the host. The proband, a 41-year-old male, was found to be homozygous for the c.6delG, p.Lys2Asnfs*17 variant in ICOS, encoding the inducible T cell co-stimulator. This variant was located inside the 5 Mb of runs of homozygosity on 2q33.2. RNA-Seq confirmed the deleteriousness of the ICOS variant in three skin biopsies revealing significant downregulation of ICOS and its ligand, ICOSLG. Reads unaligned to the human genome were applied to 926 different viruses, and α-HPV57, β-HPV107, β-HPV14 and β-HPV17 were detected. Collectively, we describe a previously unrecognized inborn error of T cell immunity to HPVs, indicating that autosomal recessive ICOS deficiency can underlie RWs, emphasizing the immunological underpinnings of RWs at the nexus of human and viral genomic variation.

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Bi-layered skin construct with gene modified keratinocytes reduces UVR DNA damage

N. Mahajan[1], A. Gorkun[1, 2, 3], M. Wu[1], K. Willson[1], A. M. Jorgensen[1], S. Soker[1], A. Atala[1]

[1]WFIRM, Wake Forest Institute for Regenerative Medicine, Winston-Salem, North Carolina, United States, [2]Pathology and Pathophysiology, FGBNU Naucno-issledovatel'skij institut obsej patologii i patofiziologii, Moskva, Moskva, Russian Federation, [3]Institute for Regenerative Medicine, Pervyj Moskovskij gosudarstvennyj medicinskij universitet imeni I M Secenova, Moskva, Moskva, Russian Federation

Ultraviolet radiation (UVR) is among major contributors to skin cancer in humans. Production of melanin serves as a main defense mechanisms against UVR damage. This pigment, which gives color to hair, skin and eyes, protects the underlying tissue from UVR damage. However, in some patient populations, this protection mechanism is not effective, leaving patients highly susceptible to cancer. Similarly, mycosporine like amino acids (MAAs), which are commonly expressed in marine algae, have been shown to serve a protective role in UVR-induced DNA damage. Cyanobacterium Cylindrospermum stagnale has a gene cluster of five genes (mylA-mylE) for MAA biosynthesis. The purpose of this study is to determine if human cells engineered to express MAA in situ will provide protection from UVR. MylA-mylE (MAA) was cloned into a lentivirus vector and used to transduce human dermal keratinocytes. Subsequently, bi-layered skin constructs with MAA-expressing, recombinant keratinocytes and fibroblasts were fabricated in fibrinogen hydrogel. The constructs were exposed to UVB radiation over four days, and then maintained for 11 days in culture media. Live/dead staining demonstrated higher cell viability in the bi-layered skin construct with recombinant cells compared to normal cell controls. There was also reduced TUNEL activity, active caspase 3, double strand breaks, and γH2AX levels in these constructs, compared to normal cell controls. Taken together, this study demonstrates that human keratinocytes producing MAA in situ have increased UVR protection with an associated reduction in DNA damage and cellular death. This technology may be utilized as a preferred method for generation of innate UVR protection in human patients with high susceptibility to skin cancer.

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Chronic mucocutaneous candidiasis due to Candida auris and non-albicans Candida species in a family with a mild TP63-associated ectodermal dysplasia

J. Park[1, 6], L. Youssefian[1], S. Khodavaisy[2], F. Palizban[3], A. Tavasoli[1, 2], E. Kalamati[4], H. Mahmoudi[2], K. Balighi[2], M. Mesdaghi[5], A. Saeidian[1], J. Uitto[1], H. Vahidnezhad[1]

[1]Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [2]Tehran University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [3]University of Tehran, Tehran, Tehran, Iran (the Islamic Republic of), [4]Mashhad University of Medical Sciences, Mashhad, Razavi Khorasan, Iran (the Islamic Republic of), [5]Shahid Beheshti University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [6]Geisinger Commonwealth School of Medicine, Scranton, Pennsylvania, United States

Tumor protein 63, encoded by TP63, has an essential role in epidermal differentiation and ectodermal development. Autosomal dominant mutations in human TP63 result in several overlapping syndromes, with some established genotype-phenotype correlations based on the protein domain affected. Several groups have linked TP63-associated ectodermal dysplasia (ED) syndromes with varying degrees of immune dysfunction. In this study, we followed the clinical course of a daughter and father who presented with mild ED symptoms, chronic mucocutaneous candidiasis (CMC), recurrent bacterial infections, and equivocal immune function status. Whole-exome sequencing (WES) and whole-transcriptome analysis via RNA sequencing (RNA-Seq) disclosed a recurrent monoallelic mutation in TP63. Additionally, RNA-Seq data was used for detection of non-albicans Candida species, Candida auris and Candida parapsilosis, in CMC lesions of the daughter and father, respectively. Although a strong association between ED and TP63 mutations exists, the mechanism of immune dysregulation remains unclear. Our cases attest to the variable expressivity of TP63-associated disorders and the potential role of TP63 in maintaining immune system integrity. Furthermore, this study represents the first case of CMC caused by the multidrug-resistant pathogen C. auris in the context of TP63-associated ED and demonstrates the utility of RNA-Seq for concomitant mutation detection and accurate identification of rare and often misidentified pathogens.

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Epidermolysis bullosa pruriginosa, muscular dystrophy, and immune-mediated myasthenia gravis in a patient with homozygous nonsense PLEC mutation

A. Tavasoli[1], H. Vahidnezhad[1], L. Youssefian[1], A. Saeidian[1], M. Rouhani[2], Y. Nilipour[4], H. Mahmoudi[3], J. Uitto[1]

[1]Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [2]Iran University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [3]Tehran University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [4]Shahid Beheshti University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of)

A 26-year-old female presented with epidermolysis bullosa prurogompsa (EBP) since infancy, hair loss, proximal motor weakness, nasal tone speech, swallowing problem, and droopy eyes at age 20. Upon neurological examination, normal cognition, inability to walk unassisted, bilateral ptosis with full range of extraocular movements, weak gag reflex, generalized muscle atrophy, and decreased deep tendon reflexes were detected. Her electromyography revealed small polyphasic muscle unit action potentials, minimal insertional activities, myopathic changes, motor and sensory increased latency, decreased amplitude, reduced velocity, and normal F wave. These findings were compatible with myasthenia gravis (MG) and muscular dystrophy. Acetylcholine receptor antibody test revealed a titer of 2.36 nmol/L (normal range: ≤ 0.4 nmol/L). Chest CT-Scan revealed no thymoma or thymus enlargement. The titer of serum creatine phosphokinase was 422 IU/L which was higher than normal. Whole-exome sequencing disclosed a homozygous PLEC variant (NM_000445.4: c.5533C>T; p.Gln1845*). Besides, muscle biopsy and staining with Hematoxylin and Eosin, modified Gomori Trichrome, Cytochrome c oxidase + Succinate dehydrogenase, Nicotinamide adenine dinucleotide, and dehydrogenase-tetrazolium reductase showed chronic atrophic changes, necrosis/ regeneration, increased internalization of nuclei, and severe endomysial fibrosis. These pathological findings were compatible with muscular dystrophy. She was treated with a stepwise incremental dose of steroid up to 30 mg/day for 6 weeks which resulted in significant improvement of her swallowing and skin lesions in addition to ptosis. Her MG composite scale was decreased from 22 to 12. A combination of muscular dystrophy and immune-based MG and EBP are novel findings in this case.

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Mutations in different domains of bullous pemphigoid antigen-1 (BPGA1) encoded by DST result in either epidermolysis bullosa simplex or musculoskeletal and neuronal deformities-associated HSAN-VI

N. Harvey[1, 2], R. Khalesi[3], M. Garshasbi[5], E. Kalamati[4], L. Youssefian[1], H. Vahidnezhad[1], J. Uitto[1]

[1]Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [2]Philadelphia College of Osteopathic Medicine, Philadelphia, Pennsylvania, United States, [3]DeNA Laboratory, Tehran, Iran (the Islamic Republic of), [4]Mashhad University of Medical Sciences, Mashhad, Razavi Khorasan, Iran (the Islamic Republic of), [5]Tarbiat Modares University, Tehran, Tehran, Iran (the Islamic Republic of)

DST encodes bullous pemphigoid antigen-1 (BPAG1), a protein with eight tissue-specific isoforms expressed in the skin, muscle, brain, and nerves. Accordingly, mutations in this gene cause different phenotypes, including epidermolysis bullosa simplex (EBS) and hereditary sensory and autonomic neuropathy type VI (HSAN-VI). The genotype-phenotype correlation is attested to by 19 distinct mutations but not well established for both disorders. In this study, we performed next-generation sequencing (NGS) on two families with different phenotypic presentations, one fetus (P1) with musculoskeletal and neurological malformations established by prenatal ultrasound and family history, and a 15-year-old female (P2) with skin blistering. P1 had a novel homozygous nonsense mutation, DST: NM_001144769, c.3805C>T, p.R1269* within a region of homozygosity (ROH). This mutation, within the plakin domain, ablates all BPGA1 isoforms leading to novel extracutaneous phenotypes in P1. P2 has a recurrent homozygous mutation DST: NM_001723.7, c.3370C>T, p.Gln1124* that presented with giant, trauma-induced skin blisters without extracutaneous involvement. This mutation is located within the coiled-coil domain present on the skin isoform of DST, BPGA1-e, associated with EBS. In summary, we report two Iranian families with DST variants, expanding the genotypic and phenotypic spectrum of DST.

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Differential chromatin accessibility and gene expression suggest Th17 polarization in activated and skin-homing T cells

Z. Zhang, Y. Zhao, L. C. Tsoi, R. Nair, P. Stuart, X. Wen, J. Elder

University of Michigan, Ann Arbor, Michigan, United States

We generated 1,090 ATAC-seq and 1,057 T-cell RNA-seq libraries from 153 subjects, derived from 8 flow-sorted T-cell subsets (defined by CD4/CD8, CLA+/ CLA-, and 0/24h CD3/CD28 stimulation). Effects of activation and skin-homing were analyzed by DESeq2. After peak calling, 78,234 consensus peaks were present in ≥ 30 ATAC-seq libraries. A Wald test examining simple main effects identified 9,072, 3,934, and 21,174 consensus peaks as differentially accessible regions (DAR; FDR < 0.05, |log2 FC|≥ 0.585) in CD4 vs CD8, CLA+ vs CLA-, and resting vs. activated T-cells, respectively. For functional annotation, DARs were assigned to the closest genes using ChIPseeker. CD4/CD8 DARs were most significantly enriched for the KEGG pathway "Th17 cell differentiation" (FDR=2.4e-07). CLA+/CLA- DARs were most enriched for "MAPK signaling pathway" (FDR=1.5e-06) and included "Th17 cell differentiation" (FDR=4.1e-04). Activation-responsive (0/24h) DARs were most enriched for "T cell receptor signaling pathway" (FDR = 1.0e-07) and included "Th17 cell differentiation" (FDR=2.3e-06). We used the same criteria to identify differentially expressed genes (DEGs) from the RNA-seq libraries, yielding 2,795, 3,629, and 10,673 genes for CD8/CD4, CLA+/CLA-, and 0/24h, respectively. CD4/CD8 DEGs revealed top KEGG enrichment for "Cytokine-cytokine receptor interaction (CCRI)" (FDR = 8.3e-19) and included "Th17 cell differentiation" (FDR = 7.8e-03), with up-regulation of IL17A (3.2-fold), IL17F (1.8-fold), and IL22 (2.9-fold) in CD4. CLA+/CLA- DEGs also revealed top enrichment for "CCRI" (FDR=2.4e-18), with up-regulation of IL17A (3.3-fold), IL17F (2.2-fold), and IL22 (1.9-fold) in CLA+. 0/24h DEGs were also enriched for "CCRI" (FDR = 2.1e-04), with dramatic up-regulation of IL17A (109-fold), IL17F (1052-fold), and IL22 (146-fold) at 24 h. IL17A, IL17F, and IL22 were among the 479 DAR/DEG pairs identified by both the CD4/CD8 and 0/24h comparisons, and IL23R was 1 of 66 DAR/DEG pairs identified by all 3 comparisons. These results support a link between skin-homing and Th17 polarization.

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Genetic variability of viral and human genomes in a large cohort of patients with typical epidermodysplasia verruciformis

A. Saeidian[1], L. Youssefian[1], H. Mahmoudi[2], P. Mansouri[2], V. Béziat[3], J. Casanova[3], E. Jouanguy[3], J. Uitto[1], H. Vahidnezhad[1]

[1]Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [2]Tehran University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [3]The Rockefeller University, New York, New York, United States

Cutaneous human papillomavirus (HPV) infection typically manifests with isolated warts. However, some patients in familial clustering develop extensive and protracted HPV infections, primarily the β-HPV types 5 and 8, with distinct cutaneous findings. This clinical entity, epidermodysplasia verruciformis (EV), with autosomal recessive inheritance, is characterized by numerous cutaneous flat warts in childhood, which progress into squamous cell carcinomas later in life. The "typical" form of EV, not vulnerable to other infections, is caused by mutations in CIB1, TMC6, or TMC8, which impair keratinocyte-intrinsic immunity to β-HPV infection. Mutations in other genes related to T-cell development or function, have been associated with "atypical" EV in patients with other infections. We developed a whole-transcriptome sequencing-based method on RNA isolated from skin biopsies for concomitant detection of viral and human genetic determinants of cutaneous wart lesions in a cohort of 50 EV patients. This method, VirPy, can detect 926 viruses, including more than 400 HPVs, and the corresponding human mutations. Nine distinct mutations in TMC8 (n=2), TMC6 (n= 5) and CIB1 (n=2) in 12 distinct families, including 14 patients were detected. The most predominant HPV in this cohort was HPV14. In addition, the RNA-seq data were examined for variant detection and prioritization, pathogenicity confirmation, and RNA expression profiling. Besides, we identified a total of 20 different HPVs including 16 β-, three α- and one γ-HPV(HPV128) in a patient with TMC8 mutation. In summary, the utilization of RNA-Seq as a first-tier diagnostic method allowed us to simultaneously profile the transcriptome of host for mutation detection and exploring the consequences of variants of unknown significance as well as to profile the cutaneous virome of EV patients.

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Artesunate inhibits RDEB fibrosis by downregulating AKT signaling pathway

D. Woodley, D. Polyakov, B. A. Levian, Y. Hou, X. Tang, M. Chen

University of Southern California, Los Angeles, California, United States

Patients with recessive dystrophic epidermolysis bullosa (RDEB) develop multiple skin wounds that heal with extensive scarring, contractures, and mitten deformities. RDEB patients display increased pro-fibrotic TGF-β signaling, a distinct pro-fibrotic gene expression profile, and elevated inflammation genes. No specific pharmacological treatment is currently available for RDEB fibrosis. Artemisinin and its derivatives are anti-malarial medications that also exhibit both anti-fibrotic and anti-inflammatory activity in animal and cell models. In this study, we evaluated the ability of artesunate (ART), a derivative of artemisinin, to inhibit RDEB-related fibrosis using fibroblasts isolated from RDEB patients. RDEB fibroblasts treated with ART demonstrated reduced expression of multiple fibrosis markers [collagen I (C1), fibronectin (FIN), connective tissue growth factor (CTGF), alpha smooth muscle actin (α-SMA), periostin, and tenascin C] by immunoblot analysis. RT-PCR analysis of ART-treated fibroblasts also showed reduced mRNA levels for fibrosis genes [tenascin-C, CTGF, periostin, TGF-β2, and α-SMA], as well as IL-6, an inflammation gene known to stimulate fibrosis. In addition, ART reduced the levels of pro-fibrogenic TGF-β in the media of RDEB fibroblasts as assessed by ELISA. When compared to normal fibroblasts in an in vitro collagen lattice assay, untreated RDEB fibroblasts exhibited enhanced contraction activity. ART, however, reversed RDEB fibroblast hyper-contractability. Lastly, RDEB fibroblasts exhibited increased expression of pro-fibrogenic TGF-β and relevant fibrotic markers via upregulation of the PI3K/AKT intracellular signaling pathway. ART treatment of RDEB fibroblasts decreased p-AKT and abolished this activation. These data demonstrate that ART may be a non-invasive, readily available, safe, and novel therapy for reducing RDEB fibrosis and scarring and improving the quality of life of RDEB patients.

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A novel preclinical model for translating an induced pluripotent stem cell therapy for the treatment of skin diseases

M. Pavlova[2], J. C. Flores[2], S. Vieau[2], V. Balaiya[2], P. S. McGrath[3], K. A. Bush[1], A. Hopkin[1], A. Bruckner[2], I. Kogut[2], D. Roop[2], G. Bilousova[2]
[1]Avita Medical Americas LLC, Valencia, California, United States, [2]Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado - Anschutz Medical Campus, Aurora, Colorado, United States, [3]Department of Pediatrics, University of Colorado - Anschutz Medical Campus, Aurora, Colorado, United States

Induced pluripotent stem cells (iPSCs) hold promise for treating recessive dystrophic epidermolysis bullosa (RDEB). An iPSC-based therapy for RDEB involves multiple steps such as reprogramming, gene correction, iPSC differentiation, and transplantation of genetically corrected iPSC-derived skin cells onto patients. Strong evidence supports the use of epidermal sheets or composite skin grafts as final therapeutic products for the transplantation of genetically corrected iPSC-derived skin cells. However, the generation of epidermal sheets or composite grafts is a lengthy and consequently expensive process. To develop a more straightforward and cost effective skin transplantation approach, we established a novel preclinical model for grafting a human skin cell suspension onto immunocompromised mice that requires low starting cell numbers. The model involves the use of a silicone chamber and sequential transplantation of fibroblasts followed by keratinocytes. Both fibroblasts and keratinocytes are delivered in suspension in a fibrin-based gel formulation. Using this modified assay, we have successfully engrafted as low as 5 x 105 keratinocytes per 1.4 cm2 of wound area. We have also successfully engrafted iPSC-derived keratinocytes. In all cases, the grafted cells formed a multilayered stratified human epidermis. We are currently investigating strategies to deliver genetically corrected RDEB iPSC-derived skin cells, including the cell harvesting and application techniques currently used for Spray-On-Skin™ cells developed by AVITA Medical. This approach will decrease the time to patient application vs. the time and cost it takes to grow epidermal sheets and will potentially simplify iPSC therapies for RDEB and other skin diseases.

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Mendelian Susceptibility to Mycobacterial Diseases (MSMD) with autosomal dominant Interferon Gama receptor one defect: A clinical diagnostic and treatment challenge for a 13 year old ethiopian girl

N. A. Gebeyehu[3], S. J. Deribessa[1], A. Freeman[2], M. T. Demissie[3], A. M. Gebremariam[5], D. M. Engliz[4], M. W. Gebre[3], T. Y. Kidane[3]
[1]pediatrics and child health, St.Paul's Hospital Millennium Medical College, Addis Ababa, Addis Ababa, Ethiopia, [2]National Institutes of Health, Bethesda, Maryland, United States, [3]Dermatovenerology, Addis Ababa University College of Health Sciences, Addis Ababa, Addis Ababa, Ethiopia, [4]Debre Birhan Specialized Hospital, Debre birhan, Ethiopia, [5]Addis Ababa University College of Health Sciences, Addis Ababa, Addis Ababa, Ethiopia

A 13 year old female patient had multiple hospital visits since age of 6months. The most striking diagnosis was repeated mycobacterial infections; which were diagnosed at times clinically and at other times with bacteriological confirmation. She had tuberculosis affecting lymph nodes, skin and soft tissue, bone and joints, lungs and epidural and para-spinal regions. She has been treated 4 times with first line, ones with second line anti-Tb drugs and now she still taking anti-TB. She has taken all childhood vaccines, including BCG. Autosomal dominant (MedGen UID: 863300) Mendelian susceptibility to mycobacterial disease due to IFNG-R1 defect. To best of our knowledge this is the first case report of Mendelian Susceptibility to Mycobacterial Diseases secondary to IFNG-R defect from Ethiopia. Although it has been immensely challenging, our multidisciplinary team has learned a lot from the clinical presentation, diagnosis and management of this child and we are presenting this case for other clinicians to learn out of it.

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Meta-analyses of genome-wide association studies in multiethnic cohorts identify risk loci associated with hidradenitis suppurativa

H. Choquet[1], J. Yin[1], Y. Kim[2], T. Hoffmann[3], S. Saini[4], S. Shringarpure[4], 2. Team[4], E. Jorgenson[5], M. M. Asgari[2]
[1]Division of Research, Kaiser Permanente Northern California, Oakland, California, United States, [2]Department of Dermatology, Massachusetts General Hospital, Boston, Maryland, United States, [3]Institute for Human Genetics, University of California San Francisco, UCSF, San Francisco, California, United States, [4]23andMe, Sunnyvale, California, United States, 5Regeneron Genetics Center, Tarrytown, New York, United States

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease with a strong hereditary component yet its genetic architecture is poorly defined. We examined genetic risk by performing large-scale genome-wide association studies (GWAS) combining the Genetic Epidemiology Research on Adult Health and Aging, the Mass General Brigham Biobank, and the UK Biobank cohorts. We conducted two ethnic-specific GWAS meta-analyses of HS, including 561 HS cases and 571,189 controls from European ancestry and 81 HS cases and 13,832 controls from African ancestry, respectively. We replicated our findings in an independent cohort, the 23andMe Research cohort, consisting of 16,615 HS cases and 2,814,119 controls from European ancestry and 1,292 HS cases and 165,237 controls from African ancestry. We identified three genome-wide significant HS-associated loci (P<5x10-8) in European ancestry individuals, including LINC01899 long non-coding RNA (lncRNA) at 18q22, and four HS-associated loci in African ancestry individuals, including LINC01592 lncRNA at 8q13. Identified loci are near genes related to the vitamin B12 transport and metabolism (LMBRD1), skin or sweat glands development (FOXI2), repetitive self-grooming behavior in mice (CBLN2), immune or inflammatory conditions (RNF19B and AK2) or epithelial-mesenchymal transition (LINC01592). Interrogation of loci uncovered by our genetic investigations using fine-mapping and functional annotations integrative tools also prioritized variants, genes, and pathways underlying HS. Our findings improve the understanding of the genetic risk factors for HS.

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The genomic and phenotypic landscape of ichthyosis: An analysis of 1000 kindreds

Q. Sun[6,1], N. Marukian[6], S. Cheraghlou[2], A. Paller[3], M. Larralde[4], L. Bercovitch[5], J. Levinsohn[6], I. Ren[7], R. Hu[6], J. Zhou[6], T. Zaki[6], R. Fan[6], C. Tian[6], C. Saraceni[6], C. Nelson-Williams[6], E. Loring[6], B. Craiglow[6], L. Milstone[6], R. Lifton[6], L. Boyden[6], K. Choate[6]
[1]Brigham and Women's Hospital Department of Medicine, Boston, Massachusetts, United States, [2]Dermatology, New York University Grossman School of Medicine, New York, New York, United States, [3]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [4]Dermatology, Hospital Aleman, Buenos Aires, Argentina, [5]Dermatology, Brown University Warren Alpert Medical School, Providence, Rhode Island, United States, [6]Dermatology, Yale School of Medicine, New Haven, Connecticut, United States, [7]Icahn School of Medicine at Mount Sinai, New York, New York, United States

Objective: Clear genotype-phenotype associations in ichthyosis have been difficult to establish. We aimed to expand the genotypic and phenotypic spectra of ichthyosis and delineate genotype-phenotype associations. Design: We recruited an international group of individuals with ichthyosis, including participants of all ages, races, and ethnicities. Genetic analysis of saliva or blood, a phenotyping questionnaire, and standardized clinical photographs were obtained. Results: Among 1000 unrelated individuals, 266 novel variants in 32 genes were identified. Pruritus, hypohydrosis, skin pain, eye problems, skin odor, and skin infections were the most prevalent self-reported features. Collodion membrane at birth (OR 6.7; P < .001), skin odor (OR, 2.8; P = .02), hearing problems (OR, 2.9; P < .001), eye problems (OR, 3.0; P < .001), and alopecia (OR, 4.6; P < .001) were significantly associated with TGM1 mutations compared with other ichthyosis genotypes. Skin pain (OR, 6.8; P = .002), odor (OR, 5.7; P < .001), and infections (OR, 3.1; P = .03) were significantly associated with KRT10 mutations. Novel phenotypes such as EKVP caused by ABCA12 mutations were identified. Conclusions: We expanded the genotypic and phenotypic spectra of ichthyosis, establishing associations between clinical manifestations and genotypes. The findings may improve clinical assessment and assist with developing customized management plans.

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Skin multi-omics data analysis reveals in the impact of life stress on skin

B. Li[1, 2], S. Tian[2], L. Kolbe[3], Y. Zou[1], S. Wang[2]
[1]Department of Skin and Cosmetics Research, Shanghai Skin Disease Hospital, School of Medicine, Tongji University, Shanghai, China, Shanghai, China, [2]CAS Key Laboratory of Computational Biology, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China, Shanghai, China, [3]Beiersdorf AG, Hamburg, Germany, Hamburg, Germany

Previous studies showed that life stress negatively impacts skin, but the relevant mechanism is poorly understood. Here, we recruited 60 stressed and 60 relaxed people (19-29 years old) to study how stress affects the skin. Each volunteer completed a 31-items life stress questionnaire, 24-items skin questionnaire, 14-items skin trait testing, and provided a sample of suction blister fluid for quantifying 36 skin cytokines, and roofs for DNA methylome and transcriptome analysis. We found that the stressed group had more severe skin dullness than the relaxed group (P=7.36×10-6). There was no significant difference in skin cytokines between the two groups, while the stressed group had an altered skin methylome and transcriptome profile. We detected 289 differentially methylated probes (DMPs) and 10 differential expression genes. Integration of methylation and expression data revealed 7 functional epigenetic modules (P<0.05), which were involved in the glutamatergic synapse, axon guidance pathway, etc. In line with previous studies on plasma/salivary, we found that life stress also affects the glutamatergic synapse in the skin. Interestingly, the expression level of SERPINA1 was significantly associated with a DMP (r=-0.29, p=0.004, in TSS1500 of SERPINA1), and with skin dullness (β=1.88, P=0.03). SERPINA1 reportedly influences the morning plasma/salivary cortisol, a life stress marker. This study provided important insight into the impact of life stress on skin, and shed light on the relevant molecular mechanism.

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“Chimeric sorting” as an effective tool for selection of genetically modified dermal fibroblasts to be used in fundamental or applied skin research.

I. Vorobyova, E. V. Sytina, E. V. Solovieva, A. A. Panteleyev
NRC Kurchatov Institute, Moscow, Russian Federation

A technology has been developed for efficient isolation of a stable pool of HIF1a overexpressing mouse fibroblasts using a high-throughput selection of cell clones on a cellulose carrier. The pHIF1a-CBD-PDGFR genetic construct has been generated containing a EYFP reporter, a target sequence of HIF1a gene (a key factor of adaptation to hypoxia in wound healing and regeneration) and a CBD/PDGFR chimeric gene construct. The presence of CBD (cellulose-binding domain) / PDGFR (platelet-derived growth factor receptor) chimeric construct in protein-producing cells enables their specific binding to cellulose carrier thus providing a possibility for cell selection based on their adhesive phenotype (“chimeric sorting”). For genetic modification, a line of immortalized postnatal mouse dermal fibroblasts was used (dp3T3cell line). This cell line has been developed using 3T3 protocol and combines the basic features of primary dermal fibroblasts with the experimental advantages of immortal cells. The high-throughput selection of HIF1a overexpressing cells was performed using BD FACSAria™ Fusion Cell Sorter (with EYFP as a reporter) or by chimeric sorting on day 5th after dp3T3 cell transfection (electroporation) using half of transfected cells for each approach (0.9±0.2x10^6). The CBD-PDGFR driven chimeric sorting was performed on cellulose filter papers, 75g/cm2. In 3 weeks chimeric sorting allowed to obtain 3±1x10^6 stably transfected cells with changed morphology (less stellate and more elongate), while FACS sorting yielded 8 colonies with 0.6±0.3x10^5 cells each with only 3 colonies exhibiting modified phenotype. Thus, “chimeric sorting” of transfected fibroblasts on a cellulose carrier appeared to be more effective in selection of HIF1a overexpressing cells as compared to conventional FACS. The advantageous efficiency of this approach was reflected in both its specificity (low level of contamination with cells of original phenotype) and its productivity (higher yield of stably transfected cells).

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Expanded GWAS meta-analysis offers novel insights into psoriasis biology

N. Dand[1], L. C. Tsoi[2], J. Barker[1], M. A. Simpson[1], J. Elder[2], International Psoriasis GWAS Consortium[1, 2]
[1]King’s College London, London, United Kingdom, [2]University of Michigan, Ann Arbor, Michigan, United States

Genome-wide association studies (GWAS) play an important role in our understanding of psoriasis biology. We report an international effort (UK, USA, Canada, Germany, Norway, Estonia) to increase the power of psoriasis GWAS by meta-analysis of 18 case-control European-ancestry GWAS datasets (36,466 cases, 458,078 controls; cumulative effective sample size: 103,614). After stringent quality control, each dataset underwent genome-wide imputation. GWAS analyses were performed locally at collaborating centers, with standard-error weighted meta-analysis undertaken centrally. We identified 360 variants independently associated with psoriasis susceptibility at genome-wide significance (P<5×10-8), 223 outside the MHC. Merging variants <1Mb apart revealed 108 associated non-MHC genomic regions. Sixty regions map to psoriasis susceptibility loci reported previously in European populations. Thirty of these (excluding MHC) include multiple independent association signals; a few, notably at IL12B and TYK2, include many such signals. We report 49 newly associated psoriasis susceptibility regions, six with multiple independent signals. Most strongly associated were chr1lp12 within PRR5L and near TRAF6 (OR=1.25, P=5.6×10-20), chr22q12.3 near NCF4 (OR=1.11, P=1.6×10-15), and chr1p36.22 within MFN2 (OR=1.09, P=1.6×10-15). Bioinformatic analysis suggests the susceptibility loci are enriched for a range of immune functions (DEPICT), with the 49 new loci linked most strongly with B-cell proliferation (P=1.2×10-9) followed by related traits pertaining to lymphocyte regulation. Enriched cell types prominently feature NK cells and pDCs in addition to CD4+ and CD8+ T-cells (SNPsea). We undertook LD-based fine-mapping to reveal candidate causal variants, both protein-altering and putative eQTL/pQTLs (PhenoScanner); candidate genes in new loci include CPVL and IL7R (with immune roles) and POU2F3 (in skin). These results offer new insights into the genetic basis of psoriasis and will support future functional research and therapeutic target development.

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Epidermal epitranscriptomics: METTL3 dependent m6A maintains the epidermal stem cell state through regulation of chromatin-modifying enzymes

A. M. Maldonado López[1, 2], S. Huang[2], A. Anderson[2], B. C. Capell[1, 2, 3]
[1]Genetics, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [3]Penn Epigenetics Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States

The balance between epidermal stemness and differentiation requires regulated spatiotemporal changes regulation of gene expression. One emerging area of gene regulation is that of epitranscriptomics (regulated RNA modifications), which offers an additional layer of gene regulation in a spatiotemporal- and signal-dependent manner. However, its significance in healthy and diseased epidermis is poorly understood. N6-methyladenosine (m6A) is the most abundant internal mRNA modification in eukaryotes and is found to facilitate rapid transcriptome turnover during cell differentiation to maintain homeostasis. Its deposition on nascent pre-mRNA is carried out by a multicomponent writer complex that consists of catalytic subunit METTL3. To understand the role of METTL3 in the epidermis, we created mice with an epidermis-specific knockout of Mettl3 (Krt14-Cre; Mettl3 fl/fl). These mice displayed a dramatic epithelial phenotype marked by the absence of hair, altered epidermal differentiation dynamics, as well as an oral epithelium that was notable for a lack of filiform papillae and oral ulcerations. Consistent with these changes, transcriptional profiling by RNA-seq demonstrated a loss of expression of epidermal stem cell and basement membrane genes along with concomitant upregulation of keratinocyte differentiation genes. Mechanistically, our results suggest that m6A exerts these effects through its ability to directly regulate the expression of various chromatin-modifying enzymes. Collectively, these results demonstrate the broad regulatory role that the epitranscriptome can play in epidermal biology and underscore the importance of further studies examining the role of METTL3 and m6A in epidermal tissue regeneration and carcinogenesis.

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Comparative analysis of inflammatory skin diseases reveals shared and distinct gene signature profiles in lesional and nonlesional regions.

B. Martínez, S. Shrotri, K. Kingsmore, P. Bachali, A. Grammer, P. Lipsky
AMPEL BioSolutions LLC, Charlottesville, Virginia, United States

Although different inflammatory skin diseases are characterized by unique clinical features, current understanding regarding the comparative molecular features is limited. Moreover, the gene expression profiles of nonlesional uninvolved skin have not been fully delineated. To determine how the molecular profiles of inflammatory skin diseases are unique or similar between conditions, we carried out a comprehensive analysis of gene expression from cutaneous lupus erythematosus (CLE), psoriasis, atopic dermatitis, and systemic sclerosis. Gene set variation analysis (GSVA), revealed that, compared to healthy control samples, lesional samples from each condition had distinct features, but all four diseases displayed common enrichment in many inflammatory signatures, including the type 1 interferon, tumor necrosis factor, and IL-23 gene signatures. Nonlesional samples also differed from healthy control samples as well as each disease compared to the other using GSVA. Indeed, nonlesional samples proved to be more discriminatory for the specific inflammatory condition than their lesional counterparts as determined by comprehensive analyses consisting of GSVA, classification and regression tree (CART) analysis, and machine learning (ML) models. Altogether, our results suggest a model of skin pathogenesis in which patients exhibit disease-specific abnormalities in their "pre-lesional" skin; however, upon initiation of clinically apparent disease, dermal inflammatory responses may lead to similar and unique inflammatory molecular manifestations among diseases. Dissection of key inflammatory pathways enriched in both clinically involved and uninvolved skin can advance the understanding of the pathogenesis of these conditions and identify novel therapies.

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Skin barrier defect and inflammation in Netherton syndrome: Lessons from a comparative study of patients and mouse models

E. Petrova[1], J. Lopez-Gay Orts[2], M. Fahrner[3], F. Leturcq[1], C. Barbieux[1], J. de Villartay[4], H. Varet[5], J. Coppée[5], J. E. Gudjonsson[6], L. C. Tsoi[6], A. Hovnanian[1, 7, 8]
[1]*Laboratory of Genetic Skin Diseases, INSERM UMR 1163 and Imagine Institute of Genetic Diseases, Paris, France,* [2]*Institut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, Paris, France, 3Institute for Surgical Pathology, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany,* [4]*DGSI Laboratory, Institut Imagine, INSERM, Paris, France,* [5]*Institut Pasteur, Transcriptome and Epigenome Platform, Biomics Pole, Paris, France,* [6]*Department of Dermatology, University of Michigan, Ann Arbor, Michigan, United States,* [7]*Universite de Paris, Paris, Île-de-France, France,* [8]*Department of Genetics, Necker hospital for sick children, Paris, France*

Netherton syndrome (NS) is a severe autosomal recessive skin disease characterized by a compromised skin barrier, hair shaft defects, chronic skin inflammation and allergy. NS is caused by loss-of-function mutations in the serine peptidase inhibitor Kazal type 5 (SPINK5) gene. Constitutive Spink5 knock-out mice reproduce the NS phenotype, but die within few hours after birth. Here we describe the generation of a viable, epidermis-specific Spink5 conditional knock-out (cKO) mouse model, allowing the study of disease progression in NS. We combined RNAseq, proteomics, immunofluorescence microscopy and flow cytometry to study the skin and immune system phenotypes of Spink5 cKO mice and to compare them to those of NS patients and previous mouse models of NS. Comparative analyses of Spink5 cKO mice and NS patients' skin transcriptome and proteome revealed a shared skin barrier and inflammation signature that was characterized by up-regulation of serine proteases, IL-36, IL-20, and IL-17 family cytokines. The systemic inflammation in Spink5 cKO mice was driven by IL-17 signaling and resulted in thymic atrophy, which strongly correlated with phenotype severity. The Spink5 cKO mouse model and our results provide the basis for further dissecting the role of serine proteases and inflammation pathways in NS using pharmacological and/or genetic tools.

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A single-cell transcriptional gradient in human cutaneous memory T cells suppresses pathogenic Th17 inflammation

M. Taylor[1], C. Cook[1], Y. Liu[1, 2], R. Schmidt[3], A. Hailer[1, 6], J. North[1], H. Wang[4], S. Kashem[1], E. Purdom[4], A. Marson[3], S. Ramos[5], R. Cho[1], J. Cheng[1, 6]
[1]*Dermatology, University of California San Francisco, San Francisco, California, United States,* [2]*Dermatology, Xi'an Jiaotong University, Xi'an, Shaanxi, China,* [3]*Gladstone Institutes, San Francisco, California, United States,* [4]*University of California Berkeley, Berkeley, California, United States,* [5]*University of North Carolina System, Chapel Hill, North Carolina, United States,* [6]*San Francisco VA Health Care System, San Francisco, California, United States*

Cutaneous psoriasis improves with targeted pathway inhibition, but the homeostatic mechanisms that normally restrain chronic tissue inflammation remain incompletely understood. We single-cell profiled human psoriatic and normal skin resident memory T cell transcriptomes to reveal a gradated transcriptional program of coordinately regulated inflammation-suppressive genes. This program, which is sharply suppressed in psoriatic lesional skin, strikingly restricts Th17 cytokine and other inflammatory mediators on the single-cell level. We have recently shown CRISPR-based deactivation of core components of this inflammation-suppressive program replicates the elevated IL17F, IL26, and IFNG in psoriatic memory T cells deficient in these transcripts, functionally validating their influence. Combinatoric expression analysis establishes a dominant single-cell trajectory of increasingly inflamed psoriatic resident memory T cells but can also distinguish the influence of individual suppressive program transcripts on specific inflammatory mediators. Finally, we find that therapeutic IL23 blockade reduces Th17 cell frequency in psoriatic skin but fails to re-establish expression of this inflammation-suppressive transcriptional program, illustrating how treated lesions may be primed for recurrence upon withdrawal of treatment.

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The role of histone demethylase UTX on epidermal homeostasis and carcinogenesis

G. N. Pacella[1, 2, 3], A. Anderson[1], E. Ko[1, 3], B. C. Capell[1, 2, 3]
[1]*Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [2]*Genetics, University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [3]*Epigenetics Institute, University of Pennsylvania, Philadelphia, Pennsylvania, United States*

Histone modifiers are among the most highly mutated genes in all forms of cancer, with histone demethylase UTX (KDM6A) being one of the most frequent. Found on the X chromosome, UTX is one of the few genes to escape X inactivation and functions as a major enhancer regulator. UTX establishes the active enhancer landscape through its histone demethylase activity as well as its ability to complex with other activating histone modifiers. UTX is implicated in many epithelial cancers and is commonly mutated or lost in cutaneous squamous cell carcinoma (cSCC). This is supported by loss of UTX expression in cSCC upon immunohistochemical staining. UTY, the Y-linked paralog of UTX, retains minimal catalytic function but can potentially compensate for UTX loss by other mechanisms. This may account for some of the sex specific differences that have been observed in the risk and severity of cSCC, given that mutations in UTX are typically heterozygous. Despite its demonstrated role in tumor suppression across cancers, there is virtually no understanding of how it functions during epidermal homeostasis or carcinogenesis. To address these questions, we generated mice with epidermal deletions of Utx. Intriguingly, we observed that homozygous Utx knockout female mice show regions of epidermal hyperplasia, an expanded keratin 14 basal layer and increased immune invasion in the skin when compared to controls. In contrast, male mice missing their one copy of Utx, as well as heterozygous female mice lacking only one copy of Utx, appear phenotypically normal. At the transcriptional level, we find that depletion of UTX leads to numerous alterations in the expression of both immune and metabolic genes, consistent with a recent discovery that UTX senses cellular oxygen. Taken together, these data offer new insights into how UTX may be critical for maintaining epidermal homeostasis and proper gene expression, and how when lost, UTX depletion may lead to increased cancer risk.

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Kullback-Leibler divergence model to integrate genetic and genomic information to assess drug response for psoriatic patients

Q. Li[1, 2], K. He[1], M. Patrick[2], T. Tejasvi[2], H. Zhang[1], P. Stuart[2], R. Nair[2], J. E. Gudjonsson[2], J. Elder[2], L. C. Tsoi[2, 1]
[1]*Biostatistics, University of Michigan, Ann Arbor, Michigan, United States,* [2]*Dermatology, University of Michigan, Ann Arbor, Michigan, United States*

Psoriasis is an immune-mediated inflammatory and hyperproliferative skin condition affecting ~2% of the US population, with a total annual cost of around 3 billion dollars. Despite the successes of drug development, there can be significant variation in treatment response, which can correlate with patients' genetic variations and baseline skin genomic profiles. However, no study has integrated multiomic information to enhance drug response assessment, potentially because this data is rarely available from the same cohort, and current modeling techniques are limited in their ability to robustly integrate partially overlapping multi-view data. We seek to address the above limitations on a longitudinal RNA-seq cohort of 44 patients that received anti-TNF treatment with documented changes in PASI score, as well as an independent genetic cohort of 428 psoriatic patients with self-reported 5 level outcomes rating the drug prognosis. We used an advanced Kullback-Leibler divergence(KL) based integrative approach to model the multi-view information, leveraging information from genetics data to improve the drug response assessment from the genomics information. We used variant calling to identify common variations in the RNA-seq samples, and regularized regression (LASSO) to improve the identification of informative genetic and genomic markers for the complex trait sparsity structure. Compared with using genomics data alone, the integrative KL model reduced the 5-fold predictive mean squared error (MSE) by 3.8% from 2.61 to 2.51, improved the model R2 from 0.0193 to 0.459 and further identified >30 informative markers that can be used to enhance drug response prediction. Our method highlights the feasibility of using statistical techniques to analyze independent multi-modal biological data, thus providing a significant opportunity to integrate available information from different sources and improving the prognostic prediction accuracy.

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Integrative analysis using allele specific accessibility in immunocytes to unravel biological effect for complex skin-disease associated loci

L. C. Tsoi, Z. Zhang, M. Patrick, H. Zhang, R. Wasikowski, J. E. Gudjonsson, X. Wen, R. Nair, J. T. Elder
University of Michigan, Ann Arbor, Michigan, United States

Despite the success of GWAS in identifying hundreds of loci associated with different complex inflammatory skin conditions, it is not trivial to decipher their molecular mechanisms due to the cell type specific regulatory features and the presence of linkage disequilibrium. We examined the allele specific chromatin accessibility (ASA) of >8 million common genetic variations in 1,227 ATAC-seq samples from resting and activated CD4 and CD8 T cells, and myeloid dendritic cells (mDC) in ~150 individuals, revealing 54,746 heterozygous sites with high coverage (20 reads). Over 10% of these putative regulatory variations show significant allelic bias (FDR 10%) in the ATAC-seq data, and 20 of them reach genomewide significance for at least one inflammatory skin condition (atopic dermatitis, psoriasis, lupus, vitiligo, scleroderma). Notably, 7 and 4 of the variations are specific to activated and non-activated T-cells respectively, 3 are mDC specific, and 6 of them are shared across both mDC and T-cells; and these variations are enriched with transcription factor binding motifs for BATF and NFkB (p<1x10-10). eQTL analysis further highlights 19 of them are associated with gene expression levels in blood (eQTLGen), and their target genes include IFNLR1 (1p36.11 for psoriasis) TNFSF4 (1q24.3 for vitiligo) and JAK2 (9p24.1 for lupus). We performed Hi-C data and confirmed a physical looping connection between the lupus signal and the transcription start site of JAK2. Skin scRNA-seq demonstrated myeloid/T-cell specific expressions in skin for many of the gene targets of these ASA signals, including JAK2 and TNFSF4. These data highlighted that multiomic information can provide complementary insights to untangle the molecular mechanisms of disease-associated genetic variation. Ongoing study will use statistical genetic to further expand the ASA analysis to other putative causal markers mapping to GWAS signals.

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Regulatory roles of lncRNAs in psoriasis determined by single cell analysis

R. Wasikowski, M. Patrick, S. Sreeskandarajan, H. Zhang, Q. Li, A. Billi, J. E. Gudjonsson, L. C. Tsoi
Dermatology, University of Michigan Michigan Medicine, Ann Arbor, Michigan, United States

Long noncoding RNAs (lncRNAs) can harbor DNA binding domains and regulate target gene expressions by binding to their promoter regions. Although a large proportion of differentially expressed lncRNAs have been identified in psoriatic skin, a chronic inflammatory disease enriched in pro-inflammatory genes, their cell type and disease specificity are yet to be revealed. We performed scRNA-seq experiments in non-lesional skin and lesional skin from 6 psoriasis patients, and compared the expression profiles of lncRNAs against skin from 5 control samples. Most of the up-regulated lncRNA were enriched in keratinocytes (for example, MALAT1 and NEAT1), fibroblasts (MEG3), or melanocytes (KU-MEL-3). However, many of these differentially expressed lncRNAs do not yet have known functional roles. We conducted a in silico analysis to assay the DNA binding ability for 121 differentially expressed lncRNA in psoriasis lesional/non-lesional against control (FDR<=5%) within each of the 12 cell types, revealing 5,879 potential downstream protein-coding gene targets. We identified that the lncRNAs NEAT1 and MALAT1 (known to perform regulatory roles) targets include epidermal genes such as: PPP1R15A, ZFP36, and SFN, which are responsible for cell cycle regulation and cell proliferation. Notably, these target genes are all correlated with MALAT1 and NEAT only in the lesional skin, but not the non-lesional/control keratinocytes. In addition, MALAT1 is also co-expressed with other binding targets JUN, and JUNB, which are involved in pro-inflammatory response within psoriasis (R≥0.35, p<2.2e-16). Interestingly, within the control and non-lesional keratinocytes, MALAT1 expression was negatively correlated with binding targets including DMKN (pro-inflammatory), and FABP5(proliferation gene) (R<= 0.4, p<2.2e-16). Together, these findings highlight how insight from co-expression analysis can contextualize the functional role of lncRNAs within a gene regulatory network.

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ILF2 contributes to hyperproliferation of keratinocytes and skin inflammation in a KLHDC7B-DT dependent manner in psoriasis

J. Yan, Q. Sun
Shandong University Qilu Hospital, Jinan, Shandong, China

Background: The extensive involvement of interleukin enhancer binding factor 2 (ILF2) in RNA stability and inflammation response is well documented. Aberrant LncRNAs expression contributes to psoriasis development and progression. However, little is known about the role of ILF2 in psoriasis. Objectives: Aimed to explore the role of ILF2 and KLHDC7B-DT in psoriasis. Methods: Long noncoding RNA (lncRNA) expression in psoriatic tissues was measured by lncRNA microarray and qRT-PCR. Normal human epidermal keratinocytes (NHEKs), HaCaT cells, and Ker-CT cells stimulated by M5 (IL-17A, IL-22, IL-1 α, oncostatin M, and TNF-α) were used to establish a psoriasis model in vitro. Fluorescence in situ hybridization was used to detect the distribution of KLHDC7B-DT and ILF2 in the keratinocytes. The proliferative effects of KLHDC7B-DT and ILF2 on keratinocytes were demonstrated by EdU assay and flow cytometry. ELISA assay was used to detect the secretion levels of cytokines. RNA pull-down and RNA immunoprecipitation (RIP) were used to detect KLHDC7B-DT directly binds with ILF2. Western blotting was used to detect the proteins related to STAT3/JNK signaling pathways. Results: ILF2 and KLHDC7B-DT were significantly overexpressed in psoriatic tissues and M5-induced keratinocytes. They were distributed both in cytoplasm and nucleus. KLHDC7B-DT promoted the proliferation of keratinocytes and induced the secretion of IL-6 and IL-8. KLHDC7B-DT could directly bind to ILF2 and activate STAT3 and JNK signaling pathways. The expression of KLHDC7B-DT was regulated by ILF2. ILF2 knockdown inhibited M5-induced proliferation and inflammatory cytokines secretion in keratinocytes. Furthermore, we found that ILF2 promoted the keratinocytes' proliferation and inflammatory response in a KLHDC7B-DT dependent manner. Conclusions: Our findings indicated that ILF2 and KLHDC7B-DT were involved in the hyperproliferation of keratinocytes and skin inflammation in psoriasis. In addition, we verified that ILF2 regulated the expression of KLHDC7B

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Integrated analysis of acne identifies a critical role for immune fibroblastic cells (IFCs) in the pathophysiology of acne and in the action of isotretinoin.

A. O'Neill[1], M. Liggins[1], J. Seidman[1], J. E. Gudjonsson[2], T. Do[3], F. Li[1], K. Cavagnero[1], T. Dokoshi[1], J. Cheng[1], F. Shafiq[1], T. Hata[1], R. Modlin[3], R. Gallo[1]
[1]Dermatology, University of California San Diego, La Jolla, California, United States, [2]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [3]Dermatology, University of California Los Angeles, Los Angeles, California, United States

To improve understanding of acne we evaluated host cell networks in the pilosebaceous unit and variables associated with C. acnes that promote skin inflammation. Single-cell RNA sequencing uncovered unsuspected activation of specific dermal fibroblast subsets in both human acne and mice challenged by C. acnes. This transcriptional response was characterized by initiation of fat cell differentiation and expression of cathelicidin (Camp) by fibroblasts; responses that were validated by immunostaining of PREF1 and CAMP in these specialized perifollicular immune fibroblastic cells(IFCs). mRNA interactome analysis showed that these novel IFCs share a major inflammatory communication network with myeloid cells in mice and human acne. 3T3L1 fibroblasts were then used as a model for IFCs. Analysis of protein and mRNA expression by these cells and in mice injected with C. acnes showed bacterial strain-specific proinflammatory triggers associated with bacterial genomic sequence, growth conditions and plasmid incorporation, not only phylotype. Next, functional relevance of IFCs was demonstrated in C. acnes-injected mice treated with retinoic acid(RA). After RA treatment, epidermal lipid synthesis decreased while Camp expression in PREF1+ fibroblasts significantly increased ($p>0.0001$), and this associated with a decrease in acneiform lesions ($p>0.004$). Skin biopsies of human acne patients treated with isotretinoin also showed increased cathelicidin in dermal fibroblasts compared to pre-treatment. However, RA treated Camp-/- mice showed no significant decrease in C. acnes acneiform lesions despite inhibition of lipid synthesis. These observations reveal previously unsuspected functions for dermal IFCs, and novel variables associated with C. acnes, that are involved in the pathophysiology of acne.

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Integrative analysis of the skin microbiome and metabolome in psoriasis

R. Wang, R. Tao, R. Li
Dermatology, Peking University First Hospital, Beijing, Beijing, China

Psoriasis is a common, chronic and recurrent inflammatory disease. Current theories have highlighted the role of the microbiome in the pathogenesis of psoriasis. Also, abnormal metabolisms can alter disease process in terms of occurrence, development and prognosis. In this study, we performed an integrated skin microbiome-metabolome analysis to gain an insight into the pathogenesis of psoriasis. A total of 22 patients with psoriasis and 22 age- and sex-matched healthy controls were recruited. Skin swabs were collected from the scalps. DNA extraction, amplicon sequencing of the ITS1 and V3V4 16S rRNA regions, the nontargeted LC-MS-based metabolomic profiling. Our results showed that psoriatic lesions were characterized by higher relative abundances of Corynebacterium and Staphylococcus than healthy controls, but no significant alteration in the fungal diversity or fungal taxonomies were detected. Nontargeted LC-MS-based metabolomics approach showed a total of 526 metabolites were differed significantly between psoriatic and healthy scalps. The prostaglandin-related metabolites, cysteine- and methionine-related metabolites and purine-related metabolites were significantly enriched on the skin surface of patients with psoriasis. Cutibacterium and Lawsonella, which are considered as skin commensals in healthy individuals, were found to be negatively correlated with several metabolites, such as the plasma metabolites of prostaglandin F. On the contrary, the psoriasis-associated bacteria, such as Staphylococcus, Corynebacterium and Streptococcus showed opposite correlation pattern with the metabolites. In conclusion, the disease-related bacteria Corynebacterium and Staphylococcus are shown to be enriched in psoriatic lesions, together with increased metabolites of prostaglandins, amino acids and purines, which are probably associated with the inflammatory response and higher cell proliferation in psoriasis. This study indicates that specific microbes and metabolites may provide new therapeutic strategies for the treatment of psoriasis.

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Distinct transcriptomic shifts in keratinocyte subsets induced by type I interferon

K. Sakamoto[1, 2], K. Nagao[1]
[1]NIAMS, NIH, Bethesda, Maryland, United States, [2]Hamamatsu University, Hamamatsu, Japan

Type I interferons (IFN) produced in response to viral infections activate host-protective immunity but can also trigger autoimmunity. The transcriptomic landscape of keratinocyte subsets during a type I IFN response has not been systematically explored, the understanding of which could deepen our insight on how keratinocytes contribute to skin immunity. Here, we sorted interfollicular epidermis, infundibulum, isthmus and bulge keratinocytes from C57BL/6 mice treated with one-time intraperitoneal injection of poly(I:C) and performed bulk RNA seq. Transcriptome analysis from control dataset revealed that keratinocyte subsets displayed distinct transcriptomic profiles, characterized by distinct biological pathways. Analysis of poly(I:C)-treated keratinocytes revealed that type I IFN induced transcriptomic shifts in all subsets analyzed, while each subset maintained their distinct identities. To further explore gene networks and pathways involved in each subset during a type I IFN response, we utilized CEMiTool, a bioinformatics platform that enables the generation of gene modules and construction of gene interaction networks, further predicting hub genes that are central to the gene interactions. We identified 7 modules that represented distinct biological processes. Construction of a weighted and annotated gene-expression network revealed top hub genes that were predicted to interact with genes expressed in our dataset. We observed differential modular activities in keratinocyte subsets at baseline or upon poly(I:C) injection. In conclusion, transcriptomic profiling of keratinocyte subsets via bulk RNA-seq revealed distinct transcriptomic landscape of keratinocyte subsets during homeostasis and their differential responses upon exposure to type I IFN. Our data provides a valuable resource that should help deepen our understanding on how keratinocytes might boost host protective immunity or exacerbate pathological inflammation.

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Role Of TLR4 In chronic relapsing itch induced by subcutaneous capsaicin injection in neonatal rats

H. Kim[1], D. Jeong[1], Y. Jung[2, 3]
[1]Dermatology, Gil Medical Center, Gachon University College of Medicine, Incheon, Korea (the Republic of), [2]Microbiology, Gachon University College of Medicine, Incheon, Korea (the Republic of), [3]Health Science and Technology, Gachon Advanced Institute for Health Science & Technology, Incheon, Korea (the Republic of)

Despite the high prevalence of chronic dermatitis and the accompanied intractable itch, therapeutics that specifically target itching have low efficacy. Increasing evidence suggests that TLRs contribute to immune activation and neural sensitization; however, their roles in chronic itch remain elusive. Here, we show that the RBL-2H3 mast cell line expresses TLR4 and that treatment with a TLR4 antagonist opposes the LPS dependent increase in mRNA levels of Th2 and innate cytokines. The pathological role of TLR4 activation in itching was studied in neonate rats that developed chronic itch due to neuronal damage after receiving subcutaneous capsaicin injections. Treatment with a TLR4 antagonist protected these rats with chronic itch against scratching behavior and chronic dermatitis. TLR4 antagonist treatment also restored the density of cutaneous nerve fibers and inhibited the histopathological changes that are associated with mast cell activation after capsaicin injection. Additionally, the expression of IL-1β, IL-4, IL-5, IL-10, and IL-13 mRNA in the lesional skin decreased after TLR4 antagonist treatment. Based on these data, we propose that inhibiting TLR4 alleviated itch in a rat model of chronic relapsing itch, and the reduction in the itch was associated with TLR4 signaling in mast cells and nerve fibers.

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Rapid reduction in S. aureus & cytotoxins in dupilumab treated atopic dermatitis subjects

L. A. Beck[10], M. Boguniewicz[1], T. Hata[2], Z. Fuxench[3], E. Simpson[4], A. De Benedetto[10], J. Ko[5], P. Ong[6], T. Yoshida[10], R. Gallo[2], S. Lussier[7], G. David[7], P. Schlievert[8], S. Gill[10], A. Rudman Spergel[9], D. Y. Leung[1]

[1]National Jewish Health, Denver, Colorado, United States, [2]University of California San Diego, La Jolla, California, United States, [3]University of Pennsylvania, Philadelphia, Pennsylvania, United States, [4]Oregon Health & Science University, Portland, Oregon, United States, [5]Stanford University School of Medicine, Stanford, California, United States, [6]University of Southern California, Los Angeles, California, United States, [7]Rho, Inc, Cary, North Carolina, United States, [8]University of Iowa, Iowa City, Iowa, United States, [9]National Institutes of Health, Bethesda, Maryland, United States, [10]University of Rochester, Rochester, New York, United States

Atopic dermatitis (AD) severity correlates with S. aureus (SA) colonization and barrier dysfunction. To address the importance of IL-4&-13 on these parameters, the Atopic Dermatitis Research Network designed a 6wk, RDBPC trial (Dupilumab[DPL]:placebo/2:1) with sampling (at 0, 3, 7, 14, 21, 28 & 42 days [d]) to quantify SA, barrier and severity (EASI, NRS, IGA & SCORAD). Seventy-two moderate-severe adult AD subjects were randomized. There was a >7-fold reduction in SA (qPCR) on lesional (L) skin in DPL vs placebo group (1o endpoint [28d];P<0.001). SA abundance (qPCR) on L skin was reduced by 3d (P=0.019) and qPCR & culture quantification were more robustly reduced at 14-42d (P≤0.004). SA reductions (qPCR) in nonlesional (NL) skin were seen ≥7d (P≤0.033), but were greater at later timepoints (≥21d;P≤0.025). Shannon diversity increased as early as 14d (L skin) (P<0.001) in DPL group. SA cytotoxins (bioassay) at L sites were reduced by 7d (P=0.04) and more so at later timepoints (≥14d;P<0.001) and reduced at NL sites (≥21d;P≤0.01). TEWL was significantly reduced at 3 & 42d (P≤0.02) and trended downward at 28d (P=0.07) in L skin. DPL treatment was associated with a rapid reduction on L skin of SA abundance, SA derived cytotoxins and greater microbial diversity which preceded significant improvements in all AD severity measures. This is the first demonstration in a RDBPC trial that DPL improves barrier function, which was observed as early as 3d. Next steps will be to address the mechanisms responsible for changes in SA and how this impacts AD severity.

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Skin-specific stearoyl-coenzyme A desaturase 1 knockout mice are colonized by saprophytic bacteria and fungi

H. J. Pyle[1], A. G. Lone[1], M. Artami[1], M. Edwards[1], P. Raj[2], B. Zhang[2], T. A. Harris-Tryon[1]

[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Immunology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

Lipids synthesized on the skin are crucial to the antimicrobial barrier. Skin lipids also facilitate survival of lipophilic skin commensals in an otherwise dry and acidic ecological landscape. Skin-specific stearoyl-Coenzyme A desaturase 1 (SCD1) knockout mice are known to have dry, inflamed skin, with sebocyte atrophy and decreased synthesis of monounsaturated fatty acids, triglycerides, and wax diesters. Characterizing the skin microbiota in SCD1 knockout mice may facilitate better understanding of skin diseases in which alterations in lipids and the microbiota co-exist. In the present study, we used 16S rRNA and internal transcribed spacer (ITS) amplicon sequencing to compare bacterial and fungal skin microbiomes between wildtype (SCD1fl/fl) and skin-specific SCD1 knockout mice (K14Cre+/-;SCD1fl/fl) in a barrier facility. The bacterial phylum Firmicutes represented most sequences in knockout mice, while Proteobacteria represented most sequences in wildtype mice. The fungal phylum Ascomycota represented most sequences in both groups. Interestingly, saprophytic fungi including Alternaria were found in higher relative abundance in the knockout group (ANCOM analysis), and the saprophytic bacteria Sporosarcina trended toward higher relative abundance in the knockout group. As expected, lipophilic Cutibacterium trended toward lower relative abundance in the knockout group. Analysis of community diversity (Shannon index) revealed greater fungal alpha diversity in the knockout group (P<0.01), with no difference in bacterial Shannon diversity. Principal coordinates analysis (Bray-Curtis dissimilarity) showed that both bacterial and fungal communities of the knockout group were unique from the wildtype group (P<0.01, PERMANOVA). In summary, these results suggest that specific bacteria and fungi thrive in the absence of SCD1 derived lipids, while other species may require these lipids in order to colonize the skin surface.

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Development of 3D reconstructed skin models with PBMC-derived immune cells

C. Meyrignac, I. Garcia, C. Capallere, I. Imbert

Ashland Global Specialty Chemicals Inc, Covington, New Jersey, United States

Skin immune system is composed of specialized immune cells that plays an important role in skin homeostasis and protection against injury. Among these cells, Langerhans cells and macrophages are key elements of both the innate and adaptive immune response. In epidermal compartment, Langerhans cells (LCs) are immature dendritic cells involved in antigen presentation to T Cells whereas in the dermal compartment, macrophages are tissue resident professional phagocytes. Under inflammatory context, epidermis and dermis are depleted from resident Langerhans cells and macrophages through migration or cell death respectively. Attracted to the inflammatory skin site by cytokine signals, they differentiate into Macrophages or Dendritic cells depending on chemical environment. In this study, we took advantage of this mechanism to generate Langerhans cells and Macrophages type 1 (M1) and 2 (M2) from Peripheral Blood Mononuclear Cells (PBMC) derived monocytes. Then, these cells were integrated into new engineered human skin tissue models for in vitro testing. Macrophages M1 or M2 were incorporated into dermal equivalent alone or in full thickness (Dermis + epidermis) and Dendritic cells or Langerhans cells into epidermis or in full thickness. These 3D reconstructions allowed to study the tissue morphology and the expression of specific markers in order to delineate the role of each subpopulations. These new immunocompetent full thickness models are aimed to restore the physiological interplay between specialized immune cells and the main skin cell types: the keratinocytes/fibroblasts. Indeed, they are promising tools for in vitro monitoring of skin homeostasis following biofunctional ingredients or chemicals application.

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Skin microbiome in cutaneous T-cell lymphoma is associated with phototherapy treatment response

M. Hooper[1], T. LeWitt[1], F. L. Veon[1], G. Enriquez[2], J. Guitart[1], M. Burns[2], X. Zhou[1]

[1]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]Biology, Loyola University Chicago, Chicago, Illinois, United States

The cutaneous T cell lymphoma(CTCL) microbiome is poorly understood despite evidence that CTCL patients have an increased risk of skin infections with disease progression. Narrowband UVB therapy(UV) has response rates of 54-90% in early-stage disease. Here, we examined skin microbial changes of 25 UV-treated CTCL patients (20 mycosis fungoides, 2 Sézary syndrome, 3 unspecified CTCL; 14 responders [R], 11 non-responders [NR]) using 16S gene sequencing. For lesional skin(LS), microbial richness was unchanged in R pre- and post-UV, but decreased after UV in NR (Simpson, p=0.046). Post-UV richness was greater in R than NR (Shannon, p=0.016). For non-lesional skin (NLS), microbial richness did not differ with UV for R or NR (Shannon, p=0.497). Bray-Curtis analysis showed that the microbial communities in NLS of R and NR were distinct both before (p<0.01) and after (p=0.017) UV. Moreover, microbial communities populating the LS of R and NR were distinct after UV (p=0.045), but not before UV (p=0.181). Bray-Curtis did not show intraindividual differences before and after UV therapy for R or for NR in LS or NLS (p>0.35). NR were significantly enriched for Porphyromonas in LS and Acinetobacterium in NLS compared to R. R harbored high levels of Staphylococcus in LS that decreased with UV, and reduced Staphylococcus in NLS. Corynebacterium increased after UV in LS of R but not NR. CTCL skin may carry predictive signals for UV response, including greater microbial α-diversity and a specific bacterial signature. UV alters the abundances of specific bacterial taxa in LS and NLS in CTCL. Importantly, our data show that UV reverts LS microbial communities to a normal-like state in R, while NR skin show signs of continued dysbiosis. Our longitudinal work is the first to demonstrate this in CTCL and lays the groundwork for follow-up trials to assess the skin microbiome as a novel biomarker for treatment response in CTCL and as an opportunity for therapeutic intervention.

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Iron excretion through the epidermis influences host defense against C. albicans

S. Khalil, M. R. Williams, R. Gallo
Dermatology, University of California San Diego, La Jolla, California, United States

Iron is an essential nutrient that functions as a cofactor for cellular enzymatic redox reactions. Hosts and pathogens compete over iron for survival. Previous studies have implicated the skin as a unique site for iron homeostasis as the major contributing source to iron excretion from the body: Primary human keratinocytes store iron as ferritin as a function of exogenous iron addition, with modulation of their differentiation. Wildtype mice subjected to systemic iron bolus or iron chelation demonstrate changes in total epidermal elemental iron content. We hypothesized that epidermal iron stores influence both systemic iron levels and could affect cutaneous pathogen survival. To test this, C57Bl6J mice were treated with one-time systemic iron bolus (1mg/kg intraperitoneal) or treated with the iron chelator desferrioxamine (300 mg/kg intraperitoneal) and then iron stores in the epidermis and microbial survival on the skin was evaluated. Inductively coupled plasma mass spectrometry (ICP-MS) performed on dispase-split mouse skin identified significant changes in epidermal iron content by these interventions ($p < 0.0001$). After topical challenge with C.albicans, animals subjected to iron overload had decreased C. albicans CFUs ($p = 0.0014$) consistent with the clinical observation of C.albicans-associated angular cheilitis in patients with iron deficiency. However, no difference was observed in S. aureus survival on the skin after systemic iron manipulation. These findings suggest that epidermal turnover may be a regulated mechanism of iron excretion that matches epidermal behavior to systemic iron levels, with physiologic implications in some cutaneous infections.

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Better performance of live probiotic over inactivated biomass on skin density

A. Garlet[2], S. Leoty-Okombi[1], M. Gault[1], L. Aversa[1], N. Pelletier[1], C. Bonnaud-Rosaye[3], W. Chan[2], V. Andre[1]
[1]BASF Beauty Care Solutions France SAS, Lyon, Rhône-Alpes, France, [2]BASF Corp Tarrytown, Tarrytown, New York, United States, [3]BASF Beauty Care Solutions SAS, Essey Les Nancy, Lorraine, France

In a previous prevalence analysis study, we showed that lactic acid bacteria occurrence significantly decreases into the wrinkle zone of old volunteer *in vivo*. Furthermore, relative abundance analysis particularly revealed the absence of a beneficial bacteria, Lactobacillus crispatus, in the same area. This discovery drove us to develop solutions to favor more desirable and young skin phenotype through a direct contact with this valuable bacterium or an extract. In this aim, we tested two conditions including two forms of Lactobacillus crispatus: the living biomass and the inactivated biomass. We observed their respective effects in vitro. L. crispatus was prepared by fermentation in MRS medium. After growth, the biomass was collected. A part of the biomass was kept alive and freeze-dried, whereas the other part was thermally inactivated. For in vitro evaluation, collagen type 1 and 5 synthesis were evaluated in human fibroblasts. We tested three concentrations of each condition (0.01; 0.125 and 0.25 %) versus the control containing the fibroblast culture medium only. After 2 days of culture with the products, deposited mature collagen type I and 5 were measured by Delfia method. We were unable to see any activation using the inactivated L crispatus sample, whereas the living biomass induced an increase of type 1 collagen by 47% at 0.01% and 133% at 0.125%, reaching up to 179% at 0.25%. Results were significant compared to the control since the 0.125% concentration. The same tendency was obtained with collagen type V (significant +55% at 0.125% and +68% at 0.25%). These in vitro results clearly demonstrate the benefits of living bacteria compared to inactivated biomass to stimulate two major components of the skin extracellular matrix. These results corroborate the clinical results obtained on skin densification.

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How the inappropriate innate immune response of atopic dermatitis promotes dysbiosis of the skin microbiome

T. Nakatsuji, A. Butcher, S. Brinton, A. O'Neill, R. Gallo
Dermatology, University of California San Diego, La Jolla, California, United States

A hallmark of atopic dermatitis (AD) is colonization by S. aureus (SA) due to insufficient induction of antimicrobial peptides (AMPs) by Th2-skewing and loss of strains of coagulase-negative Staphylococcus (CoNS) that produce antimicrobials (AMs). These CoNS-AM+ strains normally outcompete SA from the skin microbiome. To understand the mechanism responsible for dysbiosis of the microbiome in AD, 5 strains of CoNS-AM+ and 5 strains of CoNS-AM-(without AMs), representing both S. hominis and S. epidermidis, were applied to skin of control mice or mice with AD-like Th2 inflammation induced by MC903. Survival of CoNS-AM+ strains on inflamed skin was 95% less than on controls after 48hr but CoNS-AM- strains survived equally well on non-inflamed and inflamed skin ($P<0.01$). In contrast, cathelicidin knockout mice (Camp-/-) treated with MC903 showed no loss of CoNS-AM+, suggesting this host AMP selectively eliminated CoNS-AM+ compared to CoNS-AM- strains. Conversely, genetic deletion of lantibiotics (a class of AM) from a CoNS-AM+ strain of S. hominis (ShA9) enabled its survival on inflamed skin of Camp+/+ mice and resulted in > 8-fold increased resistance to LL37 in vitro. When a mix of strains 33% SA : 37% ShA9 WT (AM+) : 30% ShA9DLanti (AM-) were applied to inflamed skin the bacterial CFU shifted over 72 hrs to enable expansion of SA and loss of CoNS-AM+ (74:5:21%). In contrast, SA was eliminated and CoNS-AM+ strains survived better on normal skin (8:46:46%). Similarly, when mixed strains were incubated in vitro with a low amount of LL37 (2μM), the proportion also shifted to an AD-like microbiome (53:5:42%). These results show that the combined activity from bacterial lantibiotics and host AMPs becomes auto-destructive to CoNS-AM+ strains on inflamed skin. We propose that low production of AMPs due to suppression by Th2-skewed conditions of AD is insufficient to inhibit SA but still selectively eliminates beneficial CoNS-AM+, thus promoting dysbiosis.

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The cutaneous deviant staphylococcus epidermidis induces skin injury via secretion of phenol-soluble modulins.

M. R. Williams[1], N. Jiang[1], S. Zhang[1], A. R. Horswill[2], R. Gallo[1]
[1]Dermatology, University of California San Diego, La Jolla, California, United States, [2]Immunology & Microbiology, University of Colorado Health, Aurora, Colorado, United States

Our group has challenged the concept that S. epidermidis (SE) is only a beneficial commensal microbe by showing that when skin colonization of SE increases, it promotes skin inflammation through production of the protease EcpA. Analysis of the SE genome has revealed that it encodes 4 genes homologous to the α-type phenol soluble modulin (PSM) toxic peptide made by S. aureus (SA), thus suggesting another mechanism by which SE can harm the skin. Principle component analysis of RNA-Seq data from primary keratinocytes (nHEKs) treated with synthetic SE PSM peptides PSMα, PSMδ, PSMε and δ-toxin (hld) revealed that the transcriptional response of keratinocytes to most SE PSMs was similar to SA PSMα3 but the response to SE PSMα was like the untreated controls. Induction of genes in the IL-17 pathway including CXCL1, CXCL2, CXCL5, CXCL8 (IL8), CCL20, TRAF6, IL-1B, and TNFa were observed and validated by qPCR. Next, isogenic mutants were constructed in SE for individual PSM genes and IL-8 release was measured by ELISA from nHEKs exposed to sterile-filtered supernatants from the wild-type (WT) or mutant isolates. PSMδ and δ-toxin (hld) deletions decreased release of IL-8 by greater than 50% while a double knockout prevented the majority of IL-8 cytokine release from nHEKs above baseline (IL-8 pg/mL: WT=1752+/-428; Δpsmδ=642+/-100; Δpsmε=1413+/-122; Δhld=724+/-163; ΔpsmδΔhld=397+/-44). To elucidate the mechanism for this response we next examined nHEK LDH release and the role of activation of cell surface GPCR receptors. IL-8 production was inhibited when nHEKs were treated with the GPCR inhibitor pertussis toxin prior to SE PSMδ (IL-8 pg/mL: Control=2004+/-453; Pertussis toxin:984+/-81). LDH release also occurred in a dose-dependent manner after exposure to synthetic SE PSMs. These data reveal a novel role for SE in driving inflammation via both cytolytic and GPCR stimulation pathways and provide further evidence that SE acts as a pathogen if enabled to produce PSMs.

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Characteristics of the gut microbiome in solid organ transplant recipients

M. V. Ha[1], I. Salem[1], H. Al-Shakhshir[1], M. Ghannoum[1], B. T. Carroll[2]
[1]Dermatology, Case Western Reserve University School of Medicine, Cleveland, Ohio, United States, [2]Dermatology, University Hospitals, Cleveland, Ohio, United States

Solid organ transplantation is the ultimate life-saving intervention in patients with end stage organ damage. However, despite revolutionary advances in transplant medicine, complications such as allograft rejection, malignancy, and disseminated infection continue be a major challenge. A growing body of evidence suggests a potential influence of microbial dysbiosis on transplant outcomes. We therefore aimed to investigate the gut microbiome of solid organ transplant recipients for comparison with healthy controls to identify possible dysbiosis. Microbial genomic data, both bacterial and fungal, was generated from fecal samples of 20 solid organ transplant recipients and 20 matched healthy controls. Analyses included testing for differences in microbial diversity measures and relatively abundant species between cohorts. The bacterial median Shannon diversity index, a measure for alpha diversity of the gut microbiome, was significantly higher in solid organ transplant samples with a median of 3.125 versus 2.449 for healthy controls ($p<0.05$). Additionally, principal coordinate analyses, based on Bray-Curtis distance, displayed significant differences in the bacterial and fungal microbiota composition between stool of transplant patients and healthy controls (PCo1 vs. PCo2, $p<0.05$). Furthermore, several alterations in abundance existed at different taxonomic levels, including a a 15-fold increased abundance of Clostridium perfringens ($p<0.0001$), a 3-fold increased abundance of Staph epidermidis ($p<0.0001$), and 2-fold increased abundance of Staph aureus ($p<0.001$) at the species level in transplant patients. Ultimately, we demonstrated that solid organ transplant recipients have gut microbiome dysbiosis, characterized by an increase in microbial diversity and several alterations in taxa abundance. This is in discordance with current trends in microbiome research that associate lower diversity with worse health outcomes; however, it supports the emerging role of the microbiome as a therapeutic target.

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Vaccinia virus utilizes claudin-1 to bind and infect keratinocytes

K. A. Leffler[1], S. R. Monticelli[2], M. C. Moran[1], B. M. Ward[2], L. A. Beck[1], M. G. Brewer[1]
[1]Dermatology, University of Rochester Medical Center, Rochester, New York, United States, [2]Micro & Immuno, University of Rochester Medical Center, Rochester, New York, United States

Atopic dermatitis (AD) patients suffer from increased susceptibility to cutaneous viral infections and diminished epidermal barrier function, including defects in TJ integrity and reduced claudin-1 (CLDN1) expression. To understand the role of CLDN1 in epidermal viral infections, we generated keratinocytes (KC) lacking CLDN1 using CRISPR/Cas9 technology. CLDN1 knockout (CLDN1KO) KC were evaluated for barrier function, differentiation, and susceptibility to vaccinia virus (VV), the causative agent of the life-threatening, AD-related complication, eczema vaccinatum. CLDN1KO cells demonstrated significantly impaired barrier function (46-66% reduction) as measured by TEER. Loss of CLDN1 did not change expression of any assessed genes important in barrier function, differentiation, or inflammatory responses. We then evaluated VV infection of CLDN1KO KC. Since CLDN1 is a known entry receptor for hepatitis C virus, we tested whether VV bound CLDN1KO KC. A significant reduction (13.3-fold) in VV binding was observed in CLDN1KO KC. Despite this observation, no changes in VV infectivity of CLDN1KO cells were observed by viral genome replication, infectious virus production, or cytopathic effect. Interestingly, we found that VV infection of wildtype KC resulted in a 2.69-fold increase in CLDN1 expression compared to uninfected KCs. Since the receptor(s) for VV are unknown, we hypothesize that CLDN1 likely functions as a high affinity VV coreceptor. In the absence of CLDN1, low affinity receptors enable VV binding and infection. This supports our observations of decreased binding during a short (1 hour) binding assay and similar viral replication over a long period of infection (24-72 hours). These results indicate that CLDN1 is important for VV binding to KC. Future studies will utilize CLDN1 overexpression to confirm its function in VV infection and CRISPR/Cas9 editing of other key CLDNs to evaluate the relative importance of CLDN1 in VV binding (and other cutaneous viruses) to KCs.

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Immunoprofiling identified a population of activated neutrophils in atopic dermatitis patient whole blood

E. L. Wambeke[1], E. R. Goedken[2], V. E. Scott[1], L. N. Miller[1]
[1]AbbVie Inc, North Chicago, Illinois, United States, [2]AbbVie Bioresearch Center, Worcester, Massachusetts, United States

Atopic dermatitis (AD) is a chronic inflammatory skin disease with several key hallmarks, including elevated Th2 cytokines, interleukin (IL)-4 and IL-13; spongiosis; barrier deficits and pruritus. The goal of this study was to characterize the immune cell populations present in circulation in AD donors compared to healthy controls. Plasma samples from AD donors had increased levels of TARC (CCL17) and IgE consistent with active disease. Peripheral blood mononuclear cells (PBMC) isolated using standard Ficoll-Paque density gradient protocols were characterized using multi-color flow cytometry. Few significant differences were observed between lymphoid and myeloid cells with the exception of an unexpected cell population that was positive for the neutrophil markers CD15 and CD16. Neutrophils are the first line of defense for the immune system for inflammation. While neutrophils are not mononuclear cells, their appearance in this preparation was notable. These low-density neutrophils have been described in other inflammatory diseases including asthma, sepsis, psoriasis, and cutaneous T-cell lymphoma. These cells were further characterized by labeling with the cell surface markers, CD66b and CD62L, which can discriminate between low-density activated (CD66b+CD62L-) and mature (CD66b+CD62L+) neutrophils. The elevated presence of the CD15+CD16+ CD66b+CD62L- activated neutrophils was confirmed in AD whole blood and absent from healthy donor whole blood. Collectively these findings show the unique presence of low-density activated CD66b+CD62L- neutrophils in AD donor whole blood samples. Defining the role of these activated neutrophils in AD may provide additional mechanistic insights into this chronic inflammatory skin disease. Disclosures: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication

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Quantitative aggregation of microbiome sequencing data provides insights into associations between the skin microbiome and psoriasis

A. Chan[2], P. T. Tran[1], J. Yang[1], D. J. Lee[1, 2, 3]
[1]Division of Dermatology, Harbor-UCLA Medical Center, Torrance, California, United States, [2]Department of Medicine, The Lundquist Institute, Torrance, California, United States, [3]University of California Los Angeles David Geffen School of Medicine, Los Angeles, California, United States

While prior studies have reported distinct skin microbiome profiles associated with psoriatic lesions and qualitative reviews have attempted to synthesize these studies, differences in how the samples were obtained and analyzed limit what can be concluded. In fact, the individual reports contradict with one another; for example, Propionibacterium and Staphylococcus were found to be significantly associated with both the psoriatic lesions and with healthy skin among these studies. This meta-analysis of all publicly available datasets utilized a uniform bioinformatics pipeline and reference database to investigate associations of the skin microbiome in psoriasis. 905 skin swab samples from six studies met criteria for high-quality filtering: 312 lesional, 262 nonlesional, and 331 healthy. After reducing batch effect and adjusting for various body sites sampled, the aggregated analysis found a higher relative abundance of S. aureus, C. simulans, and Peptoniphilus in samples from patients with psoriasis versus healthy swab samples, whereas Cutibacterium, Lactobacillus, and S. warneri were significantly higher in healthy samples. Taken together, this first meta-analysis allows more generalizable associations between the skin microbiome and psoriasis skin lesions. Noise from environmental and batch effects may obscure the influence of rarer but biologically relevant taxa. Thus, further studies are warranted to explore these differences and to elucidate the role of microbes in the pathophysiology of psoriasis and may suggest possible clinical therapies through microbiome alterations.

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Cardiovascular comorbidities are associated with increased LL37 which promotes the uptake of low-density lipoprotein into macrophages

Y. Nakamura[1], K. Nikhil[1], T. Dokoshi[1], E. Luo[2], G. Wong[2], R. Gallo[1]
[1]University of California San Diego, La Jolla, California, United States, [2]University of California Los Angeles, Los Angeles, California, United States

LL37 is increased in sera of obese patients and in patients with psoriasis and rosacea. These diseases all have significant cardiac comorbidities. Since LL37 is a multifunctional molecule that also promotes inflammation through its capacity to enhance the uptake of nucleic acids into the cytosol, we hypothesized that it may also contribute to development of cardiovascular disease by influencing cellular lipid accumulation. To test this, we first studied lipoprotein uptake and delivery into macrophages in the presence of LL37 or a C-terminal 3aa truncated peptide (LL34) by using Phrodo-labeled fluorescent LDL. LL37 and LL34 induced a 6-fold increase of LDL entry into THP-1 (P=0.017 and P < 0.001, respectively) or mouse peritoneal macrophages. LL34 also promoted a 12X increase in formation of LDL aggregates (P< 0.0001), a process that promotes LDL entry. These aggregates enlarged over time (6hr) and could be induced at LL37 concentrations as low as 313 nM, similar to LL37 serum concentrations in psoriasis and rosacea. LL34-induced LDL uptake was blocked by endocytosis inhibitors and partially decreased by targeting of SR-B1 (P < 0.01). RNA-Seq analysis, confirmed by qPCR, revealed transcriptional responses consistent with elevated cytosolic LDL including decreased expression of lipid metabolism genes such as SREBF2, HMGCR and LDLR. FACS analysis of peritoneal macrophages from transgenic mice expressing human LL37 showed elevated LDL uptake. Structure-function studies with sequential substitutions of alanine at each aa position of LL34 showed the hydrophobic alpha-helical face of LL37 is important to promote LDL uptake as well as inflammatory cytokine responses. These structural determinants are being defined by synchrotron x-ray scattering measurements of LL37 LDL complexes. Overall, our observations implicate LL37 as a contributing factor for cardiovascular comorbidities observed in some inflammatory skin disorders.

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Three-dimensional structure of a microbial nanomachine and its role in acne vulgaris.

F. S. Ahmad[1], K. N. Berry[2], C. Manithody[1], T. J. Brett[1], J. P. Henderson[1], W. H. McCoy[1]
[1]Washington University in St Louis, St Louis, Missouri, United States, [2]Boston Children's Hospital, Boston, Massachusetts, United States

Treating acne with antibiotics has led to the emergence of multidrug resistant (MDR) bacteria. MDR bacterial infections incur high healthcare costs and result in higher patient mortality. New antibiotics that target specific bacteria to avoid collateral microbiota damage are needed. Antibiotics used to treat acne reduce levels of Cutibacterium acnes. Acne-associated C. acnes genomes include putative, non-ribosomal peptide synthetase (NRPS) genes. Microbes use NRPS proteins to assemble unique metabolites. Some of these metabolites are virulence factors (e.g., Escherichia coli siderophores). Inhibiting bacterial virulence factors has been proposed as a strategy to minimize antibiotic collateral damage. No C. acnes NRPS has yet been characterized. One of the putative, acne-associated NRPS C. acnes genes (PPA-RS12630) is found almost exclusively within the Propionibacterium genus, which includes C. acnes. Based on sequence homology, we hypothesize that PPA-RS12630 is an iterative NRPS with a tri-domain structure consisting of adenylation, peptidyl carrier, and condensation domains. Microbes synthesize the siderophore vicibactin using this type of NRPS. Our group used structure prediction programs to design a PPA-RS12630 E. coli expression construct. Recombinant PPA-RS12630 was expressed, purified, and used in biochemical and structural investigations. Negative-stain electron microscopy of recombinant PPA-RS12630 demonstrated a flexible, tri-domain structure consistent with an iterative NRPS. Cryogenic electron microscopy (cryoEM) data was collected on PPA-RS12630 to determine its three-dimensional structure. The high-resolution structure of this protein will provide a road map to guide the biochemical characterization of PPA-RS12630 and other related C. acnes proteins. These investigations will help us to understand the role of C. acnes in human diseases, develop precision C. acnes therapeutics, and decrease the burden of MDR bacterial infections.

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Active ingredient from Bombax costatum (kapok tree) to preserve skin microbiota equilibrium

S. Bredif, G. Bellemere, S. Leclere-Bienfait, C. Baudouin
Innovation Research & Development, Laboratoires Expanscience, Epernon, France

Preserving skin microbiota homeostasis is of crucial importance for maintaining healthy skin. We have developed a new active ingredient from Bombax costatum flowers, this polysaccharide-rich extract was evaluated in vitro on skin models using different bacterial strains representative of cutaneous microbiota (S. aureus, S. epidermidis, S. hominis, C. acnes). The extract significantly increased keratinocyte production of innate immunity markers TLR2 (Toll-Like Receptor 2) and hBD2 (human beta-Defensin 2) evaluated by ELISA assay. In a co-culture of several bacterial strains, the extract tended to inhibit the growth of the pathogenic strain S. aureus, without significantly affecting the growth of commensal bacteria. This effect was even more significant as S. aureus was present in high proportions compared to other strains, suggesting a preventive effect toward S. aureus pathogenicity by limiting its overgrowth. The extract significantly inhibited S. aureus biofilm formation, evaluated using crystal violet dye, while it increased that of S. epidermidis. The extract inhibited S. aureus adhesion on reconstructed human epidermis (RHE) without affecting S. epidermis adhesion. Adhesion was evaluated by colony forming unit counting of adhering bacteria after topical pre-treatment by the extract. Finally, the extract protected the expression of filaggrin, desmoglein-1 and corneodesmosin, assessed by immunostaining in RHE that were incubated in presence of S. aureus secretum. We have demonstrated that a natural and plant polysaccharide-rich extract obtained from Bombax costatum is able to limit growth, adhesion and biofilm forming properties of a pathogenic strain (S. aureus), without affecting commensal bacteria. Moreover, it stimulates immune defenses and helps to preserve epidermal barrier from alteration due to S. aureus. These results show the beneficial properties of the extract to preserve skin microbiota homesostasis by promoting commensal against pathogenic bacterial strains.

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Hand hygiene for pathogen removal and resident microbiome maintenance: Paradox or oxymoron?

D. J. Yeomans[2], K. Haas[1], N. K. Nguyen[3], P. Dimitriu[3], J. Arbogast[2]
[1]Microbiology, University of Massachusetts Amherst, Amherst, Massachusetts, United States, [2]Research & Development, GOJO Industries Inc, Akron, Ohio, United States, [3]Research & Development, Microbiome Insights Inc., Vancouver, British Columbia, Canada

Hands play a critical role in the exchange of microbiota across one's body, between individuals and with the environment. Hand hygiene practices are used in everyday life to help prevent the spread of disease. All hand hygiene products, irrespective of inclusion of ingredients like antimicrobials or pre/pro/post-biotics, have the potential to alter both the acquired and the innate microbiome of the hand, albeit by different mechanisms. While the intent of hand hygiene is to reduce pathogens on the skin below the infectious dose, this must be balanced with limiting the impact on the hand's resident microbial community, where disruption may contribute to skin irritation or dermatological disease. Most prior studies have focused on pathogen reduction only. With an advancing awareness of its relationship skin health, it is critical to also expand our understanding of the impact of hygiene on the hand's resident microbiota. Here, we explore the impact of various hygiene products and protocols on the hand microbiome. We examine shifts in the microbial community following a single hygiene event, as well as provide an initial exploration into the dynamics of microbial disruption and recovery after multiple exaggerated exposures to ABHS (40 uses in 4 hours). In all explored scenarios, there is an immediate post-hygiene decrease in total microbial biomass and alpha diversity. Furthermore, our data imply that the hand resident microbial community is resilient, with recovery occurring within 3 hours after exaggerated ABHS exposure. Thus, in these exposure scenarios, the benefits of frequent hand hygiene, i.e., pathogen removal, appear appropriately balanced with a low risk of impact on the hand's innate microbiota.

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Systematic analysis of existing and novel primers toward standardization of skin microbiome research

K. Haas[1], S. Ao[3], J. Blanchard[1], J. Arbogast[2], D. J. Yeomans[2]
[1]Biology, University of Massachusetts Amherst, Amherst, Massachusetts, United States, [2]Research & Development, GOJO Industries Inc, Akron, Ohio, United States, [3]Microbiology, University of Massachusetts Amherst, Amherst, Massachusetts, United States

Amplicon-based sequencing continues to be widely used for skin microbiome research. However, standard primers in use at most sequencing centers were originally optimized for the gut, despite the fact that the gut and skin microbiomes are extremely different in composition. In fact, standard primers, such as those spanning the V3V4 region, may not adequately capture Cutibacterium or Staphylococcus - two of the most prevalent genera on human skin. Continued use of these primers could contribute toward incomparable datasets and delay advances in our understanding of the skin microbiome. Here we combine a thorough in silico analysis of existing primers, followed by optimization and development of novel primers spanning the V1V3 and V3V4 regions. We then compare the performance of 6 primer pairs in a relevant complex mock community and in clinical samples obtained from human hands. As anticipated from in silico analyses, the commonly used V1V3 primer pair 27F/534R performed well in the mock community analysis, although underrepresenting S. capitis. The novel V1V3 primer 114F coupled with an optimized 519R primer (519Ropt) also performed well, picking up S. capitis better than 27F/534R, but underrepresenting N. flavescens and S. mitis; S. epidermidis was similarly overrepresented with both primer sets. Other primers tested, including new/optimized V3V4 primers, were less accurate. C. granulosum was underrepresented by an order of magnitude with all primer pairs tested. In clinical samples extracted from three human hands using the glove juice method, 27F/534R and 114F/519Ropt generally concurred with each other across the most abundant taxa, with 114F/519Ropt offering a possible benefit of additional Staphylococcus speciation. In conclusion, it is hoped this systematic analysis will advance skin microbiome research by enabling researchers to select appropriate primers to afford better cross-study comparisons in the future.

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Staphylococcus aureus proteases trigger skin inflammation via eosinophil-derived IL-17 responses

S. J. Nolan[1], N. A. Orlando[1], C. Youn[1], D. A. Dikeman[1], C. Pontaza[1], T. Pritchard[1], S. G. Kwatra[1], A. R. Horswill[2], N. Archer[1]
[1]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Immunology and Microbiology, University of Colorado, Denver, Colorado, United States

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disorder that affects 25% of children and 7-10% of adults and an associated $5.2 billion in annual healthcare costs in the U.S. Although the precise etiology of AD is unclear, it is associated with a complex interaction of epidermal barrier defects, abundant S. aureus skin colonization, and immune dysregulation, including an eosinophilic infiltrate in the skin that correlates with disease severity. However, the role of eosinophils in AD pathogenesis is largely undefined. To address this gap in knowledge, we used an *in vivo* S. aureus epicutaneous mouse model in which a S. aureus-soaked gauze pad was applied to the back skin for 7 days in wildtype (WT) and eosinophil-deficient mice, and performed image, histologic, flow cytometric, and RNAseq analyses. We discovered that eosinophil-deficient mice had reduced skin inflammation that correlated with decreased IL-17 pathway gene expression compared to WT mice. To determine IL-17 expression in the skin, we used IL-17A-tdTomato/IL-17F-GFP dual reporter mice and found that eosinophils were the predominant IL-17 expressing cells in the inflamed skin. Furthermore, adoptive transfer of eosinophils into IL-17A/F deficient mice restored skin inflammation, but not in the presence of IL-17 neutralizing antibodies. This was relevant to humans, as AD patient skin had significantly increased presence of IL-17-expressing eosinophils compared to healthy patient skin. Lastly, S. aureus proteases were crucial for skin inflammation and the recruitment of IL-17 producing eosinophils. Taken together, we uncovered a novel mechanism whereby S. aureus proteases trigger skin inflammation via eosinophil-derived IL-17 responses, which has implications in the development of immune-based therapies against AD and potentially other inflammatory skin diseases.

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Neutrophil-intrinsic TNF receptor signaling directs immunity against staphylococcus aureus

C. Youn[1], Y. Wang[1], D. A. Dikeman[1], M. P. Alphonse[1], S. J. Nolan[1], D. P. Joyce[1], C. Pontaza[1], M. Ahmadi[1], A. Tocaj[1], L. S. Miller[1,2], N. Archer[1]
[1]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Immunology, Janssen Research and Development LLC, Spring House, Pennsylvania, United States

Staphylococcus aureus is the leading cause of skin and soft tissue infections and has become a major health burden due to the emergence of antibiotic-resistant strains. Tumor necrosis factor (TNF) is a proinflammatory cytokine that is rapidly induced upon S. aureus exposure and whose inhibition is associated with increased risk of S. aureus infections in humans. However, the contribution of TNF and cognate receptors, TNFR1 and TNFR2, to host defense against S. aureus skin infections is unclear. Therefore, to determine the host defense role of TNF signaling, we used an *in vivo* mouse model of S. aureus skin infection whereby TNF, TNFR1, or TNFR2 deficient mice and wildtype (wt) mice were intradermally injected with bioluminescent S. aureus and monitored for 14 days. TNF, TNFR1, and TNFR2 deficient mice exhibited significantly increased bacterial burdens and skin lesions compared to wt mice, suggesting that TNFR1 and TNFR2 have non-redundant roles in TNF-mediated immunity. Furthermore, we identified neutrophils (PMNs) as the predominant TNFR1 and TNFR2 expressing cells. To determine the importance of PMN-intrinsic TNFR signaling, we adoptively transferred wt PMNs into TNFR1 and TNFR2 deficient mice, which significantly reduced bacterial burdens and skin lesion sizes in the mice. We next examined the differential roles of TNFR1 and TNFR2 on PMN-mediated host defense, through which discovered that TNFR1 was crucial for PMN recruitment and skin abscess formation, whereas TNFR2 was critical for PAD4-dependent neutrophil extracellular trap formation and prevention of bacterial dissemination. Taken together, these findings indicated that TNF directed immunity against S. aureus via PMN-intrinsic TNFR1 and TNFR2 signaling, which has implications in the development of novel immune-based therapies as alternatives to antibiotic treatment against S. aureus and potentially other bacterial infections.

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Skin commensals promote optimal sebaceous gland function by engaging in an immune-sebum circuit

J. Harris[1,2], R. Choa[2], A. Uberoi[1], E. Grice[1], T. Kambayashi[2]
[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States

Sebum provides vital cutaneous functions including moisture retention and defense against the external environment, including pathogens. Despite the well-defined homeostatic functions of sebum in fortifying the skin barrier, the role of the microbiota-immune system axis in sebum regulation remains unclear. We have recently shown that the keratinocyte-derived cytokine thymic stromal lymphopoietin (TSLP) promotes sebum secretion through T cell stimulation. TSLP and T cell-deficient mice display reduced sebum secretion, suggesting an immunologic role in homeostatic sebum release. We hypothesized that skin commensals could represent a trigger for skin T cell recruitment and TSLP release, thereby controlling homeostatic sebum secretion. To test this hypothesis, we measured sebum secretion in germ-free (GF) mice and found a 50% reduction in sebum release compared to conventionally-raised specific-pathogen-free (SPF) controls ($p = 0.004$). Moreover, GF mice exhibited reduced skin TSLP expression and T cell number. Restoring TSLP expression in GF mice, via delivery of an adeno-associated viral vector, did induce T cell recruitment to the skin and to the sebaceous glands (SGs). Despite this, however, SG activity and sebum secretion was not restored. To determine if T cell function could be defective in GF mice, we adoptively transferred SPF mouse-derived T cells into GF RAG KO mice. However, SPF T cells in GF mice could still not restore sebum secretion, even with TSLP overexpression. Thus, in addition to defective TSLP expression and T cell number in the skin, GF mice harbor SGs that are not responsive to TSLP-stimulated T cells. Ongoing studies are investigating the mechanism by which the skin microbiome alters SG responsiveness to TSLP and T cells. By studying this homeostatic immune-sebum circuit, novel therapeutic targets for sebum-dysregulation diseases may be identified and utilized to improve patient health and quality of life.

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Hidradenitis suppurativa is characterized by suppression of antimicrobial effector perforin-2

W. Amornpairoj, K. Rivas, D. Chopra, J. Burgess, P. Catanuto, L. Siegfried, M. Tomic-Canic, N. Strbo, H. Lev-Tov, I. Pastar
University of Miami School of Medicine, Miami, Florida, United States

Hidradenitis suppurativa (HS) is characterized by dysfunctional immune response and robust bacterial colonization, however little is known about specific molecular events involved in disease development and progression. We previously demonstrated essential role of antimicrobial effector Perforin-2 (P-2) in the cutaneous barrier repair and control of wound infection. Here we evaluated ability of HS tissue to respond to wounding and infection through *ex vivo* wound model and cell-specific evaluation of P-2. HS tissue samples (n=14) were collected during standard of care procedures. Location-matched healthy skin specimens were used as controls (n=3). H&E staining was performed to evaluate histopathology. We aimed to establish *ex vivo* HS model and compare wound healing rates between lesional and location-matched control skin. Wounds were created through the epidermis with 3mm punch, maintained at air liquid interphase and rate of re-epithelialization was evaluated by histomorphometry. Moreover, cellular composition of the immune cell infiltrates and the expression of P-2 was determined by FISH-Flow and flow cytometry. Histopathology of HS tissue confirmed epidermal hyperplasia, elongated rete ridges, dermal inflammation, and fibrosis. Inflammation was confirmed by flow cytometry; HS lesions had higher frequency of multiple cell subsets including CD8+ T cells, GD T, B cells, macrophages, and neutrophils. However, we found suppression of P-2 in all cell subsets. High level of inflammation was also accompanied by lack of re-epithelialization in *ex vivo* wound healing model. Our data show inhibition of re-epithelialization, increased inflammation and suppression of P-2 in the tissue from HS patients. Newly established *ex vivo* HS model can enable pre-clinical testing of novel treatments, including targeting P-2. A thorough understanding of the P-2 regulation in HS could be invaluable in the development of targeted treatments or the re-purposing of existing treatments for HS by inducing P-2.

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Using swabs and scanning electron microscopy (SEM) to detect biofilms in chronic epidermolysis bullosa (EB) wounds

H. Ibrahim[1, 2], S. Kuehne[3], J. Hirschfeld[3], M. Hadis[4], A. Heagerty[5], I. Chapple[6]
[1]University of Birmingham School of Dentistry, Birmingham, United Kingdom, [2]Suez Canal University Faculty of Medicine, Ismailia, Egypt, [3]University of Birmingham Institute of Clinical Sciences, Birmingham, United Kingdom, [4]University of Birmingham Institute of Clinical Sciences, Birmingham, United Kingdom, [5]University of Birmingham Institute of Inflammation and Ageing, Birmingham, United Kingdom, [6]University of Birmingham Institute of Clinical Sciences, Birmingham, United Kingdom

EB is a group of genetic disorders that cause skin and mucous membranes fragility resulting in blisters after minor mechanical trauma, which collapse/rupture, leaving open wounds. Currently, no cure is available and management focusses on wound care and managing complications. Whether skin microbiome exist in biofilms in EB remains to be explored, which we investigate in this study. Biofilms are microbial aggregates embedded in a self- or host-produced matrix along with inflammatory cells attached to a surface. Biofilms are resistant to antimicrobial therapies and may contribute to wound chronicity and impaired healing. Acquisition of skin biopsies, the gold standard method for biofilm diagnosis, from EB patients is challenging. We report the use of cotton swabs in an attempt to visualize biofilms by SEM. Swabs were obtained from wounds, post-irrigation with saline to remove planktonic microorganisms, from patients with different EB subtypes, whose wounds failed to heal for months/years but demonstrated no obvious signs of infection. Results demonstrated clear aggregated coccoid objects measuring 0.2-0.5µm in diameter suspended in a matrix along with inflammatory cells. Besides, yeast-like structures were found forming aggregations within the previously mentioned matrix. The images imply for the first time the presence of intricate biofilms in chronic EB wounds, which may contribute to wound chronicity. Moreover, wound swabs offer a promising alternative to more invasive tissue biopsies. Further studies are needed to characterize the visualized biofilms in EB chronic skin wounds as this offers new promising therapeutic approaches in treating such recalcitrant wounds.

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Characterization of myeloid cell subsets in the tumor microenvironment of merkel cell carcinoma

S. Tabachnick-Cherny[1], T. Pulliam[1], K. Smythe[2], P. Nghiem[1]
[1]University of Washington, Seattle, Washington, United States, [2]Fred Hutchinson Cancer Research Center, Seattle, Washington, United States

PD-1 pathway blockade has changed the landscape for advanced Merkel cell carcinoma (MCC) as over 50% of patients initially respond to therapy. However, for patients who do not respond (or later recur), there is a need for additional therapies. In the US, ~80% of MCC tumors are caused by the Merkel cell polyomavirus, while ~20% are caused only by UV-induced mutations. MCC tumors are thus highly immunogenic because they express either non-self viral antigens or numerous UV-induced neoantigens. Historically, studies have been focused on adaptive immunity, and little is known about innate immunity in MCC which may play an important role in immune evasion. Myeloid cells are heterogeneous with diverse lineages and roles in the tumor microenvironment (TME). It has been challenging to accurately identify specific myeloid cell subsets. We have employed recently available technologies to accurately identify and quantitate myeloid cells in the MCC TME, to assess whether they are associated with unresponsiveness to PD-1 pathway blockade. We performed single-cell transcriptional and cell surface protein (CITE-seq) analyses on PBMC and tumor samples from 8 MCC patients. We identified two major myeloid subsets in MCC tumors: tumor associated macrophages (TAMs) that expressed genes consistent with an “M2-like” signature and plasmacytoid dendritic cells (pDCs). Both of these myeloid subsets are associated with poor prognosis across other tumor types. To explore a possible association of these myeloid subtypes to response to PD-1 pathway blockade, we integrated relevant antibodies into multiplex immunohistochemistry panels and determined their expression in tumor samples obtained prior to PD-1 pathway blockade treatment. Studies are ongoing to determine if the presence of pDCs is associated with failure to respond to treatment. Presently, our findings suggest that TAMs are abundantly expressed in MCC tumors and may thus play a role in establishing an immunosuppressive TME that promotes immune evasion.

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Murine epidermis harbors functionally distinct langerhans cell subsets

P. Dimitrion[1, 2, 3], I. Adrianto[4, 3], Y. Yao[2, 3], M. Pawlitz[1, 2, 3], I. Loveless[4, 2], H. Peng[2, 3], L. Zhou[2, 3], Q. Mi[2, 3]
[1]Wayne State University School of Medicine, Detroit, Michigan, United States, [2]Dermatology, Henry Ford Health System, Detroit, Michigan, United States, [3]Center for Cutaneous Immunology, Henry Ford Health System, Detroit, Michigan, United States, [4]Public Health/Bioinformatics, Henry Ford Health System, Detroit, Michigan, United States

Epidermal Langerhans cells (LCs) derive from embryonic myeloid progenitors at the steady-state and monocyte progenitors under inflammatory conditions. LCs have the capacity to induce both immunity and tolerance in the skin, but how a single population of LCs mediates both these functions has perplexed researchers for decades. We hypothesized that LCs in murine epidermis have functionally heterogenous subpopulations. We employed single-cell RNA sequencing (scRNAseq) and scATACseq to identify transcriptional and epigenetic heterogeneity in LCs during late embryonic development, adult steady-state and inflamed-state. We found three transcriptionally distinct clusters in adult at steady-state: ATF3hiCD207lo (cLC1), ATF3loCD207hi (cLC2), and CD207+ cells expressing keratinocyte (KC) genes (kLCs). Ingenuity pathway analysis showed LC1 had downregulated immunostimulatory pathways and LC2 had upregulated immunostimulatory pathways. LCs from ATF3 knockout mice promoted Th1/2/17 immunity in co-culture experiments, confirming the immunotolerant function of cLC1s. cLC1 and cLC2 clusters had corresponding scATACseq clusters but kLCs did not, suggesting that kLCs may acquire their KC-“fingerprint” through interactions with KCs. scRNAseq analyses of E18.5 pre-LCs and 3 weeks post UVC-treatment also identified ATF3hi and ATF3lo clusters, but kLCs were neither present at E18.5 nor after UVC treatment. Overall, our single cell analyses uncover murine epidermal LC subsets with distinct functions during late embryonic development, steady-state and inflamed-state.

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Chronic wound environment shapes virulence of human commensal bacteria

R. Verpile, M. Dinic, J. Meng, J. Burgess, H. Lev-Tov, M. Tomic-Canic, I. Pastar
University of Miami School of Medicine, Miami, Florida, United States

Staphylococcus epidermidis is one of the most abundant skin-commensal known to modulate cutaneous immune response. Emerging evidence suggests S. epidermidis isolates from healthy skin improve barrier integrity and response to wounding. However, S. epidermidis could also carry a reservoir of antimicrobial resistance genes(ARGs), adding this microbe to the list of "accidental" pathogens. Hence, we aimed to characterize S. epidermidis isolates from healthy skin and chronic wounds (CW) to evaluate their virulence potential and effect on wound healing. Shotgun metagenomic sequencing was performed to analyze presence of ARG and virulence genes in isolates from both environments. Furthermore, antimicrobial susceptibility was tested using the microdilution method. To assess virulence traits of selected isolates, biofilm formation and adhesion to components of the extracellular matrix (ECM) were performed. Human *ex vivo* wound model was used to assess the effect of S. epidermidis isolates on healing. Results pointed to the prevalence of ARG in S. epidermidis isolates from CW associated with gentamicin, ampicillin, erythromycin, norfloxacin, tetracycline, and trimethoprim resistance. This was functionally confirmed, chronic wound isolates showed higher minimal inhibitory concentration (MIC) values for these antibiotics and for benzalkonium chloride, a widely used disinfectant. All CW strains exhibited a higher ability to bind to ECM components compared to healthy skin strains. This feature of CW isolates correlates with their high biofilm formation potential in both in vitro and *ex vivo* assays. Infection of human *ex vivo* wounds showed increased accumulation of CW isolates in the wound bed suggesting the strong ability of *in vivo* biofilm formation. Our study suggests that CW microenvironment influenced selection of S. epidermidis strains with prevalence of ARG and capacity to bind to ECM and form biofilm. Our data reflects the dangers of antibiotic overuse due to the frequency of antibiotic resistance and virulent potential of S. epidermidis strains found in CW.

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Commensal induced accumulation of monocyte-derived cells in neonatal skin regulates long-term cutaneous type 17 inflammation.

M. Dhariwala, J. Okoro, T. Scharschmidt
Dermatology, University of California San Francisco, San Francisco, California, United States

Early life immune interactions help shape longer-term skin health and homeostasis. Prior work has shown that commensal microbes facilitate neonatal skin accumulation of innate and adaptive T cells, thereby promoting fundamental needs such as immune tolerance to commensals and wound healing. Comparatively little is known about commensal-myeloid cell crosstalk in neonatal skin and the functional consequences of these interactions. Using mass cytometry, we surveyed the longitudinal composition of the myeloid cell compartment in murine skin from D6 to D30 of life, in SPF, gnotobiotic and conventionalized animals. This revealed classical monocytes to be a population uniquely enriched in the skin of microbially replete versus germ-free neonates. Corroborative studies revealed that skin monocytes rapidly accumulate between D1 and D3 of life, after which their numbers gradually decline. This early monocyte wave was prevented in antibiotic-treated SPF pups as well as in Myd88-/- but not IL1R1-/- mice, suggesting a key role for tonic toll-like receptor signaling in their accumulation. To dissect the functional relevance of these cells in cutaneous biology, we developed an antibody-based regimen to temporarily deplete monocytes in the first two weeks of life (NeoDmono). scRNA sequencing revealed a heightened type 17 signature in skin T cells from D15 NeoDmono mice. Flow cytometry assays confirmed sustained elevation of IL-17A production by NeoDmono skin T cells through adulthood. This reflected a heightened response to commensal microbes as IL-17 production was significantly reduced in antibiotic-treated NeoDmono mice. While there was no visible skin pathology in NeoDmono mice under homeostatic conditions, imiquimod treatment of the ears in adulthood led to significantly increased ear swelling and neutrophils. Taken together, our data demonstrate a previously unappreciated, commensal-dependent regulatory imprinting function of cutaneous classical monocytes in the early life window.

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Importance of CD40 expression on s. aureus superantigen responses in human keratinocytes

M. C. Moran[1], M. G. Brewer[2], P. Schlievert[3], L. A. Beck[2]
[1]Microbiology & Immunology, University of Rochester Medical Center, Rochester, New York, United States, [2]Dermatology, University of Rochester Medical Center, Rochester, New York, United States, [3]Microbiology & Immunology, University of Iowa, Iowa City, Iowa, United States

S. aureus produces secreted virulence factors, including superantigens (SAgs), that activate the host immune response and cause tissue damage. Little is known about what receptors SAgs utilize on epithelial cells, and keratinocytes (KC) in particular. CD40 has recently been proposed as a potential SAg receptor for TSST-1 on vaginal epithelial cells. To address the relevance of CD40 in KC, we utilized CRISPR/Cas9 to knockout (KO) CD40 in the N/TERT2G KC cell line and established a mock-transfected wild type (WT) and clonal CD40 KO. CD40 KO was confirmed by flow cytometry, and sequence analysis. When differentiated, the CD40 KO had significantly diminished barrier (38-48% reduction compared with WT, p=0.009-0.03), as measured by transepithelial electrical resistance (TEER). We assessed how CD40 KO impacted responsiveness to purified SAgs. Clones were treated with ten SAgs individually and qPCR for the inflammatory cytokines IL-6, IL-8, and TNFa was conducted. In WT cells, SEIQ, SEIU, and TSST-1 induced the most impressive increases, with fold increase of IL-8 transcripts (over sham-treated controls) of 95.5 for SEIQ, 23.0 for SEIU and 15.0 for TSST-1. In contrast, the CD40 KO clone had an attenuated response to SEIQ (34.0; 2.8-fold less), SEIU (3.8; 6.0-fold less), and TSTT-1 (2.0; 7.5-fold less). This suggests that CD40 expression is important for optimal SAg responses, but it is unlikely that CD40 is necessary for SAgs to act on KC. Additionally, CD40 may have specificity for only certain SAgs. We have previously shown that some SAgs make KC much more permissive to viral infections. Ongoing studies are investigating the role of CD40 in SAg responsiveness to vaccinia virus and herpes simplex virus-1.

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WITHDRAWN

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Cutaneous T-cell lymphoma is associated with nasal and gut dysbiosis and altered bacterial signatures

M. Hooper[1], F. L. Veon[1], T. LeWitt[1], Y. Pang[1], G. Chlipala[3], L. Feferman[3], S. Green[4], M. Burns[2], J. Guitart[1], X. Zhou[1]
[1]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]Biology, Loyola University Chicago, Chicago, Illinois, United States, [3]Research Informatics Core, University of Illinois at Chicago, Chicago, Illinois, United States, [4]Genomics/Microbiome Core Facility, Rush University Medical Center, Chicago, Illinois, United States

The nasal and gut microbiomes are largely unexplored in cutaneous T-cell lymphoma (CTCL)—a cancer typified by growing immune dysfunction and infection risk with disease progression. As nasal and gut microbiota are increasingly tied to skin health and disease, and gut microbiota are now known to modulate immunity in cancer, characterization of these niches is essential to a complete understanding of CTCL. Nasal and stool swabs from CTCL patients (53 nasal, 38 stool) and matched healthy control (HC; 20 nasal, 13 stool) individuals were analyzed using 16S gene amplicon sequencing. Distinct microbial communities were identified between CTCL and HC nares (PERMANOVA R2=0.031, p=0.005, genus level). The genera Roseomonas, Catenococcus, Vibrio, Marinobacter, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium, and Acinetobacter were enriched in the CTCL group (p<0.002). Increased relative abundance of these taxa was associated with increased skin disease burden. Nasal α-diversity did not differ between CTCL patients and HC (Shannon p=0.33). Further tuf2 sequencing to investigate nasal Staphylococcus at the species level revealed no differences in relative abundance (including S. aureus and S. epidermidis) between groups. For stool samples, α-diversity was lower in advanced CTCL patients relative to HC (p=0.015) and reduced Eggerthellaceae and Lactobacillaceae (p<0.02, family level) differentiated patients with high skin disease burden. No differences in stool β-diversity were identified. The CTCL nasal and gut microbiomes are altered, with more pronounced dysbiotic signatures in patients with greater skin disease burden. A better understanding of these microbial communities may improve our knowledge of CTCL pathogenesis and facilitate development of novel treatments.

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Purinergic molecules in murine bone marrow-derived mast cells

R. Asakawa, Y. Ogawa, S. Shimada, T. Kawamura
Dermatology, Yamanashi Daigaku Igakubu Daigakuin Sogo Kenkyubu Igakuiki, Chuo, Yamanashi, Japan

In the skin, adenosine triphosphate (ATP) is released from various types of cells by various environmental stimuli via nonlytic mechanisms, cell damage, or acute cell death. Because ATP is a potent inducer of skin inflammation, it has to be promptly hydrolyzed for the skin to achieve homeostasis. Of note, ATP activates mast cells (MCs) through P2X7R, leading to the enhanced skin inflammation. However, MC involvement in the impairment of skin inflammation via ATP hydrolysis remains largely unknown. Thus, we sought to determine the expression of ATP-hydrolyzing molecules in MCs. Bone marrow cells recovered from female B6 mice were cultured in the presence of stem cell factor and IL-3 for 5 weeks, resulting in differentiation into CD45+FcεRI+c-kit+ bone marrow-derived mast cells (BMMCs). A culture of BMMCs with 1 mM ATP-γ-S, a non-metabolizable ATP analogue, or 1 μM ionomycin for 60 min or 10 min, respectively, induced their degranulation. PBS-treated steady-state BMMCs strongly expressed Entpd-1 (CD39) and Entpd-3, and weakly expressed Enpp-1 and CD73, but not Entpd-2, Entpd-8, Enpp-2, Enpp-3, and ALP. While ionomycin upregulated Entpd-1 (CD39), Entpd-3, and Enpp-1 expressions approximately four times, ATP-γ-S upregulated only Entpd-1 (CD39) expression approximately four times. These data suggest that MCs might participate in ATP hydrolysis via Entpd-1 (CD39) and Entpd-3. BMMCs and single-cell suspension from back skin of B6 female mice clearly express Entpd-1 (CD39) at protein levels. Steady-state BMMCs potently hydrolyze exogenous ATP. Moreover, ionomycin-stimulated BMMCs trended towards increase of ATP-hydrolyzing activity. Collectively, murine MCs express functional ATP-hydrolyzing molecules such as Entpd-1 (CD39), thereby contributing to achieve skin homeostasis.

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Robust detection of microbial patterns on different skin regions by flow cytometry

J. Kupschus[1], S. Janssen[2], A. Hoek[2], J. Kuska[1], J. Raethjens3, C. Sonntag[3], K. Ickstadt[3], L. Budzinski[4], C. Hyun-Dong[4], A. Rossi[1], C. Esser[1], K. Hochrath[1]
[1]IUF-Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany, [2]Justus Liebig Universitat Giessen, Giessen, Hessen, Germany, [3]Technische Universitat Dortmund, Dortmund, Nordrhein-Westfalen, Germany, [4]Deutsches Rheuma-Forschungszentrum Berlin, Berlin, Berlin, Germany

The skin is a habitat for commensal and pathogenic microbiota. The detection of the presence and relative abundance of bacteria and fungi, as well as disease-specific shifts in their composition is of immense interest in skin biology. Currently, deep sequencing methods are the gold standard to assess the bacterial microbiome. However, there are alternative methods available. We used the recently described method of determining bacterial communities by two-parameter flow cytometry analysis of scatter and DNA content. For analysis, we developed FlowSoFine™, an interactive, free web application. This tool works out the biological patterns from flow cytometry plots via automated gating strategies to publication ready figures. Loading and processing the raw flow cytometry data, running statistical tests and visualization through our graphical user interface can be done within an hour. To validate our method, we sampled skin (18 cm2) from the forehead, the volar arm and the toe-web spaces of 54 subjects and analyzed the samples by FlowSoFine™ and by 16S rRNA sequencing in parallel. We found that we could robustly discriminate community patterns by anatomical region. Skin microbiome analysis by 16S deep sequencing confirmed the impact of body site on microbial composition using either method. In FlowSoFine™, a non-metric multidimensional scaling (NMDS) plot is used for visualization of differences in microbial patterns between groups (e.g. treatment). Beyond that automated heatmap clustering can be applied to the samples to highlight areas with similarities within groups, which will be useful in sorting regions of interest by FACS for further down-stream analyses.

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Charting the human hair follicle microbiome: Composition, distribution, and functional impact of the bacterial metabolite, butyrate

M. B. Lousada[1, 2], J. Edelkamp[1], T. Lachnit[2], F. Jimenez[3], R. Paus[1, 4]
[1]Monasterium Laboratory, Münster, Germany, [2]Zoology, Christian-Albrechts University Kiel, Kiel, Germany, [3]Mediteknia, Las Palmas de Gran Canaria, Spain, [4]Dermatology, University of Miami, Miami, Florida, United States

Human hair follicles (HFs) harbor rich microbial communities, while dysbiosis of the HF microbiome is associated with hair diseases. Yet, the composition, distribution, and physiological functions of HF commensals for human HF growth, immunity, pigmentation and/or metabolism remain poorly explored. As a first step towards clarifying these items, healthy adult human scalp HFs were laser-capture-microdissected to extract DNA from defined HF compartments for metagenomic sequencing. Besides all known main human HF microbiota (e.g., Staphylococcus, Cutibacterium and Corynebacterium), new human HF-associated microbiota were identified, amongst them some extremophiles within the bulb region, supportive of a more selective and challenging environment. Since the distribution of microbial species differed between HF compartments, the HF-microbiome and/or microbe-microbe interplay that determines which commensals colonize defined HF niches need to be dissected. To begin exploring how commensals impact human HF physiology, scalp HFs were organ-cultured with butyrate (0.1 or 1 mM, for 6 days), a key short-chain fatty acid metabolite produced by the dominant commensal, S. epidermidis. This showed that butyrate (significantly) prolonged anagen, enhanced mitochondrial activity (MTCO1), and stimulated both autophagy (LC3b) and HF pigmentation (gp100). Butyrate also increased HF expression of OR2AT4, an olfactory receptor whose stimulation enhances HF production of the AMP dermcidin. This human HF microbiome charting study strongly suggests that HF commensals have clinically relevant key functions in human HF physiology well beyond interactions with the host immune system that invite therapeutic targeting.

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Murine mast cells, express zip7

Y. Muto, Y. Ogawa, S. Shimada, T. Kawamura
Dermatology, Yamanashi Daigaku Igakubu Daigakuin Sogo Kenkyubu Igakuiki, Chuo, Yamanashi, Japan

Skin is the third most zinc (Zn)-abundant tissue in the body, In the skin, mast cell (MC) granules are enriched in Zn. Cellular Zn levels are strictly regulated by two groups of Zn transporters: Zn transporters (ZnTs) and Zrt-, Irt-like protein transporters (ZIPs). ZnT expressions were previously determined in the bone-marrow derived MCs (BMMCs). In contrast, little is known about ZIP expressions. Thus, the aim of this study is to determine ZIP expressions in murine MCs. Rodent MCs are categorized into connective tissue MCs (CTMCs) and mucosal MCs (MMCs). The former and latter are presumed to correspond to human tryptase- and chymase-positive MCs (MCTC) and tryptase-positive MCs (MCT), respectively. CTMCs and MCTC are constitutively distributed in the skin, gut lamina propria, and pulmonary perivascular tissues, whereas MMCs and MCT are distributed in the mucosal epithelium of the gut and lungs. Fetal skin-derived MCs (FSMCs) which correspond to CTMCs were obtained as follows; skin from fetal mice was enzymatically digested with trypsin to prepare single-cell suspensions. Cells were cultured in a medium containing IL-3 and stem cell factor for three weeks. Non-adherent cells are collected and purified using c-kit microbeads. Although FSMCs express various ZIPs mRNA with varying degrees, ZIP7 mRNA was overwhelmingly expressed. BMMCs correspond to MMCs were obtained as follows; bone marrow of 8-10 w C57BL/6 female mice were isolated and cultured in the same medium with FSMCs for five weeks. In line with the result of FSMCs, only ZIP7 was highly expressed in BMMCs. Collectively, we elucidated that ZIP7 is highly expressed in both the connective tissue-type MCs and mucosal-type MCs. ZIP7 facilitates normal dermal development and suppresses endoplasmic reticulum stress. We are currently investigating ZIP7 function in MCs.

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Skin metaproteomics a key functional approach to study skin healthiness and resilience

C. Gonindard[1], S. Delaunois[1], R. Ter Halle[1], L. Guillotin[1], J. Monneuse[2], L. Coutos-Thevenot[2], I. Metton[2], H. Chajra[1], M. Frechet[1]
[1]*Clariant active ingredients, Toulouse, France,* [2]*Phylogene SA, Bernis, France*

Our skin health and its resilience against assaults (pollution, climatic conditions, pathogens) rests mainly on the epidermis and its microbiome. Indeed, the skin is colonized by commensal beneficial microorganisms called microbiome. This complex ecosystem is a shield for our skin ensuring protective functions while educating our immune system. Unfortunately, when both epidermis and microbiome are fragilized diseases occur. Thus, taking care of its epidermis and microbiome is crucial to preserve skin healthiness. Therefore, studying epidermis and its microbiome with adapted methodologies to clinical sampling might help to gain valuable insight during the development of solutions dedicated to skin health. The option we choose is metaproteomics, which has only been used in gut microbiota field. In contrast to metagenomics, metaproteomics provide valuable descriptive and functional insights simultaneously on these two allies with complex interplay: the epidermis and the microbiome. The aim of this work is to demonstrate in a randomized clinical study, the efficiency of a natural active ingredient (obtained from white biotechnology) to provide skin healthiness using metaproteomics. Skin swabs were sampled from women's faces before and after the use of a cosmetic formula. The results demonstrate that the active ingredient wasn't harmful to skin microbiome, keeping its composition stable. We identified 20042 peptides belonging to 4785 proteins. Among them, 343 proteins were significantly modulated (from human, fungi and bacterial origins) impacting positively anti-oxidant, proteostasis, metabolism, anti-inflammation and extra-cellular matrix pathways. Finally, these clinical results highlight that metaproteomics is a powerful technology allowing to demonstrate that modulating proteins expression at keratinocytes and microbiome levels lead clearly to clinical and visible outcomes: improvement of skin wrinkles, mechanical properties and skin barrier function.

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Change in c. acnes phylotype abundance and improvement of clinical parameters using a new dermocosmetic product containing myrtus communis and celastrol enriched plant cell culture extracts in patients with acne vulgaris.

C. Mias[1], M. Thouvenin[1], S. Dalmon[1], V. Mengeaud[2], V. Ribet[1], S. Bessou-Touya[1], H. Duplan[1]
[1]*R&D, Pierre Fabre Dermo-cosmetique, Toulouse, France,* [2]*Laboratoires Dermatologiques Ducray SAS, Lavaur, France*

Introduction: Cutibacterium acnes (C. acnes), the main bacterial species of the pilosebaceous unit, plays a major role in acne. Recent papers demonstrated that specific C. acnes phylotypes were correlated with the severity of inflammatory acne and reported a specific loss of C. acnes phylotype diversity in this context. Objectives: The aim of the study was to evaluate the efficacy of a new dermocosmetic product containing Myrtus communis and Celastrol enriched plant cell culture extracts on C. acnes phylotype abundance and clinical parameters in subjects with mild to moderate acne vulgaris. Materials/Methods: Efficacy on C. acnes phylotype abundance was evaluated by single-locus sequence typing sequencing after 57 days of twice daily application of the product. Clinical parameters of acne and product tolerance were also assessed. Results: Our study confirmed the link between the presence of some C. acnes phylotypes and acne. At day 57, the product had a positive impact on C. acnes phylotypes: a significant decrease of pro-pathogen phylotype IC and increased of non-pathogen phylotype IB were observed. In parallel, the clinicals results showed a significant decrease of inflammatory and total acne lesions, and a significant improvement of global acne evaluation score. The tolerance of the product was assessed very good. Conclusion: This study showed the topic modulation of C. acnes phylotype abundance by this new dermocosmetic product containing Myrtus communis and Celastrol enriched plant cell culture extracts associated with a significant clinical efficacy.

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Vaccinia immune evasion ankyrin-repeat/F-box protein WR199 mediates ubiquitination and proteasomal degradation of the DNA sensor cGAS

G. Mazo, N. Yang, Y. Wang, Z. Li, H. Pan, R. C. Hendrickson, A. Ordureau, L. Deng
Memorial Sloan Kettering Cancer Center, New York, New York, United States

Poxviruses are large cytoplasmic DNA viruses. Vaccinia virus is a replication-competent prototypical poxvirus that causes mortality in the murine intranasal infection model, a surrogate for smallpox infection. Modified vaccinia virus Ankara (MVA) is a highly attenuated vaccinia strain that is replication-incompetent and a safe vaccine against smallpox. The DNA sensor cyclic GMP-AMP synthase cGAS plays an important role in host antiviral immunity. cGAS is critical for the induction of type I IFN by modified vaccinia virus Ankara (MVA) in dendritic cells, and mice lacking cGAS are highly susceptible to vaccinia infection. We recently reported the identification of E5 and WR199 as potential inhibitors of cGAS. Upon MVA or vaccinia infection, the cytoplasmic cGAS is ubiquitinated and degraded via the proteasome-dependent pathway. E5 interacts with cGAS and promotes cGAS ubiquitination and degradation, and E5 itself is among the top ubiquitinated vaccina viral proteins after infection. To identify E5-interacting partners in infected dendritic cells, we generated two recombinant MVA viruses- one with E5 tagged with FLAG-APEX2 and the other with just FLAG-APEX2 replacing the E5R gene. Using APEX2 *in vivo* proximity labeling coupled with affinity purification and mass spectrometry in virus-infected dendritic cells (DCs), we identified WR199 as one of the interacting partners of E5. VACV mutant lacking WR199 (B18R) is attenuated in an intranasal infection model. MVA lacking WR199 (MVAΔWR199) infection of DCs fails to induce cGAS degradation. WR199 contains Ankyrin repeats at the N-terminus and an F-box at the C-terminus. MVA lacking the F-box of WR199 also fails to induce E5 and cGAS degradation. In addition, knockdown of components of the Skp1/Cullins/F-box (SCF) E3 ubiquitin ligase complex including Skp1 and Rbx1 abrogates MVA-induced cGAS degradation. Our results support a model in which vaccinia WR199 plays an important role in recruiting host E3 ubiquitin ligase to degrade both E5 and cGAS.

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A novel inhabitant of the wound microbiome promotes wound healing through IL-6-mediated re-epithelialization

E. White, A. Uberoi, E. Grice
Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States

The skin microbiome exists at the interface of all cutaneous wounds, but microbial community constituents with the potential to confer a beneficial wound healing response are virtually unexplored. We previously performed a metagenomic analysis of diabetic foot ulcer samples and unearthed one such candidate microbe, Alcaligenes faecalis, which was the fourth most abundant species in the cohort but has scarcely been studied in the context of wounds. We found that treating murine diabetic wounds with A. faecalis promotes early wound healing, as well as *in vivo* keratinocyte proliferation and migration in a transwell assay ($p<0.05$). To investigate the mechanism by which A. faecalis induces this pro-epithelialization phenotype, we tested if A. faecalis promotes production of inflammatory cytokines, as keratinocyte cytokine signaling is needed for epithelialization. Indeed, culturing keratinocytes in cell-free A. faecalis conditioned media induced a robust cytokine response, notably a 16-fold increase in IL-6 ($p<0.0001$), an important initiator of keratinocyte pro-healing activity. We found that sterile supernatant of A. faecalis, rather than bacterial-cell surface molecules, promotes keratinocyte migration and IL-6 production, and was sensitive to protease digestion. These results support the hypothesis that A. faecalis secretes a protein that improves re-epithelialization by enhancing keratinocyte IL-6 signaling. To further resolve the host response and bacterial mechanism, we performed spatial transcriptomics and dual RNA-seq on A. faecalis-treated wounds. We demonstrate that A. faecalis tunes transcriptional pathways up-and down-stream of IL-6 in both the epidermal and dermal compartments. This work elucidates how a prevalent constituent of the wound microbiome promotes wound healing. These mechanisms of host-microbial interactions during wound healing represent novel targets for the treatment of chronic wounds and the development of microbiota-derived therapies.

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Inhibition of neutrophil NETosis ameliorates UVB-induced skin inflammation and kidney injury in lupus mice

X. Lyu[1, 2], M. Li[1, 2], P. L. Zhang[3], W. Wei[2], V. Werth[1], M. Liu[1]
[1]*University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [2]*Tianjin Medical University, Tianjin, China,* [3]*Beaumont Health, Royal Oak, Michigan, United States*

Background: Ultraviolet B (UVB) triggers lupus flares by worsening skin lesions and systemic symptoms, including lupus nephritis. The effects of UVB-induced skin inflammation on kidney injury are not well understood. Neutrophil NETosis has been implicated in lupus. We have reported that UVB induces skin inflammation with recruitment of neutrophils that form NETs and display NET-associated cytokines. We also found that strengthening nuclear envelope integrity by lamin B overexpression decrease NETosis and NET-associated cytokines in the skin of UVB-irradiated lmnb1Tg mice. Others reported that neutrophils are involved in skin inflammation, kidney injury and transient proteinuria in wildtype mice with UVB exposure. However, the role of NETosis in UVB-mediated lupus flare in skin and kidneys has not been studied. Methods: We generated lupus prone mice with lamin B overexpression, and exposed the female MRL/lpr-lmnb1Tg and control MRL/lpr mice (8-week-old) to UVB at 150 mJ/cm2/day for 5 consecutive days. Results: We found that UVB exposure increased skin thickness and proteinuria, with increased infiltrates, NET formation, NET-associated IFNα and IL-17A, both in skin and kidneys of MRL/lpr mice. Interestingly, strengthening the nuclear envelope decreased NET formation and ameliorated the above inflammatory responses both in skin and kidneys, with decreased proteinuria and hypercellularity in the kidneys of MRL/lpr-lmnb1Tg mice as compared to their controls. Interestingly, the skin infiltrates ($r=0.57$, $p<0.05$) and NET formation in skin ($r=0.54$, $p<0.05$) were positively correlated with proteinuria. Importantly, hypercellularity ($r=0.65$, $p<0.01$), NET formation ($r=0.56$, $p<0.05$), NET-associated IFNα ($r=0.49$, $p < 0.01$) or IL-17A ($r = 0.47$, $p < 0.05$) in glomeruli were positively correlated with proteinuria. Conclusion: Inhibition of NET formation by overexpression of lamin B can ameliorate UVB-triggered skin inflammation, proteinuria and kidney injury in young lupus prone mice.

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Qualitative analysis of dermatophytosis in experimental *in vivo* and in vitro models relies on robust preparation of spores

E. Faway[1], C. Staerck[2], C. Danzelle[2], B. Mignon[2], Y. Poumay[1]
[1]*URPHYM-NARILIS, Faculty of Medicine, Universite de Namur, Namur, Belgium,* [2]*FARAH, Faculty of Veterinary Medicine, Universite de Liege, Liege, Belgium*

Dermatophytoses are superficial infections of keratinized structures of the host caused by filamentous fungi named dermatophytes. They are the most common mycosis worldwide, with an incidence estimated around 20% in humans and in constant increase since the last decade. Because of this high incidence and the growing emergence of antifungal resistant strains, a better understanding of the mechanisms deployed by dermatophytes to adhere on and to invade host tissues is required for further identification of therapeutic targets. Recently, several models have therefore emerged worldwide in order to study the pathogenic steps involved during dermatophytosis. However, experimental procedures used to grow and condition fungi as infectious spores were quite various, leading to diverse composition of inocula, thus rendering difficult the overall interpretation of data collected with the available models. By assessing various culture parameters, we identified growth on potato dextrose agar at 30°C and under 12% of CO2 as the optimal condition improving the sporulation and viability of several dermatophyte species frequently isolated from human lesions, including Trichophyton rubrum and Trichophyton benhamiae. The resulting suspensions are characterized by a more homogenous composition, containing exclusively unicellular spores, and in a sufficient amount for further use as inoculum on experimental models of infection, like *in vivo* mouse model, or in vitro reconstructed human epidermis (RHE). Furthermore, as demonstrated during infection on RHE, the use of these suspensions as inoculum improves adhesion ability of spores, and increases the reproducibility of keratinocyte responses. In conclusion, this research paves the way towards a standardized procedure for the production of inocula used in experimental models of dermatophytosis, leading to more uniform results and facilitating the comparison of observations between models.

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An aging-susceptible circadian rhythm controls cutaneous antiviral immunity

S. Kirchner[1, 2], V. Lei[1], J. Shannon[1, 3], D. Corcoran[4], D. Hughes[5], D. Waters[5], K. Dzirasa[5, 6, 7], J. Coers[2], A. MacLeod[1, 2, 3], J. Zhang[1, 8]
[1]*Dermatology, Duke University, Durham, North Carolina, United States,* [2]*Molecular Genetics and Microbiology, Duke University, Durham, North Carolina, United States,* [3]*Immunology, Duke University, Durham, North Carolina, United States,* [4]*Center for Genomic and Computational Biology, Duke University, Durham, North Carolina, United States,* [5]*Neurobiology, Duke University, Durham, North Carolina, United States,* [6]*Psychiatry and Behavioral Science, Duke University, Durham, North Carolina, United States,* [7]*Biomedical Engineering, Duke University, Durham, North Carolina, United States,* [8]*Pathology, Duke University, Durham, North Carolina, United States*

Aged skin is prone to viral infections, but the mechanisms responsible for this are unclear. We hypothesize that an aging circadian rhythm compromises cutaneous innate antiviral immunity. In support of this hypothesis, we observed that aged mammalian skin exhibits significant reduction in transcriptional levels of antiviral proteins (AVPs) and circadian transcriptional regulators including BMAL1 and CLOCK, *in vivo* and in vitro. Using BMAL1 and CLOCK knockout mouse models, we found that loss of circadian factors significantly reduces AVP expression in the skin. Rhythmic AVP expression was also detected in cultured human keratinocytes and significantly decreased by siRNA-mediated gene silencing of CLOCK. Importantly, augmentation of circadian rhythms using nobiletin significantly reduced Herpes simplex virus infectious output from keratinocytes and human skin explant cultures. Further, the circadian-dependent regulation of antiviral genes declined in mice lacking the type I interferon receptor (Ifnar1-/-) and in mice with CD301b+ leukocyte-targeted deletion of IL-27. These findings highlight a novel, evolutionally conserved, and age-sensitive circadian regulatory axis of cutaneous antiviral immunity involving both leukocyte-mediated and keratinocyte-intrinsic processes. Our data implicate circadian restoration as a potential adjuvant strategy for combating viral infections in aged skin.

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Is scalp microbiota different between dry and oily dandruff states?

M. Maitre
PFDCPC, Toulouse, France

Scalp microbiota is characterized by the presence of Malassezia, Cutibacterium and Staphylococcus genus. Oily dandruff (OD) is associated to known modifications in scalp microbiota's composition. What about the microbiota associated with dry dandruff (DD)? To date, the literature doesn't answer this question. The aim of this study was to compare OD scalps' microbiota vs DD scalps' microbiota, based on metagenomic data. Method: Two open studies with 16 OD subjects and 17 DD subjects with mild to moderate scaly state at baseline were done. Genomic DNA extraction and NGS sequencing from Swab sampling of the 2 groups were performed. After microorganisms' identification or absolute quantification of specific strains, different statistical analysis were run according to the nature of data. Results: OD and DD dandruff states of both volunteer groups were validated using clinical observations. Differential analysis of microbiota from the two groups showed the following significant differences: (i) OD scalps' Mycobiota is characterized by unknown species of Malasseziales order while Filobasidium genus are more present on DD scalps, (ii) Cutibacterium and Staphylococcus genus dominate OD and DD scalps' bacteria populations. (iii) under-represented microorganisms' communities are different between OD and DD states. Pelomonas, Capnocytophaga or Ralstonia genus are more present on DD scalps while Brachybacterium are preferentially observed on OD scalps. Absolute quantification results underline M. restricta is associated with OD scalps. Conclusion: According to published data, dehydration and low sebum production are characteristic of DD scalps while OD scalps display high sebum production and inflammation. Interestingly, we have shown here that scalp microbiota of these two dandruff states are different. These results highlight importance to address DD and OD scalps with specific scalp care products.

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A dynamic interplay between dermal lipolysis and adipogenesis in regulating psoriatic skin inflammation

T. Xia, R. Wu, W. Zhang, X. Zhang, S. Wu, Y. Liao, Y. Liu, L. Sun, J. Li, L. Zhang
School of Pharmaceutical Sciences, Xiamen University, Xiamen, Fujian, China

Psoriasis, a common inflammatory skin disease with a worldwide incidence of approximately 2%, is significantly associated with obesity. Obesity promotes a pathological expansion of the dermal white adipose layer (dWAT), but whether and how dWAT plays a role in shaping the dermal immune response during psoriasis pathogenesis is unclear. Here by the imiquimod (IMQ)-induced psoriasis-like mouse model, we found that IMQ triggered a transient loss of dermal mature adipocytes during the acute inflammatory phase followed by a robust dermal adipogenesis response, including proliferation of adipocyte progenitor (AP) and new adipocyte formation, during the regression phase of skin inflammation. Using adipocyte lineage tracing mouse model, we showed that IMQ application promoted dedifferentiation of mature adipocytes into a population of highly pro-inflammatory and proliferative AP cells, which can re-differentiate into new adipocytes. Furthermore, scRNAseq analyses of IMQ-treated mouse skin samples and/or in vitro culture experiments validated that activated AP cells were pro-inflammatory whereas differentiated early adipocytes were anti-inflammatory. Finally, immunohistological and scRNAseq analyses of human skin samples validated our observations from mice in human psoriatic skin. Together our results have suggested that innate immune responses of dermal adipocytes and adipocyte progenitor may play an important role in shaping dermal immune system activation during acute phase of psoriasis and in inflammation resolution during disease regression. Results from our study provide novel insights into why obesity is a risk factor for psoriasis, and targeting lipolysis and/or adipogenesis maybe a novel therapeutic approach to treat psoriasis.

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Filaggrin deficiency confers an altered early life T cell response to commensal skin bacteria

J. Gonzalez, T. Scharschmidt
Department of Dermatology, University of California San Francisco, San Francisco, California, United States

Mutations in filaggrin underlie ichthyosis vulgaris (IV) and contribute to increased risk of Atopic Dermatitis (AD), conditions typified by disruption of skin microbial communities and the cutaneous immune response. Yet, it remains unclear how neonatal skin barrier compromise in the setting of filaggrin deficiency alters the quality of commensal-specific T cells and the role of such responses in heightened skin inflammation. To test this we colonized the skin of neonatal flg-/- pups with a strain of Staphylococcus epidermidis engineered to express the model antigen 2w (S. epi-2w). Using MHC-II tetramers to identify 2w-specific CD4+ T cells, we have previously shown that wild-type (wt) mice colonized neonatally with S. epi-2w develop immune tolerance to S. epi via enrichment of commensal(2w)-specific regulatory T cells (Tregs). In contrast, flg-/- pups demonstrated a dominant effector CD4+ (Teff) response, with substantially reduced percentages of S. epi-specific Tregs in the skin-draining lymph node (LN) at D21 and in the skin after adult 2w re-exposure. scRNAseq of flg-/- and wt D12 keratinocytes did not reveal substantially altered signatures, nor did TEWL measurements, consistent with an intact inside-out barrier function. However, the skin of flg-/- neonates demonstrated elevated calcium dye penetration and IL-1 levels, suggesting potential compromise of outside-in barrier function. As pediatric AD and IV share an IL-17 signature, we examined cytokines produced by S. epi-specific Teffs in flg-/- mice and found these to be enriched for Th17 cells. To examine the consequence of this altered response to commensals, we colonized flg-/- and wt pups with S. epi-2w then re-challenged them as adults with imiquimod and S. epi-2w re-exposure. Flg-/- mice demonstrated heightened S. epi-specific Th17 and significantly more ear swelling. In summary, our work suggests that filaggrin deficiency significantly impacts the quality of the commensal-specific CD4+ response early in life, with enduring consequences for skin immune homeostasis.

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Tinea pedis: Evidence for a dysbiosis of the foot microbiome

R. Brucker, D. Bolshakov, S. Shen, N. Jovanovic, B. Sakhamuri, M. Megeressa, X. Zhang, K. Beutner
DermBiont, Inc., Boston, Massachusetts, United States

Historically, Trichophyton rubrum has been identified as the causative agent of the disease, Tinea pedis. Treatment options focus on the use of antifungals; however, antifungals have modest efficiency for clearing T. rubrum with high rates of reinfection and disease reoccurrence. Here we propose that T. pedis is not a simple fungal infection but a complex dysbiosis characterized by changes of the fungal and bacterial profile of the microbiome, with Trichophyton being an indicator of the disease. In this study, we used metagenomic sequencing to compare the microbiome composition of the interdigital toe web-space, nail bed, and plantar aspect in a population of individuals with T. pedis simple (n=26), T. pedis complex (n=25), and T. pedis moccasin (n=25) against 33 healthy volunteers and their matching anatomical sites. Metagenomic sequencing demonstrated differences in beta diversity and key species distinctions between diseased (T. pedis) and healthy states. We found that beta diversity differs greatly between these two groups, and that the microbiomes of individuals with T. pedis were significantly more similar to each other than the micobiomes of healthy individuals. There are also robust marker species denoting both T. pedis and healthy states. Specifically, Trichophyton was found in both healthy and diseased subjects but higher in abundance for T. pedis subjects. Other fungi had similar co-associations with the disease state. Bacterial co-occurrences with Corynebacterium resistens had the highest association in subjects with T. pedis, while Staphylococcus hominis and S. epidermidis were significantly higher in healthy individuals as compared to individuals with T. pedis. These results indicate that T. pedis is not a strict infection model of disease, but rather a dysbiosis associated with a community of organisms. A therapeutic approach to repair the dysbiosis has the potential for a more effective diagnostic and treatment plan for patients, with better long-term outcomes.

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Commensal microbes can regulate skin barrier through the control of tryptophan-aryl hydrocarbon receptor signaling cascade

A. Uberoi[1], C. Bartow-McKenney[1], A. Campbell[1], Q. Zheng[1], L. Flowers[1], C. Mesaros[1], C. H. Sutter[2], T. R. Sutter[2], E. Grice[1]
[1]*Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [2]*The University of Memphis, Memphis, Tennessee, United States*

Commensal microbes are critical in maintaining skin homeostasis. However, their mechanisms of crosstalk with host epithelia during barrier disruption and repair are not defined. Using germ free (GF) mice, we have recently demonstrated that microbiota is necessary and sufficient for proper differentiation and repair of the epidermal barrier. By comparing epithelial transcriptomes of GF mice to specific pathogen free (SPF) mice we found that microbiota regulate genes involved in epithelial development and differentiation. GF mice were impaired in barrier repair compared to SPF mice following tape-stripping, as measured by transepidermal water loss. We identified the aryl hydrocarbon receptor (AHR) pathway, a regulator of epidermal differentiation, as downregulated in GF epidermis. Activating AHR in GF mouse skin through a topical AHR-activator rescued impaired barrier repair function of GF skin. We found that colonization with a defined consortium of human skin commensals curated from healthy human skin restored barrier competence in AHR-dependent manner. Tryptophan (Trp) metabolites are potent AHR ligands and cornified skin envelope is a rich source of substrates for Trp metabolism by microbes. We constructed Trp metabolic enzyme profiles and mined them against healthy human skin metagenomes and microbial genomes in the defined consortium. We found motif enrichment for enzymes that metabolize Trp to indole and its derivatives, e.g., indole-3-acetaldehyde, indole propionic acid. We tested 14 indole-derived metabolites and identified n=4 therapeutic candidates that improve skin barrier in vitro in primary keratinocytes and in reconstructed human epidermis. We reveal a fundamental mechanism whereby the microbiota regulates skin barrier formation and repair through Trp metabolism, with far-reaching implications for the numerous skin disorders characterized by epidermal barrier dysfunction.

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WITHDRAWN

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Metformin and akkermansia muciniphila attenuated psoriasiform dermatitis exacerbated by short-term exposure to glucose

Z. Shi, L. Wang
Sun Yat-Sen Memorial Hospital, Guangzhou, Guangdong, China

We have previously shown that short-term feeding (<1 month) with a high-sugar, moderate-fat diet (i.e., Western diet) is enough to induce clinically and molecularly-detectable psoriasiform dermatitis (PsD), suggesting that certain diet patterns could contribute to the development of PsD. However, the role of specific dietary components like sugar remains to be elucidated. Herein, we showed that short-term exposure to glucose (4 weeks) enhances the susceptibility of mice to PsD induced by imiquimod (IMQ). After weaning, C57BL/6 mice received glucose-sweetened water (GW, 15% w/w) or normal drinking water (NW) for 1month and then was induced PsD by application of topical IMQ for 5 days. In the absence of significant body mass increase compared to NW-fed mice, GW-fed mice developed a more severe phenotype of skin inflammation, as measured by an increase in ear thickness and psoriasis severity score (≥2-fold increase), histological markers including epidermal thickness and numbers of Munro microabscess, infiltration of neutrophils (8-fold increase), and mRNA levels of neutrophil chemoattractants (e.g., CXCL1 and CXCL2). Oral administration of metformin, an anti-diabetic drug, dramatically improved skin inflammation in GW-fed mice and, to a lesser extent, in NW-fed mice. Interestingly, metformin-treated, GW-fed mice showed higher levels of Akkermansia muciniphila (A. muciniphila) in the gut, compared to both metformin-treated, NW-fed mice as well as vehicle-treated, GW-fed mice. Daily oral gavage of pasteurized A. muciniphila improved the skin inflammation in NW-fed and GW-fed mice, as measured by decreased ear thickness and psoriasis severity score (50% reduction). Thus, our data revealed the influences of glucose in inflammatory signaling in the skin, supporting a critical role of dietary components in the pathogenesis of PsD. Our results also raised the prospect of metformin and probiotic A. muciniphila as adjuvant therapies for treating psoriasis patients, especially those with excessive sugar intake.

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Modulation of cutibacterium acnes phylotypes after treatment with epilobium fleischeri extract

D. Imfeld[1], E. Klaassens[2], J. Claypool[3], M. Roche[4], R. Sfriso[1]
[1]*R&D PCA, DSM Nutritional Products AG, Kaiseraugst, Aargau, Switzerland,* [2]*BaseClear BV, Leiden, South Holland, Netherlands,* [3]*DSM Nutritional Products Ltd, Lexington, Massachusetts, United States,* [4]*Newtone, Lyon, France*

The cutaneous microbiota is being increasingly considered as fundamental to the maintenance of healthy skin. Recently we described the composition of the skin microbiota on five different facial sites (IFSCC conference 2021) and the changes after treatment with an Epilobium fleischeri (EF) plant extract. We performed a randomized and placebo-controlled clinical study with Caucasian female volunteers (n=23), involving a twice daily, facial application of the leave-on test product containing 3% of EF extract for four weeks. Microbiome samples were collected via swabbing and microbial profiling was performed by 16S rRNA sequencing. We found Cutibacterium acnes (C. acnes) being the most abundant species on all the facial sites, ranging from 90% on the forehead, down to 75% in the lateral cheek. As shown previously, sebum levels were significantly reduced ($p < 0.05$) after four weeks of active product application. Furthermore, the EF extract showed to be prebiotic, as it selectively modulated skin microbiota composition and provided a skin measurable benefit (sebum reduction). In addition, we could show a 38% reduction of facial skin porphyrins on volunteers applying the active product. Acne-associated C. acnes strains are known to secrete high level of porphyrins and inflammatory mediators. Hence, we now used the same swabbing probes and applied single-locus sequence typing (SLST) to analyze possible changes within the C. acnes population down to the specific phylotype level. We present the results of the SLST analysis showing the specific occurrence of C. acnes phylotypes and changes of phylotype distribution after the EF treatment. The results are correlated to the observed changes on porphyrin levels and conclusions will be made on the possible skin benefits obtained by the treatment.

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Gut dysbiosis is associated with the development of alopecia areata

T. Sezin[1], A. Abdelaziz[1], Y. Gupta[2], M. Isha[1], J. Chen[1], S. Brigitte[1], E. Wang[1], S. Sanna-Cherchi[2], D. Zhenpeng[1], L. Bordone[1], R. Perez-Lorenzo[1], A. M. Christiano[1]
[1]Dept. of Dermatology, Columbia University, New York, New York, United States, [2]Division of Nephrology, Dept. of Medicine, Columbia University, New York, New York, United States

Alopecia areata (AA) is a highly prevalent autoimmune disease (AD) leading to hair loss in affected individuals, in which both genetic and environmental factors likely play a role. Associations between changes in the microbiome composition and many ADs have been reported, however, a characterization of the role of gut microbiota in the development of AA has not been undertaken. To investigate the functional relevance of gut microbiota to AA development *in vivo*, we depleted the gut microbiome in the C3H/HeJ mouse model of AA with broad-spectrum antibiotics. We found that antibiotic-treated mice were protected from AA induction. Additionally, using 16S rRNA gene sequencing (16SrRNA Seq) we found that AA-affected mice show an overrepresentation of a single species of Ligilactobacillus, Ligilactobacillus murinus (L. murinus), that preceded the development of the first clinical signs of AA. Further, using L. murinus specific primers, we showed that co-housed C3H/HeJ mice protected from AA show reduced levels of L. murinus in comparison to mice affected with AA, suggesting that L. murinus is associated with the development of AA. Examining the effects of the microbiome on T cell composition, we found that gut microbiota-depleted mice had a decreased percentage of effector CD8+ T cells in skin draining lymph nodes, which we previously showed to be required to drive the pathogenesis of AA. To establish the relevance of these observations to human AA, we performed 16SrRNA Seq sequencing and metagenomics and compared gut microbiome composition in stool samples between AA patients and healthy controls (HCs). We discovered a striking gut dysbiosis in AA patients compared to HCs and identified species of Lachnospiraceae and Bacteroidaceae to be overrepresented in AA. Taken together, these findings suggest that gut microbiome dysbiosis contributes to the pathogenesis of AA and offer new avenues of treatment for this disease.

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Botanical inhibitors of staphylococcal virulence: A new path toward mitigating atopic dermatitis severity?

C. Quave
Dermatology and Human Health, Emory University School of Medicine, Atlanta, Georgia, United States

Plants have served as the fundamental basis for the human pharmacopoeia since ancient times. Today, roughly 9% of all plant life on Earth has a documented use in traditional medicine. We have studied the chemistry and antimicrobial activity of more than 700 plant species, including those used in traditional medicine in topical formulations to treat inflammatory skin diseases, such as atopic dermatitis (AD). From this work, we have identified several small-molecule natural products that act against staphylococci as quorum sensing inhibitors in the absence of growth-inhibitory effects. The presence of a high staphylococcal burden in AD flare sites has been well established in the literature. However, recent studies suggest that bacterial burden at inflamed sites may not be the most crucial skin microbiome factor contributing to disease severity, but instead, secreted microbial exotoxins likely play an essential role. In a pilot study conducted with children and young adults, we recently reported significant correlations ($P < 0.0001$) between strain-specific hemolytic capacity among Staphylococcus aureus and coagulase-negative staphylococci with AD disease severity as determined by SCORAD. Here, the activity of a novel hydroperoxyl-cycloartane (IC50: 31.7 μM) and triterpenoid acids (IC50: 2-70 μM) from medicinal plants against staphylococcal quorum sensing and subsequent virulence factor production is presented, and the potential utility of these botanical ingredients in mitigating AD through selective inhibition of staphylococcal exotoxin production is explored.

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The role of siglecs in acne pathogenesis

T. Tran[1], M. Qin[2], G. Agak[2], R. Teles[2], A. Baugh[1], T. To[1], J. Kim[2]
[1]University of California Los Angeles David Geffen School of Medicine, Los Angeles, California, United States, [2]Division of Dermatology, Department of Medicine, David Geffen School of Medicine, Los Angeles, California, United States

Acne is a disease of the PSU and several factors have been associated with its pathogenesis including the bacterium Cutibacterium acnes (C. acnes). C. acnes has been shown to trigger inflammation in acne by activating toll-like receptor 2 (TLR2). Furthermore, sialic acid-binding immunoglobulin receptors (Siglecs) on innate immune cells have also been shown to regulate immune response by TLRs. In this study we isolated punch biopsies from lesional and non-lesional skin and used single-cell RNA sequencing (scRNA-seq) to identify the macrophage immune landscape. We found that there was a higher proportion of M2 to M1 macrophages in lesional skin. We next examined for the expression of siglecs on macrophages and observed that M1 macrophages exhibited lower levels of siglec expression in comparison to M2 macrophages. Importantly, M2 cells expressed higher levels of siglecs -3, and -9. Siglec-9 has been studied before in relation to M1 and M2 gene expression and their inflammatory profile. To further understand specific functions of siglecs, we isolated human M1 and M2 macrophages and stimulated with C. acnes. We observed that M1 cells had higher production of IL-6 compared to M2 cells while M2s secreted higher levels of IL-10 in comparison to M1 cells. We next looked at the effect of blocking siglec -3 and -9 using siRNA. We observed that blocking siglec-9 in M2 macrophages had no effect on IL-6 production but had a significant decrease in IL-10 production. On the other hand, blocking of siglec-3 in M1s did not have an effect on IL-10 and IL-6. Overall, our data suggest that C. acnes induction of anti-inflammatory responses by M2 macrophages may be regulated by siglec-9. Further studies are needed to identify the precise role of siglecs in acne pathogenesis which may provide insight into the regulation of acne inflammatory pathways.

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A systems biology analysis of peripheral blood and cell subsets from patients with psoriasis and psoriatic arthritis reveals biological sex-based endotypes

H. B. Lindley[1], B. Richardson[1], J. Golden[1], B. Tamilselvan[1], W. Lin[1], D. Gruszka[1], *N. L. Ward[2], T. McCormick[3], K. Cooper[3], C. A. Cameron[1], M. Cameron[1]*
[1]Population and Quantitative Health Sciences, Case Western Reserve University, Cleveland, Ohio, United States, [2]Vanderbilt University, Nashville, Tennessee, United States, [3]Dermatology, Case Western Reserve University, Cleveland, Ohio, United States

We are conducting a systems biology analysis integrating psoriasis clinical phenotypes with molecular phenotypes to discover what cellular processes might be operant in determining psoriasis endotypes. Our goal is to promote biomarker discovery in informing more precise care of individuals with psoriasis and their comorbidities. We performed RNA-Seq on peripheral blood from 102 psoriasis and psoriatic arthritis patients and controls. Also, to assess the contribution that peripheral immune cell subsets have in psoriasis pathophysiologic states, we selected a subset of patients and controls (n=38) and sorted samples by flow cytometry into intermediate monocyte, classical monocyte and CD3+/CD4+ T cells. We analyzed the datasets by performing linear contrasts between psoriasis and controls or running linear regression analysis against over 100 clinical, treatment and demographic variables. Top significantly differentially expressed genes (DEG; $p<0.05$) for each covariate were determined along with significantly enriched pathways (GSVA). Peripheral blood gene expression signatures for each clinical variable were identified through feature selection (machine learning) to yield endotype-defining gene lists. We discovered multiple peripheral blood biomarker datasets unique to endotypes denoted by biological sex. Our analysis revealed unique interferome (IFN) and inflammasome genes associated with the most severe clinical phenotypes with intermediate monocytes exhibiting robust type I IFN pathway expression, classical monocytes exhibiting genes related to sustained inflammation, and the intersection of the two subsets consisting of type I and II IFNs. Our transcriptomic analysis identified endotype biomarkers related to the interplay between clinical and molecular phenotypes.

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Dendritic cell programming in vaccinia skin scarification

J. Hsu
Immunology and Pathogenesis, Weill Cornell Medicine, New York, New York, United States

Smallpox is one of the deadliest viral diseases in human history, killing an estimated 300 million since 1900 alone. Edward Jenner's 1796 discovery that skin inoculation with cowpox in a scarification method of delivery[i] [ii] induced immunity against smallpox led to the global eradication of the disease by 1980[iii]. In recent years, the vaccinia virus model derived from cowpox was used to demonstrate that vaccinia virus skin scarification (VACVss) is a superior immunization strategy to other methods of vaccine delivery[iv]. Relative to intradermal or intramuscular injection (i.m.), skin scarification generates optimal adaptive immunity, indicated by VACV-specific CD8+ T memory and IgG titers[v]. The cellular mechanisms behind the requirement for skin scarification have not been elucidated. To test how skin scarification confers superior protective immunity, we wanted to test how Dendritic Cell (DC) directed immunity is modified by scarification, focusing on the requirement for Batf3-dependent[vi] CD103+ dermal DC1s and expression of the DNGR1 death receptor to cross-present viral antigens and achieve successful smallpox immunity[vii],[viii],[ix],[x],[xi]. However, it is unknown how scarification contributes to modified CD103+ responses and licensing. We generated an RNA microarray dataset was generated in order to examine transcriptomic changes in DC1 cells in response to skin scarification (VACV SS)[xii]. Transcriptome analysis revealed migratory DC1s (CD103+ migratory DCs) have distinct gene modules responding to the VACVss, supporting candidate pathways to interrogate productive licensing cues.

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Tralokinumab does not affect endogenous IL13Ra2-mediated regulation of free IL-13

M. A. Tollenaere, C. Mølck, H. Heibroch Petersen, H. Norsgaard
Skin Research, LEO Pharma A/S, Ballerup, Denmark

Tralokinumab, a fully human monoclonal antibody specifically targeting the IL-13 cytokine, has demonstrated clinical efficacy and safety in patients with moderate-to-severe atopic dermatitis. Tralokinumab binds IL-13 with high affinity which prevents the interaction of IL-13 with IL-13Ra1 and thereby inhibits signaling of IL-13 through the IL-13Ra1/IL-4Ra receptor complex. Similarly, tralokinumab-bound IL-13 cannot bind to IL-13Ra2, a proposed decoy receptor which binds IL-13 and regulates extracellular IL-13 levels through receptor-mediated internalization. Considering that the affinity for IL-13Ra2 binding to IL-13 is more than 1000-fold higher compared to the affinity for tralokinumab to IL-13, it has not been fully elucidated to what extent tralokinumab interferes with the endogenous regulation of IL-13 through IL-13Ra2. In this mechanistic study, we used a range of in vitro biophysical (SPR), biochemical (HTRF) and cellular assays (high-content microscopy), to investigate the effect of tralokinumab on the interaction between IL-13 and the IL-13Ra1 and IL-13Ra2 receptors, as well as the effects on IL-13Ra2-mediated IL-13 internalization. We demonstrate that while the IL-13/tralokinumab complex is unable to bind IL-13Ra2, any excess IL-13 that is not bound by tralokinumab (i.e., free IL-13) can be bound by IL-13Ra2 and subsequently internalized, regardless of the presence of tralokinumab. In addition, we found that tralokinumab is able to displace IL-13 from the ternary IL-13Ra1/IL-13/IL-4Ra complex but is unable to displace IL-13 from IL-13Ra2. This suggests that the affinity for tralokinumab to IL-13 is sufficiently strong to compete for IL-13 binding to the signaling receptor (IL-13Ra1), but not IL-13Ra2. In summary, our study indicates that IL-13 signaling via IL-13Ra1 can be prevented by tralokinumab through binding of IL-13 and even displacing IL-13 from IL-13Ra1. Furthermore, IL-13 can be internalized through IL-13Ra2 and tralokinumab does not interfere with endogenous regulation of free IL-13 through this pathway.

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ME3183, a novel phosphodiesterase-4 (PDE4) inhibitor, showed potent suppressive effects on pathogenic cytokine expression in cultures of peripheral blood mononuclear cells (PBMC) from psoriasis patients and on psoriatic features in mouse models

I. Okura[1], C. Kaji[1], A. Kano[1], Y. Tada[2]
[1]*Meiji Seika Pharma Kabushiki Kaisha, Chuo-ku, Tokyo, Japan,* [2]*Dermatology, Teikyo Daigaku, Itabashi-ku, Tokyo, Japan*

Introduction & Objectives: PDE4 plays an important role in the immune system. ME3183, an oral PDE4 inhibitor, has shown anti-inflammatory effects in non-clinical studies. Here, we evaluated the therapeutic potency of ME3183 for psoriasis both in vitro and *in vivo* and compared it with apremilast, a marketed oral PDE4 inhibitor. Methods: In the in vitro assay, cultures of PBMC from 10 psoriasis (Ps) patients were stimulated with lipopolysaccharide (LPS) in the presence of ME3183 or apremilast, and the level of tumor necrosis factor-alpha (TNF-α) was measured. In the psoriasis model, imiquimod was applied on shaved dorsal skin for 6 consecutive days. In the arthritis model, collagen antibody was injected on Day 0 and LPS on Day 3. In both models, ME3183 or apremilast was administered twice daily, and clinical score and histological findings were evaluated. Gene expression by real-time polymerase chain reaction and cell population were analyzed in the psoriasis model and paw thickness in the arthritis model. Results: In cultures of PBMC from Ps patients, ME3183 showed a 114-fold inhibitory activity against TNF-α production than apremilast. We next evaluated the effects of ME3183 on psoriasis and arthritis *in vivo*. In the psoriasis model, ME3183 significantly ameliorated the psoriatic score, epidermal thickness, gene expressions of interleukin (IL)-17A and IL-23p19 and skin infiltration of IL-17A+ cells. In the arthritis model, ME3183 significantly suppressed the arthritis score and paw thickness. In both models, ME3183 was effective at low doses compared to apremilast. Conclusions: Our results indicated that ME3183 had potent anti-psoriatic effects via suppression of pathogenic cytokine expression. ME3183 may have higher therapeutic effects on Ps and psoriatic arthritis compared to the current oral PDE4 inhibitor. The clinical efficacy of ME3183 will be evaluated in the P2a study with Ps patients.

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Preliminary results from an escalating dose cohort study of VT30, a topically formulated phosphatidylinositol 3-kinase inhibitor (PI3K), intended as a treatment for cutaneous vascular malformations associated with underlying PIK3CA or TEK mutations

K. E. Truitt, L. Pugliese
Clinical, Venthera, Inc., Palo Alto, California, United States

Purpose: This report gives findings from an (ongoing) Phase 1b, escalating dose, clinical trial, assessing the safety and tolerability of VT30 Topical Gel as a treatment for venous, lymphatic and mixed venolymphatic lesions of the skin (VM, LM and VLM, respectively), driven by inappropriate activation of intracellular PI3K. Methods: After giving informed consent and completing screening evaluations that included a confirmation of underlying genotype (PIK3CA or TEK mutation), adult subjects with cutaneous VM, LM or VLM lesions were allocated to receive 4 weeks (wks) of Topical VT30 Gel, applied once daily to a 140 cm2 target treatment area, with lesion involvement. Four sequential, open-label escalating dose cohorts of 3 subjects each (12 total) were designed for sequential completion as follows: Cohort 1, 2 wks of 0.12% (concentration strength) gel, followed by the final 2 wks at 0.6%; Cohort 2, 2 wks of 0.6% gel, followed by the final 2 wks at 1.2%; Cohort 3, 2 wks of 1.2 % gel, followed by the final 2 wks at 2.3%; and Cohort 4, a full 4 wks at the maximum tolerated or maximum feasible dose (MTD or MFD, respectively) concentration. Results: 23 potential subjects were screened to allocate a total of 12 - 7 were positive for a PIK3CA mutation, and 5 had an underlying TEK mutation. Over 12 subjects treated in Cohorts 1 – 4, VT30 topical gel has been generally safe and tolerated. To date, patient assessments have reflected a phenotypically heterogeneous group. Investigators have reported changes consistent with an improvement in underlying vascular lesions. Adequate tissue-drug levels have been documented in association with a pharmacodynamic readout suggesting local PI3K inhibition. No circulating drug has been detected in plasma. Conclusions: VT30 Topical Gel's emerging profile is consistent with a potential treatment for patients with vascular malformations of the skin, driven by inappropriate PI3K activation.

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Molecular characterization of trametinib-induced cardiotoxicity

T. C. Beck, J. Morningstar, D. Arhontoulis, L. Guo, G. Cortney, R. Biggs, K. Moore, N. Koren, T. Petrucci, R. Mukherjee, K. Helke, S. Vaena, M. Romeo, R. Norris
Medical University of South Carolina, Charleston, South Carolina, United States

Trametinib is a MEK1 inhibitor used in the treatment of BRAF V600E or V600K mutated melanoma. Roughly 10% of patients develop a cardiomyopathy following chronic trametinib exposure. Although described clinically, the molecular landscape of trametinib-induced heart failure has not been characterized. We hypothesized that trametinib promotes widespread transcriptomic changes consistent with drug-induced cardiotoxicity. Mice treated with trametinib (1 mg/kg/day) had an average survival time was 69 days. Mice exhibited a significant reduction in ejection fraction, fractional shortening, and stroke volume, consistent with cardiomyopathy-induced heart failure. Histological analysis of the heart unveiled atrophied cardiomyocytes and disorganized myocardium with preserved sarcomere structure. Intramyocardial calcifications were observed in two animals. Bulk RNA-sequencing identified 435 differentially expressed genes (DEGs) following trametinib treatment. Over-represented DEGs were identified in several pathways, including microRNAs in cancer, PI3K-Akt signaling, focal adhesion, and p53 signaling. Upstream gene analysis predicted IL-6 as a regulator of 17 relevant DEGs. An Upstream Chemicals analysis identified doxorubicin, a cardiotoxic drug, as a chemical with comparable chemical-to-gene regulatory interactions based on patterns of downstream gene expression (80% similarity). Notably, an 11-fold downregulation of the apelin receptor (APJ) was observed following trametinib treatment. The APJ-apelin system regulates cardiac contractility and suppression of the axis is associated with doxorubicin cardiotoxicity. Studies examining the serum apelin and IL-6 levels are ongoing. The identification of serum biomarkers predictive of trametinib-induced cardiotoxicity may reduce the need for invasive cardiac monitoring and improve outcomes in patients receiving trametinib for metastatic melanoma.

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Difamilast, a new topical PDE4 inhibitor, ameliorates not only chronic allergic dermatitis but also idiopathic dermatitis induced by psychological stress in mice

N. Arichika, H. Hiyama, T. Nakashima, M. Shibamori, H. Urashima
Otsuka Seiyaku Kabushiki Kaisha, Tokushima, Japan

Difamilast is a novel PDE4 inhibitor with the strongest inhibitory activity against PDE4B among the four PDE4 subtypes. Moizerto® ointment (0.3%, 1% difamilast) has been recently approved for treatment of atopic dermatitis (AD) in Japan. Exogenous antigen stimulation or psychological stress can trigger or exacerbate AD symptoms. In this study, we evaluated the therapeutic effects of difamilast, tacrolimus, and betamethasone valerate on two different mice models of dermatitis: one was chronic contact hypersensitivity (CCH) induced by repeated application of hapten every 2 days for 52 days, the other was idiopathic dermatitis due to persistent self-scratching induced by application of hapten three times a week for 16 weeks, followed by individual housing (social isolation) for 6 months. Efficacy was assessed by repeated topical application of each drug for 4 or 6 weeks after each dermatitis established. In the CCH model, difamilast ointment group significantly improved skin hyperplasia from 0.03% to 3% dose-responsibly with inhibition of inflammatory cell infiltration such as T cells, eosinophils, and neutrophils. Betamethasone valerate ointment (0.12%) improved skin hyperplasia more strongly than 3% difamilast ointment. Tacrolimus ointment (0.1%) temporarily improved skin symptoms, but eventually had no effect. In the idiopathic dermatitis model, difamilast (1%, 3%) significantly improved skin symptoms with a decrease in MIP-1 alpha and MIP-2 content. Tacrolimus (0.1%) slightly improved skin symptoms, while betamethasone valerate (0.1%) exacerbated it. Notably, difamilast ameliorates both CCH and psychological stress-induced idiopathic dermatitis. Betamethasone valerate worsened the idiopathic dermatitis probably due to skin atrophy as steroid's side effect and tacrolimus had weak effects in both models. Therefore, difamilast is expected to be a possible treatment for AD symptoms such as chronic allergic dermatitis and psychological stress-exacerbated idiopathic dermatitis.

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Synthetic melanin nanoparticles improve wound healing

D. Biyashev[1], Z. E. Siwicka[2], M. Demczuk[1], U. Onay[1], S. Evans[1], N. Collins-McCallum[2], N. Gianneschi[2], K. Lu[1]
[1]*Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States,* [2]*Chemistry, Northwestern University, Evanston, Illinois, United States*

Excessive release of reactive oxygen species (ROS) during acute skin injury has detrimental effects on wound healing. Since natural melanin is an efficient scavenger of radical species, we hypothesized that a topical treatment consisting of synthetic melanin nanoparticles (SMNP) would benefit the healing process. We utilized the mouse chemical skin injury model to test our hypothesis, using nitrogen mustard (NM) for injury induction. Topical treatment with SMNP resulted in significantly improved wound healing as evidenced by the rate of wound area reduction ($p=0.006$ at day 3, $n=17$), decreased edema ($p=0.003$ at day 2, $n=9$-10), and time of eschar detachment compared to the vehicle group ($p=0.005$, $n=9$-10). Using the ultraviolet radiation-induced injury model, we confirmed that the positive effects of SMNP treatment were not due to the unlikely potential adsorption of residual NM by SMNP. Mechanistically, SMNP treatment increased the activity of superoxide dismutase (SOD) in the skin compared to the vehicle group ($p=0.04$, $n=3$-7). Inhibition of Cu-Zn SOD with small molecule inhibitor ATN-224 at least partially reversed the beneficial effects of SMNP on wound healing *in vivo*. Further, immune array analysis showed predominant downregulation of inflammatory and apoptotic pathways in SMNP-treated animals. Accordingly, the SMNP treatment reduced ERK1,2 phosphorylation ($p=0.048$, $n=3$), decreased expression of MMP-9 ($p=0.04$, $n=3$-5), and decreased TUNEL staining ($p=0.01$, $n=3$) compared to the vehicle group. Preliminary data obtained using human skin explants show that treatment with SMNP decreases histopathologic features associated with chemical injury, such as blistering and pyknotic nuclei. Overall, our data suggest that synthetic melanin mimics can potentially be developed as a topical therapy for injured skin

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Lebrikizumab allows interleukin (IL)-13 membrane binding and subsequent internalization through the decoy receptor IL-13 receptor alpha 2 (Rα2)

I. Wulur, R. Van Horn, A. Ryuzoji, A. Okragly, C. Patel, R. Benschop
Eli Lilly and Company, Indianapolis, Indiana, United States

Lebrikizumab (lebri) is a novel, monoclonal antibody that selectively targets IL-13 and prevents formation of the IL-13 receptor alpha 1 (Rα1)/IL-4 receptor alpha (Rα) heterodimer receptor signaling complex. A previous crystal structure report showed that lebri does not interfere with binding of IL-13 to the IL-13 Rα2 decoy receptor. In contrast, other IL-13 antibodies tralokinumab and cendakimab had been reported to inhibit IL-13 binding to both IL-13Rα1 and IL-13Rα2. We wanted to investigate whether lebri binding to IL-13 interfered with IL-13 binding to IL-13Rα2 and the subsequent internalization. Through competitive binding experiments using surface plasmon resonance, we confirmed that lebri can bind to the tralokinumab/IL-13 and cendakimab/IL-13 complexes. These data showed that lebri binds to IL-13 at a different epitope. First, we induced IL-13Rα2 expression on HaCaT cells, a spontaneously transformed keratinocyte cell line from adult human skin, using co-treatment of IL-13 and TNF-alpha. Then, we fluorescently stained these cells and confirmed the increased expression of IL-13Rα2. Using live-cell confocal imaging, we observed that IL-13 can bind IL-13Rα2 and is internalized into the cells. These experiments were conducted in the presence of an IL-13Rα1 inhibitory antibody to ensure the observed binding is only through IL-13Rα2. Importantly, we also observed binding and internalization of the IL-13/lebri complex, while IL-13/tralokinumab and IL-13/cendakimab complexes do not bind to the IL-13Rα2 receptor and are not internalized into the cells. The internalized IL-13/lebri complex colocalized with a lysosome marker, indicating that it is likely to be degraded in lysosomes. In summary, lebri allows IL-13 to bind and internalize through the IL-13Rα2. This mode of action differentiates it from tralokinumab and cendakimab, since lebri allows natural clearance of IL-13 levels through IL-13Rα2.

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***In vitro* activity of efinaconazole against terbinafine and itraconazole resistant and susceptible dermatophyte, candida, and mold clinical isolates.**

A. Gamal[1, 2, 5], M. Elshaer[3, 1], B. Elewski[4], M. Ghannoum[1, 2]
[1]*Dermatology, Case Western Reserve University, Cleveland, Ohio, United States,* [2]*Dermatology, University Hospitals, Cleveland, Ohio, United States,* [3]*Clinical Pathology, Mansoura University Faculty of Medicine, Mansoura, Egypt,* [4]*Dermatology, UAB Hospital, Birmingham, Alabama, United States,* [5]*Dermatology, October 6 University Hospital, 6th of October, Giza, Egypt*

Recently, numerous cases of dermatophytosis resistant to terbinafine were reported. These cases have been increasing in frequency, with no improvement even with treatment using systemic therapies. Our objective was to determine the antifungal activity of efinaconazole compared to fluconazole, itraconazole, and terbinafine against dermatophyte, Candida, and mold clinical isolates In vitro. Activity of antifungals was assessed using minimum inhibitory concentration(MIC) and minimum fungicidal concentration(MFC). Isolates tested included susceptible and resistant clinical isolates of Trichophyton mentagrophytes (n=16), T. rubrum(n=43), T. tonsurans (n=18), T. violaceum (n=4), Candida albicans (n=55), C. auris (n=30), Fusarium sp., Scedosporium sp., and Scopulariopsis sp. (n=15 for each). Against dermatophytes, efinaconazole was the most active antifungal compared to the other agents tested with MIC50 and MIC90 (Concentration that inhibited 50% and 90% of strains tested, respectively) of 0.002 and 0.03 µg/ml, respectively. While fluconazole, itraconazole and terbinafine showed MIC50 and MIC90 of 1 and 8 µg/ml, 0.03 and 0.25 µg/ml, and 0.031 and 16 µg/ml, respectively. Against Candida isolates, MIC50 and MIC90 of efinaconazole were 0.016 and 0.25 µg/ml, respectively. In contrast, fluconazole, itraconazole and terbinafine had MIC50 and the MIC90 of 1 and 16 µg/ml, 0.25 and 0.5 µg/ml, and 2 and 8 µg/ml, respectively. Against different mold species, efinaconazole MIC ranged between 0.016 and 2 µg/ml, compared to a range from 0.5 to higher than 64 µg/ml for the comparators. In conclusion efinaconazole showed potent activity against a broad panel of Candida, mold and dermatophyte isolates. Furthermore, Efinaconazole demonstrated high activity against itraconazole and terbinafine resistant isolates.

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Biodegradable bioadhesive nanoparticle delivery of chemotherapy for the treatment of cutaneous malignancies

J. Chang, H. Suh, J. Lewis, M. Bosenberg, W. Saltzman, M. Girardi
Yale University, New Haven, Connecticut, United States

Encapsulation of chemotherapeutic agents within biodegradable nanoparticles (NP) allows for gradual drug release from the polylactic acid core. Bioadhesive, degradable NPs can be retained at the site of administration, maximizing local effects while minimizing systemic side effects. Our bioadhesive NP (BNP) loaded with chemotherapy have previously been proven efficacious in the treatment of murine PDVC57 squamous cell carcinoma (SCC) tumors, with increased intratumoral (i.t.) retention of the active agent. SBI-111 is a potent anti-tumor agent but with considerable systemic toxicity. We examined the efficacy of i.t. injection of BNP encapsulated SBI-111 (BNP-111) in the pre-clinical treatment of melanoma and SCC. We determined the in vitro efficacy of SBI-111 in YUMMER 1.7, an immunogenic melanoma cell line (IC50=0.108±0.05μM) and in PDVC57 (IC50= 0.055±0.3μM). We then assessed the therapeutic effect of BNP-111 *in vivo* with YUMMER1.7 tumors established in C57Bl/6 mice. They received a single, low dose i.t. injection of BNP-111 or vehicle and were sacrificed 14 days after treatment. BNP-111 treated tumors had significantly reduced tumor volume (vehicle: 589.3mm3 ±391.2, BNP-111: 108.6 mm3 ±151.7, p=0.0095). Histologic examination of tumor cross section demonstrated substantial decrease in tumor area following treatment with BNP-111 (9.23mm2 ±4.28, p = 0.012) compared to vehicle (51.53mm2± 13.04) and revealed tumor resolution in 50% of BNP-111 treated mice. To determine the degree to which co-injection with immunomodulator CpG may increase the anti-tumor effectiveness of BNP-111, mice bearing PDVC57 SCC tumors received i.t. BNP-111 + CpG, CpG alone, BNP-111 alone, or vehicle and were sacrificed 14 days after treatment. Combination of BNP-111 + CpG substantially increased the percent of SCC free mice (70%) in comparison to CpG (20%) or BNP-111 (10%) alone. Our results suggest that BNP-111 can be used to induce cutaneous tumor regression and that co-injection with immunostimulatory CpG will provide efficient and wide-spread regression of skin neoplasms.

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Topical calcipotriol plus imiquimod immunotherapy for non-keratinocyte skin cancers

M. Azin[1, 2], K. H. Ngo[1, 2], S. Demehri[1, 2]
[1]*Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States,* [2]*Cutaneous Biology Research Center, Massachusetts General Hospital, Boston, Massachusetts, United States*

Non-keratinocyte cutaneous malignancies including breast cancer cutaneous metastasis and melanoma in situ are often poor surgical candidates due to their anatomical location, size and the morbidities associated with surgery. Imiquimod, a toll-like receptor 7 agonist, is used for the treatment of these cutaneous malignancies, which activates the innate immunity in the skin. However, imiquimod's modest effect on the activation of adaptive immunity limits its efficacy as a monotherapy. We have previously demonstrated that topical induction of thymic stromal lymphopoietin (TSLP) cytokine by calcipotriol protects against skin and breast cancer development by directly activating T cell immunity. Herein, we demonstrate that the combination of a topical TSLP inducer (i.e., calcipotriol and retinoic acid) with an innate immune cell activating agent (i.e., imiquimod and STING agonist) leads to robust antitumor immunity against cutaneous malignancies. Two applications of topical calcipotriol plus imiquimod reduced subcutaneous MMTV-PyMTtg breast tumor growth compared with calcipotriol or imiquimod alone ($p = 0.011$). This tumor suppression was associated with increased induction and proliferation of CD4+ and CD8+ T cells in the tumor microenvironment. Following subcutaneous injection of B16-F10 melanoma cells into flanks of wild-type mice, topical treatment with calcipotriol plus imiquimod and retinoic acid plus Imiquimod blocked subcutaneous melanoma growth ($p < 0.05$). Interestingly, the combination of calcipotriol with another innate immune cell activator, DMXAA (STING agonist), also effectively inhibited subcutaneous melanoma growth ($p = 0.019$) and induced tumor infiltrating CD4+ and CD8+ T cells. These findings highlight the synergic effect of topical TSLP induction in combination with innate immune cell activation as a novel immunotherapy for cutaneous malignancies.

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CXCL9, CXLC10, and CXCL11 differentially form CXCR3-regulated G protein:β-arrestin signaling complexes

J. S. Smith[1], K. Zheng[1], D. Eiger[2], T. Pack[2], S. Rajagopal[2]
[1]*Harvard Medical School, Boston, Massachusetts, United States,* [2]*Duke University School of Medicine, Durham, North Carolina, United States*

Chemokine receptors (CKRs) are G protein coupled receptors (GPCRs), a super-family of transmembrane receptors that are the target of ~1/3 of FDA-approved medications. CKRs are critical regulators of the immune response, in part through their central role in chemotaxis, and therefore play a role in nearly every inflammatory skin condition. Despite their critical role in regulating inflammation, few FDA-approved medications target CKRs. The purpose of this study was to identify CKR signaling pathways that could provide new opportunities for drug development. We focused on the CKR CXCR3 which is expressed primarily on effector T cells and plays a critical role in the Th1 immune response. We found that GPCRs, including CXCR3, can promote direct interactions between G proteins and β-arrestins. We confirmed this interaction through a variety of biochemical and biophysical techniques, including bioluminescent resonance energy transfer (BRET) and immunoprecipitation. Surprisingly, these interactions were only appreciable with the Gi/o protein-family, and not other Gα protein subtypes. This newly appreciated Gi:β-arrestin complex appears distinct from other canonical G protein and β-arrestins GPCR signaling pathways. Interestingly, only CXCL11, and not CXCL9 or CXCL10, could form this Gi:β-arrestin complex downstream of CXCR3 ($p<0.05$), suggesting distinct signaling roles for CXCR3 chemokines. Our results reveal a signaling paradigm in which GPCRs can promote formation of Gi:β-arrestin signaling complexes and where different ligands for the same receptor activate distinct signaling pathways. Specifically, the Gi:β-arrestin pathway broadens our understanding CKR signal transduction and suggests different chemokines for the same receptor have distinct functions. Understanding the signaling differences between CXCL9, CXCL10, and CXCL11 is critically important for targeting drugs towards CXCR3, and the findings are likely applicable to other CKRs.

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Induction of type 17 collagen decreases ultraviolet b-induced cellular senescence in human htert/ker-ct keratinocytes

T. Ansary, K. Kamiya, M. Hossain, M. Komine, M. Ohtsuki
Dermatology, Jichi Ika Daigaku, Shimotsuke, Tochigi, Japan

Cellular senescence is an irreversible growth arrest caused by oxidative stress and DNA damage and ultraviolet radiation mediated cellular damage is responsible for skin photoaging. The Type 17 collagen (COL17A1) expressed in epithelial hemidesmosomes plays a crucial role in maintaining hair follicle stem cells. Apocynin, (1-(4-Hydroxy-3-methoxyphenyl)ethan-1-one)) is a chemical inducer of Collagen 17A1 which promotes the Collagen 17A1 protein synthesis. Our unpublished data showed that klotho mutant (kl/kl) mice, which is a premature aging model, display increased senescence associated-β-galactosidase (Sa-β-Gal) activity, p16 and phosphorylated histone H2AX (γH2AX) expression along with deceased Collagen 17A1 expression. Here, we aimed to study whether induction of COL17A1 by apocynin can decrease Ultraviolet B (UVB)- induced cell senescence or not. hTert/KER-CT keratinocytes were pretreated with apocynin (10 μM, 20 μM, 40 μM) for 24 hours and exposed to UVB (100 J/m2) using a UVB lamp. After 3 hours, the expression of COL17A1, p16, and γH2AX was measured by immunofluorescence and qRT-PCR, cellular senescence was measured by SA-β-gal staining, and propidium iodide (PI)/RNase solution was used to measure cell cycle analysis. In our study, UVB exposure decreased COL17A1 expression in the epidermal basal cells, increased Sa-β-Gal activity, G1 arrest, p16 expression, and γH2AX expression ($P<0.001$). Apocynin restored the COL17A1 expression and reduced senescence, cell cycle arrest and expression of γH2AX and p16 in human keratinocytes ($P<0.001$). Although the mechanism is not yet understood, we can speculate that apocynin restores COL17A1 expression under UVB stress and thus maintain the epidermal stem cell functionality. By maintaining the stem cell's homeostasis apocynin may also reduce senescence and decrease DNA damage.

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Melatonin and its metabolites act as agonists on the AhR and can interact with the PPARγ

A. T. Slominski[1], T. Kim[1], R. M. Slominski[1], S. Qayyum[1], Y. Song[1], Z. Janjetovic[1], W. Placha[2], K. Kleszczynski[3], V. Atigadda[1], Y. Song[1], C. Raman[1], R. Reiter[4]
[1]*University of Alabama at Birmingham, Birmingham, Alabama, United States,* [2]*Jagiellonian University, Krakow, Poland,* [3]*University of Munster, Munster, Germany,* [4]*UT Health at San Antonio, San Antonio, Texas, United States*

Melatonin is widely detected in nature. It has pleiotropic activities, mediated by interactions with high affinity G-protein coupled MT1 and MT2 receptors, or which at high concentration (≥1 μM) are MT1 and MT2 independent. The AhR is a perfect candidate for a receptor mediating latter effects because melatonin has structural similarity to its natural ligands. Our molecular modeling, using homology crystal structure of the AhR has identified melatonin and its metabolites as excellent candidates to act on the AhR. Specifically, melatonin, 6(OH)Mel and AFMK share the same LBD as its natural ligands. Analysis of the binding free energy of the compounds showed that melatonin and its metabolites bind to the AhR, in similar manner as its natural ligands. These in silico analyses were validated experimentally. Thus, using the commercial Assay System, we demonstrated that melatonin and its indolic and kynuric metabolites act as agonists on the AhR with EC50 at range 10-4-10-6 M. This was confirmed by transcriptional stimulation of CYP1A1 promoter. Finally, melatonin and its metabolites stimulated AhR translocation from the cytoplasm to the nucleus in human keratinocytes as documented using Image Stream II cytometry and WB analyses of cytoplasmic and nuclear fractions of HaCaT keratinocytes. Since docking of melatonin and its metabolites to the LBD of the PPARγ using Schrodinger software showed similar scores to the known ligand, we performed binding studies using commercial TR-TFRET kit to assay the interaction of the ligand with the LBD of the PPARγ. These showed agonistic activity of melatonin, 6(OH)Mel and AFMK, however, with low affinity (EC50 was at 10-4 M range). In conclusion, melatonin and its indolic and kynuric metabolites can act as the agonists on the AhR and can interact with PPARγ at high concentrations.

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The effect of topical 5-azacytidine in irritant and allergic contact dermatitis

Y. Ogawa, Y. Muto, S. Shimada, T. Kawamura
Dermatology, University of Yamanashi, Yamanashi, Japan

DNA methylation is one of major epigenetic mechanisms by which regulate a panel of gene expressions. An old but a revisiting compound, 5-azacytidine, is a pyrimidine nucleoside analog of cytidine that can inhibit DNA methyltransferase, induce cell differentiation, and has direct cytotoxicity on abnormal bone marrow hematopoietic cells. Thus, systemic administration of 5-azacytidine has been approved for the treatment of myelodysplastic syndrome and acute myeloid leukemia. However, the effect of topical 5-azacytidine on skin inflammation is largely unknown. To this end, we utilized croton oil (CrO)-induced irritant contact dermatitis (ICD) model and 2,4-dinitrofluorobenzene (DNFB)-induced allergic contact dermatitis (ACD) model. At day -1, vehicle or 1 mg of 5-azacytidine solution was applied to each ear. At day 0, 1% CrO was applied to both ear, and ear thickness was measured over time. Interestingly, the ear swelling response to CrO in 5-azacytidine-treated ear was significantly increased and prolonged compared with that of vehicle-treated ear. In DNFB-induced ACD model, topical application of 1 mg of 5-azacytidine solution to the abdomen one day before the sensitization with 0.5% DNFB did not alter following ear swelling. In sharp contrast, topical application of 1 mg of 5-azacytidine solution to the ear of 0.5% DNFB-sensitized mice one day before the elicitation with 0.2% DNFB significantly decreased ear swelling compared with that of vehicle applied ear. These data suggest that topical 5-azacytidine differently influences ICD and ACD as well as sensitization phase and elicitation phase of ACD. Topical 5-azacytidine must modify a bunch of gene and protein expressions. However, it could be a therapeutic option of ACD.

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New class of mTOR inhibitor stabilizes intermediate filament networks in severe epidermolysis bullosa simplex keratinocytes.

K. McGuire, A. McCormick, D. Huey
BioMendics, LLC, Rootstown, Ohio, United States

Epidermolysis Bullosa Simplex (EBS) is a rare genodermatosis caused by mutations in either KRT5 or KRT14. Severe EBS (i.e., EBS Dowling-Meara) is characterized by extremely fragile skin with frequent and severe herpetiform blistering. Genetic mutations cause poor keratin 5/14 dimerization resulting in mechanically weakened intermediate filament (IF) networks that collapse into intracellular aggregates upon stress (e.g., scratching, UV). Enhanced cellular removal and degradation of mutant keratins through stimulation of autophagy and the UPS system has the potential to strengthen the IF networks, reducing blistering and erosions. Previously, UPS cochaperones (e.g., 4-phenylbuterate) were shown to decreased keratin aggregation but also decreased keratinocyte adhesion and migration. The purpose of this research was to evaluate if BM-3103, (4-Hydroxy 4'-methyoxytolan), a known wound healing promoter, anti-inflammatory, and autophagy inducer, could remove mutant keratins/aggregates thereby strengthening the IF network without adversely affecting cell survival, adhesion, or migration. Assays tested on patient derived EBS keratinocytes included: (1) Assessment of cytotoxicity, adhesion, and migration effects of BM-3103 using MTT, LDH, and scratch assays. (2) Autophagy induction (LC3-II conversion) and keratin aggregation (heat stress assay). There were no signs of cytotoxicity, loss of cell adhesion or slowed migration. Cells were exposed to escalating concentrations of BM-3103 for 24 hrs then heat shocked (43°C for 30min) generating an abundance of aggregates visualized by immunofluorescent microscopy. Untreated keratinocytes displayed keratin aggregate formation in 72% of KRT5 and 87% of KRT14 mutant cells. Treatment with BM-3103 reduced the incidence of keratin aggregates to 16% ($p<0.01$) of KRT5 and 24% ($p<0.01$) KRT14 mutant cells. BM-3103 is the first compound identified that is capable of stabilizing IF networks in keratinocytes from severe EBS patients. A clinical trial is ongoing to assess if BM-3103, can heal blistering areas and strengthen EBS patient skin.

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A formulation containing extracts of two herbs and two algae is a clean, green alternative for multiple ingredients used in topical therapeutic products.

C. R. Thornfeldt[2,1]
[1]*CT Derm, P.C., Fruitland, Idaho, United States,* [2]*Episciences Inc, Boise, Idaho, United States*

A novel mixture of peptide dominant extracts of Acacia and Maca herbs with Chlorella and Clamydomonas algae provide several of the desired functionalities for ingredients in topical formulations for skin care and therapeutic products. A 10 subject randomized *in vivo* trial compared this herbal/algal test formulation containing these four extracts of repairing of the epidermal permeability barrier after tape stripping. Then after a single application at four different sites of the test formulation(TF), 100% petrolatum(P) and 100% mineral oil(MO), Tewameter measurements were taken at 4 time points between 20 minutes and 6 hours. TP produced 34.2% repair at 20 minutes for a trend of statistical significance of $p=0.063$ and 41.9% repair at 6 hours which is statistically significant $p<0.044$. P produced 34.6% repair at 20 minutes for $p<0.009$ and 41.2% repair at 6 hours for $p<0.016$. MO did not produce statistically significant improvement until 2 hours with 22.7% for $p<0.02$ then at 6 hours with 26.5% improvement for $p<0.03$. A second, 10 subject randomized *in vivo* trial compared TF with prescription 2.5% hydrocortisone ointment(2.5HC) in reducing UVL induced erythema. After UVL exposure a single application of TF and 2.5HC were applied to different sites. They were assessed by spectrophotometer two hours later. Both TF and 2.5HC produced statistically significant reduction in erythema, but there was no difference between the two. TF induced an 11.5% reduction of $p<0.02$ while 2.5HC induced a 13.0% reduction of $p<0.043$. Conclusions: The first trial suggests that TF to be equal in efficacy to pure petrolatum in repairing an abnormal permeability barrier. The second trial suggests TF is equal in anti-inflammatory effect to prescription hydrocortisone 2.5%. These results indicate the versatility for using TF as a clean, green substitute for multiple ingredients used in topical formulations instead of those derived from Oil and Gas Industry.

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Topical application of gemcitabine chemotherapy generates microvesicle particles in a platelet-activating factor-receptor- and acid sphingomyelinase-dependent manner

A. Thyagarajan[1], R. Johnson[2], J. B. Travers[1], R. P. Sahu[1]
[1]Pharmacology and Toxicology, Wright State University Boonshoft School of Medicine, Dayton, Ohio, United States, [2]Department of Orthopedics and Plastic Surgery, Wright State University Boonshoft School of Medicine, Dayton, Ohio, United States

Chemotherapy has remained the mainstay for the treatment of multiple types of cancers. In particular, topical use of chemotherapy has been used for skin cancers. Though effective, topical chemotherapy has been limited due to adverse effects such as local and even systemic toxicities. Given our ongoing studies that exposure to pro-oxidative stressors, including therapeutic agents induces the generation of extracellular vesicles known as microvesicle particles (MVP) which are dependent on activation of the Platelet-activating factor-receptor (PAFR), a G-protein coupled receptor present on various cell types, and acid sphingomyelinase (ASMase), an enzyme required for MVP biogenesis. Based upon this premise, we tested the hypothesis whether topical application of gemcitabine will induce MVP generation in human skin. Our *ex vivo* studies using human skin explants demonstrate that gemcitabine treatment results in MVP generation in a dose-dependent manner in a process blocked by PAFR antagonist and ASMase inhibitor. To confirm the mechanisms, we employed PAFR-expressing and deficient (Ptafr-/-) mouse models as well as mouse deficient in ASMase enzyme (Smpd-1-/-). Similar to the findings using human skin explants, our studies demonstrate that gemcitabine-induced MVP release in WT mice was blunted in Ptafr-/- and Smpd-1-/- mice. These findings demonstrate a possible mechanism by which local chemotherapy can signal systemically, in a process that is dependent upon the PAFR-ASMase pathway. Future studies can test the abilities of blocking MVP release on the effectiveness and tolerability of topical chemotherapy.

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Harvest time of hibiscus flower extract affects collagen boosting activity in skin cells emphasizing the importance of plant/flower harvest time to build potent extracts

K. Corallo[1], J. Trivero[1], C. Chen[1], N. Pernodet[1, 2]
[1]Estee Lauder Companies, New York, New York, United States, [2]Stony Brook University, Stony Brook, New York, United States

Nature and plants create and produce many bioactive molecules. When considering plants such as flower extracts as a source of potent molecules, many studies attempted to optimize the extraction processes. However, very little of this work considers the importance of timing and determining when flowers are at their most potent peak and the best time to harvest them in order to get the highest yield of potent molecules for skin benefits. With limited resources and time, plants have adapted to optimize their survival by producing specific molecules. As time goes and plants age, many of these molecules either disappear or get damaged, and different ones are being produced. It is well known that living organisms like plants, humans, or even bacteria have circadian clocks that are synchronized with the 24-hour cycle on Earth. These clocks influence molecule production and release. We have studied extracts from an exclusive hibiscus flower depending on time of harvest and correlated with biological activities on skin cells. Over a total flower lifetime of 3 days, 3 extracts were produced exclusively from blooming to mature flowers. These 3 extracts were characterized and tested for collagen production in skin cells. We demonstrated that the timing of harvest was critical to obtain the highest level of collagen production. Moreover, when the optimized extract was combined with our exclusive moringa extract, which uses a patented process to increase the isothiocyanate content, and additional collagen inducers, we were able to further increase collagen production. This study emphasizes the precision of nature's power and the critical role timing can play, and the importance of considering the role of time in identifying an optimal flower harvest time to create highly potent flower extracts to deliver skin benefits.

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Biological and in-silico characterization of pyrazole and pyrazolone derivatives: Identifies novel anti-skin cancer, antioxidant and tyrosinase inhibitory agents.

S. T. Boateng[1], T. Roy[1], K. Torrey[2], S. Banang-Mbeumi[1], D. Aryal[3], S. Murru[1], H. Ma[2], J. C. Chamcheu[1]
[1]University of Louisiana at Monroe, Monroe, Louisiana, United States, [2]University of Rhode Island, Kingston, Rhode Island, United States, [3]Edward Via College of Osteopathic Medicine, Monroe, Louisiana, United States

The psychosocial burden akin to cutaneous conditions such as cancer and hyperpigmentation disorders, make their management very sensitive. Current therapeutic interventions, are associated with side-effects, cost, invasiveness and bioavailability issues. Herein, 26 new pyrazole and pyrazolone compounds were synthesized via microwave-assisted reactions and their pharmacokinetic parameters ADMET predicted by in-silico methods. In addition, these compounds were evaluated for their anti-proliferative, antioxidant, anti-tyrosinase effects. Many potent pyrazole and pyrazolone hits were identified with low-micromolar IC50 values against non-melanoma cells A431 and SCC-12 ($p<0.001$), and melanoma cells SKMel-28 and A375 ($p<0.01$) with several-fold affinity over control cells (HaCaT) than Cisplatin, a known anticancer drug. The most potent hit significantly reduced scratch wound healing and colony formation and induced apoptosis as evidence by modulation of cleaved caspase 3, 9, PARP levels and Bax/Bcl-2 ratio ($p<0.01$). Tyrosinase inhibition assay identified nine effective derivatives with up to 98% inhibition compared to the control Arbutin with 58% ($p<0.001$). Using antioxidant assay, six active derivatives with superior scavenging activity than quercetin control ($p<0.01$), but comparable to ascorbic acid were identified. Employing the Swiss Target Prediction and SwissADME databases, several derivatives with good binding affinities to the targets COX-1/2, FLT3, VEGFRI, MAPK-4, and JAK-1 were identified, which also exhibited excellent intestinal, oral and topical absorption properties. In summary, these novel and promising anti-cancer, anti-oxidant and tyrosinase inhibitory compounds warrant further characterization and investigation in the management of skin cancer and hyperpigmentation disorders.

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Dual targeting mTOR and autophagy by fisetin alleviates psoriasis-like responses induced by TNF-α and IL-17A in vitro and imiquimod-induced dermatitis *in vivo*

T. Roy[2], S. Banang-Mbeumi[2], S. T. Boateng[2], R. Chamcheu[2], L. Kang[3], S. Huang[1], J. C. Chamcheu[2]
[1]LSU Health Shreveport, Shreveport, Louisiana, United States, [2]Univ. Louisiana Monroe, Monroe, Louisiana, United States, [3]VCOM Louisiana, Monroe, Louisiana, United States

Recently, the central mTOR pathway has emerged as a clinically relevant player in the pathogenesis of psoriasis. Our previous study has shown that fisetin, a dietary polyphenol, possesses prodifferentiation and mTOR inhibitory properties, and suppresses psoriasis-like responses in vitro. However, the effects of fisetin on psoriasis *in vivo* and the underlying mechanisms are unclear. Here, we evaluated fisetin's effects on cytokines(IL-6/22 and TNF-A/IL-17A) and anti-CD3/CD28 stimulated keratinocytes(HEKa) and CD4+T cells compared to rapamycin, a known inhibitor of mTOR. We observed that fisetin and rapamycin alone or in-combination inhibited($p<0.001$) mTOR activity in HEKa and the secretion of IL-17A and IFN-γ in CD4+T cells co-cultured with HEKa, and increased levels of autophagy markers LC3A/B and Atg5. RNA-Seq analysis, identified 12713 differentially expressed genes(DEGs) in the fisetin-treated($p<0.0001$) compared to 7374 DEGs in the rapamycin-treated($p<0.001$)groups, with differently expression patterns the PI3K/Akt/mTOR-pathways, psoriasis, and epidermal development genes. Using kinase assay and in silico molecular modeling, we observed high binding-affinity of fisetin to IL-17A, Rac1, and PIK51α, which are dysregulated psoriasis targets. In imiquimod(IMQ)-induced mouse psoriasis model, topical application of fisetin($p<0.001$) exhibited a better effect than rapamycin($p<0.05$) in reducing skin inflammatory responses, Akt/mTOR phosphorylation levels, and modulating autophagy and oxidative stress in skin lesions. Overall, our data demonstrate that fisetin potently inhibits psoriasis targets, upregulates autophagy and alleviates IMQ-induced murine psoriasis-like disease. Our findings suggest that fisetin either alone or as an adjuvant to existing therapies, has a great potential for treatment of psoriasis and other skin inflammation.

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REDD1/DDIT4 regulates the glucocorticoid receptor function in human keratinocytes

D. Chudakova[1], P. Bhalla[1, 2], G. Baida[1], I. Budunova[1, 2]
[1]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]SBDRC, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Glucocorticoids (Gcs) are widely used for inflammatory skin diseases. However, Gcs are also notorious for adverse effects such as skin atrophy. The glucocorticoid receptor (GR) is a transcription factor mediating Gcs action. Previously, we found that GR target gene REDD1 is causatively involved in atrophic effects of Gcs, and that REDD1 inhibitors could protect skin from steroid hypoplasia. In this work, we searched for the mechanisms underlying REDD1 effects on GR function using HaCaT REDD1 KO keratinocytes generated by CRISPR/Cas9 approach. Transcriptome analysis revealed that induction of GR target genes (FKBP51, SGK1, GILZ, BIRC3, and KLF9) by glucocorticoid fluocinolone acetonide (FA) in REDD1 KO was markedly lower than in Cas9-control cells. Interestingly, induction of mitochondrial GR targets, MT-ND2 and MT-CYTB, by FA was also decreased in REDD1 KO cells compared to control. This suggested the existence of novel feed forward loop in GR activation via REDD1. Next, we assessed whether a) REDD1 KO affected GR expression at RNA/protein levels, and b) modified the major steps in GR activation: GR phosphorylation at activation site Ser211 and GR nuclear import. Surprisingly, the difference between REDD1 KO and control cells in terms of GR expression and activation was rather marginal. We also did not find direct protein-protein interaction between GR and REDD1 using co-immunoprecipitation (co-IP). It is known that GR function is strongly regulated by chaperones including immunophilins FKBP51 and FKBP52. Specifically, FKBP51 inhibits GR activity via altered nuclear translocation and inhibition of interaction between GR and its ligands, and potentially may affect GR-DNA binding. We found that FKBP51 protein levels were increased in REDD1 KO keratinocytes, while FKBP52 levels did not change significantly. The ongoing experiments are focused on the evaluation of FKBP51 interaction with GR in the absence of REDD1 and the effect of FKBP51 knockdown on response of REDD1 KO cells to FA.

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Krafft temperature of surfactants in vehicles for roflumilast and pimecrolimus cream and effects on skin tolerability

D. R. Berk, D. W. Osborne
Arcutis Biotherapeutics, Inc., Westlake Village, California, United States

Creams and lotions use emulsifiers to create a physically stable vehicle for topically applied dosage forms. The minimum temperature required for an anionic emulsifier to form micelles, which can cause lipid extraction from skin and result in irritation, is known as the Krafft temperature (TK). A cream or lotion formulated with emulsifiers having a TK above safe water exposure temperatures (approximately 50°C) cannot irritate skin due to epidermal lipid extraction. Roflumilast cream, a novel, once-daily, topical formulation of a highly potent phosphodiesterase-4 inhibitor in development for treatment of several dermatologic conditions, demonstrated favorable tolerability in clinical trials. The purpose of this study was to determine TK for the two primary anionic emulsifiers used in the vehicle of roflumilast cream (dicetyl phosphate and ceteth-10 phosphate) and commercial-grade sodium cetostearyl sulfate (used in pimecrolimus cream). 0.0012% (wt/wt) solutions (near but not below the critical micellization concentrations [CMC] of the alkyl phosphate emulsifiers) were mixed in 20-mL glass scintillation vials. The vial was placed into a water bath and the temperature increased by 1-2°C until the solid emulsifier completely dissolved and foam was visible after shaking. The TK was fine-tuned by returning the samples to ambient condition, observing precipitation of the solid emulsifier, placing them in a water bath just below the previously observed TK, and increasing temperature by 0.5°C increments. TK was 58°C for dicetyl phosphate, 53°C for ceteth-10 phosphate. The TK of an aqueous solution cetostearyl sulfate mixed at its CMC (0.0075% wt/wt) was 41°C. These results indicate the anionic emulsifiers in roflumilast cream vehicle cannot function as surfactants and extract epidermal lipids from the stratum corneum whereas sodium cetostearyl sulfate can extract epidermal lipids in treated skin exposed to warm (>41°C) water. These results may explain, in part, why roflumilast cream and its vehicle have favorable tolerability and low rates of local irritation.

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The synergistic effect of retinyl propionate and hydroxypinacolone retinoate on skin early aging

R. Ye[1, 2], M. Hong[1], Q. Wang[3], Y. Xie[4], L. Du[1]
[1]Inertia Shanghai Biotechnology Co., Ltd, Shanghai, China, [2]DermaHealth Shanghai Biotechnology Co., Ltd, Shanghai, China, [3]Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, China, [4]National Clinical Research Center For Child Health, The Children's Hospital, Zhejiang University School of Medicine, Hangzhou, China

Early aging is become increasingly important as the life expectancy rise rapidly. The occurrence of early aging is influenced by both extrinsic and intrinsic factors: the lower production rate of collagen and elastic fibers, the breaking of elastic fibers and collagen fibers caused by UV-induced metalloproteinases. Early aging shows a decline of the skin at multi-levels, which needs multi-effect active substances. Retinol and its derivatives are the most effective anti-aging substances. By increasing the synthesis of collagen and inducing dermal elastin and fibers, retinol and its derivatives effectively reduce wrinkles and improves skin firmness as well as elasticity. However, high concentrations of those substances can easily irritate the skin, while low dosages cannot achieve the effect. In this study, we demonstrated a composition of retinol derivatives with a strong synergistic effect and less skin irritation. We screened a range of ratios of hydroxypinacol retinoate and retinol propionate combinations and found the composition of hydroxypinacolone retinoate to retinol propionate at the ratio of 5:9 with the best performance. We administered 5 μg/mL HPR, 9 μg/mL retinyl propionate and their combination to cells, extracted total cell RNA at 24 h, 48 h, and 72 h, and detected the expression of collagen-related mRNA by qPCR. We showed that hydroxypinacol retinoate (HPR) and retinol propionate synergistically increase the expression of type IV collagen, CRBP-1, RARB, and elastin to more than 300 times. In conclusion, we demonstrated that the application of the retinol derivative composition in cosmetics and/or skin care products can significantly enhance the structural protein of the extracellular matrix of the skin with a lower application amount , less skin irritation and better anti-aging effect.

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***In vitro* evaluation of skin distribution and human dermal papilla cells proliferation for minoxidil 5% topical lotion hydrogel**

G. Song, K. Ip, Y. Liu, D. Banov, A. Bassani, M. Carvalho
Research and Development, Professional Compounding Centers of America, Houston, Texas, United States

For decades, the treatment of reference for androgenetic alopecia has been the topical application of minoxidil 2% or 5%, which is commercially available as a solution and a foam. When compounded, minoxidil may be incorporated in variable strengths to a topical base in order to meet the individual needs of men. An alternative topical compounded formulation was developed to include minoxidil in a proprietary hydrogel that is alcohol-free to reduce the skin irritation, and does not require pH adjustment to facilitate the compounding process. In vitro studies were conducted to investigate the proliferation of human dermal papilla cells upon treatment with minoxidil 5% topical lotion hydrogel. Additional in vitro studies evaluated the distribution of minoxidil across the skin. The commercial product of reference was used as positive control. For the proliferation analysis, the bromodeoxyuridine staining assay identified the proliferating human dermal papilla cells upon 24 hours of exposure by minoxidil 5% (commercial product versus compounded formulation), using fluorescence detection. The proliferation effect of minoxidil was significant in both minoxidil commercial (RFU 181,446 ±38,171, p=0.021) and minoxidil compounded (RFU 183,109±24,416, p=0.006), when compared to the negative control (untreated cells). For the skin distribution, the Franz Skin Finite Dose Model evaluated the percutaneous absorption of minoxidil into the dermis and receptor medium using skin samples from two male Caucasian donors. Following a skin integrity test and 3 hours of diffusion, the minoxidil was extracted from the dermis layer and analyzed by UPLC. It was shown that the percutaneous absorption into the dermis and receptor medium was similar for the two minoxidil products. The in vitro performance of the minoxidil 5% topical lotion hydrogel was comparable to the commercial product of reference. As such, the easy to compound, alcohol-free formulation is a promising therapeutic alternative in androgenetic alopecia.

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Retinoic acid receptor-related orphan receptor (ROR) agonists impact cellular responses to UV radiation in human keratinocytes

W. Cvammen[1], M. G. Kemp[1, 2]
[1]*Pharmacology & Toxicology, Wright State University Boonshoft School of Medicine, Dayton, Ohio, United States,* [2]*Research, Dayton VA Medical Center, Dayton, Ohio, United States*

Retinoic acid receptor-related orphan receptor (ROR) agonists have been shown to regulate circadian rhythms in cultured cells and to improve various disease outcomes in animal models. Because expression of the core nucleotide excision repair protein XPA is governed by the circadian clock, we examined whether a natural and synthetic ROR agonist (nobiletin and SR1078, respectively) impact XPA expression and cellular responses to UV radiation in human keratinocytes. Interestingly, though these compounds have little effect on total XPA expression, they both reduce the expression of the endopeptidase cathepsin L (CTSL), which is known to act on several nuclear proteins, including at the C-terminus of XPA under defined cell lysis conditions. We further show that NOB and SR1078 slow keratinocyte proliferation to varying extents and that while NOB protects cells from the lethal effects of UVB radiation, it acts as a photosensitizer in response to UVA radiation. Thus, these results help to better characterize the potential functions of ROR agonists in human keratinocytes exposed to UV radiation.

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Development of LY3454738, an agonistic antibody to human CD200R

A. Koester[1], S. C. Potter[1], D. I. Ruiz[2], K. D. Werle[1], S. P. Bauer[3], D. R. Witcher[3], L. Malherbe[4], J. Rhoden[5], S. Demarest[2]
[1]*Immunology, Eli Lilly and Company, San Diego, California, United States,* [2]*Biotechnology, Eli Lilly and Company, San Diego, California, United States,* [3]*Biotechnology, Eli Lilly and Company, Indianapolis, Indiana, United States,* [4]*Toxicology, Eli Lilly and Company, Indianapolis, Indiana, United States,* [5]*ADME, Eli Lilly and Company, Indianapolis, Indiana, United States*

CD200R is an immune receptor of the IgG family that is primarily expressed on cells of the myeloid lineage and was recently identified as a marker for Th2 biology (Blom et al 2017). *In vivo* studies with knockout mice of either the receptor or its ligand, CD200, have demonstrated that it is an inhibitory receptor capable of negatively regulating immune responses. Previous work using agonistic antibodies to mouse CD200R showed inhibition of mast cell activation in vitro and *in vivo* (Cherwinski et al 2005) as well as efficacy in multiple preclinical models of autoimmune diseases. We developed an agonistic antibody to the human CD200 receptor to downregulate the immune system in multiple human inflammatory conditions. LY3454738, is a humanized IgG4 monoclonal antibody that was derived from a rabbit antibody discovered by immunizing rabbits with alternating soluble extracellular domain (ECD) of hCD200R and cyno CD200R protein. The antibody was selected based on desired properties for agonism and cross-reactivity to cyno CD200R. In vitro LY3454738 demonstrated inhibition of FcγR induced cytokine secretion from a human myeloid cell line as well as inhibition of primary mast cell activation. *In vivo* the antibody demonstrated efficacy in a humanized mouse model of contact hypersensitivity as well as passive cutaneous anaphylaxis in cynomolgus monkeys. After demonstrating safety and tolerability in a phase 1 trial in healthy volunteers, LY3454738 is currently being studied in patients with atopic dermatitis. The poster will describe properties and functional activities of the clinical drug candidate.

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A novel curcumin-harmine-isovanillin compound inhibits the growth of actinic keratoses by suppressing MAPK and PI3K-AKT signaling

Z. Bordeaux[1], J. Choi[1], K. Lee[1], G. Braun[2], J. Deng[1], V. Parthasarathy[1], M. P. Alphonse[1], C. West[3], S. G. Kwatra[1], M. Kwatra[2]
[1]*Johns Hopkins Medicine Department of Dermatology, Baltimore, Maryland, United States,* [2]*Anesthesiology, Duke University School of Medicine, Durham, North Carolina, United States,* [3]*Genzada Pharmaceuticals USA, Inc, Hutchinson, Kansas, United States*

Actinic keratoses (AK) are premalignant cutaneous lesions with limited treatment options. Here we investigate GZ21T, a novel anti-tumor agent composed of curcumin, harmine, and isovanillin, in the treatment of AK. The pro-apoptotic effects of GZ21T on EGF-stimulated HaCaT cells were assessed by Annexin-V assay, and the mechanism was clarified by western blot. Treated cells were subjected to RNA-sequencing and reverse phase protein array. Differentially expressed genes (DEGs) were calculated using DESeq2 for R. Gene set enrichment analysis (GSEA) was performed using fgsea for R, and gene set variation analysis (GSVA) was conducted to evaluate GZ21T's effect on pathways implicated in the pathogenesis of AK. Enrichment analysis was conducted on hypophosphorylated proteins using Enrichr. SKH-1 mice were exposed to UVB (500 J/m2) five times weekly for ten weeks and were subsequently treated with 0.25 grams topical GZ21T or control cream five days per week. An increase in the percentage of HaCaT cells in early ($p=0.041$) and late apoptosis ($p=0.029$) was observed with increasing GZ21T concentrations, which was partially mediated by PARP cleavage. After 40 days, GZ21T treated mice showed decreased lesion count ($p=0.028$) and tumor surface area ($p=0.026$). We identified 92 DEGs between control and GZ21T treated HacaT cells, with upregulation of TP53 and NOTCH1 tumor-suppressor genes. GSEA showed that GZ21T downregulates KRAS and mTOR signaling. GSVA revealed upregulation of transforming growth factor-beta (TGF-β) secretion and NOTCH signaling with downregulation of MAPK1 and 3 activation ($p<0.05$ for all). Proteomic analysis demonstrated downregulation of ErbB, mTOR, HIF-1, and PI3K-AKT signaling. In conclusion, GZ21T inhibits the growth of AK through suppression of targets related to MAPK, PI3K-AKT, HIF-1, and mTOR signaling.

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Selective inhibition Of fibro-inflammatory kinase TAK1: A potential therapeutic strategy to ameliorate systemic sclerosis

S. Bale[1], P. Verma[1], B. Yalavarthi[1], S. Arthur Scarneo[2], T. A. Haystead[2], S. Bhattacharyya[1], J. Varga[1]
[1]*University of Michigan, Ann Arbor, Michigan, United States,* [2]*Duke University, Durham, North Carolina, United States*

Fibrosis leads to failure of the skin, lungs, and other organs in systemic sclerosis (SSc); accounts for substantial morbidity and mortality; and lacks effective therapy. Recent findings implicated TGFβ-activated kinase 1 (TAK1), triggered by TGF-β and toll-like receptor signaling, in SSc pathogenesis. The objectives were to evaluate the activation of TAK1 signaling axis in SSc patients and to evaluate the antifibrotic ability of pharmacological TAK1 blockade in organ fibrosis. HS-276, a drug-like novel small molecule inhibitor of TAK1 was screened for its anti-fibrotic potential in cell cultures using foreskin, adult and SSc skin fibroblasts, and in bleomycin induced skin and lung fibrosis model. HS-276 treatment ameliorated dermal and pulmonary fibrosis and reduced the expression of several pro-fibrotic mediators in bleomycin-treated mice compared to vehicle-treated control. Importantly, HS-276 induced the regression of pre-established organ fibrosis. HS-276 abrogated TGF-β1-induced activation of collagen synthesis and myofibroblasts differentiation in explanted normal skin fibroblasts (neonatal and adult) and in constitutively active SSc fibroblasts. The antifibrotic effects of HS-276 were accompanied by reduced phosphorylation of p38 MAPK and JNK signaling as well as reduced expression IL-6 and other inflammatory markers. These findings implicate the significance of TAK1 signaling pathway in SSc skin and lung fibrosis and identify HS-276 as a potential antifibrotic agent for the treatment of SSc and other fibrotic diseases.

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Microarray patch-based adenovirus vaccines enable effective skin immunization

S. C. Balmert, Z. G. Ghozloujeh, C. Carey, T. L. Sumpter, G. Erdos, E. Korkmaz, L. Falo Jr.
Dermatology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States

Recombinant live adenovirus (Ad) vectors represent a readily modular vaccine construct that can be engineered to include any antigen of interest, thereby enabling rapid vaccine development against a myriad of infectious pathogens, including coronaviruses. However, current approaches used for delivery, storage, and distribution of Ad vaccines hinder their full potential for effective global immunization campaigns. Here, we developed simple, effective, practical, and needle-free Ad vaccines based on microarray patches (MAPs) to enable sustainable mass vaccination against SARS-CoV-2. Rational formulation of live Ads with nonreducing sugars and mechanically strong carbohydrates into dissolvable MAPs enabled effective skin-targeted delivery of Ads and efficient cutaneous transduction as determined by *in vivo* live imaging of mice following skin application of MAPs integrating Ad vectors with a reporter gene. Immunogenicity assessment of dissolving MAP-based Ad vectors encoding SARS-CoV-2 proteins in mice demonstrated that skin immunization via MAP delivery of Ad-vectored COVID-19 vaccines elicited robust antigen-specific antibody responses, and these antibodies led to virus-specific neutralization activities, which were enhanced compared to those obtained with traditional intramuscular immunization. Furthermore, MAP delivery of Ad-vectored vaccines elicited potent cell-mediated immune responses, including polyfunctional virus-specific CD8+ and CD4+ T-cell responses in spleens and lungs of immunized mice, as determined by intracellular cytokine staining and flow cytometry, as well as antigen-specific cytotoxic T-cell responses in spleens of mice, as determined by lytic assay. Collectively, our results suggest that dissolvable MAPs could enable the development of skin-targeted Ad-based vaccines that may increase the effectiveness of global immunization programs for SARS-CoV-2 and other existing or future pathogens.

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Pharmacological inhibition of the chemokine receptor CX3CR1 promotes hair growth

Y. Chang[1, 2], Z. Dai[1], A. M. Christiano[1, 3]
[1]*Dermatology, Columbia University Vagelos College of Physicians and Surgeons, New York, New York, United States,* [2]*Dermatology, Air Force Medical University, Xi'an, Shaanxi, China,* [3]*Genetics & Development, Columbia University Vagelos College of Physicians and Surgeons, New York, New York, United States*

Macrophages are located around hair follicles in normal skin and typically peak in density during the mid-telogen stage of the hair cycle. Hair follicles chemotactically attract macrophages and T cells and are in range to regulate hair follicle stem cells (HFSCs) quiescence, proliferation, and differentiation during physiologic and injured states. The expression of the fractalkine receptor CX3CR1 has been identified as a key regulator of macrophage and T cell migration and function at sites of inflammation. To define the role of CX3CR1 expressing immune cells in hair cycle, we analyzed the single-cell RNA sequencing of the dermal CD45+ immune cells during early (p45), mid (P50 and P63), and late (P80) telogen stages of the hair cycle in C57/B6 mice. The scRNA-seq analysis revealed that CX3CR1 expression is tightly restricted in the TREM2+ macrophage subset and cytotoxic CD8+ T cells, and CX3CR1+TREM2+ macrophages were detected around mid-telogen HFSCs using immunofluorescence. We previously showed that Oncostatin M (OSM)-producing TREM2+ macrophages are sufficient for maintaining quiescence of HFSCs, and TREM2+ macrophages inhibition using pexidartinib, a small-molecule inhibitors of macrophage receptors Csf1r, was effective at initiating anagen at P60 in C57/B6 mice. Here, we observed that the highly selective CX3CR1 inhibitor AZD8797 effectively depleted TREM2+ macrophages and initiate local anagen at the point of administration within 21 days by topical application and within 14 days via subcutaneous injection at P60 telogen skin, whereas no hair growth was evident in vehicle control mice. Our data indicate that CX3CR1 plays an important role in the migration of OSM-producing TREM2+ macrophages into telogen skin and blocking CX3CR1 signaling pathway through small molecule inhibition represents a novel axis to induce hair growth.

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An *in vitro* psoriasis model for high throughput screening

A. Pappalardo[1], P. Vasilikos[1], M. Nathaniel[1], Z. Guo[1], H. E. Abaci[1], A. M. Christiano[1, 2]
[1]*Dermatology, Columbia University Vagelos College of Physicians and Surgeons, New York, New York, United States,* [2]*Genetics and Development, Columbia University Vagelos College of Physicians and Surgeons, New York, New York, United States*

Psoriasis is a common inflammatory dermatosis, affecting up to 3% of the US population. It presents with thick erythematous papules and plaques, characterized by abundant scaling, occasional itch and a variable pattern of distribution. Psoriasis is a multifactorial disease with a strong genetic component and is associated with Th1/Th17 skewing, but the specific etiology is unknown. Notably, psoriasis does not occur spontaneously in small mammals, which makes the development of new and effective drugs a challenging process. To address the lack of an *in vivo* model, we developed a scalable in vitro platform using human skin constructs (HSCs) that recapitulates several histological and biochemical features of the disease, and can be used in high throughput screening. The model can be generated using either human primary cells or iPSC-derived keratinocytes and fibroblasts. The psoriatic-like phenotype was induced by supplementing the medium with serum derived from the culture of activated psoriatic CD4+ lymphocytes polarized to Th1 and Th17 subsets. We tested the therapeutic efficacy of 12 different drugs (Apremilast, Cyclosporin A, Methotrexate, Apremilast, Ruxolitinib, Tofacitinib, Abrocitinib, Ustekinumab, Celeoxib, Meloxicam, Sulfasalazine, Teriflunomide) in a 12-well plate format, and can be modified to a 96-well plate format in a high throughput setting. Using ELISA we quantified the concentration of several key cytokines such as IL-8 and CCL20 in the culture medium. We further characterized the effect of the drug treatments on reversal of the psoriatic histological phenotype with immunofluorescent microscopy, assessing key markers of the disease such as Ki67, keratin 16, involucrin, filaggrin and loricrin. Our findings demonstrated the utility of 3D skin models to recapitulate key phenotypic features of skin inflammatory diseases, and can be adapted to efficiently screen drug libraries in a high throughput setting.

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UVB-mediated DNA damage induces matrix metalloproteinases to promote photoaging in an AhR- and SP1-dependent manner

D. J. Kim[1], A. Iwasaki[1], A. L. Chien[2], S. Kang[2]
[1]Yale School of Medicine, New Haven, Connecticut, United States, [2]Johns Hopkins Medicine, Baltimore, Maryland, United States

It is currently thought that ultraviolet B (UVB) radiation drives photoaging of the skin primarily by generating reactive oxygen species (ROS). In this model, ROS purportedly activates AP-1 to upregulate matrix metalloproteinases (MMPs) 1, 3, and 9, which then degrade collagen in the dermis to produce wrinkles. However, these MMPs are expressed at relatively low levels in the skin and correlate poorly with wrinkles in mice, suggesting that another mechanism may be more directly associated with photoaging. Here we show that MMP2, which degrades type IV collagen in the dermal-epidermal junction, is abundantly expressed in human skin, increases with age in sun-exposed skin, and correlates robustly with aryl hydrocarbon receptor (AhR), a transcription factor directly activated by UV-generated photometabolites that can suppress repair of bulky DNA lesions. Through mechanistic studies with HaCaT keratinocytes, we found that AhR, SP1, and other pathways associated with DNA damage are required for the induction of both MMP2 and MMP11 (another MMP implicated in photoaging) mRNA, but not MMP1/3. Consistent with these findings, DNA damage by topoisomerase inhibitor was sufficient to induce MMP2/11 but not MMP1/3/9. Lastly, we found that chronic UVB treatment in hairless mice generated a distinct band-like pattern of MMP2 in the epidermis of mice, while topical treatment with AhR antagonists vitamin B12 and folic acid ameliorated UVB-induced wrinkle formation and dampened MMP2 expression in the skin. From these data, we propose an expanded model of photoaging whereby UVB-mediated DNA damage induces MMP2 through DNA damage in the epidermis, ultimately causing focal damage to the underlying type IV collagen in the basement membrane, while UV-induced ROS leads to the degradation of type I and type III collagen in the dermis. This model not only provides a molecular basis for the complex changes underlying photoaging, but also underscores the therapeutic potential of AhR modulation in preventing photoaging.

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RNA methylation facilitates the repair of UV-induced DNA damage and suppresses photocarcinogenesis

Z. Yang[1], S. Yang[1], Y. Cui[1], J. Wei[2], P. Shah[1], G. Park[1], X. Cui[2], C. He[2], Y. He[1]
[1]University of Chicago Division of the Biological Sciences, Chicago, Illinois, United States, [2]University of Chicago Division of the Physical Sciences, Chicago, Illinois, United States

UV-induced DNA damage is repaired by nucleotide excision repair (NER) that corrects bulky helix-distorting DNA lesions across the whole genome and is essential for preventing mutagenesis and skin cancer. Here we show that METTL14 (methyltransferase-like 14), a critical component of the m6A RNA methyltransferase complex, promotes the repair of UV-induced DNA damage through regulating m6A mRNA methylation-mediated translation of the NER factor DDB2 and suppresses UV-induced skin tumorigenesis. Ultraviolet irradiation down-regulates METTL14 protein through NBR1-dependent selective autophagy. METTL14 knockdown decreases NER and DDB2 abundance. Conversely, overexpression of wild-type METTL14, but not its enzymatically inactive mutant, increases NER and DDB2 abundance. METTL14 knockdown decreases m6A methylation and translation of the DDB2 transcripts. Adding DDB2 reverses the DNA repair defect in METTL14 knockdown cells, indicating that METTL14 facilitates NER through regulating DDB2 m6A methylation and translation. Similarly, knockdown of YTHDF1, an m6A reader promoting translation of m6A modified transcripts, decreased DDB2 protein levels. Both METTL14 and YTHDF1 bind to the DDB2 transcript. In mice, skin-specific heterozygous METTL14 deletion increases UV-induced skin tumorigenesis. Furthermore, METTL14 as well as DDB2 is down-regulated in human and mouse skin tumors and by chronic UV irradiation in mouse skin, and METTL14 level is associated with the DDB2 level, suggesting a tumor-suppressive role of METTL14 in UV-associated skin tumorigenesis in association with DDB2 regulation. Taken together, these findings demonstrate that METTL14 is a target for selective autophagy and acts as a critical epitranscriptomic mechanism to regulate the repair of UV-induced DNA damage and suppresses photocarcinogenesis.

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Phototherapy-induced IFNκ drives type I IFN induced anticancer responses in CTCL

Z. Yu[1], A. Gehad[1], J. Teague[1], J. Crouch[1], K. Yu[1], J. O'Malley[1], T. Kupper[1], T. Benezeder[2], J. E. Gudjonsson[3], J. M. Kahlenberg[3], M. Sarkar[3], P. Vieyra-Garcia[2], P. Wolf[2], R. A. Clark[1]
[1]Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Medizinische Universitat Graz, Graz, Steiermark, Austria, [3]University of Michigan Medical School, Ann Arbor, Michigan, United States

Study of Stage I-II mycosis fungoides (MF) skin lesions from 14 patients by gene expression profiling demonstrated reduced expression of six type I IFNs compared to healthy human skin. Psoralen plus UVA therapy (PUVA) induced increased levels of type I IFNs that were associated with CD8 T cell recruitment into tumors, expression of antigen specific T cell activation genes and depletion of malignant T cells. MF skin lesions expressed significantly higher levels of two negative regulators of IFN production, IRF2 and IFI35; IRF2 levels correlated with the number of malignant T cells in skin. IRF2 is known to antagonize epithelial production of type I IFNs. Indeed, we found reduced expression of ULBP2, a marker of epithelial type I interferon production. Immunostaining confirmed reduced epidermal production of type I alpha IFNs that was reversed by PUVA therapy. IFNκ is an epithelial type I interferon induced by UVB that triggers production of other type I IFNs and is responsible for the flaring of cutaneous lupus after sun exposure. We studied human keratinocytes treated with UVA with or without psoralen. IFNκ enhanced keratinocyte production of type I IFNs and downstream interferon genes in response to UVA. IFNκ knockout keratinocytes had markedly reduced type I IFNs induction after UVA, illustrating the key role of epithelial IFNκ in boosting type I interferon production. In summary, we find that type I IFNs are markedly reduced in MF lesions, that phototherapy induces epithelial type I interferon production via INFκ and that this increase is associated with effective tumor clearance. Our results suggest other medications that induce epithelial type I IFNs, including MEK or EGFR inhibitors, may be effective therapies for CTCL.

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Translation and growth pathways are directly influenced by autoimmune regulator (Aire) in skin keratinocytes

R. P. Feehan, K. R. Patrick, T. Gao, R. Hobbs
Dermatology / Immunology & Mol. Bio, Penn State College of Medicine, Hershey, Pennsylvania, United States

In response to environmental stressors, such as ultraviolet B (UVB) radiation, keratinocytes activate a stress response marked by coordination between signaling pathways associated with proliferation, DNA damage, and apoptosis. Herein, we have identified autoimmune regulator (AIRE), which is classically thought of as a transcriptional regulator, as a key influencer of apoptosis, the DNA damage response (DDR), and surprisingly protein synthesis in stressed skin keratinocytes. Initially, we created keratinocyte cell lines stably and inducibly expressing AIRE (either wild-type or with function blocking mutations) fused to the BirA* biotin ligase and subjected these cells to a proximity based biotinylation screen (BioID) to identify potential AIRE binding partners. Using isobaric labeling (iTRAQ) coupled to tandem mass spectrometry, we identified that the AIRE binding partners most affected by function blocking AIRE mutations included UVB-associated proteins related to apoptosis, DNA damage, and protein translation. To validate these findings, UVB-irradiation (25 mJ/cm2) of CRISPR-Cas9 mediated AIRE knock-out (KO) keratinocytes showed increased apoptotic signaling (BIM, BID, NOXA, FOXO3a, & FasL) and cell death (Cleaved Caspase-3 -8 -9 and Cleaved PARP) compared to wild-type cells. The DDR (P-CHK1, P-CHK2, P-H2B, P-ATM, P-ATR, & γH2AX) was also increased in keratinocytes that lacked AIRE following exposure to UVB (25 mJ/cm2) or peroxide treatment (100 μM H2O2, 30 min). Exposure to UVB radiation concurrently increased protein translation pathways (P-S6K, P-S6, & P-4EBP1) in AIRE KO cells. Using puromycin incorporation (3 μM), we found global protein synthesis was similarly increased in AIRE KO cells following a 2h serum pulse. This influence of AIRE on protein translation indicates a novel non-nuclear function for AIRE. Altogether, our data reveal AIRE to be a coordinate regulator of multiple UVB-induced cell stress response pathways in skin keratinocytes and helps to expand our understanding of AIRE's function in skin photobiology.

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Skin aging: Investigation of a synergistic effect between cigarette smoke and sun rays using tissue-engineered skin substitutes

A. Grenier[1, 2], P. J. Rochette[1, 3], R. Pouliot[1, 2]
[1]*Centre de Recherche en Organogénèse Expérimentale de l'Université Laval/LOEX, Axe Médecine Régénératrice, Centre de recherche du CHU de Québec-Université Laval, Quebec, Quebec, Canada,* [2]*Faculté de pharmacie, Universite Laval, Quebec, Quebec, Canada,* [3]*Département d'ophtalmologie et ORL-chirurgie cervico-faciale, Universite Laval Faculte de medecine, Quebec, Quebec, Canada*

Skin aging is influenced by two distinct processes: intrinsic and extrinsic aging. The latter regroups the external factors influencing skin aging, including cigarette smoke and sun rays. While these factors, studied separately, are known to cause premature skin aging, the effects of their synergy have been poorly characterized. The objective of this project is to assess the harmful impact of this synergy on skin aging. Healthy skin substitutes were produced according to the self-assembly method and then exposed to cigarette smoke extract (CSE), followed by irradiations at different doses of UVA (5, 10 and 20 kJ/m2). CSE was obtained by capturing cigarette smoke using an AGI-30 impinger. The skin substitutes were analyzed by histology, immunofluorescence, dot blot (collagen-I, collagen-III, collagen-IV, elastin) and western blot (p38 MAPK, ERK1/2, JNK). A decrease in the living epidermis thickness was observed following exposure to CSE combined with 20 kJ/m2 of UVA. A significant decreased in collagen-III and collagen-IV expression was observed with the immunostainings when skin substitutes were exposed to CSE and then irradiated, which correlates with a decrease in pro-collagen synthesis and an increase in the activity of MMP-1. Finally, the ERK1/2 signaling pathway seems to be involved in this synergy. This work demonstrates a synergistic effect between cigarette smoke and sunlight on the expression of proteins altered during skin aging and it would be in part caused by an activation of the ERK1/2 signaling pathway.

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Development and assessment of nanoparticle-encapsulated sunscreens

B. Yu[1], H. Suh[2], A. Zhou[1], S. Tomer[1], K. Shin[2], M. Swallow[1], J. Lewis[1], W. Saltzman[2], M. Girardi[1]
[1]*Dermatology, Yale School of Medicine, New Haven, Connecticut, United States,* [2]*Biomedical Engineering, Yale School of Engineering and Applied Science, New Haven, Connecticut, United States*

The risk of developing skin cancer from ultraviolet radiation (UVR) can be mitigated with sunscreens. Safety and aesthetic concerns about commercial sunscreens have led to our development of a bioadhesive nanoparticle (BNP) sunscreen platform. Building upon prior work encapsulating FDA-approved agents avobenzone and octocrylene, we have now also encapsulated EU-approved agents diethylamino hydroxybenzoyl hexyl benzoate (DHHB) and octinoxate (OCX) with 50% and 30% loading, respectively. Nonadhesive nanoparticles (NNPs) are produced initially and can be converted to BNPs, which possess an aldehyde-rich corona that can bind epidermal amines. To assess bioadhesion, 0.01 mg/cm2 of BNPs-DHHB and OCX were applied to Vitro-Skin and underwent continuous stirring in a water bath. 100% of BNPs were retained after 1 hour compared to 35-58% of NNPs. The in vitro SPF of NNPs-DHHB and OCX was 100. Furthermore, photostability testing demonstrated that encapsulation maintains DHHB stability upon UVR exposure. To complement in vitro testing of BNP sunscreens, *in vivo* studies are needed to examine their ability to prevent UVR-induced mutagenesis, which occurs through direct and indirect mechanisms. In the direct pathway, photons absorbed by DNA cause immediate damage including formation of a key mutagenic DNA photoproduct: the cyclobutane pyrimidine dimer (CPD). CPDs also result from indirect DNA damage mediated by non-DNA chromophores that may compromise DNA long after UVR exposure ends, forming so-called dark CPDs (dCPDs). We explored two methods for quantifying dCPDs in murine skin following a single, acute UVA (100kJ) exposure: ELISA and immunofluorescent staining. Both methods demonstrated dCPDs peaking ~30 min after UVA exposure and provide a way to assess sunscreen efficacy in preventing UVR-induced DNA damage in living skin. Evaluating *in vivo* performance over chronic exposure protocols will support in vitro characterization in optimizing a BNP sunscreen formulation.

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Far ultraviolet-C radiation from a filtered KrCl lamp does not result in migration of Langerhans cells in human skin

M. J. Conneely[1], D. Grussu[1], S. K. Hirata Tsutsumi[1], P. O'Mahoney[2], S. H. Ibbotson[2, 3], E. Eadie[3], R. P. Hickerson[1]
[1]*Biological Chemistry and Drug Discovery, University of Dundee School of Life Sciences, Dundee, Dundee, United Kingdom,* [2]*University of Dundee School of Medicine, Dundee, Dundee, United Kingdom,* [3]*Ninewells Hospital Photobiology Unit, Dundee, United Kingdom*

There is global interest in both the beneficial and detrimental health effects of ultraviolet-C (UVC) radiation in the wavelength range 200-230 nm (known as Far-UVC). Technology using Far-UVC is proposed as a highly effective control measure for reducing the transmission of COVID-19. Far-UVC, and other wavelengths of UVC, are well-known to efficiently inactivate pathogens in air and on surfaces. Although studies have shown irradiation of skin with 254 nm UV results in DNA damage in the epidermal basal layer, irradiation with Far-UVC (222 nm) shows minimal DNA damage and only in the granular layer, which is comprised of non-proliferating keratinocytes. Therefore, accumulation of these DNA photoproducts would not be expected to be associated with cancer risk. It has also been shown that even high doses of Far-UVC exposure to human skin do not induce erythema. However, the effects of Far-UVC on the immune system are, to the best of our knowledge, unknown. It is well-reported that both ultraviolet B (UVB 280-315 nm) and ultraviolet-A (UVA 315-400 nm) have effects on cutaneous Langerhans cells (LC), inducing migration from the epidermis to the draining lymph nodes, thereby suppressing skin immune function. Here we present data generated in a range of skin types (Fitzpatrick II-V) demonstrating little or no impact of Far-UVC on the cutaneous immune system, as assessed by Langerhans cell migration, at doses of up to 3,000 mJ/cm2 (US daily limit is 450 mJ/cm2). These results support the safety of filtered Far-UVC use, which could have a transformative effect on public health, allowing effective virus inactivation and reduction of transmission independent of human behavior. Conflict of interest disclosure: the authors state no conflict of interest. However, MJC and RPH are directors of Ten Bio Ltd, a company focused on developing human skin explant models.

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Development of a natural product-based sunscreen

A. Zhou, B. Yu, J. Lewis, M. Girardi
Dermatology, Yale School of Medicine, New Haven, Connecticut, United States

Sunscreens are a cornerstone of skin cancer prevention; however, currently available sunscreens have limitations. Concerns regarding current organic sunscreen agents include photodegradation, potential disruption of endocrine pathways, and paradoxical generation of damaging reactive oxygen species (ROS). Recent studies by the FDA have shown that organic sunscreens permeate into systemic circulation at levels warranting further safety testing, highlighting the importance of investigating alternative strategies for safe and effective photoprotection. Towards this goal, we previously conducted high-throughput screening of natural compounds for photoprotective potential, quantifying UV spectral absorbance, photostability, cytotoxicity, and ROS generation. From a pilot set of 915 compounds, we prioritized 11 candidates of interest for confirmatory photostability testing and follow-on in vitro SPF testing at 5wt% in P2 formulation. Top performers were diosmin (DIO; SPF 2.7), isoliquiritigenin (IQL; SPF 2.5), ferulic acid (FA; SPF 3.1), and cytisine (CYT; SPF 6.3). To create a broad-spectrum formulation, these agents were assessed individually and in combination for in vitro SPF and critical wavelength (λcrit), and *in vivo* in C57BL/6J mice for protection against ultraviolet radiation (UVR)-induced cyclobutane pyrimidine dimer (CPD) formation and irritant contact dermatitis potential. 10% DIO + 10% FA + 5% CYT was the best performer of our test combinations, with a mean in vitro SPF of 48.6 (SD 3.2), λcrit of 373, and 92.4% CPDs blocked (SD 3.5%). Other promising combinations utilizing varying concentrations of FA and CYT with DIO or IQL also demonstrated λcrit>370 and SPF>15, i.e. the FDA minimum requirements for decreasing the risk of skin cancer. None of the combinations induced irritant dermatitis as assessed by gross and histologic examination after 5 repeated applications to shaved dorsal skin every 2 days. Collectively, these data demonstrate proof of concept for high throughput screening as a method of identifying candidate alternative sunscreen agents toward the formulation of a natural product-based sunscreen.

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Painless photodynamic therapy for actinic keratosis: Enhancement of innate and adaptive immune responses by 5-fluorouracil pretreatment in a murine model

S. Anand[1, 2, 3], L. Heusinkveld[3], L. Lefatshe[1], E. Maytin[2, 1, 3]
[1]Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio, United States, [2]Dermatology and Plastic Surgery Institute, Cleveland Clinic, Cleveland, Ohio, United States, [3]Cleveland Clinic Lerner College of Medicine, Cleveland, Ohio, United States

Painless photodynamic therapy (pPDT) to treat actinic keratoses (AK) involves application of a photosensitizer followed by immediate exposure to light; this causes little-to-no pain, yet results in effective lesion clearance by inducing long-term immune responses. In this investigation, we show that pretreatment of AK lesions with 5-fluorouracil (5FU), a popular chemotherapeutic agent with immune-modulatory effects, causes significant enhancement of PDT-related immune responses in 5FU-treated lesions. Hairless mice with AK lesions (generated by repeated UVB exposure for 20 weeks) were treated topically with 5FU or with vehicle for three days prior to application of topical ALA followed immediately by light (Blu-U) exposure. Lesions were harvested for time-course analyses of intra-tumoral immune responses after PDT with or without 5FU. Our immunofluorescence data showed increased recruitment of innate immune cells, i.e., neutrophils (Ly6G+) and macrophages (F4/80+), which peaked at 72 hours and 1-week post pPDT, respectively, and was greater in 5FU treated lesions. Also, enhanced infiltration of activated T cells (CD3+) throughout the time course, and of cytotoxic T cells (CD8+) approximately 1 - 2 weeks post pPDT, was observed in 5FU treated lesions. In addition to its effects on innate and adaptive immunity, 5FU pretreatment significantly reduced the presence of cells expressing, the immune checkpoint marker PD1, at ~72 hours post pPDT, which should favor an anti-tumor immune response by promoting inflammatory, cytotoxic T cell activity. Our results suggest that a combination of these two cancer therapies (5FU and pPDT), each individually known to induce long-term anti-tumor immune responses in addition to their primary effects on cancer cells, may synergize to provide better management of AK in the dermatology clinic.

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Theabrownin in black tea suppresses UVB-induced MMP-1 expression in HaCaT keratinocytes

H. Kim[1, 2], E. Kim[1], E. Lee[1], N. Park[1], Y. Hong[1], J. Jung[1]
[1]Amore-Pacific Research and Development Center, Yongin, Gyeonggi-do, Korea (the Republic of), [2]Department of Chemistry and Cosmetics, Jeju National University, Jeju, Jeju, Korea (the Republic of)

Theabrownin is a heterogeneous polyphenolic compound, obtained from fermented dark teas, with many bioactive functions. However, the role of theabrownin in skin photoaging remains unclear. In this study, we investigated whether theabrownin modulates ultraviolet B (UVB)-induced matrix metalloprotease-1 (MMP-1) expression in HaCaT keratinocytes. Theabrownin exerted antioxidant properties, which resulted in scavenging of intracellular reactive oxygen species generated by UVB irradiation in HaCaT cells. Consequently, theabrownin inhibited UVB-induced extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK) phosphorylation, which are activated by oxidative stress. Theabrownin also suppressed the downstream signaling pathway of mitogen-activated protein kinase (MAPK) and the nuclear accumulation of activator protein-1 (AP-1), which is an essential transcription factor for UVB-stimulated MMP-1 expression. Based on these results, we conclude that theabrownin can serve as a novel anti-photoaging compound for the skin.

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UVB-irradiated keratinocyte-derived extracellular vesicles induced STING and inflammasome mediated proinflammatory responses

Y. Li[1, 2], T. Vazquez[1, 2], M. Ogawa-Momohara[1, 2], V. Werth[1, 2]
[1]CMCVAMC, Philadelphia, Pennsylvania, United States, [2]University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: Ultraviolet B irradiation (UVB) contributes to skin inflammation. As UVB mostly affects the epidermis, the crosstalk between epidermis and dermis in the response to UVB needs investigation. Extracellular vesicles (EVs), lipid bilayer membrane vesicles secreted by cells, can carry lipids, proteins and nucleic acids to mediate signal transduction. Stimulator of interferon genes (STING) and inflammasome activation-mediated pyroptosis play critical roles in immunity and inflammation. EVs derived from UVB-irradiated keratinocytes might trigger STING and inflammasome-mediated proinflammatory responses. Methods: HaCaT cells were irradiated with UVB, then cultured for 24 hours and the supernatant was harvested for EV collection. EVs were isolated by ultracentrifugation and used to stimulate dermal cells with/without inhibitors. The supernatant was harvested for ELISA and the lysed cells were collected for Western blot. WT and STING KO mice were treated with/without UVB for 5 days. Dorsal skin were collected for histological analysis. Results: UVB irradiated HaCaT cells released more extracellular vesicles with small EV surface markers (1.78x109/mL vs. 3.31x108/mL). KEV-UVB triggered more interferonb (IFNb) release from macrophages than fibroblasts(111.1±21.45 vs. 4.85±0.72 pg/mL $P<0.05$ n=3). Inhibition of the STING signaling pathway attenuated KEV-UVB triggered IFNb (13.18±6.38 vs. 111.1±21.45 pg/mL $P<0.05$) and interleukin 1b (IL1b) (60.52±11.41 vs. 325.2±62.68 pg/mL $P<0.05$) production in macrophages, while suppression of the inflammasome pathway attenuated KEV-UVB triggered IL1b (22.00±5.01 vs. 34.67±5.85 pg/mL $P<0.05$) but not IFNb (636.9±165.1 vs. 680.8±147.1 pg/mL $P>0.05$) in macrophages. Conclusions: KEV-UVB were mediators of inflammation, and triggered both STING and inflammasome-mediated cytokine release. Inflammasome worked as downstream of STING during KEV-UVB-mediated proinflammatory response. Targeting STING and inflammasome might be potential therapeutic approaches for UVB-induced skin inflammation.

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Intrinsic versus extrinsic skin aging: Extrinsically differ from intrinsically aged human skin fibroblasts in their metabolic adaptive responses and by carrying a signature of catastrophic failure.

S. Schneider[1], M. Pollet[1], M. Majora[1], S. Faßbender[1], A. Marini[1], J. Hüsemann[1], M. Knechten[1], H. Schwender[2], J. Krutmann[1, 3, 4]
[1]IUF – Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany, [2]Mathematical Institute, Heinrich Heine University Duesseldorf, Duesseldorf, Germany, [3]Medical Faculty, Heinrich Heine University Duesseldorf, Duesseldorf, Germany, [4]Human Phenome Institute, Fudan University, Shanghai, China

Aging of human skin results from genetic (= intrinsic) factors, which affect all parts of human skin, and non-genetic (= extrinsic) factors such as solar radiation, which impact only selected skin areas. For both, age-dependent changes in dermal fibroblasts are relevant. To better understand the relationship between intrinsic and extrinsic skin aging, we analyzed dermal skin fibroblasts which were obtained from intrinsically (NHDFINT) versus extrinsically (NHDFEXT) aged human skin of the same donors of one of three age groups (young, middle-aged and old). Proteome analysis revealed that age-dependent changes (from young to middle-aged to old) in protein diversity and abundance followed a linear kinetic in NHDFINT, but developed in a non-linear way in NHDFEXT with almost no change between young versus middle-aged and a dramatic alteration from middle-aged to old. In addition, functional enrichment analysis uncovered that (i) NHDFINT and NHDFEXT from middle-aged donors strikingly differed in their metabolic adaptive responses and that (ii) NHDFEXT isolated from old donors carried a signature of catastrophic failure, which was not present in donor-matched NHDFINT. Of note, treatment of NHDFEXT from middle-aged, but not from old donors with the NAD+ precursor β-nicotinamide mononucleotide rescued their metabolic alterations and resulted in a phenotype which resembled age group-matched NHDFINT. Thus, ageing-associated adaptive response alterations are crucially affected by extrinsic factors in human skin fibroblasts, and their targeting might alter the ageing phenotype.

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Antioxidants suppress pro-inflammatory markers induced by ultraviolet radiation and rural pollution in normal human keratinocytes

A. Ortiz[1], H. Sun[1], M. Matsui[3], T. Carle[2], D. Gan[2], L. Gildea[2], M. Costa[1]
[1]Environmental Medicine, NYU Grossman School of Medicine, New York University, New York, New York, United States, [2]Mary Kay Inc, Dallas, Texas, United States, [3]Skin biology research group, Teaneck, New Jersey, United States

Exposure to ultraviolet radiation (UVR) and airborne pollution, particularly in the form of particulate matter sized 2.5 µm (PM2.5), is associated with signs of skin aging and inflammation. Damage from UVR and PM2.5 is believed to be the result of oxidative stress, therefore, we examined the effects of a novel composition containing three antioxidants in an in vitro model system in order to better understand their potential. We focused in particular on rural sources of PM2.5 in order to obtain information related to this source of PM2.5. A combination of resveratrol, niacinamide and GHK peptide (AOx mix) reduced UVR-induced oxidative stress in normal human epidermal keratinocytes and cells exposed to rural PM2.5 alone and in combination with UVR. The AOx mix increased expression of NRF2 significantly in the presence of PM2.5 and UVR, indicating a protective response. RNAseq analysis demonstrated that exposure to ssUV and rural PM downregulates various NRF2-inducible genes and that the antioxidant mix increased transcription of genes that participate in protective antioxidant functions, such as HMOX1, PRDX1, and TXNRD1. The AOx mix was also effective at preventing increases in CYP1A1 and IL-6 after exposure of cells to a combination of UVR and PM2.5. These results indicate that a novel topical antioxidant preparation may reduce cellular damage that leads to extrinsic skin aging, not just due to UVR, but also exposure to airborne pollution.

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Ultraviolet A mediates the keratinocytes supranuclear melanin cap formation via opsin 3

L. Yinghua, Z. Wen, W. Yu, S. Xiaoping, D. Xian, G. Yangguang, Z. Wei, H. Lu
Department of Dermatology, Affiliated Hospital of Guizhou Medical University, Guiyang, China

Background: the human keratinocytes supranuclear melanin cap acts as a microparasol to protect the nucleus from ultraviolet (UV) induced DNA damage. UV induced cytoplasmic dynein expression mediates supranuclear melanin cap formation. However, the molecular mechanism of keratinocytes responding to UV irradiation and mediating melanin cap formation remains unclear. Opsins(OPNs) belong to the G protein-coupled receptors superfamily and are photosensitive receptor proteins mediating phototransduction by translation of absorbed photons to cellular responses. OPN3 is a member of the opsin family and is widely expressed in mammalian tissues. Its absorption spectrum and function remain unclear. Objective: To investigate whether OPN3 can mediate UVA to induce keratinocytes supranuclear melanin cap formation. Methods: We detected the expression of OPN3 in human primary keratinocyte and HaCaT by real-time fluorescence quantitative PCR and Western blot, and observed the localization of OPN3 by confocal microscopy. silencing OPN3 with small interfering RNA technology and lentivirus transfection technology, ultraviolet irradiation After, the melanin cap formation was observed by Fontana-Masson silver method protocol. And the mechanism of related signal pathway is studied by Western blot. Results: OPN3 is the important light sensor in keratinocytes responsible for UVA mediated supranuclear melanin cap formation. OPN3-mediated melanin cap formation required Ca2+-dependent Gai protein-coupled receptor and cyclic adenosine monophosphate (CAMP) signal transduction, thus contributing to the UVA-induced AKT phosphorylation to upregulated cytoplasmic dynein expression, and providing evidence of OPN3 function in mammalian phototransduction. Conclusion: our study provides insights into the molecular mechanisms by which human keratinocytes respond to UVA radiation supranuclear melanin cap Formation and may further reveal the physiological role of skin response to light.

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The impact of the spectral composition of long-wavelength ultraviolet A1 and visible light on cutaneous biologic effects

M. S. Ceresnie[1], J. Maghfour[1], K. El Dairi[1], M. Mokhtari[2], I. H. Hamzavi[1], H. W. Lim[1], I. Kohli[1]
[1]*Photomedicine & Photobiology Unit, Department of Dermatology, Henry Ford Hospital, Detroit, Michigan, United States,* [2]*Oakland University William Beaumont School of Medicine, Auburn Hills, Michigan, United States*

Background & Aim: Recent studies have demonstrated visible light and long-wavelength UVA1 (VL+UVA1, 370-700 nm) to cause erythema in light skin and synergistically increased pigmentation in dark skin subjects.1, 2 Spectral compositions of VL+UVA1 may further impact these biologic effects. Yet, no phototesting guidelines exist, thus hindering the development of reliable sunscreens protective against this part of sunlight. The objective of this study was to optimize the spectral output of VL+UVA1 as a step to standardize the assessment of protection from VL+UVA1. Methods: Four subjects with Fitzpatrick skin phototype (SPT) I-III were enrolled in this prospective pilot study. Two VL+UVA1 light sources were used: one with 2% UVA1 and another with 4% UVA1. to match more closely that measured in sunlight. Subjects were irradiated with each light source at 320 J/cm2. Clinical scoring, diffuse reflectance spectroscopy (DRS), and colorimetry were performed immediately, 24 hours, 7 days, and 14 days after irradiation. Results: In all subjects, irradiation with VL+ 4% UVA1 resulted in a stronger cutaneous response than that with VL+ 2% UVA1, showing an average 4-fold and 3-fold increase in immediate erythema and delayed pigmentation, respectively. These results were supported by colorimetry measured Δ a*, Δ ITA, and DRS measured relative dyschromia. Conclusion: These preliminary results indicate that the spectral composition of VL+UVA1 impact cutaneous responses and an output resembling sunlight should be strongly considered when standardizing sunscreen phototesting guidelines. This will enable a realistic and standardized design for the evaluation of sunscreen photoprotection within this spectrum.

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Macrophage depletion preserves dermal collagen in UVB exposed mice

M. Sharma[1, 2], T. Vazquez[1, 2], V. Werth[1, 2]
[1]*Dermatology, CMCVAMC, Philadelphia, Pennsylvania, United States,* [2]*Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States*

We previously reported that anti-TNFα antibodies (etanercept) block UVB-induced recruitment of neutrophils and macrophages (MΦ) into the dermis in mice. Paradoxically, etanercept accelerates loss of dermal collagen. To examine the role of MΦ in dermal collagen alteration during UVB exposure, we depleted MΦ in mice. C57BL/6J mice were treated with (0.15 ml) of Clophosome-A-Clodronate Liposomes (CCL) i.p. twice, 5 and 3 days before UVB exposure. We had 4 groups- sham, sham+CCL, UVB- and UVB+CCL-treated mice. Mice were UVB-irradiated (100 mJ/cm2/d for 5d) and sacrificed 3h after the last exposure. Skin sections were stained with picrosirius red for collagen fibers. Under circular polarized light, picrosirius red differentiates collagen fibers as red (mature) versus green (thin). In UVB-irradiated mice, red fibers were decreased compared with non-irradiated controls ($p<0.01$). UVB+CCL mice showed more red fibers relative to UVB-treated mice ($p<0.001$). UVB increased collagen fragmentation compared to controls. CCL treatment inhibited UVB-induced collagen fragmentation compared to UVB-treated mice. Procollagen, decorin, and TGF-β protein levels increased in UVB+CCL mice ($p<0.001$) compared to UVB-treated mice. MMP-13 was significantly decreased in UVB+CCL mice compared to UVB-treated mice ($p<0.001$). We further investigated which subtypes of MΦ, M1 or M2, play an important role in maintaining collagen. Imaging mass cytometry showed that MΦ trended to increase after UVB irradiation, as compared to controls and UVB+CCL-treated mice. Overall, we identified CD80+ and iNOS+ M1Φ. Overlapping of these markers suggest M1Φ are one population of cells. M2 MΦ were either CD206+ or ARG-1+, suggesting the presence of two distinct population of M2 MΦ . CD206+ M2 macs were increased with UVB relative to sham ($p<0.05$) and were decreased in UVB+CCL compared to UVB-treated mice ($p<0.05$). In conclusion MΦ depletion maintains mature collagen, with increased levels of procollagen, decorin, TGF-β, and decreased MMP13, suggesting MΦ exert a pro-inflammatory effect in UVB-irradiated skin.

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CP31398, which reverses UV-induced p53 mutations, does not undo ultraviolet radiation-induced immune suppression

M. Sherwani[1], H. Rashid[1], Y. Kwon[1], M. Athar[1], N. Yusuf[1, 2], C. A. Elmets[1, 2]
[1]UAB, Birmingham, Alabama, United States, [2]VA Clinic Birmingham, Birmingham, Alabama, United States

Chronic, excessive exposure to solar ultraviolet radiation is the major causative agent for cutaneous squamous cell carcinomas. Mutations in p53 are essential for these cancers to develop. UV radiation is also immunosuppressive and is an additional requirement for UV-induced SCCs to develop. Mutations in p53 can be reversed with the compound CP31398, which results in fewer SCCs in mice exposed to a UV radiation skin carcinogenesis protocol. It is unknown whether p53 mutations contribute to photoimmunosuppression. The purpose of this study was to determine if the UV immunosuppressive effects could be reversed by pretreatment with CP31398. A local UVB regimen consisting of UVB radiation (200 mJ/cm2) for 4 days followed by sensitization with the hapten 2, 4, dinitrofluorobenzene (DNFB) was employed for photoimmunosuppression. To determine the role of CP-31398, we treated the shaved dorsal skin of C57BL/6 mice with CP-31398 (1.25 mg/mouse) or vehicle cream, 30 min before each UVB treatment. Treatment with CP-31398 did not abrogate the immunosuppressive effect of UV. These findings suggest that although p53 mutations are responsible for UV-induced SCCs, they are not necessary for UV-induced immunosuppression.

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Inflammasome activation in human keratinocytes and mouse epidermis by ultraviolet radiation

S. Talley, E. M. Campbell, M. Denning
Loyola University Chicago Stritch School of Medicine, Maywood, Illinois, United States

Ultraviolet B radiation (UVB) is a ubiquitous environmental toxin that causes extensive skin damage resulting in inflammation, photoaging and cancer. UVB is known to induce inflammasome activation and a sterile inflammation associated with cytokine release (e.g. IL-1β, IL-18) and immune cell infiltration. We developed circularly permutated luciferase reporters for caspase-1 activation to assess inflammasome activation by UVB in vitro and *in vivo*. HaCaT keratinocytes transduced with caspase-1 reporters encoding either the caspase-7 or Gasdermin D cleavage sequence became active after exposure to UVB, with maximum caspase-1 activation 9 hours post-UVB with 15 mJ/cm2. UVB-induced caspase-1 activation was inhibited by the pan-caspase inhibitor zVAD (p=0.0002) and the multi-kinase inhibitor Dabrafenib (p=0.002), but not by the pyroptotic pore blocker LaCl3, reactive species scavenger N-acetylcysteine, or intracellular calcium chelator BAPTA-AM. Transfection of Poly I:C induced caspase-1 activation (>80-fold) and resulted in morphological cell death, consistent with the ability of toll-like receptor activation to trigger inflammasome activation and pyroptosis. To evaluate if UVB can induce inflammasome activation *in vivo*, transgenic mice expressing the caspase-7 cleavage sequence reporter were exposed to 50-75 mJ/cm2 UVB and assessed for skin bioluminescence. Caspase-1 activation was observed at 6 hours after UVB exposure, but returned to baseline by 24 hours. This data is surprising given that mouse keratinocytes are reported to not express inflammasome proteins or activate inflammasomes, and the influx of immune cells into the skin peaks 24-72 hours after UVB exposure. Thus, the mechanism of UVB-induced inflammasome activation in mouse skin is unclear. Since inflammation is considered to be a significant driver of tissue damage and cancer formation, understanding how UVB activates skin inflammasomes in both acute and chronic scenarios will provide opportunities to circumvent UVB-induced inflammation and the detrimental photobiology that ensues.

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Validated method for assessing the effects of blue light on human skin

R. Kala, N. Heiberger, H. Mallin, S. Wheeler, A. J. Langerveld
Genemarkers, Kalamazoo, Michigan, United States

The COVID pandemic caused an increase in virtual meetings & work from home scenarios that resulted in people spending increased time in front of computer screens & electronic devices. Studies have shown that blue light can produce cytotoxic effects, primarily through the production of reactive oxygen species & increased inflammation. However, the topic has been controversial with some studies claiming no adverse effects of blue light on the skin. Methods for testing the effects of blue light using in vitro testing models are lacking. Our work was conducted in order to develop a reproducible, validated testing method for assessing the effects of blue light on the skin. We designed a custom blue-light box that can be used to generate blue light at 460 nm wavelength. We performed a series of studies to optimize the dose and timing of the exposure & skin-culture conditions. Our work demonstrates that 6 hours of daily blue light for 5 consecutive days (total 30 J/cm2) produced a dose-and-time dependent decrease in skin health in 3D full thickness in vitro skin tissues. In addition, gene expression data showed an increase in the expression of genes that regulate inflammation and oxidative stress pathways (IL1A, IL6, CXCL8, COX2, CYP1B1, & NQO1) & a decrease in the expression of genes that maintain skin barrier and integrity (KRT1, KRT10, LOR, DSC and Collagen). Genes regulating skin aging & hydration including MMP1 & FLG were also regulated by exposure to blue light. Enzyme-linked immunoassays were performed to confirm changes in specific proteins. Exposure to blue light significantly increased 8-hydroxy-2' -deoxyguanosine, a marker for oxidative stress, & MMP1, markers for photoaging. Immunohistochemistry staining was performed to confirm changes in Collagen, Filaggrin & NQO1 protein expression in skin tissue. Our results showed that consistent blue light exposure produced skin damage via alterations in key biological pathways. This work provides a new, reproducible in vitro testing method for assessing the effects of blue light on human skin using gene expression, protein ELISA and IHC staining.

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Regulation of XPC binding dynamics and global nucleotide excision repair by p63 and vitamin D receptor

C. Wong[1], D. H. Oh[1, 2]
[1]Dermatology Research Unit, San Francisco VA Health Care System, San Francisco, California, United States, [2]Department of Dermatology, University of California San Francisco, San Francisco, California, United States

p63 and the vitamin D receptor (VDR) play important roles in epidermal development and differentiation, but their roles and relationship in the response to ultraviolet (UV) radiation are unclear. Using TERT-immortalized human keratinocytes expressing shRNA targeting p63 in concert with exogenously applied siRNA targeting VDR, we assessed p63 and VDR's separate and combined effect on nucleotide excision repair (NER) of UV-induced 6-4 photoproducts (6-4PP) with a focus on the XPC DNA damage recognition protein. Knockdown of p63 reduced VDR and XPC expression relative to non-targeting controls, while knockdown of VDR had no effect on p63 and XPC expression. Upon UV irradiation through filters with 3 μm pores to create spatially discrete spots of DNA damage, keratinocytes depleted of p63 or VDR exhibited slower removal of 6-4PP than control cells over the first 30 minutes. Co-staining of control cells with antibodies to XPC revealed that XPC accumulated at DNA damage foci, peaking within 15 minutes and gradually fading over 90 minutes as NER proceeded. In either p63- or VDR-depleted keratinocytes, XPC over-accumulated at spots of DNA damage so that 50% more XPC was retained at 15 minutes and 100% more XPC was retained at 30 minutes than in control cells, suggesting dissociation of XPC after binding was also delayed. Concurrent knockdown of VDR and p63 resulted in similar impairment of 6-4PP repair and XPC over-accumulation, but even slower release of XPC from DNA damage sites such that almost 200% more XPC was retained relative to controls at 30 minutes post-UV. These results suggest that VDR accounts for some of p63's effects in delaying 6-4PP repair associated with over-accumulation and slower dissociation of XPC, though p63's regulation of basal XPC expression appears to be VDR-independent. The results are consistent with a model where XPC dissociation is an important step during NER, and further link two important regulators of epidermal growth and differentiation to NER.

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Variations in *in vivo* visible light phototesting methodologies

I. Kohli[1, 2]

[1]*Dermatology, Henry Ford Health System, Detroit, Michigan, United States,* [2]*Physics and Astronomy, Wayne State University, Detroit, Michigan, United States*

While previously regarded as nonsignificant with minimal to no photobiologic effects, visible light (VL) has now been shown to have biologic effects on skin in subjects with all skin phototypes. However, currently there are no standardized guidelines to perform VL phototesting. A review of the published *in vivo* VL phototesting methodologies was performed to compare the various phototesting parameters. The methodologies were found to vary at multiple levels including spectral output of the irradiation sources, irradiance level, dosage, single vs. multiple exposures, assessment methods, assessment time points after irradiation, and calculation procedure for VL protection factor (VL-PF) among other variables. Variations in these parameters can cause significant differences in biologic response to VL. Burning and blister formation with VL irradiation reported in some studies, at doses that have been reported to be well tolerated in others, can be attributed to this. The growing interest in VL photobiology warrants careful investigation of the impact of above listed parameters. This in turn will aid in development of a standardized protocol enabling efficient comparisons among studies and establishment of adequate VL-PF.

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Differences in tumor thickness-specific incidence of cutaneous melanoma

M. Chen[1], I. de Vere Hunt[2], E. John[3], M. Weinstock[4], S. Swetter[1], E. Linos[1]

[1]*Dermatology, Stanford Medicine, Stanford, California, United States,* [2]*Program for Clinical Research and Technology, Stanford University, Stanford, California, United States,* [3]*Epidemiology and Population Health, Stanford Medicine, Stanford, California, United States,* [4]*Dermatoepidemiology, Providence VA Medical Center, Providence, Rhode Island, United States*

The recent incidence of cutaneous melanoma across different thicknesses in the United States is not well described. Our aim was to evaluate recent trends in the incidence of melanoma by tumor thickness and examine associations of sex, race/ethnicity, and socioeconomic status (SES) with thickness-specific incidence. We analyzed 187,487 patients with a new diagnosis of cutaneous melanoma in the Surveillance, Epidemiology, and End Results (SEER) Registry from 2010 to 2018. We calculated age-adjusted incidence rates of melanoma by tumor thickness and annual percentage change (APC) in incidence rates. Analyses were stratified by sex and race/ethnicity. We evaluated associations with SES in 134,359 melanoma patients diagnosed from 2010 to 2016. Melanoma incidence was higher in men compared to women across all thickness groups. Individuals in lower SES quintiles, Black patients, and Hispanic patients were more likely to be diagnosed with thicker tumors. Between 2010 and 2018, there was no significant increase in incidence of cutaneous melanoma across the full population (APC, 0.39; 95% CI, -0.40, 1.18). The incidence of thickest melanomas (>4.0 mm) increased between 2010-2018, with an APC of 3.32 (95% CI, 2.06 to 4.60) overall; 2.50 (95% CI, 1.27 to 3.73) in men, and 4.64 (95% CI, 2.56 to 6.75) in women. For thinner tumors (≤1.0 mm, >1.0 – 2.0 mm, >2.0 – 4.0 mm), incidence peaked in 2013 through 2015 and decreased since then until 2018. This is the first study showing potential stabilization of overall melanoma incidence rates in the US after nearly a century of continuous increase in incidence. Low SES, Black, and Hispanic groups have a higher proportion of thick melanoma. The continued rise in incidence of thick melanoma is unlikely to be due to overdiagnosis given the stability of thin melanoma rates.

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Association of race with thickness in cutaneous melanoma

J. Brown-Korsah[1], C. Blebea[3], M. Ming[2], E. Chu[2]
[1]Case Western Reserve University School of Medicine, Cleveland, Ohio, United States, [2]Dermatology, Penn Medicine, Philadelphia, Pennsylvania, United States, [3]Internal Medicine, Penn Medicine, Philadelphia, Pennsylvania, United States

Prior studies examining racial disparities in cutaneous melanoma have found that Black patients (BPs) present at a later stage than non-Hispanic White patients (NHWPs). However, previous studies have not assessed whether there are differences in the thickness of primary melanoma lesions between BPs and NHWPs, nor have they examined in detail socioeconomic status (SES) and primary body location (PBL) as potential explanations for the stage differences noted. This study sought to determine whether there are differences in the thickness distribution between melanoma of BPs and NHWPs matched for histologic subtype, controlling for SES and PBL. A retrospective, case-control study of melanoma patients diagnosed within the University of Pennsylvania Health System between 1986 and 2016 was performed. BPs were matched by subtype to four NHWPs diagnosed within 10 years of each BP. Univariate and multivariate logistic regression were performed. Forty-one BPs with melanoma fulfilled eligibility criteria. The BPs were mostly male (22 [53.7%]) and had a mean age at diagnosis of 63.4 (SD=16.5) years. Of the 41 BPs, 30 (73.2%) BPs had invasive lesions. The median thickness for invasive lesions for BPs and NHWPs were 2.25mm (IQR=4.28) and 0.57mm (IQR=1.05) (p=<0.001), respectively. On univariate analysis, BPs were more likely to have a thickness of >4.00mm (24.4% of BPs vs. 1.8% of NHWPs; OR = 21.76, 95% CI: 5.99 – 104.70). On multivariate analysis of invasive melanomas, controlling for PBL and SES, BPs were more likely to have a thickness of >4.00mm when compared to NHWPs (OR=12.8; 95% CI: 2.32 – 85.18). BPs with melanoma presented with a greater thickness compared to NHWPs, when controlling for SES and primary body location. Our findings indicate that there might be a delay in diagnosis of melanoma in the Black community not explained by SES differences or issues related to body site such as visibility of lesions. This may indicate directions for public health messaging and research initiatives.

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Targeting a novel carbohydrate that serves as an immune checkpoint and angiogenic regulator for treatment of melanoma

J. Chung, V. Ramani, P. D. Cruz, K. Ariizumi
Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

We discovered DC-HIL/Gpnmb receptor expressed by melanoma and myeloid-derived suppressor cells (MDSC), with 2 functions, immune checkpoint and angiogenesis, both mediated by its binding to syndecan-4 (SD4) on activated T cells and select endothelial cells (EC), respectively. Using microarray technology, we now report that DC-HIL's precise ligand on SD4 is a rare heparan sulfate (rHS) saccharide sulfated highly at positions 2-O/6-O. Consistently with this discovery, HS sulfotransferases required for rHS synthesis rises markedly during T-cell activation, and knocking-down gene expression by specific shRNA abrogated DC-HIL binding to T cells/EC. Deletion mutant analysis showed removal from DC-HIL of parts of its N-terminal HS binding motif and of an Ig-like domain abrogated completely its binding to rHS. Importantly, we showed rHS to completely block binding of DC-HIL to T-cells/EC and to neutralize DC-HIL's angiogenic activity *in vivo*. To study rHS expression and function, we created a specific mAb (1F6). IHC-labeling with 1F6 showed rHS expression by select EC in normal lung and heightening of this expression in metastatic lung. Resting human T cells highly expressed CD44 in the absence of rHS and SD4, whereas all 3 markers were present in activated T cells. 1F6 precipitated SD4 and CD44 from extracts of activated (but not resting) T cells, suggesting T-cell activation is required for both glycoproteins to acquire rHS. Crosslinking IF6 in human T cells (n=4) strongly inhibited the CD3 response (3H-cpm and IFN-g, IC50 of 8 nM) without inducing apoptosis. Finally, infusion of 1F6 (10 µg i.p. 2x/wk) into mice s.c. implanted with B16 melanoma inhibited tumor growth and reduced lung metastasis (p<0.01) vs. combined anti-PD1/VEGF mAb. 1F6's better outcomes correlated with more T-cells, less MDSC infiltration, and reduced microvessel densities within the tumor. Thus, our new sugar target for treating melanoma appears superior to current immune checkpoints, due to synergy of immune and vascular attributes.

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Inhibition of PAI-1 blocks PD-L1 endocytosis and improves the response of melanoma cells to immune checkpoint blockade

C. Lee[1, 2], Y. Tseng[1], W. Chen[3], J. Yang[3], H. Tzeng[3]
[1]Dermatology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, [2]Dermatology, Chang Gung University College of Medicine, Taoyuan, Taiwan, [3]Institute for Translational Research in Biomedicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan

Immune checkpoint molecules, especially PD-1 and its ligand PD-L1, act as a major mechanism of cancer immune evasion. Although anti–PD-1/PD-L1 monotherapy increases therapeutic efficacy in melanoma treatment, only a subset of patients exhibits long-term tumor remission, and the underlying mechanism of resistance to PD-1/PD-L1 inhibitors remains unclear. In this study, we demonstrated that cell surface retention of PD-L1 is inversely correlated with PAI-1 expression in vitro, *in vivo*, and in clinical specimens. Moreover, extracellular PAI-1 induced the internalization of surface-expressed PD-L1 by triggering clathrin-mediated endocytosis. The endocytosed PD-L1 was transported to lysosomes for degradation by endolysosomal systems, resulting in the reduction of surface PD-L1. Notably, inhibition of PAI-1 by pharmacological inhibitor with tiplaxtinin led to elevated PD-L1 expression on the plasma membrane, both in vitro and *in vivo*. Strikingly, targeting PAI-1 by tiplaxtinin treatment synergizes with anti–PD-L1 immune checkpoint blockade therapy in a syngeneic murine model of melanoma. Our findings demonstrate a role for PAI-1 activity in immune checkpoint modulation by promoting surface PD-L1 for lysosomal degradation and provides an insight into the combination of PAI-1 inhibition and anti–PD-L1 immunotherapy as a promising therapeutic regimen for melanoma treatment.

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Superiority of artificial intelligence compared to dermatologists and primary care providers in the diagnosis of malignant melanoma

J. M. Anderson[1, 2], I. Tejani[3], T. Jarmain[3], L. Kellett[3], R. Moy[2]
[1]The University of Texas Health Science Center at San Antonio, San Antonio, Texas, United States, [2]Research Department, Moy Fincher Chipps Facial Plastics and Dermatology, Los Angeles, California, United States, [3]Triage Technologies Inc, Toronto, Ontario, Canada

The early diagnosis of skin cancer, especially melanoma, significantly reduces morbidity and mortality; however, most skin lesions are not initially evaluated by dermatologists and some patients may require a referral from a primary care provider to be examined by a specialist. This study sought to determine the performance of Triage Snap, an artificial intelligence (AI) algorithm, in classifying lesions as benign or malignant to determine whether AI could assist in the triage of skin cancer cases. A set of 100 dermoscopic images (80 benign and 20 biopsy-verified malignant melanoma) from the International Skin Imaging Collaboration was assessed by AI, dermatologists (n=4), family physicians (n=7), and primary care mid-level providers including both NPs and PAs (n=12). The images were classified as benign or malignant by the AI or medical provider. The AI significantly outperformed both the dermatologists and the primary care providers in determining the malignancy of lesions. The AI had greater specificity, positive predictive value (PPV), and accuracy compared to all 3 groups (p<.05). There was no significant difference in sensitivity between the AI (80%), dermatologists (75%), and family physicians (78%), but the AI was more sensitive compared to mid-level providers (61%) (p<.05). The AI's high accuracy (92%) and PPV (.8) seen in this study demonstrates that AI is a reliable triage tool for medical providers. Further, AI may decrease the morbidity and mortality associated with skin cancer by shortening the time to correct diagnosis.

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Performance monitoring of a streamlined and scalable non-invasive gene expression assay for pigmented lesions

K. Kaur, T. Allen, K. Hill, M. D. Howell, B. Jansen, J. Rock, L. Clarke, C. Ibarra
DermTech Inc, La Jolla, California, United States

The DermTech Pigmented Lesion Assay (PLA) is a qPCR-based assay that aids in the detection of melanoma by assessing up-regulation of two melanoma-associated genes, LINC00518 and PRAME, in skin samples collected by non-invasive adhesive patches. This study is to establish assay performance characteristics of the PLA post optimization, and to investigate the prognostic potential of the assay by comparison of qPCR amplification cycle in PLA positive samples with the histopathologic diagnoses of cutaneous pigmented lesions. RNA isolated from non-invasively collected skin cells was analyzed from seventy-five pigmented lesions. The PLA was optimized to simplify the laboratory workflow and increase scalability of the assay through a series of process improvements including RNA quantification by 1-step qRT-PCR (vs. reverse transcription followed by qPCR) and assay miniaturization. PLA result was determined by evaluating qPCR amplification of LINC00518 and PRAME multiplexed with housekeeping genes β-Actin and PPIA respectively. Samples that demonstrated over-expression of LINC00518 and PRAME relative to the housekeeping genes were considered PLA positive. Blinded to the PLA results, biopsy specimens from the pigmented lesions were examined and classified by a dermatopathologist as invasive melanoma (n=22); melanoma in situ (MIS) (n=26); benign nevus (24); or other (n=3). All twenty-two lesions classified as invasive melanoma by histopathology were PLA-positive, with an average LINC00518 Ct of 32.2 and PRAME Ct of 30.9. Seventeen of the twenty-six samples classified as MIS were PLA-positive, with an average LINC00518 Ct of 33.8 and PRAME Ct of 33.2. Of the twenty-seven samples not classified as melanoma, five were PLA-positive, with an average LINC00518 Ct of 34.4 and PRAME Ct of 35.8. The results indicate that PLA Ct values are highly correlated with histopathologic diagnoses. The optimized PLA assay demonstrated a sensitivity of 100% for invasive melanoma and 65% for MIS, with an overall specificity of 81%.

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Cdk2: A marker for invasive melanoma

N. R. Love[1], M. Kiuru[1, 2], E. Simmons[1, 3]
[1]Dermatology, University of California Davis, Sacramento, California, United States, [2]Pathology, University of California Davis, Sacramento, California, United States, [3]Case Western Reserve University School of Medicine, Cleveland, Ohio, United States

Background: Although cyclin-dependent kinase 2 (CDK2) is known to be upregulated in certain cancers, its spatial expression pattern in melanocytic tumors has remained unknown. Objective: To characterize CDK2 expression in benign and malignant melanocytic proliferations. Methods: We performed CDK2 immunohistochemistry (IHC) on normal skin and a tumor panel comprising over 200 congenital nevi, dysplastic nevi, melanomas in situ, and invasive melanomas. We recorded their staining patterns, intensities, and compared these to expression of melanoma marker PRAME (Preferentially expressed Antigen in Melanoma). Results: Serial antibody dilutions revealed that CDK2 IHC reliably stains dermal-epidermal junction melanocytes in both lesional and non-lesional skin. Melanocytic nevi progressively lost CDK2 expression in the dermis (>90%), whereas the melanocytes of malignant melanoma retained strong CDK2 in their invasive aspects (>90%). PRAME and CDK2 expression were highly correlated in invasive melanoma. Conclusion: CDK2 is expressed by malignant melanocytes in the deep dermis, suggesting a putative role during melanomagenesis. Deep dermal CDK2 positivity is highly specific and sensitive for invasive melanoma, and thus, CDK2 IHC may be a useful ancillary test for diagnosing challenging melanocytic tumors.

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Determining intra-tumoral heterogeneity and immune escape mechanisms in melanoma using spatial transcriptomics

Y. Lim[1], S. Kang[2], H. Kim[3], J. Mun[1], M. Roh[4], N. Gulati[5], H. Yang[6], J. Moon[7], C. Won[6], C. Park[2]
[1]Department of Dermatology, Seoul National University Hospital, Jongno-gu, Seoul, Korea (the Republic of), [2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea (the Republic of), [3]Genomic Medicine Institute, Medical Research Center, Seoul National University College of Medicine, Seoul, Korea (the Republic of), [4]Department of Dermatology, Yonsei University College of Medicine, Seodaemun-gu, Seoul, Korea (the Republic of), [5]Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, New York, United States, [6]Department of Dermatology, Asan Medical Center, Songpa-gu, Seoul, Korea (the Republic of), [7]Samsung Genomic Institute, Samsung Medical Center, Gangnam-gu, Seoul, Korea (the Republic of)

Melanoma, like other cancers, originates from a single clone. As the cancer progresses, the tumor thickens and immune evasion ensues. However, few studies have investigated the mechanisms involved in these processes using spatial transcriptomics. We aimed to elucidate genetic and transcriptional heterogeneity in relation to melanoma thickness and to explore the molecular mechanisms related to immune escape. Five punch-biopsied fresh tissue samples and saliva were obtained from a single patient and used for paired whole exome sequencing. The subsequent excision specimen was prepared for formalin-fixed paraffin-embedded block and profiled using spatial transcriptomics. DNA-level mutation accumulation was commensurate with the thickness of the melanoma. Spatial transcriptome analysis indicated that immune evasion involves changes in antigen presentation as well as defects in β2-microglobulin and human leukocyte antigen class I processing. Levels of these molecules, which are critical for T cell recognition of tumor antigens, tended to significantly decrease as the melanoma depth increased. We also found that the expression of caspases and interferon alpha inducible protein-encoding genes inversely correlated with tumor thickness. The study was based on sampling a lesion from a single patient. Spatial transcriptomics can increase our mechanistic understanding of tumor immune escape.

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Comparison of S100A8 and PRAME as biomarkers for diagnosing melanoma

J. Hai[1], S. L. Wong[1], Y. Li[3], D. L. Migloretti[3], M. A. Fung[1, 2], M. Kiuru[1, 2]
[1]Dermatology, University of California Davis Health System, Sacramento, California, United States, [2]Pathology and Laboratory Medicine, University of California Davis Health System, Sacramento, California, United States, [3]Public Health Sciences, University of California Davis Health System, Sacramento, California, United States

Early diagnosis of melanoma is crucial to improved patient prognosis but can be difficult to achieve from histopathology alone. PRAME is used increasingly in dermatology as an ancillary tool to help differentiate between benign vs. malignant melanocytic tumors. S100A8 is another biomarker found to be expressed in melanoma-associated epidermal keratinocytes, but its diagnostic utility has not been compared to other biomarkers, including PRAME. In this retrospective case-control study, we compared S100A8 and PRAME immunohistochemistry (IHC) in melanocytic nevi and melanoma (n=209). An S100A8 and PRAME IHC score were assigned to each tumor sample, indicating the proportion of tumor-associated epidermis stained or the proportion of tumor cells stained, respectively. S100A8 IHC scores were previously reported (Kiuru et al., 2021). We analyzed diagnostic accuracy in detecting melanoma and melanoma in situ by using receiver operating characteristic curves, which showed an area under the curve (AUC) of 0.8326 for S100A8 and 0.8741 for PRAME. These AUCs were both significantly greater than chance, or AUC=0.5 ($p<0.001$), but not significantly different from each other ($p=0.22$). For S100A8, when a positive test was defined as a score of 4 or 5 (>50% of tumor-associated epidermis stained), the sensitivity was 42.42%, and specificity was 98.18%. For PRAME, when a positive test was defined as a score of 3 (>50% of tumor stained), the sensitivity was 79.80%, and the specificity was 87.27%. When a positive test was defined as >50% of tumor stained for both S100A8 and PRAME, sensitivity was 39.39%, and specificity was 99.09%. Thus, both biomarkers are useful for accurately detecting malignant melanocytic tumors. When combined with PRAME, S100A8 increases specificity, demonstrating the utility of S100A8 when interpreted alongside other histopathological features and ancillary tests.

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Comparison of soluble proteins from skin sections of acne and TCA induced postinflammatory hyperpigmentation and erythema

N. Karaman-Jurukovska[1], I. H. Hamzavi[2], I. Kohli[2], C. Nicholson[2], T. Mohammad[2], A. Nahhas[2], T. Braunberger[2], M. Matsui[1], T. Mammone[1]
[1]Estee Lauder Companies, New York, New York, United States, [2]Department of Dermatology, Henry Ford Health System, Detroit, Michigan, United States

Postinflammatory hyperpigmentation (PIH) is an acquired hypermelanosis occurring after cutaneous inflammation or injury that can arise in all skin types, but more frequently affects skin-of-color. The differences in the ethology of PIH and Postinflammatory erythema (PIE) in skin of color were evaluated from soluble protein extracts collected from skin section samples, using Somascan protein kit1.3 k (n=5). The skin samples were collected from selected gluteal TCA-induced lesions and truncal acne pustules, of either PIH or PIE, at day 28 post initial evaluation. Differences between proteins (FDR<0.05) from PIH and PIE were analyzed with STRING version 11.5 and analysis points toward involvement of JAK/STAT signaling pathway and enhanced IL17 signaling in PIH compared to PIE lesions (OSM, CSF3, IL10RA, IL12RB2, IL10RB, IL3, CSF2, IL17D, IL17F, IFNA2, IFNA10, CRLF2, IL5RA, TYK2, IL12RB1, PRLR, GHR). The involvement of JAK/STAT signaling pathway has been described for some chronic cutaneous inflammatory conditions and acne. A higher occurrence of dermal remodeling proteases and inhibitors were found in PIE (MMP1, MMP2, MMP7, TIMP2) indicating a dermal remodeling phase at the time of excision. Concurrently, elevated levels of IL-1β, and TGF-β (critical for triggering and continuing differentiation programs of naïve CD4+ T cells to IL-17 secreting Th17 cells) in PIH samples suggests continuing promotion of macrophage infiltration and sustained inflammation. In addition to MMP13 and MMP16, the protein Keap1 was found to be increased in the PIH samples. Keap1, a repressor of master cellular defense against oxidative and electrophilic stresses, has been reported to be involved in the imbalance of proteolysis that can lead towards premature aging and in a senescent phenotype of endothelial cells. The sustained inflammation with excess of Keap1 protein might contribute to an altered proteostasis and ethology of PIH.

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Depletion of senescent cells improves targeted therapy outcome in melanoma

R. Perez-Lorenzo[1], E. Y. Lee[1], S. O. Erjavec[1, 2], A. M. Christiano[1, 2]
[1]Dermatology, Columbia University, New York, New York, United States, [2]Genetics and Development, Columbia University, New York, New York, United States

Cellular senescence is a non-proliferative but viable state that can be induced by oncogene expression and anti-cancer therapeutics, such as BRAF inhibition, and has long been considered a beneficial tumor suppressive mechanism. However, senescent cells express a well-characterized senescence-associated secretory phenotype (SASP), which may contribute to an immunosuppressive microenvironment and an increased malignant phenotype, leading to tumor recurrence and metastasis. To investigate the role of senescent cells in tumor growth, we treated B1610 (B16) melanoma cells in vitro with the senolytic drug ABT737 or vehicle control and injected 5x104 viable cells into syngeneic mice. We found that senolytic pre-treated B16 cells grew into significantly smaller tumors compared to controls. Tumors from senolytic-treated cells had significantly fewer senescence-associated β-galactosidase and p16 expressing cells, which correlated with decreased SASP expression, and reduced tumor infiltration of myeloid-derived suppressor cells. We next treated BRAF mutant human melanoma cells in vitro with the BRAF inhibitor PLX4720, and found a significant increase in the number of senescent cells and expression of SASP members such as IL-1β, IL-6, and IL-8, a phenomenon known as therapy-induced senescence. Further, there was a significant increase in apoptotic cells when treated with a combination of PLX4720 and ABT737, suggesting that this combination may be more effective for tumor eradication than BRAF inhibition alone. We then asked whether eliminating senescent cells with senolytic drugs enhanced tumor control by BRAF inhibition anti-cancer therapy. We treated YUMM1.7 subcutaneous tumors with PLX4720 and the senolytic drug ABT263 and showed a durable repression of tumor regrowth compared to controls. Our findings suggest that therapy-induced senescence is a potential mechanism for acquired resistance and evasion of anti-tumor immune responses, which can be overcome by depleting senescent cells with senolytic drugs.

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Reprogramming the tumor microenvironment by a second-generation recombinant modified vaccinia virus Ankara

S. Liu[1], G. Mazo[1], N. Yang[1], T. Zhang[2], S. B. Tariq[1], Y. Wang[1], D. Hirschhorn-Cymerman[1], L. Ji[1], A. Tan[2], J. Wang[3], W. Yan[4], J. Choi[4], A. Rossi[1], J. Z. Xiang[2], M. O. Li[1], T. Merghoub[1], J. D. Wolchok[1], L. Deng[1]
[1]Memorial Sloan Kettering Cancer Center, New York, New York, United States, [2]Weill Cornell Medicine, New York, New York, United States, [3]Genvira Biosciences, Ottawa, Ontario, Canada, [4]IMVAQ Therapeutics, Sammamish, Washington, United States

Immune checkpoint blockade (ICB) therapy has brought hope to many cancer patients, but the response rate is low in many cancer types, and acquired resistance to ICB can develop over time. Oncolytic viruses are promising therapeutic agents for advanced cancers. Modified vaccinia virus Ankara (MVA) is an attenuated, replication-deficient poxvirus safe for human use, making it a favorable platform for cancer immunotherapy. Our first-generation recombinant MVA has shown promising antitumor efficacy in multiple murine tumor models due to the deletion of the E5R gene (encoding an inhibitor of the DNA sensor cGAS) from the MVA genome and the insertion of two membrane-anchored transgenes – Flt3L and OX40L, which leads to the activation of the host innate and adaptive antitumor immunity. Here in this study, we engineered our second-generation recombinant MVA (MQ833) with the deletion of two more viral immune evasion genes – E3L and WR199, and the insertion of IL12 anchored to the extracellular matrix to mitigate toxicity. Intratumoral (IT) delivery of MQ833 resulted in an 80-100% cure in the mouse B16-F10 melanoma model, which is dependent on nucleic acid-sensing and IFN signaling pathways. Single-cell RNA sequencing analysis revealed that IT MQ833 injection reprogrammed the tumor microenvironment into an immune-stimulating state, by activating CD8+ and CD4+ T cells, depleting regulatory T cells, recruiting and activating neutrophils, and polarizing M1 macrophages. Interestingly, MQ833 treatment cured 70% of B2m knock-out melanomas likely due to combined effects of IL-12 and type I and II IFN. Loss of MHC-I is the most common mechanism of tumor resistance to ICB. Hence, our results support the use of MQ833 for ICB-resistant tumors.

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Identification and functional implication of variants associated with skin pigmentation in Chinese population

X. Cai[1], X. Liu[2], Y. Zhao[1], K. Xu[2], W. Li[1], Z. Yang[2], S. Wang[1]
[1]Chinese Academy of Sciences Shanghai Institute of Nutrition and Health, Shanghai, Shanghai, China, [2]Academy of Medicine Science, Zhengzhou University, Zhengzhou, China

Skin pigmentation is a major feature of skin, and a marker associated with beauty, health and aging. Among the past genome-wide association studies on skin pigmentation, a relatively small proportion have focused on Chinese population. Large-scaled studies based on measured skin pigmentation phenotypes are particularly rare in Chinese and other East Asian populations. In the Jidong cohort study (N = 3,544), we measured the skin pigmentation on low-exposed area with colorimeter and performed genome-wide scans on skin color and melanin index. We identified associated loci on 9p22.2, 10q26.11, 12q21.33, 15q12.6 and 15q13.1, and validated in independent cohorts. Functional annotation and luciferase report assays revealed evidence for the top SNP on 12q21.33 functioning as an enhancer, and regulating pigmentation-related gene TMTC3, although in previous studies, this top SNP has been mapped to the nearby gene KITLG, another well-known pigmentation-related gene. From publicly available database, we found further evidence suggesting regulation pathway on how TMTC3 altering skin pigmentation. This study provided additional insight into the genetics underlying skin pigmentation.

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Non-redundant roles for GLUT1 and GLUT3 in melanoma and primary melanocytes

R. Yang[1], D. Yu[1], J. Kim[3], E. Lee[1], R. Mahapatra[1], R. C. Wang[1, 2]
[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Harold C. Simmons Cancer Center, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [3]Quantitative Biomedical Research Center, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

The GLUT1 transporter mediates glucose uptake in most tissues and is upregulated in both inflammatory and malignant skin diseases. Most melanomas overexpress both GLUT1 and GLUT3, but it is unclear whether the closely related transporters are functionally redundant. Consistent with its function in keratinocytes, GLUT1 knockdown in melanoma cells (UACC-62 and Mel-juso) inhibited glucose uptake and cell proliferation. Notably, GLUT3 knockdown did not impair glucose transport yet showed even more dramatic effects on cell proliferation and morphology. Fractionation revealed the preferential localization of GLUT1 to the plasma membrane while GLUT3 preferentially localized to endosomes. RNA-sequencing, Western blotting, and immunofluorescence revealed an essential role for GLUT3 in signal transducer and activator of transcription 3 (STAT3) activation, which has been implicated in melanoma growth and metastasis. Deletion of GLUT1 or GLUT3 in primary mouse melanocytes through Tyrosinase-cre expression confirmed a critical role for GLUT3 in STAT3 activation and non-redundant roles for GLUT1 and GLUT3 in pigmentation and melanocyte proliferation *ex vivo*. These studies identify unique functions for GLUT1 and GLUT3 in melanoma and melanocytes and highlight the GLUT3-STAT3 signaling axis as a potential therapeutic target in melanoma.

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Inverse genetic risk between cutaneous melanoma, basal cell carcinoma, squamous cell carcinoma and vitiligo

S. Rashid[1, 2], N. Klebanov[3], M. Shaughnessy[3], M. Daly[4, 5, 6], M. Artomov[4, 5], H. Tsao[1, 3]
[1]Dermatology, Wellman Center for Photomedicine, Boston, Massachusetts, United States, [2]Boston University School of Medicine, Boston, Massachusetts, United States, [3]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [4]Analytical and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts, United States, [5]Broad Institute, Cambridge, Massachusetts, United States, [6]Helsingin yliopisto Suomen molekyylilaaketieteen instituutti, Helsinki, Uusimaa, Finland

Several epidemiological studies have reported an inverse association between vitiligo and skin cancers such as cutaneous melanoma, basal cell carcinoma and squamous cell carcinoma. There are conflicting reports whether this association is a consequence of increased immunosurveillance observed in vitiligo patients or rather a shared genetic contribution. The latter hypothesis however, is not currently sustained by available evidence. Herein, we compared 23 vitiligo-associated variants (P<5x10-8) from a recently published genome-wide association study and identified three variants—contained within the TYR, MC1R-DEF8, and RALY-EIF252-ASIP-ACHY-ITCH loci—that have been associated with increased risk for melanoma, basal cell carcinoma, and squamous cell carcinoma and a concurrent decreased risk for vitiligo. We then subjected GWAS summary statistics for vitiligo and each skin cancer to an unbiased two-sample mendelian randomization analysis. We observed a protective role for vitiligo in the development of cutaneous melanoma (effect size=-6.78x10-4; p=5.41x10-2), basal cell carcinoma (effect size=-1.29x10-3; p=1.77x10-3), and squamous cell carcinoma (effect size=-2.19-4; p=1.81x10-3). When the vitiligo-associated signature was subjected to the same analysis, a significantly increased protective relationship was observed for each skin cancer (melanoma effect size=-0.511; p=2.41x10-5; basal cell carcinoma effect size=-0.454; p=4.36x10-7; squamous cell carcinoma effect size=-0.489; p=5.59x10-10). Our results support a causal inverse genetic relationship between these skin cancers and vitiligo, and reveal potential therapeutic targets for the prevention and effective treatment of vitiligo-associated skin cancer.

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Identification of experimental therapeutics overcoming NRAS-based BRAFi-resistant malignant melanoma targeting brain metastases in a bioluminescent murine model

J. Jandova[1], S. Park[1], M. Corenblum[2], L. Madhavan[2], J. Snell[1], G. T. Wondrak[1]
[1]Pharmacology and Toxicology, University of Arizona, Tucson, Arizona, United States, [2]Department of Neurology, University of Arizona, Tucson, Arizona, United States

Molecularly targeted therapeutics have revolutionized the treatment of BRAFV600E-driven malignant melanoma, but the rapid development of resistance to BRAF kinase inhibitors (BRAFi) presents a significant obstacle. Here, we describe the identification of experimental melanoma therapeutics overcoming NRAS-based BRAFi-resistance employing a stringent genetic model of BRAFi-resistance, i.e. isogenic melanoma cell lines that differ only by NRAS mutational status (BRAFi-sensitive A375-BRAFV600E/NRASQ61 versus BRAFi-resistant A375 BRAFV600E/NRASQ61K). As a result of unbiased candidate screening, the clinical antimalarial mefloquine (MQ) was the only apoptogenic agent causing BRAFi-resistant A375 melanoma cell death at low micromolar concentrations. Comparative gene expression-array analysis (A375-BRAFV600E/NRASQ61 versus A375-BRAFV600E/NRASQ61K) revealed that MQ is a dual inducer of endoplasmic reticulum (ER) and redox stress responses that precede MQ-induced loss of viability. ER-trackerTM DPX fluorescence imaging and electron microscopy indicated ER swelling, accompanied by rapid induction of ER stress signaling (phospho-eIF2α, XBP-1s, ATF4). Fluo-4 AM-fluorescence indicated the occurrence of cytosolic calcium overload observable within seconds of MQ exposure. In a bioluminescent murine model employing intracranial injection of A375-Luc2 (BRAFV600E/NRASQ61K), an oral MQ regimen efficiently antagonized growth of brain metastases. Taken together, these data suggest feasibility of identifying valid candidates for drug repurposing aiming at chemotherapeutic elimination of malignant melanoma cells targeting BRAFV600E/NRASQ61K cells even if metastasized to the brain.

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Exploring the synergic effects of a plant and a peptide on hair follicle pigmentation

D. Broadley[1], M. van Lessen[1], A. Takeoka[3], R. Arai[4], K. Suzuki[4], A. Abe[4], T. Nagahama[4], A. Takaoka[4], W. Funk[2], H. Erdmann[5], T. Bíró[1], M. Bertolini[1]
[1]Monasterium Laboratory Skin & Hair Research Solutions GmbH, Munster, Nordrhein-Westfalen, Germany, [2]Clinic for Plastic, Aesthetic and Reconstructive Surgery Dr. Dr. med. Funk, München, Bavaria, Germany, [3]Cooperative Researcher, CellLab, NatureLab. Co., Ltd., Tokyo, Japan, [4]Research & Development Headquarters Self-Medication, Taisho Pharmaceutical Co., Ltd., Tokyo, Japan, [5]Kosmed Klink, Hamburg, Germany

Canities is an age-linked loss of bulb melanocyte and hair follicle (HF) pigmentation. Canities can significantly alter the physical appearance of a person and negatively impact their well-being, which drives the search and demand for new anti-greying agents. αMSH is pro-pigmentary hormone of the melanocortin family, which stimulates melanogenesis through its target receptor, MC1. GreyverseTM (Gv) is a biomimetic synthetic peptide derived to mimic αMSH and is an established promoter of HF melanogenesis with redox properties. Preliminary studies revealed that cotreatment of Gv and Paeonia Suffruticosa root bark extract (PS) synergically induces mitochondrial E3 ligase (MITOL) and melanogenesis in human melanocytes in vitro. Thus, this study investigated the additive effects of PS and Gv on hair pigmentation *ex vivo*. HFs were cultured in serum-free medium, supplemented with PS and/or Gv for 4 days, then assessed for protein expression and enzymatic changes associated to melanogenesis. Quantitative immune-histomorphometry revealed that PS and Gv cotreatment led to an additive increase in melanin content (p<0.05) that derived from a synergic increase in Gp100 expression (p<0.05), an increase in tyrosinase activity, and αMSH and TYRP expression within the hair follicle pigmentary unit. Taken together, PS and Gv showed synergic effects in promoting hair pigmentation, primarily through melanosome maturation. Thus, this combinational treatment deserves further pre-clinical and clinical exploration for the reversal of hair greying.

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Transcriptomic and bioinformatic evidence for disctict roles of p38 isoforms in cutaneous melanoma

S. K. Sandhu, N. Adusumilli, A. Kiss, A. J. Friedman, T. Efimova
The George Washington University, Washington, District of Columbia, United States

The p38 kinases mediate cell adaptation to stresses and play varying roles in tumorigenesis. However, the p38 isoform-specific roles in cutaneous melanoma (SKCM), particularly in context of clinically important driver mutations, remain elusive. We used bioinformatic tools to analyze the p38 isoform expression in TCGA SKCM subtypes defined by the presence of BRAF or NRAS driver mutations. We found that p38β, p38γ and p38δ expression levels were increased, while p38α levels were decreased in BRAF-mutated (MT) compared to BRAF wild-type (WT) metastatic SKCM. In contrast, p38δ expression was decreased in NRAS-MT relative to NRAS-WT metastatic SKCM. None of these correlations were significant in primary SKCM. p38γ expression negatively correlated, while p38δ levels positively correlated with the CD8+ T cell and B cell abundance across BRAF- and NRAS-MT SKCM. Moreover, p38γ expression negatively correlated with the biomarkers for CD8+ T cells, while p38δ expression positively correlated with the biomarkers for CD8+ T cell, B cell, dendritic cell and other immune cell populations, as well as T cell exhaustion markers across BRAF- and NRAS-MT SKCM subtypes. The positive correlation of p38δ expression with T cell exhaustion markers may have implications for the potential prospect of targeting p38δ in the SKCM TME to boost antitumor immunity. The protein-protein interaction network analysis revealed the top-ranked p38γ-interacting genes SNTA1 and ROBO1 known to promote cancer cell migration and melanoma angiogenesis, respectively. Interestingly, the top-ranked p38δ-interacting gene GPX4 is known to promote BRAF MT melanoma resistance to BRAF inhibitors. Notably, based on drug sensitivity analysis, high p38δ expression correlated with resistance to 15 drugs or small molecules, including BRAF inhibitor. In summary, our data support distinct roles for p38s in different genomic subtypes of SKCM and at different disease progression stages, raising the possibility of the isoform-specific targeting for a more individually tailored approach in treatment of SKCM.

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Repurposing bortezomib for improved treatment of melanoma by exploiting immunogenic cell death

S. M. Daignault-Mill, D. Moi, R. J. Ju, B. Zeng, B. Gabrielli, L. Spoerri, R. Dolcetti, N. K. Haass
The University of Queensland, Saint Lucia, Queensland, Australia

Immunogenic cell death (ICD) constitutes a prominent pathway for the activation of the immune system against cancer, which in turn determines the long-term success of anticancer therapies. Only a few agents can elicit bona fide ICD, including some clinically established chemotherapeutics such as the proteasome inhibitor bortezomib, as demonstrated in malignant myeloma and mantle cell lymphoma, but not yet in melanoma. We have shown in melanoma that bortezomib induces NOXA-dependent apoptosis. Here, we show that bortezomib indeed causes ICD in vitro through induction of endoplasmic reticulum stress, autophagy and apoptosis and through translocation and/or secretion of damage-associated molecular patterns (DAMPs). Vaccination with bortezomib-treated dead melanoma cells induced tumor immunogenicity *in vivo*, as evidenced in a significant reduction/delay after challenge with live cells. Intralesional injection of bortezomib synergized with subsequent systemic treatment with immune checkpoint inhibition using CTLA-4 and PD-1 antagonists. Re-challenge demonstrated long-term protection through bortezomib combined with immune checkpoint inhibition. Polyfunctional T cell assays revealed that intralesional bortezomib injection generates a tumor-specific T cell response. Finally, immune checkpoint inhibitor-resistance was reverted by bortezomib-induced immunogenicity. In summary, bortezomib induces ER stress and apoptosis, enhances ICD markers (DAMPs) in vitro and is immunogenic *in vivo*. Bortezomib-induced ICD is a good strategy to recruit the inflammatory immune response. Bortezomib-induced ICD enhances response to immune checkpoint inhibitors, even in ICI-resistant tumors. We propose intralesional injection of bortezomib combined with systemic CTLA-4 andPD-1 antagonists to improve immune therapy in melanoma.

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Immunoprevention of dysplastic nevi in mice

M. Sherwani[1], B. N. McDaniel[1], N. Yusuf[1,2], C. A. Elmets[1,2]
[1]UAB, Birmingham, Alabama, United States, [2]VA Clinic Birmingham, Birmingham, Alabama, United States

Melanoma, an aggressive malignancy of melanocytes, is responsible for more deaths than any other skin cancer. Despite the fact that there is a long lag period before premalignant dysplastic nevi become invasive melanomas, there has been little progress in developing new methods for melanoma prevention. Using an animal model of melanomagenesis, we examined whether administration of anti-PD-L1 antibodies, an effective immunotherapy for advanced and metastatic melanoma, could be employed for immunoprevention. When mice were pretreated with Abs to PD-L1 prior to topical administration with a regimen that produces dysplastic nevi (treatment of C3H/HeN mice with topical DMBA and TPA), significantly fewer dysplastic nevi developed at 25 weeks compared to animals given with isotype control Abs. Treatment with ant-PD-L1 Abs was associated with a significant increase in CD8+ T-cells that produced IL-17 (0.4% vs. 0.78%) and IFN-γ (0.98 vs. 2.47%) compared to animals that had been were pretreated with isotype controls. There was also a decrease in CD11b+, Gr1+ (3.28% vs. 1.84%) and CD4+CD25+Foxp3+ (8.93 vs. 3.56) cells. These findings indicate that treatment with ant-PD-L1 Abs may be an effective for immunoprevention of dysplastic nevi in individuals who are at risk for melanoma.

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Planar cell polarity gene frizzled 6 promotes melanoma metastasis by regulating canonical Wnt signaling and EMT pathways

B. Dong[1], L. Simonson[1], S. Vold[1], E. Oldham[1], L. Barten[1], N. Ahmad[1,2], H. Chang[1]
[1]University of Wisconsin-Madison, Madison, Wisconsin, United States, [2]William S Middleton Memorial Veterans Hospital, Madison, Wisconsin, United States

The planar cell polarity (PCP) pathway controls tissue polarity during development by regulating the directional movement of cells and coordinating neighboring cells to the tissue axes. Increasing evidence suggests that it also plays active roles in cancer by promoting tumor cell migration and invasion. Here, we show that Frizzled6 (FZD6), one of the core PCP genes, is overexpressed in multiple melanoma cell lines and human samples. Knockdown or knockout of FZD6 does not affect cell proliferation, but significantly reduces the invasive ability of melanoma cells. In addition, we have found that knockout of Fzd6 dramatically reduces lung metastasis in the Pten/BRaf mouse model of melanoma. Mechanistic studies in vitro and *in vivo* reveal a surprising involvement of canonical Wnt signaling and EMT pathway in the FZD6-mediated invasive phenotype. Our study not only provides insights into the role and mechanism of FZD6 in melanoma progression but also sets the stage for additional studies to target FZD6 as a therapeutic tool to block the metastatic spreading of melanoma cells.

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Melanoma and the protective role of phosphorylated α-syn (pSer129) to prevent malignant degeneration

R. A. Norman[1], M. E. JImenez-Capdeville[2], I. Rodriguez-Leyva[3], E. Chi-Ahumada[2], F. Garcia-Ortega[2], L. Gil[4], S. A. Niño[2]
[1]Nova Southeastern University, Fort Lauderdale, Florida, United States, [2]Universidad Autonoma de San Luis Potosi, San Luis Potosi, Mexico, [3]Universidad Autonoma de San Luis Potosi - Facultad de Medicina, San Luis Potosi, Mexico, [4]Universidad Alfonso X el Sabio, Villanueva de la Canada, Comunidad de Madrid, Spain

Background:The misfolding and prion-like propagation of the protein alpha-synuclein(α-Syn) is the leading molecular signature in Parkinson's disease(PD). It has a protective role in the nucleus, where it is recruited to DNA double-strand break sites to promote DNA damage response and repair. The protein α-Syn is also highly expressed in melanoma. Elevated levels correlate with poorer survival to melanoma and α-Syn expression is required for tumor growth in experimental models. Phosphorylated α-Syn(pSer129) is associated with enhanced nuclear localization. Hypothesis:Nuclear pSer129 plays a physiologic role of genomic protection in skin cells. Reduced DNA binding induces severe transcriptional deregulation leading to uncontrolled proliferation in melanoma. Objective:To demonstrate the reduced presence of pSer129 in the nuclei of melanoma cells. Methodology:Twenty skin biopsies for pathological analysis were immunostained for pSer129. The positivity of the nuclei was quantified using a mathematical algorithm in 40X photomicrographs. Biopsies included four each of Stages 2, 3, and 4 Clark's(Anatomic) Level melanomas and eight Melanoma in situ (lentigo maligna). Each was stained with Mart-1 and HMB45 and in some p16. Ages ranged from 26-80 years old. Results: Biopsies from early stage melanoma in situ showed a remarkable loss of pSer129 immunopositivity in nuclei as compared with non-affected adjacent skin cells and later stage melanoma cells. Conclusion:The phosphorylated form of α-Syn accumulates in the nucleus of healthy cells. Although melanoma cells express high levels α-Syn, mostly in the cytoplasm, the disappearance of pSer129 from the cell nuclei is closely related to the beginning of malignant degeneration. These results support a protective role of pSer129 of gene expression in skin cells.

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A nuclear cAMP microdomain suppresses tumor growth by hippo pathway inactivation

M. Drozdz[1], A. S. Doane[1], R. Alkallas[2], G. Desman[1], R. Bareja[1], M. Reilly[1], J. Bang[1], M. Yusupova[1], J. You[1], J. Wang[1], A. Verma[1], K. Aguirre[1], E. Kang[1], I. Watson[2], O. Elemento[1], E. Piskounova[1], T. Merghoub[3], J. Zippin[1]
[1]Weill Cornell Medicine, New York, New York, United States, [2]McGill University, Montreal, Quebec, Canada, [3]Memorial Sloan Kettering Cancer Center, New York, New York, United States

cAMP signaling pathways are critical for both oncogenesis and tumor suppression. cAMP signaling is localized to multiple spatially distinct microdomains, but the role of cAMP microdomains in cancer cell biology is poorly understood. We developed a tunable genetic system that allows us to activate cAMP signaling in specific microdomains. We uncovered a previously unappreciated nuclear cAMP microdomain that functionally activates a tumor suppressive pathway in a broad range of cancers by inhibiting YAP, a key effector protein of the Hippo pathway, inside the nucleus. We show that nuclear cAMP induces a LATS-dependent pathway leading to phosphorylation of nuclear YAP solely at serine 397 and export of YAP from the nucleus with no change in YAP protein stability. Thus, nuclear cAMP inhibition of nuclear YAP is distinct from other known mechanisms of Hippo regulation. Pharmacologic targeting of specific cAMP microdomains remains an untapped therapeutic approach for cancer, and since Hippo pathway deregulation can lead to oncogenesis and chemotherapeutic resistance, drugs directed at the nuclear cAMP microdomain may provide new avenues for the treatment of cancer.

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Genome-wide scans identified genetic variants associated with facial aging traits quantified by deep learning methods

F. Wang[1, 2, 3], Q. Qi[1], Z. Li[4], X. Hu[2, 3], R. Ye[2, 3], L. Du[2, 3], S. Wang[1]
[1]CAS Key Laboratory of Computational Biology, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China, [2]Department of Science, Inertia Shanghai Biotechnology Co., Ltd, Shanghai, China, [3]Department of Science, DermaHealth Shanghai Biotechnology Co., Ltd, Shanghai, China, [4]Xiamen Meitueve Technology CO., Ltd, Xiamen, China

As life expectancy and aging population increase worldwide, facial aging is becoming progressively important. The occurrence and progression of facial aging are strongly influenced by genetic factors. Previous studies have identified a number of genetic variants associated with facial aging phenotypes using traditional image analysis and human assessments. In this study, we developed a deep learning framework to precisely measure 4 facial aging phenotypes (lacrimal sulcus, pigmentation spots, wrinkle forehead, and wrinkle under eyes). We then derived the phenotypes in 7,347 Han Chinese from two independent cohorts: Jidong cohort (N=5,036) and the National Survey of Physical Traits cohort (NSPT, N=2,311). A genome-wide scan in the Jidong cohort identified eight genomic regions showing genome-wide significant association with facial aging, including four previously unreported loci (i.e. 10q21.3, 9q21.12, 13q14.2 and 22q12.2) and four previously known genes: PPARGC1B, BNC2, MFSD12 and MC1R. All the eight loci were successfully replicated in the NSPT cohort at nominal significance. Meta-analysis of all samples further enhanced the significance level of the novel associations on 10q21.3, 9q21.12, and 22q12.2 (P=3.65×10-13, 2.82×10-8 and 5.00×10-9, respectively). In conclusion, our study identified novel genetic risk factors for facial aging traits using deep learning-based measurement in Han Chinese.

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Long-term melatonin treatment stimulates human epidermal pigmentation and melanocyte number

A. Sevilla[1], J. Chéret[1], R. Paus[1, 2, 3]
[1]University of Miami School of Medicine, Miami, Florida, United States, [2]Centre for Dermatology Research, The University of Manchester, Manchester, Manchester, United Kingdom, [3]Cutaneon, Hamburg, Germany

Melatonin, the evolutionarily ancient methoxyindoleamine, has long captured the attention of investigators since the discovery of its regulation of mammalian fur and amphibian skin pigmentation, as well as its extrapineal synthesis and receptor expression in human skin. Yet, melatonin's role in human skin and its impact on melanocyte physiology remain unclear. Therefore, we asked whether melatonin stimulates or inhibits epidermal pigmentation through its effects on melanin content, tyrosinase activity, as well as melanocyte number and proliferation in their natural cutaneous habitat with interactions with surrounding keratinocytes, at 2 different time points (three and six days of culture) and concentrations (50, 100, and 200 ug/mL). Our preliminary data show that after three days of culture, melatonin tendentially decreases tyrosinase activity in a dose-dependent manner and tendentially decreases the number of proliferative melanocytes without affecting the number of melanocytes present in the epidermis. Interestingly, after six days of culture, the different concentrations of melatonin decrease the in situ tyrosinase activity. However, melatonin increases intraepidermal melanin content correlated with an increased number of melanocytes. However, after six days of culture, melatonin at 200 ug/mL stimulated melanocyte proliferation. Taken together, our data showed that melatonin's regulatory effects on epidermal pigmentation and melanocyte number and their proliferation *ex vivo*, are depending on the concentration used and the duration of treatment. Short-term pulse therapy with inexpensive hormone promises to target hyperpigmentary cutaneous disorders while long-term therapy at high concentration may help treating hypopigmentary cutaneous disorders such as vitiligo by increasing the intracutaneous pool of melanocytes and restoring a physiological level of pigmentation.

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CNN-based histopathology image analysis for early-stage melanoma recurrence

G. Wan[1, 2], M. DeSimone[1], F. Liu[3], N. Nguyen[1], B. Leung[1], M. S. Choi[1], A. Bruce[1], A. M. Stagner[1], C. Lian[1], E. Russell-Goldman[1], M. Jiao[3], D. Zhen[2], J. Zhao[2], J. Gil[5], I. Németh[4], G. Marko-Varga[5], S. G. Kwatra[6], K. Yu[2], Y. Semenov[1, 2]
[1]Mass General Brigham, Boston, Massachusetts, United States, [2]Harvard University, Cambridge, Massachusetts, United States, [3]Stevens Institute of Technology, Hoboken, New Jersey, United States, [4]University of Szeged, Szeged, Hungary, [5]Lund University, Lund, Sweden, [6]Johns Hopkins University, Baltimore, Maryland, United States

Emerging data suggests that the majority of melanoma mortality occurs in patients with recurrence of disease that was initially early-stage (Stage I or II). Thus, there is a need for prognostic tools to identify patients at high risk of recurrence. This study examines the capability of weakly-supervised Convolutional Neural Networks (CNNs) for detecting prognostic signals of early-stage melanoma recurrence using whole-slide histopathology images (WSIs). We collected 224 WSIs from 58 patients with recurrence and 203 WSIs from 58 patients without recurrence and a minimum of 5-year follow-up. Each gigapixel WSI was processed into 512 x 512 pixel patches. We annotated regions of tumor for 50 patients and performed a two-step analysis. First, a model was trained to classify melanoma and non-melanoma patches. Second, a model was trained to differentiate between patches at high- and low-risk of recurrence. We compared performance of two CNN architectures, VGG16 and ResNet50, using 5-fold cross-validation. The median study follow-up for non-recurrent tumors was 6.5 years. Both architectures successfully identified melanoma patches: VGG16 (ACC 94.5%, AUC 98.9%) and ResNet50 (ACC 95%, AUC 99%). For recurrence prediction, VGG16 achieved slightly better patient-level performance (ACC 85.3%, AUC 93.5%) compared to ResNet50 (ACC 85.3%, AUC 91.3%). We demonstrate that a state-of-the-art CNN pipeline can accurately detect prognostic signals for early-stage melanoma recurrence using only WSIs. Our patch-based approach achieved these results without requiring detailed region of interest annotation. This approach can be used to automatically identify tumor areas with high-risk features, enabling subsequent molecular analyses of these regions to understand specific mechanisms for recurrence.

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Controlling mTORC1 activity as a novel therapeutic strategy for managing human hair growth and pigmentation

T. Suzuki[1], J. Chéret[1, 2], F. D. Scala[1], J. Gherardini[1, 2], J. O'Sullivan[1], G. Epstein-Kuka[3], A. J. Bauman[4], C. Demetriades[5], R. Paus[1, 2, 6]
[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States, [2]Monasterium Laboratory Skin & Hair Research Solutions GmbH, Munster, Nordrhein-Westfalen, Germany, [3]Foundation for Hair Restoration, Miami, Florida, United States, [4]Bauman Medical Group, Boca Raton, Florida, United States, [5]Cell Growth Control in Health and Age-Related Disease, Max Planck Institute for Biology of Ageing (MPI-AGE), Cologne, Germany, [6]CUTANEON, Hamburg, Germany

The mechanistic Target of Rapamycin Complex 1 (mTORC1) exerts multiple physiological functions, including nutrient sensing and the control of metabolism, proliferation, senescence and Wnt signaling. Yet, the role of mTORC1 activity in human hair follicle (HF) biology remains unknown. Since patients with a loss-of-function mutation of the key endogenous inhibitor of mTORC1 activity, tuberous sclerosis complex (TSC), can show poliosis, we probed the hypothesis that mTORC1 regulates human HF pigmentation, using HF organ culture. When healthy human anagen scalp HFs were treated *ex vivo* with the key mTORC1 inhibitor, rapamycin, this significantly stimulated HF melanin production, gp100 expression, tyrosinase activity and the dendricity of gp100+ HF melanocytes. Interestingly, this was associated with increased production of a-MSH by keratinocytes, notably in graying HFs, in some of which rapamycin even re-stimulated melanogenesis. In contrast, excessive intrafollicular mTORC1 activity induced by TSC2 knockdown significantly reduced HF pigmentation. (Of note, this provides the first siRNA assay system for functionally interrogating the unclear role of TSC in human organ physiology). Moreover, rapamycin prolonged anagen and inhibited hair matrix keratinocyte apoptosis *ex vivo*, thus expanding the window of opportunity for repigmenting gray hair, which can only occur during anagen. Thus, we show here that mTORC1 activity plays a fundamental role in the control of both, human hair pigmentation and HF cycling. This invites one to explore rapamycin and rapalogs for the management of human hair growth and pigmentation disorders.

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Melanoma persister cells survive CD8 T cell attack and seed acquired resistance to immunotherapy

M. X. Wang, B. E. Mauch, F. Araujo Hoffman, S. H. Harris, C. P. Lathrop, A. F. Williams, M. J. Hangauer
Department of Dermatology, University of California San Diego, La Jolla, California, United States

Clinical data indicate that acquired resistance occurs in nearly half of cancer patients who initially respond to immunotherapy. The molecular processes by which immunotherapy sensitive tumors survive and acquire resistance are poorly understood. For targeted therapy and chemotherapy, acquired resistance is thought to involve the survival of quiescent "persister" cells which form a reservoir that can seed tumor regrowth. For immunotherapy, little is known about the cancer cells which survive initially effective immunotherapy. We hypothesize that a melanoma persister cell population survives direct CD8 T cell attack and seeds subsequent tumor relapse. To explore this question, we have developed models in which primary human CD8 T cells transduced with retrovirus expressing a melanoma antigen-specific T cell receptor (TCR T cells) are cocultured with human melanoma cells which endogenously present the cognate antigens. To focus on the persister cell setting, we expose the melanoma cells to continually refreshed TCR T cells for 12 or more days after which melanoma cell death largely ceases and residual quiescent surviving persister cells remain. Upon TCR T cell removal, the surviving melanoma cells regrow but become resensitized to subsequent retreatment with TCR T cells. This reversibility of melanoma tolerance to TCR T cells indicates a nonmutational mechanism. Upon further extended coculture (> 1 month), a minority of persister cells regrow into proliferating colonies which are irreversibly resistant to TCR T cells. We also observe similar persister cell formation and regrowth upon treatment with purified cytokines IFNgamma and TNFalpha indicating that "bystander" persister cells may also form distal from sites of direct CD8 T cell attack. Together, these findings reveal that melanoma persister cells tolerate direct CD8 T cell attack and may contribute to acquired resistance to immunotherapy.

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EPAC-mTORC1 signaling regulates proliferation of primary melanoma cells and loss of dependence on EPAC signaling correlates with melanoma progression

M. K. Singh[1], A. Krishnan[1], A. I. Bhasker[1], C. I. Rodriguez[1], E. Castro-Perez[1], M. Ndiaye[1], N. Ahmad[1, 2], H. Khan[1], S. M. Schieke[1, 2], V. Setaluri[1, 2]
[1]Dermatology, University of Wisconsin-Madison, Madison, Wisconsin, United States, [2]William S Middleton Memorial Veterans Hospital, Madison, Wisconsin, United States

Exchange Proteins directly Activated by cAMP (EPACs) belong to a family of RAP guanine nucleotide exchange factors (RAPGEF). EPAC1/2 (RAPGEF3/4) activate RAP1 and the alternative cAMP signaling pathway. We previously showed that the differential growth response of primary and metastatic melanoma cells to cAMP is mediated by EPAC. However, the mechanisms responsible for this differential response to EPAC signaling are not understood. In this study, we show that pharmacological inhibition or siRNA-mediated knockdown of EPAC selectively inhibits the growth and survival of primary melanoma cells by downregulation of cell cycle proteins and inhibiting the cell cycle progression independent of ERK1/2 phosphorylation. EPAC inhibition results in upregulation of AKT phosphorylation but a downregulation of mTORC1 activity and its downstream effectors. We also show that EPAC regulates both glycolysis and oxidative phosphorylation, and production of mitochondrial reactive oxygen species, preferentially in primary melanoma cells. Employing a series of genetically matched primary and lymph node metastatic (LNM) melanoma cells, and distant organ metastatic melanoma (MM) cells, we show that the LNM and MM cells become progressively less responsive and refractory to EPAC inhibition suggesting loss of dependency on EPAC signaling correlates with melanoma progression. Analysis of TCGA dataset showed that lower RAPGEF3, RAPGEF4 mRNA expression in primary tumor is a predictor of better disease-free survival of patients diagnosed with primary melanoma suggesting that EPAC signaling facilitates tumor progression and EPAC is a useful prognostic marker. These data highlight EPAC signaling as a potential target for prevention of melanoma progression.

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Endogenous DOPA inhibits melanoma through suppression of CHRM1 signaling

M. Doepner[1], C. Natale[1], I. Lee[1], R. Brathwaite[1], S. Venkat[2], S. Kim[3], Y. Wei[4], C. Vakoc[4], J. Katzenellenbogen[3], B. Katzenellenbogen[3], M. Feigin[2], T. W. Ridky[1]
[1]University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Roswell Park Comprehensive Cancer Center, Buffalo, New York, United States, [3]University of Illinois at Urbana-Champaign, Urbana, Illinois, United States, [4]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, United States

Melanoma risk is 30 times higher in people with lightly pigmented skin compared to those with darkly pigmented skin. Here we show that this difference results from more than melanin pigment and its associated ultraviolet radiation (UVR) shielding effect. Using primary human melanocytes representing the full human skin pigment continuum and several preclinical melanoma models, we show that cell-intrinsic differences between dark and light melanocytes regulate melanocyte proliferative capacity, overall cellular differentiation state, and susceptibility to malignant transformation, independently of melanin and UV exposure. We determined that these differences result from dihydroxyphenylalanine (DOPA), a melanin precursor synthesized at higher levels in melanocytes from dark skin. Although DOPA was not previously known to have specific signaling activity, we used both high throughput pharmacologic and genetic *in vivo* CRISPR screens to determine that DOPA limits melanocyte and melanoma cell proliferation by inhibiting the muscarinic acetylcholine receptor M1 (CHRM1), a G Protein-Coupled Receptor (GPCR) not previously known to interact with DOPA, nor to affect melanoma pathobiology. Pharmacologic CHRM1 antagonism in melanoma leads to depletion of c-Myc and FOXM1, both of which are proliferation drivers associated with aggressive melanoma. In preclinical mouse melanoma models using both immune deficient and syngeneic immune competent mice, pharmacologic inhibition of CHRM1 or FOXM1 inhibited tumor growth. CHRM1 and FOXM1 may be new therapeutic targets for melanoma.

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Novel immune-related proteins differentially expressed in melanoma

G. Chhabra, J. Thornton, S. Su, M. Ndiaye, N. Ahmad
Dermatology, University of Wisconsin-Madison, Madison, Wisconsin, United States

Novel immune-related targets hold promise to improve melanoma treatment and patient survival. Using bioinformatics, we previously identified 21 immune-related molecules differentially expressed in metastatic melanoma and have significant correlation with patient survival. We shortlisted five candidate molecules that have been implicated in pathogenesis of other cancer types, however, none has been explored in melanoma. These are (i) MZB1, a B cell-specific cochaperone. (ii) SAMSN1, a negative regulator of B-cell activation that regulates cell polarization. (iii) NCKAP1L, which controls lymphocyte activation and proliferation, and phagocytosis by neutrophils/macrophages. (iv) KLK8, an epidermal protease involved in the skin barrier proteolytic cascade. (v) EBI3, which functions in innate immune responses. In this study, we aimed to validate the expression profile of these five molecules at protein level in clinical melanoma tissues along with S100, a melanoma biomarker. We performed fluorescent immunostaining of a human melanoma tissue microarray (ME2082c; US Biomax) consisting of 208 tissues cores (128 primary melanoma, 64 metastatic melanoma, 8 adjacent skin and 8 normal skin) followed by multispectral Vectra Imaging coupled with inForm software analysis. We observed significantly increased expression ($p<0.05$) of KLK8, SAMSN1 and EBI3 in S100-positive tumor cells compared to S100-negative cells. Further, MZB1 and NCKAP1L protein levels were significantly increased ($p<0.05$) in melanoma primary and metastatic tissues compared to normal skin. KLK8 and SAMSN1 protein expression showed increasing trend in melanoma tissues. Interestingly, EBI3 expression was not changed between the melanoma and skin tissues. Further, we quantified MZB1 protein levels using Simple Western and observed significantly increased levels in A375, G361, HS294T, WM115 melanoma cells compared to normal melanocytes as well as in tumors obtained from BrafV600E/PtenNULL mice (n=5) compared to tumor adjacent skin. Overall, our study suggest that MZB1 and NCKAP1L could be potential new targets in melanoma.

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Combination of cysteamine and isobionic-amide in a new formulation to decrease epidermal pigmentation

C. Hartman[1], S. Shah[2]
[1]Skin Wellness, Homewood, Alabama, United States, [2]Scientis US, Trinity, Florida, United States

Hyperpigmentation disorders are characterized by an overproduction of melanin. Treatments for pigmentary disorders either disrupt the biosynthesis of melanin or are cytotoxic to melanocyte. Cysteamine a well-studied depigmenting agent inhibits melanin synthesis at several steps in the melanogenesis pathway. It is now through the development of a novel complex combining stabilized cysteamine (st. Cys) with isobionic-amide, forming a new technology that results in a more efficacious skin depigmenting activity. Isobionic-amide is part of the vitamin B3 family that inhibits the synthesis of melanin by blocking the transfer of melanosomes into keratinocytes. Our in vitro studies have shown isobionic-amide to be non-cytotoxic and more potent than the depigmenting agent, niacinamide. The combined depigmenting activities of st.Cys and isobionic-amide may offer a novel, safe, and efficacious approach to the inhibition of melanogenesis and melanosomal transfer resulting in a reduction of melanin. Cell culture studies were conducted comparing the isobionic-amide or niacinamide to evaluate cellular viability, melanin synthesis and melanosomal transfer inhibition. Melanocytotoxicity was only seen in very high concentrations (500 µm) of isobionic-amide compared to niacinamide in B16 cells. In BDVII cells, keratinocytotoxicity was observed at a high concentration of 200 µm compared to Niacinamide. Isobionic-amide significantly reduced melanin synthesis and the melanosomal transfer from melanocytes to keratinocytes in our in vitro model. Isobionic-amide is a safe, non-toxic ingredient that inhibits melanogenesis by targeting melanin biosynthesis and melanosome transfer inhibition to keratinocytes resulting in a potent depigmentation coupled with an increase in pigment correction.

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Effects of UV on melanosome pH and metabolism

Z. Eraslan, D. Zhou, Q. Chen, J. Zippin
Weill Cornell Medicine, New York, New York, United States

Ultraviolet (UV) radiation is a cause of skin cancer and aging and has been shown to affect a variety of biological phenomena. Whereas UV radiation can induce local hormones that affect melanin synthesis, the mechanisms by which UV radiation directly affects melanocyte biology and melanin synthesis, independent of DNA mutation, remain poorly understood. In this study, we examined the effects of UV radiation on melanosomal pH and metabolism in melanocytes and melanoma cells in vitro. We demonstrate that UV radiation elevates melanosomal pH in a soluble adenylyl cyclase (sAC) and reactive oxygen species (ROS) dependent manner. Furthermore, we observe that UV radiation leads to a variety of other metabolic changes in melanocytes with various biological implications. Last, we observed by using LC-MS that UV exposure brings about immediate changes in the level of specific amino acids which have a crucial role in melanin synthesis. Overall, our results highlight that direct UV radiation of melanocytes reprograms cellular metabolism and melanosomal pH to enhance melanin synthesis.

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Downregulation of CTLA4 in tumor and blood samples is associated with a worse prognosis in malignant melanoma

P. Vaddi[1], D. G. Osborne[1], N. K. Williams[1], D. Ravindran Menon[1], A. Nicklawsky[2], D. Gao[2], Z. Zhai[1], M. Fujita[1]

[1]Dermatology, University of Colorado Denver School of Medicine, Aurora, Colorado, United States, [2]Biostatistics and Informatics, University of Colorado Denver School of Medicine, Aurora, Colorado, United States

CTLA-4 is a negative regulator of T lymphocyte activation, and the inhibition of CTLA-4 is used to treat melanoma but has limited efficacy. We measured CTLA4 mRNA expression in melanoma using TCGA tumor data and found the association of decreased CTLA4 with a worse patient prognosis. Because CTLA-4 is constitutively expressed in regulatory T (Treg) cells and upregulated in non-Treg T cells, we measured CTLA4 in blood samples. Whole blood samples from an Australian cohort (170 melanoma patients and 103 healthy controls) were measured for CTLA4 by qRT-PCR and analyzed using Kaplan-Meier (K-M), log-rank test, Cox proportional hazards model, and multivariate linear regression model. Unlike reported data of other cancers, blood CTLA4 was decreased in melanoma patients than in healthy controls. K-M survival curve demonstrated the association of low blood CTLA4 with worse patient survival. While such a trend was not evident in primary melanoma patients, the analysis of metastatic melanoma patients showed an association. This trend was confirmed using another cohort from the United States (246 melanoma patients). To understand immune cell subsets responsible for the downregulated CTLA4 in melanoma, we fractionated blood samples and found downregulated CTLA4 in T cells in melanoma patient blood. Consistent with these results, Treg cells from healthy human PBMCs showed decreased CTLA4 and CTLA-4 surface protein expression when cultured with condition media from human metastatic melanoma cell lines but not primary melanoma cell lines. In summary, we demonstrate that CTLA4 is lower in tumor and blood samples of melanoma patients, and the reduction correlates with a worse prognosis of melanoma patients, especially in metastatic melanoma. Our unpredicted and novel findings call for further investigation into the role of CTLA4 in Treg cell biology, tumor immunity, and cancer therapy.

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Clinical and histopathologic risk factors for early-stage melanoma recurrence

B. Leung[1], N. Nguyen[1], M. S. Choi[1], M. DeSimone[3], 1, G. Wan[1], S. Zhang[1, 2], A. Rajeh[1], M. Amadife[1], K. Tang[1], D. Zhen[1], J. Phillipps[1], R. Jairath[1], N. Alexander[1], A. M. Stagner[1, 2], C. Lian[2, 3], I. Németh[4], J. Gil[5], G. Marko-Varga[5], K. Yu[2], Y. R. Semenov[1, 2]

[1]Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States, [3]Brigham and Women's Hospital, Boston, Massachusetts, United States, [4]University of Szeged, Szeged, Hungary, [5]Lund University, Lund, Sweden

Emerging data suggest that the majority of patients who die from melanoma experience recurrence of disease that is early-stage (Stage I or II) at the time of diagnosis. Therefore, we aim to understand the clinical (demographics, medical history, surgical margins) and histopathologic (synoptic features) characteristics that influence early-stage melanoma recurrence utilizing a multi-institutional database comprising of 1,244 patients with 1,342 early-stage primary cutaneous melanomas from 2000-2020. A multivariable Cox proportional hazards model with clustering adjustment was used to analyze the risk for melanoma recurrence. Harrell C's concordance index was used to assess the goodness of fit. 331 (24.7%) of melanomas recurred within the study period. Among the recurrent melanomas, 52% of patients progressed to develop metastatic disease and 39.9% were deceased at end of follow-up. In univariate modeling for risk of melanoma recurrence, Breslow depth, AJCC stage, and tumor mitotic rate had the highest Harrell's C (0.8, 0.77, and 0.77 respectively) and were at significantly higher risk of melanoma recurrence. Multivariate modeling demonstrated that individuals who are older at diagnosis (HR 1.02, p<0.001), in the second quartile of income range of $77,484-$99,677 (HR 1.36, p=0.046), Clark's level above 4, melanoma stage above 2A, with the presence of tumor-infiltrating lymphocytes (HR 1.51, p=0.02), and presence of mitoses (HR 2.09, p=0.001) were at higher risk of melanoma recurrence. The risk factors identified above, particularly mitotic rate, can guide clinicians in risk stratifying patients with early-stage melanoma for increased surveillance to detect recurrence.

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WITHDRAWN

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Epigenetic regulation of Slamf6 expression in the immune response to melanoma

G. Micevic[1], R. Flavell[2], M. Bosenberg[1]

[1]Dermatology, Yale School of Medicine, New Haven, Connecticut, United States, [2]Immunobiology, Yale University, New Haven, Connecticut, United States

Many patients with advanced melanoma do not mount a successful anti-melanoma immune response, become refractory to checkpoint inhibitors, and unfortunately succumb to their disease. There is a great unmet need for improved mechanistic understanding of the anti-melanoma immune response, as well as novel strategies to improve long term survival. Failure of the anti-melanoma immune response has been linked to the establishment of a hypofunctional/exhausted T-cell state, with at least two distinct T-cell populations. One is a pre-exhausted/progenitor population which has replicative potential, is marked by low expression of programmed death 1 receptor and can respond to immune checkpoint blockade. The other is a terminally exhausted population marked by high expression of programmed death 1 receptor and does not respond to immune checkpoint therapy. Recently, the immunoglobulin family receptor Slamf6 was found to specifically mark the pre-exhausted cells, but not terminally exhausted T-cells. Epigenetic changes play important roles in establishing and maintaining T-cell exhaustion. The role of Slamf6 expression during T-cell exhaustion, and specifically the mechanism of Slamf6 loss during the transition from a pre-exhausted to a terminally exhausted state are unknown. We investigated the mechanism of Slamf6 silencing during antigen specific T-cell responses in melanoma. Our data suggest that Slamf6 expression undergoes significant epigenetic regulation during terminal T-cell exhaustion and we identified the epigenetic drivers of Slamf6 silencing. We found that Slamf6 expression is associated with melanoma response to immunotherapy and melanoma-specific overall survival. Further functional studies are needed to fully elucidate the role of Slamf6 in T-cell exhaustion, therapy resistance and evaluate potential epigenetic approaches to improve melanoma immunotherapy.

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Effect of sun exposure on distribution of nevi and melanoma in Caucasian and skin of color individuals

J. Harvey[1], J. Besch-Stokes[2], P. Bhullar[1], B. Boudreaux[1], P. Puri[2], K. Severson[1], M. Buras[3], C. Costello[1], M. R. Pittelkow[1], A. R. Mangold[1]
[1]Dermatology, Mayo Clinic Arizona, Scottsdale, Arizona, United States, [2]Mayo Clinic Alix School of Medicine, Scottsdale, Arizona, United States, [3]Division of Clinical Trials and Biostatistics, Department of Quantitative Health Sciences, Mayo Clinic Arizona, Scottsdale, Arizona, United States

Relatively little is known about clinical and epidemiologic characteristics of nevi and melanoma in skin of color minorities (SOCM) compared to Caucasians. We conducted a prospective study between 3/2017-8/2018 of 211 patients that volunteered for a skin cancer screening examination. The distribution of melanocytic neoplasms was recorded. Surveillance, Epidemiology, and End Results Program (SEER) data was used to identify melanoma data from 2000-2017. Most nevi were in sun-exposed areas (n=2,214, 66.0%) with the majority in intermittent locations (63.5%; chronic sun exposure: 35.0%; non-sun-exposed: 1.5%). On average, Caucasians had 3.0 more nevi in sun-exposed locations compared to non-sun-exposed locations (95% CI 2.73, 3.26, p <0.0001). SOCM had 6.17 more nevi in sun-exposed areas compared to non-sun-exposed (95% CI 6.05, 6.31; p <0.0001). Most melanomas (n=210,844, 65.2%) occurred in sun-exposed areas. Caucasians had 33,000 more melanomas per 100,000 patients with melanoma in sun-exposed areas (95% CI, 32,600, 33,300; p <0.0001) compared to SOCM. African Americans were the only race to have more melanoma in non-sun-exposed locations than chronic. Caucasian's nevi:melanoma ratio in sun-exposed areas was 22 (95% CI 21.7, 22.3); 33.2 in non-sun-exposed areas (95% CI 32.8, 33.6). In SOCM, the nevi:melanoma ratio in sun-exposed areas was 14 (95% CI 13.9, 14.1); 10.9 in non-sun-exposed areas (95% CI 10.8, 11). The various nevi:melanoma ratios across body locations and sun exposure supports different pathways of nevogenesis and carcinogenesis. The different environmental and genomic drivers of nevi and melanoma warrants further study.

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Evaluation of diagnosis diversity in artificial intelligence datasets

M. Chen[1], V. Rotemberg[2], J. Lester[3], R. Novoa[1, 4], A. Chiou[1], R. Daneshjou[1]
[1]Dermatology, Stanford Medicine, Stanford, California, United States, [2]Dermatology, Memorial Sloan Kettering Cancer Center, New York, New York, United States, [3]Dermatology, University of California San Francisco, San Francisco, California, United States, [4]Pathology, Stanford Medicine, Stanford, California, United States

Artificial intelligence (AI) algorithms are increasingly used for clinical tasks within dermatology. We conducted a study of potential biases in dermatology AI algorithms by analyzing the diagnoses included for training and/or testing AI algorithms that may lead to bias against patients with skin of color or women. Our systematic literature review of AI datasets applied to dermatologic tasks from January 1, 2015, to March 1, 2021 included 120 articles with a partial or full breakdown of disease distribution. We observed 240 dermatologic diseases after aggregating diseases by previously reported multilevel taxonomies. The most frequently identified high-level diagnostic groupings were malignant (83 papers) and benign (74 papers) pigmented lesions. We further identified diagnoses commonly seen in or with higher prevalence in patients with skin of color and women. The diagnoses in skin of color seen in the highest number of papers were acne (12 papers) and vitiligo (4 papers). For comparison, two diagnoses more commonly diagnosed in fair skin individuals included melanoma (83 papers) and basal cell carcinoma (44 papers). Four of the fourteen skin of color diagnoses were included in zero papers. For diagnoses highly represented in women, atopic dermatitis (6 papers) and lupus (6 papers) had the most papers. We also determined the number of confirmed unique images of our diagnoses of interest across all papers. The diagnoses in skin of color and women had a median number of confirmed unique images of 301 and 2,386, respectively. Our analysis illustrates that dermatologic diagnoses of particular salience in skin of color or women were less common in AI papers than diagnoses more common in fair skin. To have fair algorithmic performance across the entire population, researchers should prioritize generating data for diagnoses that are common or enriched in patients with skin of color and women.

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Non-sunscreen photoprotection methods are associated with lower serum 25-hydroxyvitamin D, with Asian and Black individuals showing less decrease in 25-hydroxyvitamin D with shade seeking: NHANES 2011-2016

J. Tsai, A. L. Chien
Dermatology, Johns Hopkins Medicine, Baltimore, Maryland, United States

Individuals with skin of color (SOC) have increased epidermal melanin that protects against photocarcinogenesis but increases the risk of vitamin D deficiency. Whether recommendations on photoprotection should differ for SOC is an important public health question, and few studies have examined the association between photoprotection and serum 25-hydroxyvitamin D [25(OH)D] levels in SOC populations, particularly in Asian individuals. We analyzed the data of 8269 adults aged 20-59 years (3050 Non-Hispanic White [NHW], 2001 Hispanic, 1042 Non-Hispanic Asian [NHA], 1848 Non-Hispanic Black [NHB], 328 other race/multiracial) from the National Health and Nutrition Examination Survey, 2011-2016. After adjusting for survey cycle, season, age, sex, race-ethnicity, education, income to poverty ratio, body mass index, smoking, alcohol use, physical activity, vitamin D intake, and other photoprotection methods, sunscreen use was not associated with significant change in 25(OH)D (0.317 nmol/L/unit increase in frequency, p=0.393). Lower 25(OH)D was seen with increased shade seeking (-2.304 nmol/L/unit increase in frequency, p<0.001) and long sleeve use (-0.874 nmol/L/unit increase in frequency, p=0.009). Heterogeneity of effects by race-ethnicity was present for shade seeking (likelihood ratio test [LRT], p=0.030) but not sunscreen or long sleeve use (LRT, p=0.438 and p=0.265). When stratified by race-ethnicity, the association between shade seeking and decreased 25(OH)D was stronger in NHW individuals (-2.877 nmol/L/unit increase in frequency, p<0.001) than in NHA (-0.734 nmol/L/unit increase in frequency, p=0.044 vs. NHW) and NHB participants (-0.486 nmol/L/unit increase in frequency, p<0.001 vs. NHW). In summary, we found that long sleeve use and shade seeking, but not sunscreen use, were associated with lower 25(OH)D across all participants, with NHA and NHB individuals showing less decrease in 25(OH)D with shade seeking, which may shift the risk-benefit ratio of shade seeking in these populations.

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Metabolic syndrome in black patients with cutaneous lupus erythematosus – a pilot study

H. W. Chen[1], J. L. Coias[1], J. Raman[1], B. Adams-Huet[2], I. Neeland[3], B. F. Chong[1]
[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Population and Data Science, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [3]Cardiology, University Hospitals, Cleveland, Ohio, United States

Cutaneous lupus erythematosus (CLE) is an autoimmune skin disease that can disproportionately impact patients with skin of color. Metabolic syndrome (MetS) is a result of chronic, low-grade inflammation known to be prevalent in patients with systemic lupus erythematosus (SLE), but little is known regarding this association in CLE. We performed a cross-sectional study of a convenience sample of 78 patients with CLE enrolled in the University of Texas Southwestern Cutaneous Lupus Registry to determine the prevalence of MetS, as defined by three out of five modified Adult Treatment Panel III criteria. Patients with SLE or other autoimmune skin diseases were excluded from the study. Chi-squared tests and multivariable logistic regression were utilized to quantify associations between race/ethnicity and MetS. The study cohort comprised of 53% Black, 8% Hispanic and 1% Asian patients. Among Black patients, we found a non-significant increased frequency of MetS amongst Black CLE patients (53.7%) relative to matched Black controls (39.0%) stochastically derived from the Dallas Heart Study (p=0.18). Compared to Black controls, Black CLE patients more frequently had low HDL (56% vs 31%, p=0.02) and hypertriglyceridemia (31% vs 13%, p=0.03). Amongst CLE patients, Blacks were at increased risk for MetS (OR 4.96 [1.81-15.0], p=0.003) and risk for MetS increased in older Black patients (OR 2.16 [1.03-4.83] per 10 years of age, p=0.05). We report a higher frequency of MetS in our CLE cohort compared to controls, particularly in Black patients of increasing age. These findings highlight the importance of prompt diagnosis of CLE in Black patients and suggest increased screening for cardiovascular risk factors may be warranted.

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Incidence and characteristics of acral lentiginous melanoma in Asian Americans and Pacific Islanders

A. H. Wei[1, 2], D. X. Zheng[1, 2], C. R. Cullison[1, 2], B. T. Carroll[2]
[1]Case Western Reserve University, Cleveland, Ohio, United States, [2]University Hospitals, Cleveland, Ohio, United States

Acral lentiginous melanoma (ALM) is a rare but aggressive subtype of cutaneous malignant melanoma (CMM). While uncommon in White individuals, ALM represents a higher proportion of CMM in skin of color (e.g. Asian Americans). Patients of color have worse CMM outcomes and ALM-specific survival compared to White patients, yet the reasons for these differences remain unclear. Given its rarity, there have been limited population-based studies of ALM incidence and tumor characteristics among Asian Americans and Pacific Islanders (AAPI). We analyzed all histologically confirmed cases of ALM among AAPI and non-Hispanic Whites (NHW) in the Surveillance, Epidemiology, and End Results specialized AAPI database (1990-2014). We collected incidence data, and patient (sex, age, race/ethnicity) and tumor (location, ulceration, Breslow depth, pathologic stage, sentinel node positivity) characteristics. Student's t and chi-square tests were used to compare ALM between AAPI and NHW individuals. ALM incidence rates were similar between AAPI and NHW (1.5 per 1,000,000 person-years vs. 1.8 per 1,000,000 person-years, P=0.092). ALM composes 11.8% (205/1,732) of all CMMs in AAPI compared to 0.7% (2,017/281,754) in NHW; this relatively high proportion of ALM was observed across Asian ethnic groups. Compared to NHW, AAPI ALM tumors were significantly more common in men (54.6% vs. 47.1%, P=0.041), were higher stage (P<0.00001), more often ulcerated (42.4% vs. 33.3%, P=0.027), and more often sentinel node positive (42.1% vs. 31.7%, P=0.020) at diagnosis. Our study includes the largest AAPI ALM cohort to date. We confirm that ALM composes a larger proportion of CMM in AAPI than in NHW, and identified that this trend is consistent across Asian ethnic groups. ALM incidence rates were similar between AAPI and NHW, yet ALM was diagnosed at more advanced stages in AAPI, which may explain differences in survival. Our findings underscore a need for earlier clinical detection of ALM within the AAPI population.

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Human skin ethnicity: Contribution of tissue engineering to develop cosmetic ingredients

M. Arcioni, L. Restellini, C. Serre, C. Capallere, I. Imbert
Ashland Global Specialty Chemicals Inc, Sophia Antipolis, France

Recent publications describe various skin disorders in relation with phototypes and aging. The highest phototypes (III to VI) are more sensitive to acne with the appearance of dark spots due to the inflammation induced by Cutibacterium acnes (previously named Propionibacterium acnes). Ethnic variations of skin dryness with aging due to a lower activity of specific enzymes involved in the maturation of lipids of the stratum corneum were also reported. To observe and understand these cutaneous disorders, tissue engineering is a perfect tool. Since several years, pigmented epidermis with melanocytes derived from specific phototypes have allowed developments of in vitro models for biological investigations. In this study, several in vitro models were developed to study various skin disorders associated with phototypes and aging. Treatments with biofunctionals and natural ingredients were further investigated in these models to help decrease effects of acne, inflammation and cutaneous dryness. Hyperpigmentation and increase of inflammatory cytokines release were observed on the reconstructed pigmented epidermis after the application of C. acnes, and pollutant (PM10). Experimental investigations combining tissue engineering and molecular biology were conducted to modify genetically the cells in order to decrease the expression of the targeted proteins. In this study, a silencing of Glucocerebrosidase was performed to decrease the maturation of lipids involved in the epidermal barrier function in order to create a 3D skin dryness-like model. Results suggest that treatment with Myrciaria Dubia fruit extract helps to prevent and decrease the negative effects of C. acnes and pollutants on skin. A decrease of hyperpigmentation and inflammation, as well as an increase of lipid content were observed in the respective 3D models. These new in vitro models are essential tools to investigate ethnic skin focused ingredients.

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Race and its impact on inpatient calciphylaxis outcomes

S. Lanyi[1], F. Nutan[1], F. Nutan[2, 1]
[1]Virginia Commonwealth University, Richmond, Virginia, United States, [2]East Carolina University, Greenville, North Carolina, United States

Within the past several years, critical race theory has received heightened attention from the cultural, social, and political world. Similarly, the medical community has reevaluated many longstanding theories about race and associated risk factors for disease states as well as correction factors for calculations such as eGFR. We sought to determine if there may be an inherent disparity in the diagnosis, understanding, and outcomes of race as it pertains to calciphylaxis. In our retrospective cohort study, patients with biopsy proven calciphylaxis (n=26) admitted to a VCU between 2010-2020 were analyzed for multiple variables including known risk factors, comorbidities, and demographics. From our cohort, 18 black patients were diagnosed with biopsy proven calciphylaxis compared to 8 white patients. The mortality rate was 46%, consistent with current literature. Stratified by race, mortality in black patients was 61% compared to 12.5% in white patients. With hemodialysis (HD) being a known risk factor for calciphylaxis, it was observed that the mortality rate for those on HD was 53%. A total of 15 black and four white patients were on HD with a mortality rate of 47% for the black patients on HD compared to 25% in the white patients. In previous studies, calciphylaxis has been predominantly described in the white population as a risk factor, which is in contrast to the findings of this study. This points to a sociocultural health disparity rather than race, as a risk factor for the development of calciphylaxis in line with the critical race theory. HD status is associated with a higher mortality rate within the sample population, with black patients demonstrating both higher rate on HD (0.83 blacks vs 0.5 whites) and higher mortality rate of those on HD. These findings shed light on the need to reevaluate race not as a biological construct, but as a social construct that creates barriers to maximal treatment outcomes. Future studies are needed to include a broader region within the US to increase the power of this study and to help remove these antiquated theories from our health system.

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Using quantitative colorimeter measurements to assess the reliability of fitzpatrick skin typing in darker skin tones

T. Dyson[1, 2], S. Alcorn[3], C. Aguh[1]
[1]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Eastern Virginia Medical School, Norfolk, Virginia, United States, [3]Radiation Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

The Fitzpatrick skin type (FST) scale is a subjective method used for skin type classification, but data suggests that it may not be as accurate in those with darker pigmentation. Colorimeter scales can be used to provide more objective classifications of skin type. Colorimeter measurements of L*, a*, and b* variables signify pigmentation, position on the red/green spectrum, and position on the yellow/blue spectrum, respectively. In this study, we sought to correlate objective colorimeter measurements with subjective FST. A total of 8 patients, 4 with FST IV and 4 with FST V were recruited and assigned FST based on survey responses. Colorimeter measurements for L*, a*, and b* variables were obtained in the lower outer quadrant of the right breast, the upper outer quadrant of the left breast, and the lower outer quadrant of the left breast for each participant. Using Stata software, two sample t-tests were performed comparing the L*, a*, and b* variables of each breast quadrant between the FST IV and V participants. No statistically significant difference was found for the variables of the lower outer quadrant of the right breast or the lower outer quadrant of the left breast. Analysis demonstrated a statistically significant difference in only the L* variable (p-value = 0.0403) of the upper outer quadrant of the left breast for FST IV participants in comparison to FST V participants with no statistical differences in the a* and b* variables. There are no major differences in the colorimeter values in patients with Fitzpatrick skin types IV and V. However, the statistical significance noted in the upper outer quadrant may be due it being the most photoprotected area and future clinical assessment would benefit from focusing on identifying FST based on photoprotected areas only, especially in darker skin types.

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Evaluation and efficacy of skin of color dermatology education among underserved adolescents

N. Wong[1], K. Williams[1], S. Tolliver[2], D. Mehregan[2]
[1]Wayne State University School of Medicine, Detroit, Michigan, United States, [2]Dermatology, Wayne State University School of Medicine, Detroit, Michigan, United States

Melanoma education among underserved adolescents is limited as per a review of current literature. This study focused on educating and promoting knowledge of melanoma pertaining to skin of color, in addition to common dermatological conditions with the target population of underserved high students in Detroit Michigan. Detroit Michigan was chosen because it has a large African American population. Students in underserved areas are at a greater disadvantage when it comes to exposure to dermatology and knowledge of dermatology-related conditions. The conditions that were presented included hyperpigmentation, hair loss, skin cancer, hidradenitis suppurativa, atopic dermatitis, and acne. Students received a presentation pertaining to melanoma and common dermatological conditions seen within skin of color patients. Before the presentation, they completed a pre-survey that was used to assess adolescents' knowledge, attitudes, and behavior regarding melanoma and other common dermatological conditions in skin of color. One month later, students were asked to complete a post-survey to assess the efficacy of the presentation. Long-term goals include widespread implementation of this program to increase awareness of melanoma in teenagers with skin of color and improve the 5-year survival rate of minorities to equal the industry standard. Our review demonstrates statistically significant improvements in knowledge, attitudes, and planned behaviors toward the medical profession and skin knowledge among the high school students who participated in the pre-program survey and post-program survey. Amid the favorable results, a 23%, 32%, and 20% improvement over pre-program results in SPF knowledge, acne myths, and skin cancer recognition (p.<001), as well as a 45% increase in intent to use sunscreen (p.<001). This program demonstrated a meaningful impact and showed potential to promote awareness and changed behaviors in relation to melanoma in skin of color individuals.

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Average RGB color value to categorize skin color of dermatology images

J. E. Lamb[1], A. X. Stone[1], Z. Li[3], J. Hu[4], A. J. James[2]
[1]University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States, [2]Dermatology, UPMC, Pittsburgh, Pennsylvania, United States, [3]Computer Science, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [4]Electrical & Computer Engineering, University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Visual learning equity is a health justice effort to increase the diversity of skin color in images used for dermatology education. However, visual assessment of skin color is subjective and varies based on viewers' personal characteristics and contextual situation. Here, we aim to create an objective measure of assessing skin color using the average red, green, and blue (RGB) color values of image pixels to categorize images. The RGB color model is additive and RGB values increase as skin color lightens from black to white. We manually cropped 200 images of skin conditions from preclinical lecture slides to only contain the unaffected, background skin of the patient. The RGB value of each image pixel was calculated and an average RGB number for the entire image was computed. To classify the average RGB values into human skin color groupings we used the NIS Skin Color Scale as a reference. We determined the average RGB value for each point on the NIS Skin Color Scale and defined the average RGB from points 1-2 as light/white skin, 3-5 as medium/brown skin, and 6-10 as dark/black skin. Two reviewers also visually categorized the 200 images using the NIS Skin Color Scale with the same skin color groupings. The percent agreement between the visual scores of the two reviewers was 83% with a Cohen's kappa of 0.64. The average RGB categorization method showed high reliability with the reviewers' visual scores, with a percent agreement of 82.5% and Cohen's kappa of 0.67. There was a strong linear relationship between the average RGB value of the image and reviewers' visual NIS Skin Color Scale numeric score (Spearman's rho = -0.78, p<0.001). Extracting average RGB color value from image pixels is a reliable method of categorizing the skin color of images. This measure can be used to objectively determine representation of skin color in dermatology training images as we strive for equitable inclusion of all skin colors.

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Impaired follicular Nrf2 signaling: Potential early therapeutic target in hidradenitis suppurativa

M. Kerns[1], X. Xing[2], J. E. Gudjonsson[2], A. S. Byrd[3], S. Kang[1]
[1]Dermatology, Johns Hopkins University, Baltimore, Maryland, United States, [2]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [3]Dermatology, Howard University, Washington, District of Columbia, United States

Hidradenitis suppurativa (HS) is a chronic debilitating disease characterized by inflammatory nodules and sinus tracts in areas rich in terminal hair follicles and apocrine glands. Lifestyle factors associated with oxidative stress, such as obesity and smoking, worsen symptoms. An early step in HS is follicular dilation and occlusion with keratin, which results in follicular rupture and a robust inflammatory response. Nrf2 signaling is a key regulator of follicular response to environmental redox insults and keratin expression. Herein, we utilized indirect immunofluorescence (IF) to assess levels of total and phosphorylated (active) Nrf2 and its inhibitor Keap1 in HS lesional skin (n=10) and sex-matched healthy controls (n=4). There was a decrease of IF signal for all three targets in HS hair follicles compared with controls. Next, we examined whether keratin 16 (K16) may provide a potential mechanistic link between dysfunctional Nrf2 signaling and hyperkeratinization of HS hair follicles. K16 is a stress-inducible target and regulator of Nrf2 signaling. Moreover, K16 overexpression in a tissue-specific transgenic mouse model results in aberrant keratinization of the outer root sheath. We found an upregulation of both K16 and its type II keratin binding partner K6 in the hyperplastic outer root sheath of HS hair follicles and sinus tracts. This suggest that impaired Nrf2 signaling, possibly by virtue of its bidirectional regulatory relationship with K16, may contribute to follicular hyperkeratinization and occlusion in HS. Given growing evidence of the efficacy of topical Nrf2 inducers and attractiveness of early intervention in HS, this work opens up new promising therapeutic avenues for a clinically devastating disease with limited treatment options.

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Patient race is associated with diagnostic uncertainty for psoriasis among dermatologists

F. Ahmed, R. Fitzsimmons, E. Chu, D. B. Shin, J. Takeshita
University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States

Dermatologists are less confident in diagnosing psoriasis among racial/ethnic minority individuals compared to White individuals, according to survey data. This may contribute to racial/ethnic disparities in psoriasis treatment and disease burden. The aim of our study was to assess diagnostic uncertainty for psoriasis among dermatologists in clinical practice by patient race/ethnicity. We performed a cross-sectional study of adult patients with at least one outpatient dermatology visit in the University of Pennsylvania Health System and a diagnosis of psoriasis between 2010 and 2019. Our primary outcome was the performance of a skin biopsy for psoriasis, which served as an indicator for clinician's uncertainty in making a clinical diagnosis. Multivariable logistic regression was performed to evaluate the association between patient race/ethnicity and skin biopsy for psoriasis. Our study included 10,008 patients. Mean (standard deviation) age was 55.7 (16.8) years; 56% were female. The racial/ethnic distribution was 70.6% White, 13.1% Black, 4.9% Asian, and 3.2% Hispanic. Skin biopsy to diagnose psoriasis was performed among 477 (4.8%) patients. The proportion of skin biopsies performed was highest among Black patients (9.9%) followed by Asian (4.7%), White (4.1%), and Hispanic (3.7%) patients. In adjusted analyses that controlled for patient sociodemographic, dermatologist, and other factors, Black patients (odds ratio [OR] 2.0, 95% confidence interval [CI] 1.5–2.7) and those with more years of follow-up (OR 1.2, 95% CI 1.1–1.2) were each more likely to receive a skin biopsy than White patients and those with shorter follow-up time, respectively. Patients who had at least one visit with a psoriasis specialist were also more likely to have received a skin biopsy than those who had none (OR 1.3, 95% CI 1.0–1.5). Our study findings suggest that, in clinical practice, dermatologists are less confident in making a psoriasis diagnosis among Black versus White patients and further support the need for greater diversity in dermatology education.

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Metabolomic profiling of cutaneous lupus erythematous

L. Abbas[1], G. Barber[1], H. Vu[2], L. Cai[2, 3], R. C. Wang[1], B. F. Chong[1]
[1]Department of Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Children's Medical Research Institute, Core Metabolomics, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [3]Department of Population and Sciences, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

Cutaneous lupus erythematosus (CLE) is a chronic autoimmune disease that disproportionately impacts patients with skin of color. Metabolic reprogramming plays a critical role in modulating the innate and adaptive immune response, but its role in cutaneous autoimmune conditions, like CLE, is less well studied. Thus, untargeted liquid chromatography-mass spectrometry metabolomics was performed to characterize the skin and serum of CLE patients. Eleven CLE patients (8 Black and 2 Hispanic Whites) and 14 normal controls were recruited from outpatient dermatology clinics at the University of Texas Southwestern Medical Center and Parkland Health and Hospital System in Dallas, TX. Unpaired t-tests were performed to compare disease samples to controls. Fourteen serum samples (9 CLE vs. 6 control) and 13 skin samples (5 CLE vs. 8 control) were analyzed. CLE patients were found to have 11 metabolites of differential abundance in the skin but only 2 in the serum. CLE skin showed increased levels of citrulline (fold change (FC)=1.15, p=0.02) and uracil (FC=1.79, p=0.04), and down-regulation of cyclic adenosine diphosphate (cADP) (FC=0.83, p=0.04), nicotinamide mononucleotide (NMN) (FC=0.75, p=0.016), and nicotinamide adenine dinucleotide (NAD+) (FC=0.86, p=0.016). Confirmatory qRT-PCR assays were performed for enzymes involved in NMN and NAD+ metabolism, CD38 and NT5E. CD38 (mean FC=26.94) and NT5E (mean FC=7.84) were found to be significantly upregulated in 5 CLE skin vs. 5 normal skin biopsies, which support decreased levels of NMN and NAD+ in CLE skin. These findings suggest abnormalities in nicotinamide metabolism in CLE, which may have novel biomarker and treatment implications.

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Racial differences in melasma risk factors and treatment patterns

E. D. Getachew[1], J. Yoon1, K. Young[1], B. Leung[3], N. Nguyen[3], A. Mostaghimi[2], Y. Semenov[1, 3], N. Theodosakis[3]
[1]Harvard Medical School, Boston, Massachusetts, United States, [2]Brigham and Women's Hospital, Boston, Massachusetts, United States, [3]Massachusetts General Hospital, Boston, Massachusetts, United States

Melasma is a common acquired hyperpigmentation disorder disproportionately affecting women of color. Though its cause remains unknown, several predisposing risk factors have been identified. Our investigation sought to better define racial and ethnic differences in risk factors, treatment patterns, and incidence of melasma in the US through a large case-control analysis. We queried the TriNetX database representing 40 US healthcare organizations for adults diagnosed with Melasma using ICD-10 code L81.1 (n=22,110). Controls were matched by age, sex and race. We explored gravidity, hyperthyroidism, and exposure to exogenous hormones and hyperpigmenting drugs as risk factors. We also analyzed treatment with topical steroids and retinoids, hydroquinone (HQ), tranexamic acid (TA) and azelaic acid. Median age of diagnosis was lowest in Whites (41.5) and highest in Blacks (54.1). Hispanics and Whites had increased hormone exposure (both 19%), while Blacks had the highest exposure to hyperpigmenting drugs (46%). Asians and Blacks had significantly increased risk of melasma compared to Hispanics and Whites across all risk factors: gravidity history (OR=1.4, p=0.001 and OR=1.4, p<0.001), hyperthyroidism history (OR=1.8, p=0.005 and OR=2.4, p<0.001), exogenous hormones exposure (OR=2.6, p<0.001 and OR=2.6, P<0.001) and hyperpigmenting drugs exposure (OR=2.8, p<0.001 and OR=2.2, p<0.001). Notably, across all races, history of exogenous hormones was a stronger risk factor than gravidity history. Hyperpigmenting drugs were also a significant risk factor across races raising the possibility of drug-induced hyperpigmentation contributing to melasma. For therapy, Blacks were most likely (34%) and Hispanics least likely (26%) to receive tretinoin. HQ and TA treatment rates were lowest in Whites at 30% and 2.5% respectively. Overall, our results indicate significant racial differences in the incidence of melasma, melasma risk due to various risk factors and treatment patterns.

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Top skin of color publications in dermatology

B. Cooper[1], J. Anderson[2], M. Laughter[3], C. Presley[4], J. Albrecht[5], R. Dellavalle[6, 7]
[1]Rocky Vista University College of Osteopathic Medicine, Parker, Colorado, United States, [2]Pathology, Stanford University, Stanford, California, United States, [3]Transitional Year Residency, The University of Texas at Austin Dell Medical School, Austin, Texas, United States, [4]Dermatology, Lehigh Valley Health Network, Allentown, Pennsylvania, United States, [5]The University of Utah School of Medicine, Salt Lake City, Utah, United States, [6]Dermatology, University of Colorado - Anschutz Medical Campus, Aurora, Colorado, United States, [7]Dermatology Service, VA Eastern Colorado Health Care System, Aurora, Colorado, United States

Recent advances in dermatology have aimed to be more inclusive of Skin of Color (SoC) to better serve the general public. This study compares the metrics and content of top publications regarding SoC in dermatology. Web of Science was utilized to measure an article's citation count while Altmetrics was employed to measure the amount of online media attention surrounding published research papers by way of the Altmetric Attention Score (AAS), with a higher AAS denoting a greater amount of online attention. The purpose of this study is to evaluate the relationship of a research article's citation count and the amount of attention it receives through platforms such as news outlets, blogs, and twitter. It attempts to discover if what the public finds to be important is congruent with the SoC content being discussed in high impact dermatology research papers. The significance of this is due to the influence high impact research papers have on clinical practice, as these studies often guide clinical recommendation. By doing so, dermatologists can recognize the general public's engagement in SoC research topics while also practicing under the recommendations given through high impact SoC research papers. Our study demonstrated a statistically significant difference in several of the categories compared, including the AAS (P=0.02), news outlet mentions (P=0.008), and twitter mentions (P=0.02). As social media platforms continue to grow and be utilized to spread medical information and current publications, Altmetric may be able to capture the attention of the general public better than WoS to further the academic work of SoC in dermatology.

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Simultaneous targeting of MAPK and PI3K signaling via CK2 inhibition in melanoma

R. Perez-Lorenzo[1], S. Stoyanov[1], A. M. Christiano[1, 2]
[1]Dermatology, Columbia University, New York, New York, United States, [2]Genetics and Development, Columbia University, New York, New York, United States

Acral melanoma (AM) occurs in a much higher proportion in darker-skinned individuals compared to cutaneous melanoma, and is the most common type of melanoma in patients of African and Asian descent. AM is associated with activating mutations in KIT or inactivating mutations in NF1. These tumors respond poorly to available targeted therapies and immune checkpoint blockade, underscoring the urgent need for therapeutics to improve overall survival of patients with metastatic disease. The protein kinase CK2 is upregulated in several types of cancer, including melanoma, and especially in AM. CK2 over-expression contributes to the malignant phenotype and is associated with resistance to targeted therapy. We investigated the potential of CK2 inhibition to overcome resistance to MEK inhibitors (MEKi) in preclinical models of AM. In vitro treatment of NF1-null human melanoma cells with a small-molecule CK2 inhibitor (CX4945) resulted in reduced cell viability and downregulation of PI3K and MAPK-ERK signaling, as demonstrated by dephosphorylation of AKT (S129 and S473) and ERK (p44/42 MAPK; Thr202/Tyr204). Interestingly, the downregulation of MAPK signaling correlated with increased levels of TYRP-1 and MITF, suggesting increased antigenicity in response to treatment. CX4945 also had a synergistic effect reducing cell viability and increasing apoptosis, with the MEKi trametinib and the BRAFi PLX4720. Further, in colony formation assays, CX4945 significantly reduced the appearance of trametinib-resistant colonies in NF1-null cutaneous melanoma, as well in AM-derived cell lines. To demonstrate translational relevance of these findings, we treated NF1-null human melanoma cells grown as xenografts in nude mice with CX4945, and noted a significant reduction in tumor growth that resulted in stable disease. Together, our results suggest that the combination of CK2 inhibitors with MEK inhibitors, and potentially other tyrosine kinase inhibitors, may improve therapeutic outcomes in difficult to treat malignant melanomas of acral origin.

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Environmental justice & cutaneous sarcoidosis.

A. A. Guevara[2], C. Ortiz[2], A. J. James[1]
[1]Dermatology, UPMC, Pittsburgh, Pennsylvania, United States, [2]School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Cutaneous sarcoidosis is an idiopathic granulomatous disease with variable systemic involvement and no known cure or prevention. In the U.S., Black people disproportionately develop sarcoidosis and have higher mortality rates than all other populations. Studies show genetic predisposition and ill-defined environmental associations may combine to heighten disease risk. To investigate the role of environmental exposures, we compared geographic data of cutaneous sarcoidosis cases to the Environmental Protection Agency's Environmental Justice Areas (EJA), areas defined by census tracts determined to be vulnerable to environmental pollutants. We conducted a retrospective medical record review of 111 adult patients at the University of Pittsburgh Medical Center with documented biopsy-confirmed, clinical diagnoses of cutaneous sarcoidosis. We reviewed demographic, comorbidity, medication, social/family history, and exposure data. 52.2% of patients identified as Black, 45.9% White, and 0.9% Asian. 64% identified as female and 36% male. Mean age at diagnosis was 47.8 years with younger mean age for Black vs. White patients (45.8 vs. 50.5, P=0.04). 58.6% of patients lived in EJAs. Black patients were more likely to live in EJAs (81.0% vs. 31.4%, P<0.001) and have systemic disease (82.7% vs. 62.7%, P=0.02) than White patients. Of 6 deceased patients, 5 (83.3%) identified as Black, and all resided in EJAs. EJA residence was associated with mortality (P=0.007) and cardiac sarcoidosis (P=.007) in our cohort overall and with more widespread disease in White patients (P=.049). In summary, EJA residence is associated with cutaneous sarcoidosis and mortality, particularly in Black patients who have an earlier age at diagnosis and increased risk of systemic involvement. Future analysis of specific environmental exposures among prevalence clusters will be important to identify and correct corresponding racial health disparities in cutaneous and systemic sarcoidosis.

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Efficacy of topical LXR agonist in the treatment of primary cicatricial alopecia in Scd1−/− mouse

J. Kim[1], J. Huang[1], E. Wang[1], A. M. Christiano[1, 2]
[1]Department of Dermatology, Columbia University, New York, New York, United States, [2]Department of Genetics and Development, Columbia University, New York, New York, United States

Primary cicatricial alopecias (PCAs) are heterogeneous disorders characterized by the replacement of hair follicle (HF) structures by fibrous tissue, leading to permanent hair loss. The underlying pathogenesis and treatment options are poorly characterized due to the lack of preclinical research models. The asebia mouse, which harbors mutations in the stearoyl-CoA desaturase 1 (Scd1) gene, was previously proposed as a model for PCA, however, the immunopathogenic mechanisms underlying permanent hair loss in Scd1−/− mice are largely unknown. We found that intraepithelial CD207+ Langerhans cells (LCs) were significantly increased primarily within the HF isthmus and infundibulum epithelium, whose dendrites surrounded caspase-8-driven apoptotic HF epithelial cells. Flow cytometry revealed that a CD45+CD207mid LC population expanded within epithelial compartments in Scd1−/− mice, which was not observed in Scd1+/− mice. Thus, hair loss in Scd1−/− mice shares multiple immunologic features with human central centrifugal cicatricial alopecia (CCCA). Systemic treatment with anti-CD207 antibody markedly attenuated the progression of hair loss, suggesting intraepithelial LCs as immunopathogenic driver in Scd1−/− mice. We postulated that correction of the lipid synthesis defect in Scd1−/− mice would reverse the CD207+ LC-mediated pathogenic process. The liver X receptor (LXRs) is considered a master regulator of cholesterol biosynthesis. Topical treatment with a LXR agonist (GW3965) showed promising efficacy in the attenuation of progressive hair loss by reducing intraepithelial CD45+CD207mid LC expansion. We established the Scd1−/− mouse as a preclinical PCA model in which intraepithelial CD207+ LCs induce immune-mediated permanent hair loss. Our findings invite further clinical evaluation of LXR agonists as novel potential therapeutic agents in the treatment of PCA.

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A polygenic risk score uncovers racial and genetic differences in susceptibility to prurigo nodularis in patients of African ancestry

C. Vasavda[1], G. Wan[2], C. Lu[2], N. Sutaria[1], N. Nguyen[2], M. Szeto[1], W. Adawi[1], J. Deng[1], V. Parthasarathy[1], Z. A. Bordeaux[1], M. Taylor[1], M. Marani[1], K. Lee[1], M. P. Alphonse[1], S. Kang[1], Y. Semenov[2], A. Gusev[3], S. G. Kwatra[1]
[1]Dermatology, Johns Hopkins Medicine, Baltimore, Maryland, United States, [2]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [3]Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States

Prurigo nodularis (PN) is an understudied inflammatory skin disease characterized by intensely pruritic, hyperkeratotic nodules. Unfortunately, current treatments are often inadequate and there are no FDA-approved therapies. PN also disproportionately affects black patients, who experience more fibrotic nodules and suffer from increased systemic inflammation, comorbidity development, and mortality. However, it is unknown whether genetic, socioeconomic, or environmental factors contribute to this disparity. We thus developed a polygenic risk score (PRS) for PN to identify genetic risk factors that underlie PN, calculated from 309 cases and 198,740 controls within the FinnGen Biobank. Our calculated PRS reliably predicted a diagnosis of PN in the Partners BioBank, an independent cohort of 171 PN cases and 30,686 controls (OR 1.33 [95% CI 1.09-1.61], P = 0.00466). Through genome-wide association (GWA) analyses, we identified a significant A-to-G variant (P < 5 × 10−8) on chromosome 20 near the gene PLCB4 and another strong signal within an intron of TXNRD1 (rs34217906; P = 6.4 × 10−7). Finally, we discover that black patients are at greater genetic risk of developing PN (OR, 2.63 [95% CI 1.49-4.61, P = 0.00078]). When incorporating race as a covariate with the PRS, the new adjusted model was significantly associated with PN (P = 0.00466). This association was more significant with race compared to race and ancestry (P = 0.07394). As race is a sociocultural construct and not a genetically-bounded category, our findings implicate both genetic and environmental factors that may underlie some of the observed racial disparities in PN.

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Representation of fitzpatrick skin phototype in dermatology surgical textbooks

D. Porras Fimbres[1], C. Rundle[1], C. Presley[2], C. Stamey[1]
[1]Duke University School of Medicine, Durham, North Carolina, United States, [2]Lehigh Valley Health Network, Allentown, Pennsylvania, United States

The United States (US) is becoming more ethnically and racially diverse, yet dermatology textbooks do not reflect this change. These textbooks are destitute in Skin of Color (SoC) representation, despite a growing heterogenous US population. In a field where images are critical for diagnosis, the lack of darker skin representation can be a barrier to adequate care for SoC populations. This study aims to quantify and qualitatively describe the representation of Fitzpatrick skin phototypes in core dermatology textbooks. Three textbooks were selected: Surgery of the skin: procedural dermatology, Dermatologic surgery, and Facial reconstruction after Mohs surgery. All photographs of patients were recorded, and skin type was classified according to the Fitzpatrick skin types I-VI. Animated images and those in which discerning skin type was challenging (e.g. images of cadavers or mucosa) were excluded. To limit bias, skin type was assessed visually without consulting figure captions. Photo topic and site were recorded. Images collected for each category in the Fitzpatrick scale were compared based on number and topic. 1620 patient images were collected, of which 38.77% represented skin type I, 45.86% type II, 8.77% type III, 3.46% type IV, 1.79% type V, and 1.36% type VI. Skin type III-IV composed 12.2% of the total 1620 images, emphasizing the need for medical textbooks to improve the representation of patients with SoC. Keloids and dyspigmentation comprised the dermatologic pathologies represented by SoC in all reviewed images. Conditions such as nonmelanoma skin cancer and actinic keratosis, procedures such as skin flaps and grafts, and therapies including cosmetic interventions, repairs after excisions, and photodynamic therapy were mostly represented by skin type I-II and few were classified as V-VI. Darker skin types were found to be disproportionally underrepresented in the textbooks analyzed. Exposure to conditions in SoC is critical for accurately diagnosing and surgically treating dermatoses in all patients.

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Clinical trials in hidradenitis suppurativa and psoriasis under-represent certain gender, ethnic, and racial groups

C. Greif[1, 2], R. S. Gibson[1, 2], A. B. Kimball[1, 2], M. L. Porter[1, 2]
[1]Clinical Laboratory for Epidemiology and Applied Research in Skin (CLEARS), Boston, Massachusetts, United States, [2]Dermatology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States

Hidradenitis suppurativa (HS) and psoriasis (PsO) are inflammatory skin conditions with distinct demographic profiles. We sought to determine whether gender, ethnic, and racial profiles of clinical trial patients match those of HS and PsO populations, although it is unknown whether demographics change treatment response. We identified completed phase II-III clinical trials starting after January 1, 2010 from the US National Library of Medicine (ClinicalTrials.gov) on systemic therapies for HS and PsO in adults. We found 142 PsO and 10 HS trials and collected gender, ethnicity, and race of participants. We combined trial patients for each disease and used chi square analysis to determine whether demographic profiles differed between the trial and general patient populations. For HS, we used demographic estimates from a study of 13,885 US HS patients (Kilgour et al. 2021). For PsO, we used estimates from studies of 27,220 (Stern et al. 2004) and 12,625 (Armstrong et al. 2021) US survey respondents, including 601 and 379 with PsO, respectively. We combined these datasets to estimate gender and used the latter study to estimate ethnicity and race for PsO. HS and PsO trials under-represented females (HS: 63.7% [95% CI: 61.4-66.0%] vs. 73.5%, p<0.001; PsO: 33.0% [95% CI: 32.6-33.4%] vs. 57.6%, p <0.001). HS trials under-represented Hispanic (3.8% [95% CI: 1.4-6.2%] vs. 12.0%, p <0.001) and Black patients (9.1% [95% CI: 6.3-11.8%] vs. 20.3%, p<0.001). HS trials over-represented White patients (76.6% [95% CI: 72.5-80.6%] vs. 59.9%, p<0.001), and PsO trials over-represented Asian patients (12.4% [95% CI: 11.9-12.8%] vs. 4.3%, p<0.001). Other differences were 4% or less. Limitations include comparing trial populations to US demographics, as some trials were international, and excluding trials on topical therapies, likely skewing our sample toward more severe patients.

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Quantitative and qualitative analysis of dermatologic surgery literature from 2019 – 2022

C. Diamond[1], J. Albrecht[4], C. Rundle[2], C. Presley[3], C. Stamey[2]
[1]Duke University School of Medicine, Durham, North Carolina, United States, [2]Dermatology, Duke University Health System, Durham, North Carolina, United States, [3]Division of Dermatology, Lehigh Valley Health Network, Allentown, Pennsylvania, United States, [4]The University of Utah School of Medicine, Salt Lake City, Utah, United States

According to the United States Census Bureau, 4/10 Americans identified as a race or ethnicity other than Caucasian between 2010 and 2020. Despite this finding, 47% of dermatologists and dermatology residents felt their training was deficient in skin of color (SoC) content. Prior studies have found that there is a paucity of SoC images in general dermatology education; however, there are no studies examining the representation of SoC in dermatologic surgery literature. Here, we assessed all articles published in Dermatologic Surgery from January 1, 2019, through January 15, 2022, and appraised published images. The aggregate number of articles, article type, patient media (i.e. image or video), skin location, and Fitzpatrick skin types were collected. Articles without images were not screened for SoC content. A total of 1420 articles were evaluated, resulting in 721 articles, outlining a total of 1253 distinct patients, which contained pertinent patient images or videos. Of these, 126 (17.5%) articles discussed SoC patients, containing a total of 551 SoC patients. The most common Fitzpatrick skin type II (51.3%), followed by types III (26.7%), IV (10.4%), I (5.8%), V (4.7%), and VI (1.0%), respectively. The most common SoC topic was vitiligo and cosmetics (face lifts, botox, scarring and laser use). There was no SoC representation in Reconstructive Conundrum articles, nor representation in topics such as NMSC. Our current assessment of Dermatologic Surgery demonstrates a vast underrepresentation of SoC patients. As patient populations become more diverse, greater attention should be given to SoC representation in dermatologic surgery literature to provide high quality dermatologic care to these populations.

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A qualitative exploration of melanoma awareness in black communities

I. de Vere Hunt[1], S. Owen[1], A. Amuzie[2], V. Nava[1], A. Tomz[1], L. A. Barnes[1], J. Lester[3], S. Swetter[1], E. Linos[1]
[1]Stanford University, Stanford, California, United States, [2]Indiana University, Bloomington, Indiana, United States, [3]University of California San Francisco, San Francisco, California, United States

People with skin of color are more likely to have advanced melanomas at diagnosis, with strikingly lower 5-year melanoma survival rates among Black (70%) compared to white patients (92%). Yet, survey data suggests many people of color have not been provided health information about melanoma skin cancer. We sought to explore Black people's perspectives on melanoma risk using in-depth qualitative interviewing. We conducted 26 semi-structured interviews (July–August 2021) over video or telephone with individuals self-identifying as Black or African American. We conducted an inductive thematic analysis of the full interview transcripts. Of the 26 participants, 12 were male and 14 female, with an average age of 43 years (range 18-85). Participants were from 11 different states. The following key themes were extracted:1) Lack of understanding of term 'melanoma': many participants did not know that melanoma was a type of cancer, with responses including 'that's a nice science term' and 'melanoma does not register'. 2) Do not feel at risk of melanoma: most participants did not feel at risk of developing melanoma, with one explaining 'I've never known anyone Black with skin cancer'. Misconceptions about the role of sun exposure were pervasive. 3) Surprise that melanoma can occur on palms, soles and nails: many participants were 'shocked' to hear that the palms, soles and nail beds were the most common sites for melanoma in Black people. 4) Skin cancer awareness messages don't apply to or include Black people:one participant explained on recalling messages they had seen about skin cancer, 'I never thought that it pertained to me 'cause it would always be like, you know, white people'. We highlight a need for focused health information on melanoma designed specifically for Black communities. We delineate two key messages for focused messaging: 1) skin cancer can and does occur in Black people; 2) high-risk sites in Black people include the palms, soles and nail beds.

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Variability in bullous pemphigoid disease area index scoring in patients of color

D. Mustin[1], E. Cole[2], T. DeGrazia[2], R. Feldman[2]
[1]Emory University School of Medicine, Atlanta, Georgia, United States, [2]Dermatology, Emory University, Atlanta, Georgia, United States

The Bullous Pemphigoid Disease Area Index (BPDAI) is an objective measure with activity scoring divided into erosions/blisters, erythema/urticaria, and damage. Emory Autoimmune Blistering Disease (AIBD) Clinic treats many patients with darker skin tones and the erythema/urticaria component can be challenging in patients with darker skin. In this study, we explored the effect of Race and Fitzpatrick skin type (FST) on BPDAI total activity score (BPDAI-TAS) and pruritus component scores (BPDAI-PCS) based on a retrospective chart review of BP patients from 2014 to 2020. 107 patients were included in the analyses: most subjects were White (68.2%), followed by Black (31.8%). Although Black patients had higher BPDAI-TAS compared to White patients, this association was not significant (22.9±23.9 vs. 18.3±18.1; p=0.261). BPDAI erosions/blistering (BPDAI-EB) and damage (BPDAI-D) were higher in Black patients (20.2±23.9 vs. 11.7±15.0; p=0.028, and 4.5±3.3 vs. 1.5±2.4; p=0.000, respectively). In contrast, BPDAI urticaria/erythema (BPDAI-UE) was lower in Black patients (1.8±3.6 vs. 4.0±7.2; p=0.090). Similar findings were observed in BPDAI by FST: patients with FST V-VI demonstrated higher scores for BPDAI-TAS, BPDAI-EB, and BPDAI-D, but lower scores for BPDAI-UE. Black patients demonstrated higher levels of anti-BP180 IgG (71.9±75.5 vs. 37.9±49.1; p=0.009) while Black patients and FST V-VI reported greater BPDAI-PCS (19.4 ± 9.2 vs. 14.3 ± 9.2; p=0.024). This study demonstrates racial differences in relation to BP disease activity. Black patients have higher BPDAI-EB, pruritus scores and increased levels of autoantibodies. BPDAI-UE scores were lower in Black patients potentially from underappreciating the urticaria/erythema in darker skin. BPDAI-D is more common in darker skin patients, but it is not clear how this relates to disease activity. BPDAI needs to be interpreted carefully in the context of skin tone and future research may require alternative tools to capture disease severity in darker skin types.

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Evaluation of racial differences in prescription rates of systemic medications for psoriasis and psoriatic arthritis

R. S. Gibson[1], P. Salian[1], A. B. Kimball[1, 2], M. L. Porter[1, 2]
[1]Dermatology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States

Prior studies have suggested that black patients are less likely to receive biologics compared with white patients. The objective of the study was to analyze the demographics and prescription rates of systemic medications. We conducted a review of Beth Israel Deaconess Medical Center claims database from the Jan 1, 2010–Dec 31, 2021 for patients that had ICD-9 or ICD-10 codes for psoriasis or psoriatic arthritis. The total number of patients with the diagnosis of psoriasis or psoriatic arthritis was 9,406 patients (male 49.7%; female 50.3%) with an average age of 52.5. The self-reported race for the patients included white (6,561, 69.8%), Asian (518, 5.5%), black (474, 5.0%), unknown (1,374, 14.6%), and other (479, 6.2%). A total of 6,767 systemic medications were prescribed for 43.4% of white patients, 43.0% of black patients, 34.9% of Asian patients, 36.3% of the other racial group, and 25.3% of the unknown racial group for either psoriasis or psoriatic arthritis. The systemic medications prescribed included prednisone (30.1%), TNF-α inhibitors (24.7%), methotrexate (17.9%), apremilast (5.8%), IL-17 inhibitors (5.5%), IL-12/IL-23 inhibitors (4.4%), IL-23 inhibitors (3.8%), acitretin (3.2%), mycocphenolate (1.7%), JAK inhibitors (1.4%), azathioprine (1.2%), and cyclosporine (0.3%). Using a chi-square test, there was not a significant difference in the prescription rates of systemic medications between white patients and black patients (X2=0.02, p-value=0.89). Limitations of our study include not being able to assess underlying disease severity or patient compliance. Our data does not show a statistically significant difference in the prescription rates of systemic medications between white and black patients suggesting that racial disparities may have decreased over time, possibly as a result of increased access and patient willingness to utilize systemic medications with longer term safety data and better safety profiles.

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Assessment of allergic contact dermatitis and patch testing in skin of color patients

A. Burli[1], H. Maibach[2]
[1]Dermatology, University of Rochester Medical Center, Rochester, New York, United States, [2]Dermatology, University of California San Francisco School of Medicine, San Francisco, California, United States

Objectives: Skin of color patients face important health issues relevant to dermatologists, such as allergic contact dermatitis; however, there is a lack of information surrounding common allergens causing contact dermatitis that disproportionally affect skin of color patients, as well as interpreting patch testing in this population. Methods: Covidence, Embase, MEDLINE, PubMed, Web of Science, and Google Scholar were searched to identify relevant articles studying allergic and irritant contact dermatitis in skin of color patients. Results: Certain allergens such as paraphenylenediamine (PPD), imidazolidinyl urea, dimethylol dimethyl hydantoin, and thioureas had differential sensitivities in Black vs White Patients. The most common positive reactions in African American patients included PPD (10.9%), balsam of Peru (12.3%), bacitracin (13.0%), fragrance mix (18.1%), and nickel (27.5%). When interpreting patch test results in patients with higher Fitzpatrick skin types, positive patch tests presented with lichenification and hyperpigmentation, rather than erythema and vesicles. Furthermore, characteristic bright red or pink hues for positive results may appear violaceous or faint pink. Conclusion: Awareness of the common allergens associated with allergic contact dermatitis in patients of skin of color can help guide patch testing as an important diagnostic tool. Further research must be conducted regarding contact dermatitis in this patient population, especially given the lack of data surrounding Hispanic, Asian and Pacific Islander, and Native American patients. Limitations include the low sample of manuscripts studying this topic.

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Acne-related quality of life differs by race/ethnicity and sex

G. Santos Malave[1], R. Fitzsimmons[2], D. B. Shin[2], J. Takeshita[2]
[1]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]University of Pennsylvania, Philadelphia, Pennsylvania, United States

Little is known about racial/ethnic differences in the impact of acne on quality of life (QoL). We aimed to evaluate acne-related QoL by race/ethnicity among individuals in the U.S. We performed a cross-sectional analysis of baseline data from phase III clinical trials for acne. Study inclusion criteria were: (i) published between 2014 and 2021, (ii) included U.S. sites, (iii) measured acne-related QoL using the Acne-QoL instrument. Only data from U.S. participants were included. The primary outcome was acne-related QoL as measured by Acne-QoL domain scores (self-perception, role-emotional, role-social, and acne symptoms); lower scores indicate greater impact on QoL. The primary independent variable was race/ethnicity categorized as White (reference), Black, Asian, Hispanic/Latino, or other race. Multivariable linear regression was performed to evaluate the association between race/ethnicity and Acne-QoL scores. Effect modification by sex was identified so results were stratified by sex. The study included 3,328 participants. The racial/ethnic distribution was 49.1% White, 29.7% Hispanic/Latino, 14.7% Black, 3.8% Asian, and 2.8% other race; mean (standard deviation) age was 20.3 (7.2) years; 58.9% were female. Across all domains, females had lower mean adjusted Acne-QoL scores (12.0 to 13.3) compared to males (15.4 to 17.5). In adjusted analyses that accounted for basic demographic and acne severity information, Hispanic/Latino males reported greater impact on QoL than White males in all domains [self-perception (β coefficient -2.95; 95% confidence interval -3.86, -2.04), role-emotional (-3.43; -4.33, -2.52), role-social (-2.87; -3.62, -2.12), acne symptoms (-1.97; -2.63, -1.32)]. Hispanic/Latino females also reported greater impact on QoL than White females in all domains except for self-perception (β coefficient range -0.99 to -1.11; $p<0.05$). Our findings highlight sex and racial/ethnic differences in the QoL impact of acne with larger burdens reported by women and Hispanic/Latino individuals, respectively.

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Use of low-level light therapy in management of central centrifugal cicatricial alopecia

M. K. Cook, B. Feaster, J. J. Subash, J. Larrondo, A. J. McMichael
Dermatology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States

Background: Central Centrifugal Cicatricial Alopecia (CCCA) is a scarring hair loss that predominately affects women of African descent. The etiology is unknown, however is proposed to involve genetic, inflammatory, and environmental factors. Treatment includes anti-inflammatory therapies, such as intralesional steroids, topical steroids, and oral antibiotics. However, effective, safe medical therapy is often a challenge for many patients. Low-level red-light therapy has effectively improved androgenetic hair loss by promoting anti-inflammatory factors, however, its use in CCCA management is not well understood. Objective: To investigate the potential use of low-level light therapy for the management of CCCA. Methods: Five African American women with a biopsy-proven diagnosis of stage II-IV CCCA were enrolled in this prospective clinical trial. All patients were required to be on a stable treatment regimen for at least three months prior to enrollment, and regimens were kept constant throughout the study period. Each patient was instructed to use the REVIAN RED all-LED Light Cap for 10 minutes per day. Patients' hair loss was assessed with follow-up visits at 2,4 and 6-months using digital photography, patient self-assessments of symptoms, and clinician evaluations of severity. Results: The mean age of patients was 53.4 years old, the average duration of disease was 12 years, and baseline stages of disease severity ranged from IIB-IVA. Of the three patients that have completed 4 months of treatment thus far, there was a subtle improvement in hair loss in patients 2 and 3, and no increased hair loss in patient 1. Conclusion: Low-level light therapy could serve as a safe and effective treatment option for patients with CCCA. Overall, patient satisfaction with treatment was high, with patients referring to the cap as a convenient and effective option for their hair loss. To our knowledge, this is the first study on the use of low-level light therapy for CCCA management.

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A comparison of scholarly productivity among matched dermatology applicants by underrepresented in medicine status

D. X. Zheng[1], J. Narang[1], K. Schrom[1], A. Sarfo[1], J. Scott[2], V. Nambudiri[3], T. Sharma[1]

[1]*Dermatology, University Hospitals, Cleveland, Ohio, United States,* [2]*Dermatology, Johns Hopkins Medicine, Baltimore, Maryland, United States,* [3]*Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States*

Introduction: Research output is an important metric for evaluating dermatology residency applicants. Although dermatology is among the least racially/ethnically diverse specialties, efforts have been made to increase representation among trainees underrepresented in medicine(UIM). Yet, UIM students often face barriers to pursuing research relative to their non-UIM peers. We evaluated the association of UIM status with scholarly productivity among matched dermatology applicants. Methods: We analyzed a cohort of allopathic medical students matching into dermatology residency in the years 2009, 2011, 2016, and 2018. The primary outcome was mean number of peer-reviewed indexed publications attained prior to residency, determined by applicant Scopus profiles. The predictor of interest, UIM status, was defined as self-reported African American or Native American race, or Hispanic ethnicity. Multivariable Poisson regression was used to determine the association between UIM status and publication output. Results: Our cohort comprised 952 matched dermatology applicants, 87 (9.1%) of whom self-identified as UIM. Non-UIM applicants published a higher mean total number of publications (3.6 vs. 2.3 for UIM, P=0.007) and dermatology-related publications (3.1 vs. 2.1 for UIM, P=0.02) prior to residency. After controlling for PhD status and medical school ranking, UIM applicants had on average 0.8 fewer indexed publications than non-UIM applicants. There was no difference in match rates between UIM (49%) and non-UIM (53%) applicants (P=0.27). Conclusions: While UIM dermatology applicants published fewer papers prior to residency than non-UIM applicants, they did not match at significantly lower rates. As scholarly productivity may assume even greater importance in applicant evaluation with the transition of USMLE Step 1 to pass/fail scoring, it will be important to ensure that UIM students are not disproportionately impacted.

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Assessment of pain, pruritus, and quality of life in persons with keloids

A. Knowles, O. Akinseye, O. Martinez Luna, C. Goodsett, Y. Darwish, D. Glass

Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

Keloids are the result of an aberrant wound healing response that leads to scar tissue expanding above and beyond the boundaries of the original cutaneous injury. An estimated 2-4% of the population has keloids, with persons of color having an increased predisposition. Besides being cosmetically disfiguring, keloids can be both painful and pruritic, significantly affecting patients' quality of life (QoL). Our aim was to assess the extent to which these keloid-associated symptoms (pain and pruritus) affect QoL. We conducted a cross-sectional survey of a cohort of patients enrolled in a keloid registry. 64 participants with keloids and 27 controls were included in the study. Participants completed both a general health related QoL instrument (Short Form- 36), and a skin specific QoL questionnaire (Dermatology Life Quality Index). Pain and pruritus Visual Analog Scales (VAS) were utilized to assess the severity of pain and pruritus the participants experienced with their keloids. Compared with controls, those with keloids had significantly decreased quality of life. 30 persons with keloids (47%) reported a moderate or greater effect on their quality of life, expressed as a DLQI score >5. There was a moderately positive correlation between pain and pruritis VAS scores and DLQI scores in persons with keloids. Furthermore, participants with keloids who took oral pentoxifylline as monotherapy for 5 weeks showed an improvement in pain, pruritus and QoL compared to baseline, suggesting that oral pentoxifylline improves keloid-associated pain and pruritus. Study limitations include a small sample size and unmatched comparison groups. In conclusion, keloid associated pain and pruritus significantly affect QoL comparably to other chronic dermatologic conditions. Physicians treating patients with keloids should strive for symptom relief to improve QoL.

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Psoriasis and psoriatic arthropathy in diverse U.S. adult cohort: All of us research program

M. M. Tran, I. H. Moseley, E. A. George, E. Cho

Dermatology, Brown University Warren Alpert Medical School, Providence, Rhode Island, United States

Psoriasis (PsO) is a chronic immune-mediated skin condition with possible joint involvement. Few U.S.-based studies on PsO and psoriatic arthropathy (PsA) have prioritized recruiting diverse populations. Using the ongoing All of Us (AoU) Research Program, one of the largest databases with a sizeable proportion of participants from historically underrepresented groups in biomedical research, we conducted a preliminary cross-sectional analysis to estimate the burden of PsO and PsA across several demographic groups. This study includes adults >18 years old enrolled through Health Care Provider Organizations and Direct Volunteer sites. Linking data from electronic health records (EHRs), surveys, and physical measurements at enrollment, we estimated PsO and PsA prevalence by age, race, gender, sexual orientation, education, income, insurance, body mass index (BMI), and smoking. Odds ratios (OR) and 95% confidence intervals (CI) were calculated with multivariate logistic regression adjusted for the aforementioned variables. Out of 329,038 participants in AoU v5, there were 251,597 participants (76.5%) with available EHRs. Of these, there were 150,158 (45.6%) with skin of color (SoC) and 5,479 (2.4%) with a PsO diagnosis. Nearly a quarter (22.0%) of PsO patients had PsA. Odds of PsO and PsA were lower among Blacks (PsO OR 0.32; CI, 0.28-0.36, PsA 0.20; 0.15-0.26) and Hispanics (PsO 0.77; 0.71-0.84, PsA 0.74; 0.61-0.89) compared to Whites. Age and BMI were linearly associated with increased PsO and PsA prevalence. Higher odds of disease were found in former smokers (PsO 1.30; 1.22-1.39, PsA 2.15; 1.33-3.78). Lower odds were observed in uninsured adults (PsO 0.43; 0.35-0.52, PsA 0.37, 0.22-0.58) and those with less than a high school degree (PsO 0.72; 0.63-0.82, PsA 0.65; 0.47-0.87). Using the AoU dataset, we identified lower rates of PsO and PsA in participants with SoC, lower education levels, and no health insurance. This suggests PsO and PsA underdiagnosis in these underserved populations, possibly due to limited dermatologic care access.

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Alopecia areata in underrepresented groups: Preliminary analysis of the All of Us research program

I. H. Moseley, E. A. George, M. M. Tran, E. Cho

Dermatology, Brown University Warren Alpert Medical School, Providence, Rhode Island, United States

Background: Alopecia areata (AA) is an autoimmune condition characterized by patchy, nonscarring hair loss. Few studies of AA in the United States have adequately included participants from underrepresented groups. The All of Us (AoU) Research Program is ongoing and aims to build a diverse database to promote elucidation of health disparities by prioritizing underrepresented groups during data collection. We used the latest data release to evaluate the burden of AA among underrepresented groups defined by the novel AoU framework. Methods and Results: AoU includes adults over 18 who enroll using a digital interface either as direct volunteers or through participating health care provider organizations. We linked surveys and electronic health record (EHR) data to estimate the prevalence of AA in underrepresented groups defined by race, ethnicity, physical disability, sexual orientation/gender identity (LGBTQIA+), income (annual household income ≤$35,000), and education (less than a high school degree). All of Us v5 includes 329,038 participants. Of these, 251,597 (76.5%) had EHR data and 752 were diagnosed with AA (prevalence, 0.30%; 95% CI, 0.28–0.32). We used multivariate logistic regression adjusted for age, race/ethnicity, sex, health insurance status, physical disability, and history of autoimmune disease to estimate the adjusted odds ratio (OR) for AA diagnosis in each underrepresented group. Compared to Whites, Blacks and Hispanics had higher odds of AA (OR, 1.72; 95% CI, 1.39-2.11 and 2.13; 95% CI, 1.74-2.59, respectively). Lower odds of AA were observed in participants with less than a high school degree (0.80; 95% CI, 0.59–1.08), household income ≤$35,000 (0.67; 95% CI, 0.54-0.83), and no health insurance (0.35; 95% CI, 0.20-0.56). Conclusions: Among AoU participants, Blacks and Hispanics had increased odds of AA compared to Whites. Decreased odds of AA diagnosis among participants with lower education and income levels may reflect limited access to dermatologic care and potentially higher levels of undiagnosed AA.

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A closer look: Understanding the role of lesional fibroblasts in hidradenitis suppurativa

A. S. Byrd[1], J. Richert-Jones[2], C. Carmona-Rivera[3], M. J. Kaplan[3], G. A. Okoye[1], V. M. Harvey[2], J. Chan[2, 4]

[1]Dermatology, Howard University College of Medicine, Washington, District of Columbia, United States, [2]Skin of Color Research Institute, Hampton University, Hampton, Virginia, United States, [3]NIH, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, United States, [4]Foundational Sciences, Dr. Kiran Patel College of Osteopathic Medicine, Nova Southwestern Univer., Clearwater, Florida, United States

Hidradenitis suppurativa (HS), is a debilitating skin disorder with subcutaneous abscess-like nodules, boils, and sinus tracts appearing in skin folds. Despite the seriousness of the condition, limited data defining the etiology/pathogenic pathway exists. Moreover, few studies, including basic science and clinical trials, have been done amongst African-Americans (AA), a population with high disease prevalence. Our previous work has shown that neutrophil extracellular trap (NET) formation prolongs chronic inflammation leading to potential autoimmunity. Fibroblasts are the predominant cells responsible for wound closures and fibrosis, as well as a major producer of ECM proteins. Within HS lesions, prominent fibroblasts may be a major contributing factor of disease progression. Thus, lesional fibroblasts were isolated from HS patients (HSFs) and characterized for cellular defects. Using normal AA skin fibroblasts (NFs) and HSFs, we initially examined their growth factor and glucose dependence, in parallel with our study using isolated AA keloid fibroblasts (KFs). We found that both NFs and KFs can survive well without serum. Contrastingly, HSFs were not sensitive to reduced glucose, but were highly sensitive to serum withdrawal. In a 24h incubation, only ~43-60% of HSFs survived, while >80% of NFs remain attached. This serum-dependence may provide an entry point to determine whether growth factor pathway inhibitors may be used to target lesional HSFs without altering NFs. Ongoing studies will define the mechanistic differences underlying HS lesion formation. In combination with our understanding of potential NET-mediated autoimmunity, these data may provide additional means to co-target immune cells and lesional HSFs for effective treatment.

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Study design of a phase 3b, multicenter, randomized, double-blind, placebo-controlled trial of guselkumab (GUS) in patients with skin of color who have moderate to severe plaque and/or scalp psoriasis (VISIBLE)

A. Alexis[1], T. Bhutani[2], A. J. McMichael[3], O. Choi[4], D. Chan[4], K. Rowland[4], L. Gao[4], L. Park-Wyllie[4], A. Rodriguez[5], C. Kindred[6], S. Desai[7]

[1]Weill Cornell Medicine, NY, New York, United States, [2]Univ California San Francisco Med Center, San Francisco, California, United States, [3]Wake Forest School of Medicine, Winston-Salem, North Carolina, United States, [4]Janssen, Horsham/Spring House, Pennsylvania, United States, [5]Nashville Skin, Nashville, Tennessee, United States, [6]Kindred Hair & Skin Center, Columbia, Maryland, United States, [7]Univ Texas SW Med Center, Dallas, Texas, United States

Racial/ethnic differences exist in psoriasis (PsO) clinical presentation, post-inflammatory pigment alteration, disease severity, and quality of life impact. However, data remain limited on many PsO clinical characteristics and treatment response in skin of color (SOC) patients (pts). Here we describe the Phase 3b VISIBLE study, the first large-scale prospective PsO biologic study to enroll pts across the full spectrum of SOC. Study feasibility assessments included comparison of efficacy endpoints between SOC and White pts in prior GUS trials that included 7%-23% pts of non-White race. Literature was reviewed to identify other PsO biologic trials with sufficient SOC pt inclusion for formal statistical comparisons of efficacy and areas of unmet need. VISIBLE is a 112-week double-blind, placebo (PBO)-controlled study that will include ~200 pts that all self-identify their race as non-White, comprising 2 cohorts: 1) moderate to severe body PsO and 2) moderate to severe scalp PsO, both randomized to GUS or PBO; PBO→GUS at Week16. The entire skin-type spectrum will be represented (Fitzpatrick skin types I-VI; ≥50% types IV-VI). Coprimary endpoints are proportions of pts achieving Psoriasis Area and Severity Index 90 response and Investigator Global Assessment (IGA)=0/1 for the body cohort and Psoriasis Scalp Severity Index 90 response and scalp-specific IGA=0/1 for the scalp cohort at Week16. A rich combination of clinical efficacy, photos, skin sampling, labs, transcriptomics, genomics, and pt-reported outcomes will facilitate cutting-edge translational science to help fill scientific gaps related to PsO treatment in SOC pts.

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Clinical characteristics of black patients with mycosis fungoides and sézary syndrome: A retrospective cohort study

S. M. Talluru, I. M. Cataluna, C. Samuel, S. Rozati

Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Prior studies suggest Black patients with Mycosis Fungoides and Sézary syndrome (MF/SS) have higher incidence rates and poorer prognosis. In this ongoing retrospective study, we sought to characterize Black MF/SS (B) patients compared to White MF/SS (W) patients to elucidate these disparities. We retrospectively reviewed 434 MF/SS patients (W 56%; B 44%) seen at a single center between 2011-2021. The Black MF/SS cohort had significantly earlier onset (W 59 yrs vs B 51 yrs), female predominance (W 40%; B 65%), and higher nodal stage (W 86% vs B 76% were N0) (p<.01). No racial differences were found for max LDH, large cell transformation (LCT), or initial tumor/blood stage. Having higher max LDH was associated with inferior survival in both cohorts. In the Black MF/SS cohort, no significant differences were found between gender and overall stage on initial presentation, tumor stage, or LCT. Black females were diagnosed earlier than Black males (mean age 49 vs 54 yrs; p<.05). Black patients with late stage (IIB-IVB) had higher rates of bacteremia and greater max LDH (509) compared to early stage Black patients (289) (p<.001). Univariate analysis within the Black MF/SS cohort revealed age >60, advanced stage, higher LDH, LCT, and bacteremia were associated with inferior survival (p<.01), whereas gender, age at diagnosis, smoking, and alcohol history were not. Our study's large Black MF/SS cohort expands the limited available data for non-White CTCL populations and confirms Black MF/SS patients, particularly Black females, are younger at the time of diagnosis. Clinical characteristics such as high LDH levels and bacteremia were associated with inferior survival in this cohort and may be harbingers of disease progression. While we further dissect laboratory and clinical features (e.g., folliculotropic and hypopigmented variants) in our study cohorts, these findings reinforce the need for more research regarding the clinical heterogeneity of MF/SS in Black patients to inform personalized clinical care.

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Instagram lip fillers trends: A cross sectional analysis of hashtags

N. Vashi[1], M. Maymone[2], H. d. Garza[1]

[1]Dermatology, Boston University, Boston, Massachusetts, United States, [2]Dermatology, Brown University, Providence, Rhode Island, United States

Background: Mass media platforms like Instagram have the ability to contribute to society's perception of what is considered beautiful in this day and age, particularly the desirability of full lip sizes, and result in unrealistic delineations of beauty. Additionally, "influencers" such as Kylie Jenner have a strong impact over social media, and advertising may lead to increasing demand for lip enhancement cosmetic procedures and unfulfilled expectations from cosmetic procedures. Objective: To examine popular Instagram posts that utilize cosmetic lip filler hashtags, survey the presence of skin of color, and analyze social media's influence of lip size to consider how beauty, in particular lip volume, is delineated in this era of Instagram. Methods & Materials: In this cross-sectional study, the top 9 Instagram posts with the highest total engagement for the hashtags #lipfiller and #lipfillerbeforeandafter was recorded for 7 consecutive days. Quantitative and qualitative data including the number of comments, likes, views, Fitzpatrick skin type, and lip ratios were recorded. Results: The average top to bottom lip ratio was larger for #lipfiller (1:1.15) and #lipfillerbeforeandafter (1:1.14). The top total engagement posts for both hashtags had a higher percentage of women with skin types I-III (93.33%, #lipfiller; 82.35%, #lipfillerbeforeandafter) compared to skin types IV-VI (6.67%, #lipfiller; 11.76%, #lipfillerbeforeandafter). Posts for both hashtags had a high percentage of aesthetic claims (74.4%), advertised a product in the text (90.32%), and advertised clinical or professional services (85.48%). All (100%) of the #lipfillerbeforeandafter top posts advertised and focused on a product or service. Only 12.9% of the #lipfiller posts involved celebrities. Conclusion: Dermatologists should educate and raise awareness to the misperceptions of beauty created by Instagram and emphasize individualistic counseling for cosmetic procedures.

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Racial differences in inflammatory biomarkers in hidradenitis suppurativa patients

M. Taylor, V. Parthasarathy, J. Deng, Z. A. Bordeaux, K. K. Lee, M. P. Alphonse, S. G. Kwatra

Dermatology, Johns Hopkins University, Baltimore, Maryland, United States

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disorder that is characterized by a high systemic inflammatory load and disproportionate disease prevalence among African American patients. It is unclear whether there are racial and ethnic differences in systemic inflammation among HS patients. We analyzed data from All of Us, an NIH-funded research program that enrolls diverse individuals throughout the US and contains demographic, electronic health record, and laboratory data. We identified individuals with HS based on ICD-9-CM code 705.83, ICD-10-CM code L73.2, and SNOMED-CT concepts. We analyzed racial and ethnic differences in erythrocyte sedimentation rate (ESR; units = mm/h) and C-reactive protein (CRP; units = mg/L) levels using t-tests and multiple linear regression. There were a total of 1,129 individuals with HS and the majority were female (81.2%), white (48.8%) or black (46.6%) race, and non-Hispanic ethnicity (81.8%), with a mean age of 47.8 years. Among HS patients, ESR was significantly elevated in black patients compared to white patients (44.1 [95% CI: 39.8-48.4] vs. 27.9 [95% CI: 24.9-31.0]; $p < 0.001$). Similarly, CRP levels were elevated in black versus white HS patients (39.5 [95% CI: 30.2-48.8] vs. 20.7 [95% CI: 15.8-25.6]; $p = 0.001$). Black race remained significantly associated with elevated ESR ($\beta = 22.9$; $p < 0.001$) and CRP ($\beta = 18.2$; $p = 0.002$) in multiple linear regression models, adjusted for age, ethnicity, and gender. Significant differences in ESR or CRP levels between Hispanic and non-Hispanic HS patients were not observed. In summary, systemic inflammatory biomarkers were elevated among black patients with HS when compared to patients of other races and ethnicities. Increased systemic inflammation may be associated with greater disease burden as well as a greater risk for subsequent development of associated disease comorbidities.

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Retinol: A better solution for skin anti-aging than retinyl palmitate

J. Hwang, Y. Na, A. Park, H. Kim, W. A. Park
Amorepacific Corporation, Jung-gu, Seoul, Korea (the Republic of)

The retinoids are a well-known anti-aging ingredients that show a clinical efficacy for wrinkle improvement. Retinol has been applied to many products as a representative ingredient for skin anti-aging cosmetics for many years. Recently, the use of retinol derivatives such as retinyl palmitate or retinyl propionate is increasing. The introduction of these derivatives is intended to use a more stable and to lower the risk of skin irritation. However, a technology to increase the stability of retinol has been developed and the problem has been sufficiently overcome. In this study, we conducted experiments comparing retinol and retinyl palmitate, a well-known retinol alternative, regarding the efficacy on skin anti-aging related markers in vitro. The efficacy of retinol on procollagen I expression, a representative dermal component for skin elasticity was increased in a dose-dependent manner in normal fibroblasts. Retinyl palmitate also induced procollagen I expression, but the effect of retinol was higher significantly. As a result of evaluating hyaluronic acid(HA) expression in normal keratinocytes, retinol showed a significant increase in HA secretion whereas retinyl palmitate did not induce HA secretion. The expression of MMP-1 increased by TNFα was reduced when retinol was treated, and retinyl palmitate had no inhibitory effect. Therefore, the efficacy of retinol was confirmed even in inflammatory aging in this study. In terms of antioxidant efficacy, retinyl palmitate had a lower radical scavenging activity compared to the high radical scavenging activity of retinol. Although not shown in this study, the antioxidant efficacy of retinol was superior to that of retinyl propionate. In conclusion, retinol can be expected to have an obvious effect than its derivatives by affecting the skin anti-aging targets. Retinol is a still attractive anti-aging ingredient in cosmetics.

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An *in-vitro* keratinocyte model for the evaluation of skin damage induced by 5G radiation.

F. Havas[1], M. Cohen[1], M. Reynier[2], G. Percoco[2], L. Peno-Mazzarino[2], J. Attia-Vigneau[3]
[1]IFF - Lucas Meyer Cosmetics, Yavne, Israel, [2]Bio-EC, Longjumeau, France, [3]IFF - Lucas Meyer Cosmetics, Toulouse, France

With the rapid spread of next-generation cellular communication technology, wireless bands between 3 – 100 GHz (5G), are increasingly in use in major population centers. The electromagnetic radiation involved remains non-ionizing, however it is of higher energy compared to existing cellular or wi-fi bands; and, this technology's range is shorter – so that more emitter stations will be needed, placed closer to one another, and thus also closer to the user. This will result in higher net EM energy exposure. There is no clear consensus on the risks inherent in this exposure yet, but there is concern among the public and the scientific community, based on indications that this radiation can affect immune function, trigger inflammatory responses, and influence gene expression linked to protein folding, oxidative stress, tissue / ECM matrix turnover, and more. Further, some skin appendages have been shown to act as antennae for this radiation, which may lead to localized damage. To better understand the effects of this radiation on skin, we irradiated a normal human epidermal keratinocyte culture using a 6 GHz signal generator. Biological effects were evaluated by ELISA or immunostaining. After a remarkably short 1-h exposure time, clear effects were observable in several indicators of keratinocyte function, including: an increase in key inflammatory cytokine IL1-alpha; a reduction in collagenase inhibitor TIMP1; an increase in wound healing and epidermal differentiation facilitator ANGPLT4; and an increase in SA100A9, involved in immune recruitment during injury. These results indicate a clear influence of 5G irradiation on the keratinocytes, suggesting induced changes in skin homeostasis which may be consistent with a state of injury and damage response. In upcoming studies, we hope to be able to show these effects in more depth, and propose some possible solutions for daily users of 5G technology.

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Tyrosine kinase 2 inhibition rescues hair follicles from IL-12–mediated immune privilege collapse and reverses the induction of human alopecia areata in a humanized mouse model

J. Edelkamp[1], T. Rouille[1], J. Kim[2], A. Keren[3], J. Viola-Söhnlein[1], L. Gao[2], A. Rossi[4], F. Jimenez[5], A. Gilhar[3], R. Paus[1,6], M. Bertolini[1], I. M. Catlett[2]
[1]Monasterium Laboratory Skin & Hair Research Solutions GmbH, Munster, Nordrhein-Westfalen, Germany, [2]Bristol Myers Squibb Co, Princeton, New Jersey, United States, [3]Technion, Haifa, Israel, [4]Sapienza Universita Editrice, Rome, Lazio, Italy, [5]Mediteknia Hair Transplant Clinic and Hair Lab, Las Palmas de Gran Canaria, Spain, [6]University of Miami School of Medicine, Miami, Florida, United States

Alopecia areata (AA), an immune-mediated hair follicle (HF) disorder, leads to hair loss due to collapse of the HF's physiologic immune privilege (IP), induced by elevated IFNγ levels and a Tc1-driven inflammatory response dominated by T and NK cells. Given that IL-12 signaling is crucial for IFNγ production by immune cells, we explored for the first time the role of IL-12 and tyrosine kinase 2 (TYK2)-mediated signaling in the induction and prevention of HF IP collapse via a selective TYK2 inhibitor (BMS-986202). In human scalp HF organ culture, treatment with IL-12+IL-18 upregulated MHC-I/II MICA/B expression in the hair bulb, increased the intrafollicular number of CD3+ or CD56+ cells, and selectively upregulated expression of IFNγ-inducible genes. Treatment with BMS-986202 prevented and restored the IL-12+IL-18–induced IP collapse and secretion of IFNγ, suggesting that local IL-12 receptor signaling was required for the induction of HF-IP collapse. More IL-12RB2+ cells were found around the bulb of acute lesional scalp HFs of AA patients vs healthy individuals. In the humanized AA mouse model, BMS-986202 restored HF IP in human HFs *in vivo*, as shown by reduced MHC-I/II expression, inhibited HF keratinocyte apoptosis, and promoted human hair regrowth, as indicated by increased anagen V/VI stages. Collectively, our data point toward involvement of IL-12 signaling in early stages and maintenance of AA pathogenesis through stimulation of IFNγ production from resident IL-12RB2+ immune cells, and eventually HF-IP collapse. Thus, inhibition of TYK2-dependent IL-12–mediated signaling represents a potential pharmacologic strategy for AA.

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Rethinking alopecia scores: Correlating measures of hair loss with established photographic scoring systems

C. Gudobba[1], N. Rodriguez[1], T. Ogunleye[2], S. C. Taylor[2], G. Cotsarelis[2], E. Bernardis[2]
[1]Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]University of Pennsylvania Department of Dermatology, Philadelphia, Pennsylvania, United States

Alopecia scoring systems have traditionally been designed to assess and track severity and progression of specific types of alopecia. Each scoring system's design and clinical validation is limited to only one alopecia type (e.g., Severity of Alopecia Tool score for alopecia areata (AA), Sinclair visual analogue scale for female pattern hair loss (FPHL), Olsen top extent scale for central centrifugal cicatricial alopecia (CCCA)), even though all forms of alopecia share a common feature of decreasing hair density. We propose that a scoring system that measures percent hair loss would be useful across different types of hair loss. We tested correlations between the AA, FPHL, and CCCA scoring systems and the underlying percent hair loss. On a dataset of 284 top-view scalp images taken from 250 subjects, 3 raters manually scored each image using the 3 different scoring systems. After an intraclass correlation analysis demonstrated reliability between the raters, we established a final consensus set of 3 scores per image. Using regression models, we analyzed the score correlations within and across alopecia types. Our results suggest that there is a precise and quantifiable correlation between these scoring systems and the underlying hair loss percent: both CCCA and FPHL scoring systems show a logarithmic relationship with respect to percent scalp area affected (a proxy for the AA scale), resulting in an R2 of 0.793 and 0.804, respectively. Additionally, the CCCA and FPHL scoring systems show a linear relationship with each other, resulting in an R2 of 0.963. These findings suggest that distinct alopecia scoring systems measure percent hair loss as a common underlying feature, and that an automated algorithm quantifying this percent hair loss from photographs may be designed and applied to all forms of hair loss.

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Inhibition of class I HDACs preserves hair follicle inductivity in postnatal dermal cells

M. Park[1], S. Jang[2], J. Chung[2, 1], K. Kim[2], O. Kwon[2, 1], S. Jo[2]
[1]Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea (the Republic of), [2]Dermatology, Seoul National University Hospital, Seoul, Seoul , Korea (the Republic of)

Induction of new hair follicles (HFs) may be an ultimate treatment goal for alopecia; however, functional cells with HF inductivity must be expanded in bulk for clinical use. In vitro culture conditions are completely different from the *in vivo* microenvironment. Although fetal and postnatal dermal cells (DCs) have the potential to induce HFs, they rapidly lose this HF inductivity during culture, accompanied by a drastic change in gene expression. This suggests that epigenetic regulation may be involved. Of the various histone deacetylases (HDACs), Class I HDACs are noteworthy because they are ubiquitously expressed and have the strongest deacetylase activity. This study revealed that DCs from postnatal mice rapidly lose HF inductivity and that this reduction is accompanied by a significant decrease in histone H3 acetylation. However, MS-275, an inhibitor of class I HDACs, preserves HF inductivity in DCs during culture, increasing alkaline phosphatase activity and upregulating HF inductive genes such as BMP4, HEY1, and WIF1. In addition, the inhibition of class I HDACs activates the Wnt signaling pathway, the most well-described molecular pathway in HF development, via increased histone H3 acetylation within the promoter region of the Wnt transcription factor LEF1. Our results suggest that class I HDACs could be a potential target for the neogenesis of HFs.

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Tracing the proliferative dynamics of dermal papilla (DP) progenitors during adult hair follicle (HF) regeneration

Y. Jiang, P. Myung
Yale University, New Haven, Connecticut, United States

The dermal papilla (DP) instructs the cyclical regeneration of the adult hair follicle (HF). The HF grows in anagen, regresses in catagen and rests in telogen. The DP expands in cell number as the HF regenerates, which is critical for HF growth and regulating hair shaft size. Currently how the DP expands is not clear. As the DP is considered to be a quiescent population, the prevailing theory is that the DP expands from proliferative cells that reside outside the DP. We performed EdU pulse chase experiments to track when and where DP progenitors divide and contribute to the expanding DP. Interestingly, we found that two different populations contribute to DP expansion. In early anagen, dermal cup cells that reside outside of DP proliferate and migrate into the DP. In late anagen, a subset of DP cells is reactivated to proliferate to expand the DP. The proliferative dermal cup cells trace to the lower DP and proliferative DP cells trace to the upper DP. Surprisingly, the proliferative dermal cup cells exit from DP after a hair cycle is finished while the proliferative DP cells are retained, suggesting differential behavior and potential of these two populations contributing to the DP. Based on our finding, we suggest a two-stage model for DP expansion. In the first stage, the DP itself is quiescent and dermal cup cells surrounding the DP proliferate to expand the DP. In the second stage, the DP starts proliferating to expand itself. This suggests that DP cells are not terminally differentiated but rather have self-renewing capacity. Our results reveal a previously unknown potential for DP self-renewal. Further, this paves the way to understand signals that regulates DP regeneration, which can help to expand DP cells in vitro for clinical applications.

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Decomposing a deterministic path to hair follicle dermal niche formation: The intersection of two morphogen gradients

G. Strickland[1], R. Qu[2, 3], K. Gupta[1], Y. Jiang[1], D. Dong[1], C. Saez[1], P. Weng[1], M. Taketo[4], Y. Klugar[2, 3, 5], P. Myung[6, 3, 7]
[1]Neurology, Yale School of Medicine, New Haven, Connecticut, United States, [2]Computational Biology and Bioinformatics Program, Yale University, New Haven, Connecticut, United States, [3]Pathology, Yale School of Medicine, New Haven, Connecticut, United States, [4]Kyoto Daigaku, Kyoto, Japan, [5]Applied Mathematics, Yale University, New Haven, Connecticut, United States, [6]Dermatology, Yale School of Medicine, New Haven, Connecticut, United States, [7]Yale Stem Cell Center, Yale University, New Haven, Connecticut, United States

Organ formation requires coordinating signals to time proliferation, specify cell fates, and shape tissue. Tracing these events and the signals that drive them remains a challenge, as intermediate states across many critical transitions are unresolvable over real time and space. Here, we designed a unique computational approach to decompose a non-linear differentiation process into biological meaningful components to resolve the signals and cell behaviors that drive a rapid transition during hair follicle dermal condensate genesis. Combining scRNA-sequencing with genetic perturbation, we reveal that proliferative Dkk1+ progenitors transiently amplify over a short timeframe to become quiescent dermal condensate cells by the mere spatiotemporal patterning of Wnt/b-catenin and Sonic Hedgehog signaling gradients. Together, they deterministically coordinate a rapid transition from proliferation to quiescence, cell fate specification, and morphogenesis. Moreover, genetically re-patterning these gradients reproduces these events autonomously in “slow-motion” across more intermediates that resolve the process. This integrated analysis unravels two morphogen gradients that intersect to coordinate events of organogenesis.

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Particulate matter induces inflammatory response in human outer root sheath cells via oxidative stress-dependent MAPK and JAK-STAT signaling pathways

H. Choi, H. Lee, J. Na, C. Huh, J. Shin
Dermatology, Seoul National University Bundang Hospital, Seongnam, Korea (the Republic of)

Particulate matter (PM), a major air pollutant has been demonstrated to cause intracellular inflammation by inducing reactive oxygen species (ROS) generation in human keratinocytes. Although PM has been shown to be associated with various skin disorders, including atopic dermatitis, eczema, and skin aging, there have been few studies on the effect of PM on hairs. The aim of this study was to investigate the potential role of the oxidative stress-dependent MAPK and JAK-STAT signaling pathways in the inflammation of outer root sheath (ORS) cells induced by PM. The human ORS cells were treated with PM at various doses for 3 to 24 hours. Intracellular ROS was detected using a chemical fluorescent probe by a fluorescence plate reader. Gene and protein expressions of aryl hydrocarbon receptor (AhR), p38, JNK, JAK1, JAK2, JAK3, STAT1, STAT3, STAT5, and cyclooxygenase 2 (COX-2) were determined using reverse transcription-polymerase chain reaction and western blotting, respectively. The levels of prostaglandin E2 (PGE2) and proinflammatory cytokine expression, including that of matrix metalloproteinases (MMPs) and interleukins (ILs) were analyzed using enzyme-linked immunosorbent assay. PM increased ROS generation in human ORS cells via activation of AhR. PM also induced activation of p38, JNK, and STAT3 and increased level of inflammatory mediators and cytokines including COX-2, PGE2, IL-1α, IL-1β, IL-6, IL-8, and MMP-1. Pretreatment with N-acetyl-L-cysteine attenuated the activation of signaling pathways and increase of cytokines. Also, pretreatment with inhibitors of MAPK and JAK-STAT pathways decreased the cytokine levels stimulated by PM. These results suggest that PM can cause inflammatory response in human ORS cells via oxidative stress-dependent MAPK and JAK-STAT signaling pathways which might impair hair growth.

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Ceramide-1-phosphate accelerates premature skin aging in skin fibroblasts exposed to diesel particulate extract

K. Shin[1, 2], S. Kim[1], Y. Choi[1], S. Back[1], H. Lee[2], B. Kim[1], J. Kim[1], Y. Uchida[1, 3], K. Park[1]
[1]Hallym University, Chuncheon, Gangwon-do, Korea (the Republic of), [2]LaSS Lipid Institute, LaSS Inc, Chuncheon, Korea (the Republic of), [3]Northern California Institute for Research and Education, San Francisco, California, United States

Extrinsic factors, such as air pollutants and ultraviolet irradiation (UVR), accelerate premature aging, and a correlation between levels of pollutants and skin aging symptoms has been reported. Diesel particulate matter, a major source of air pollution, helps initiate some diseases, including skin diseases. We recently demonstrated that diesel particulate extract (DPE) activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), followed by activation of sphingomyelinase (SMase), leading to ceramide (Cer) production in cultured normal keratinocytes (KC). We characterized that increases in a Cer-dependent lipid mediator induces apoptosis. Using normal human dermal fibroblasts (HDF), here we investigated whether/how DPE accelerates premature skin aging. Non-toxic concentrations of DPE increased cell senescence marker ß-galactosidase activity in HDF. Following DPE exposure, we found increases in mRNA/protein expression and in matrix metalloprotease (MMP)-1 and MMP-3 activities, which are all associated with skin aging. NOX was activated by DPE and resulted in increased Cer production due to SMase activation in HDF. We next asked whether Cer or its metabolites (Cer-1-phosphate [C1P] and sphingosine-1-phosphate [S1P]) promotes MMP-1/MMP-3 activation. We found that inhibition of Cer kinase, but not sphingosine kinase suppressed MMP-1/MMP-3 activation, and we identified that C1P increases MMP-1/MMP-3 activation via initiation of an arachidonate pathway by activating cPLA2, followed by STAT1- and STAT3-dependent transactivation. We confirmed increases in type 1 collagen degradation in HDF via a DPE-mediated C1P-dependent pathway. NOX is also activated by not only DPE, but other external/internal stressors; i.e., UVR, cigarette smoke, and inflammatory cytokines. Our evidence above points to C1P playing a critical role in skin aging in response to diverse stressors.

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Guiding skin organoid generation via extracellular matrix cues and spatially controlled morphogen gradients

E. Jeon, A. Pappalardo, L. Sorrells, H. E. Abaci
Dermatology, Columbia University Irving Medical Center, New York, New York, United States

Recent advances in generating human organoids from embryoid bodies (EBs) of induced pluripotent stem cells (iPSCs) provide a promising tool for modeling human organ development and disease. A major limitation of current organoids developed in petri-dishes is that the EBs are surrounded symmetrically on all sides by growth factors and extracellular matrix (ECM), leading to uncontrolled spontaneous organization of the tissue. The goal of this study was to precisely guide the self-organization and differentiation of iPSCs-derived EBs into anatomically-relevant skin organoids via ECM cues and spatially-controlled morphogen gradients. Firstly, we evaluated the performance of chemically-defined hydrogels based on dermis-relevant ECM molecules, including collagen types I and IV, laminin, fibronectin, vitronectin, hyaluronic acid, and heparin. We found that collagen type I hydrogels supplemented with laminin and fibronectin promoted the growth and generation of skin organoids with significantly increased pigmentation and hair follicle generation, compared to culture medium suspension and Matrigel. Next, we developed a 3D-bioprinted hydrogel-based microfluidic device that can create asymmetrical cross-gradients of differentiation factors, including bone morphogenetic protein 4 and fibroblast growth factor 2 in a single EB resolution. Under these steep gradients, the EBs showed spatial patterning via polarized differentiation into distinct skin cell lineages and appendages. Interestingly, skin organoids adopted an elongated shape in the device, as opposed to their standard spherical shape, leading to the generation of mature skin organoids by maximizing the oxygen and nutrients transport. Overall, the ability to tailor the extracellular microenvironment and morphogen gradients in the EBs will generate physiologically-relevant skin organoids which may have a transformative impact on our understanding of human skin morphogenesis and developmental skin diseases.

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Aire deficiency induces upregulation of JAK-STAT signaling in keratinocytes and results in alopecia areata-like lesions in mice

N. Maglakelidze, T. Gao, R. P. Feehan, R. Hobbs
Dermatology, Microbiology & Immunology, Penn State College of Medicine, Hershey, Pennsylvania, United States

Alopecia areata (AA) is an autoimmune hair loss disorder with no cure. We report here that C57BL/6J female mice with germline deficiency in autoimmune regulator (Aire-/-) spontaneously developed AA-like lesions (n=61/98; 62%). Aire-/- lesions exhibited AA hallmarks such as miniaturized hair follicles (HFs), decreased hair width (-29%, p<0.0173) and length (-27%, p=0.0002), and increased lymphocytic infiltrate at the hair bulb (+191% in CD8+ T cells, p=0.0546) relative to body site matched Aire+/+ tissue. Anagen HFs are targeted by CD8+ T cells in AA due to overexpression of MHC class I and upregulation of JAK-STAT signaling. Immunohistology confirmed overexpression of MHC class I (+45%, p=0.0385) and STAT1 (+68%, p=0.0032) in Aire-/- lesions. To explore the keratinocyte-autonomous role of Aire in AA and JAK-STAT signaling, we generated CRISPR/Cas9-mediated AIRE-/- keratinocyte clonal cell lines. AIRE-/- keratinocytes displayed increased JAK1 (+21%) and JAK2 (+60%) activation and elevated STAT1 expression (+114%) after IFN-γ treatment (50 ng/mL, 30 min) relative to AIRE+/+ cells by immunoblot. We also observed substantial downregulation of PIAS1 (-43%), a STAT1 inhibitor and AIRE binding partner, and SOCS1 (-63%) and SOCS2 (-20%), JAK inhibitors, in AIRE-/- keratinocytes. Microscopy confirmed increased nuclear STAT1 localization (+24%, p=0.0011) and decreased nuclear PIAS1 (-47%, p <0.0001) in AIRE-/- keratinocytes compared to AIRE+/+ cells after IFN-γ treatment. Overall, our data suggest that AIRE deficiency in keratinocytes leads to an AA-like molecular signature, AIRE functions as a suppressor of JAK-STAT signaling in keratinocytes, and the Aire-/- mouse can be used as a new model to study AA. Given that patients with AIRE mutations display an array of ectodermal dystrophies (including AA), our findings provide a framework towards understanding the role of Aire in skin and HF biology with hopes of uncovering pathogenic mechanisms in AA and AIRE deficiency disorders.

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Stem cell niche architecture dictates hair progenitor distribution and differentiation

H. Wei[1], T. Xin[1], V. Greco[1, 2]
[1]Genetics, Yale School of Medicine, New Haven, Connecticut, United States, [2]Dermatology & Cell Biology, Yale Stem Cell Center, Yale Cancer Center, Yale School of Medicine, New Haven, Connecticut, United States

Stem cells and their nearby niche cells generate an organ architecture essential to its function. For hair production, the hair follicle remodels its epithelial architecture through stem cell behaviors under the instruction of a juxtaposed fibroblast niche -- dermal papilla. Although dermal papilla's signaling crosstalk with hair follicle epithelium is well-characterized, little is known about how dermal papilla's physical architecture is organized to support epithelial stem cells. Here, we leveraged live mice imaging, 3D reconstruction, and genetic manipulations to understand how dermal papilla fibroblasts remodel its niche architecture in orchestration with the stem cell progeny -- hair progenitors. We find that dermal papilla fibroblasts develop broad membrane protrusions to form an upward polarized niche architecture enclosed by hair progenitors. Importantly, this remodeling process strengthens niche's architectural integrity such that dermal papilla retained enclosed even after genetic fibroblast depletion. Furthermore, we discovered that fibroblast TGFβ signaling is required to actively maintain the niche architecture. Dermal papilla fibroblasts that could not receive TGFβ signals relocated from within the epithelium precociously while retaining their niche identity. Orchestrated with the relocating niche fibroblasts, hair progenitors redistributed inside out concurrently. Interestingly, the redistributed hair progenitors still perform regenerative behaviors such as proliferation and differentiation, yet their progeny could not maintain normal differentiation and generated shorter hair shafts. Altogether, our work reveals cellular and molecular mechanisms that organize the hair follicle niche architecture. It further highlights that niche's architectural remodeling dictates stem cell distribution and differentiation. We propose that organ shape and functional diversity may result from transformable niche architectures and their scalable mechanic-chemical crosstalk with stem cells.

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Investigating the effects of two peptides on the ECM of skin cells from males with collagen, fibronectin and hyaluronic acid-boosting activities for anti-aging benefits

K. Stafa[1, 3], B. Deng[2], D. Collins[1], R. Cao[2], N. Pernodet[1]
[1]Estee Lauder Companies, New York, New York, United States, [2]Shanghai Research Laboratory, Estee Lauder Companies, Shanghai, China, [3]LAB SERIES Research Center, New York, New York, United States

Skin is made of different layers, but the dermal layer provides “cushioning” and most of the mechanical properties of the skin with its protein matrix. The extracellular matrix (ECM) is an intricate, 3D network or “mesh” made of a plethora of proteins, whose major components are Collagen, Elastin, Fibronectin and Hyaluronic Acid (HA) among others. Briefly, collagen provides cell adhesion and tensile strength, elastin imparts elasticity to tissue subjected to repeated stretch whereas HA is the key molecule involved in attracting and binding water molecules to keep skin moisturized, hydrated, and plump. Lastly, fibronectin assists in the attachment and migration of skin cells, functioning like a “biological glue” to keep collagen fibers, elastin fibrils and HA polysaccharidic units together in a “matrisome.” These fibrous proteins work in concert to provide tensile strength, architectural structure, and organization. As we age, ECM components decay leaving skin saggy, less elastic, dehydrated, and more prone to wrinkles, therefore, there is a continuous need to support the core matrix with potent and highly efficient active ingredients and peptides. Historically, most in vitro studies have been carried out on skin cells from females. However, male consumers are becoming increasingly knowledgeable of sophisticated skin care products and are seeking high-performing, solution-driven male beauty products. Therefore, we specifically tested the effects of two peptides in modulating collagen, elastin, fibronectin and HA in two Normal Human Dermal Fibroblasts (NHDF) cells from 28- and 68-year-old male donors. Here, we present our results in inducing collagen, elastin, fibronectin and HA, hence, aiding to keep ECM at optimal functionality to support mechanical male skin properties and help against sagging and lines and wrinkles.

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An approach to hair loss in hijab-wearing individuals in primary care

S. Dahak[1], J. Koblinski[1], L. Krueger[2]
[1]The University of Arizona College of Medicine Phoenix, Phoenix, Arizona, United States, [2]Dermatology, Emory University, Atlanta, Georgia, United States

Objectives: To identify the physical findings of hair loss in women who wear the hijab. To illustrate findings on examination that warrant additional care by a dermatologist and suggested treatment recommendations. Hair loss in hijab-wearing individuals is a cause of major concern for patients and families. Early diagnosis and treatment are crucial to prevent permanent scarring alopecia and improve the quality of life in these patients. Hijab is a cloth worn by Muslim women to cover their hair and preserve their modesty. The earliest description of hair loss in individuals who wear the hijab found in the literature was in a 1980 Letter to the Editor found in the Archives of Dermatology. They described the frequent complaint of hair loss in Libyan women who wore a headscarf wrapped tightly around the scalp. Fear of offending patients and lack of training on how to approach hijab-wearing individuals serves as a barrier to timely diagnosis of traction alopecia in this population. Additionally, many hijab-wearing patients do not broach the topic of hair loss due to feelings of embarrassment and the fear of violation of modesty. This can be mitigated by approaching the encounter with sensitivity while minimizing assumptions, and ensuring that the patient has control over their modesty. When performing the physical exam, ensure the patient’s modesty and limit anxiety of being seen without hijab by placing a sign outside the door informing staff to not enter during the encounter. Additionally, add an additional barrier by utilizing a curtain and minimizing the number of male staff in the room. Counseling is essential to prevent the progression of traction alopecia. The patient should be encouraged to avoid placing hair in tight hairstyles under the hijab and releasing the hair from the updo when able to. Potential treatments can include minoxidil, antibiotics, or intralesional corticosteroids. In severe cases of scarring traction alopecia, hair transplantation may be the only restorative treatment option.

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UV-induced reduction in polycomb repression promotes epidermal pigmentation

M. Li[1], P. Flora[1], H. Pu[2], C. Bar[1], J. Silva[3], I. Cohen[4], P. M. Galbo[5], H. Liu[5, 6], X. Yu[7], J. Jin[7], H. Koseki[8, 9], J. A. D’Orazio[2], D. Zheng[5, 10], E. Ezhkova[1]
[1]Department of Cell, Developmental, and Regenerative Biology, Icahn School of Medicine at Mount Sinai Black Family Stem Cell Institute, New York, New York, United States, [2]The Markey Cancer Center, Department of Toxicology and Cancer Biology, Department of Pediatrics, University of Kentucky College of Medicine, Lexington, Kentucky, United States, [3]Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, New York, United States, [4]The Shraga Segal Department of Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev Faculty of Health Sciences, Beer Sheva, Southern, Israel, [5]Department of Genetics, Albert Einstein College of Medicine, Bronx, New York, United States, [6]INSERM, Paris, Île-de-France, France, [7]Mount Sinai Center for Therapeutics Discovery, Departments of Pharmacological Sciences and Oncological Sciences,, Icahn School of Medicine at Mount Sinai Tisch Cancer Institute, New York, New York, United States, [8]Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan, [9]AMED-CREST, Yokohama, Japan, [10]Departments of Genetics, Neurology, and Neuroscience, Albert Einstein College of Medicine, Bronx, New York, United States

Ultraviolet (UV) radiation is a prime environmental stressor that our epidermis is exposed to on a daily basis. To avert UV-induced damage, epidermal stem cells (EpSCs) become pigmented via a process of heterotypic interaction between melanocytes and EpSCs, however the molecular mechanisms of this interaction are not well understood. In this study we show that the function of a key chromatin regulator, the Polycomb complex, was reduced upon UV exposure in human and mouse epidermis. Genetic ablation of key Polycomb subunits in murine EpSCs, mimicking depletion upon UV exposure, results in an increased number of epidermal melanocytes and subsequent epidermal pigmentation. Genome-wide transcriptional and chromatin studies show that Polycomb regulates the expression of UV-responsive genes and identify type II collagen as a critical secreted regulator of melanogenesis and epidermal pigmentation. Altogether, our findings show how UV-exposure induces Polycomb-mediated changes in EpSCs to affect melanocyte behavior and promote epidermal pigmentation.

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The RNA-based reprogramming of renal epithelial cells derived from recessive dystrophic epidermolysis bullosa patients into induced pluripotent stem cells

A. Frieman[3, 1], N. Diette[3, 1], P. S. McGrath[3, 4], A. Bruckner[3, 1, 2], I. Kogut[3, 1], G. Bilousova[3, 1]
[1]Dermatology, University of Colorado - Anschutz Medical Campus, Aurora, Colorado, United States, [2]Children’s Hospital Colorado, Aurora, Colorado, United States, [3]University of Colorado Gates Center for Regenerative Medicine, Aurora, Colorado, United States, [4]Pediatrics, University of Colorado - Anschutz Medical Campus, Aurora, Colorado, United States

No effective therapies are available for recessive dystrophic epidermolysis bullosa (RDEB) at this time. Reprogramming adult cells into induced pluripotent stem cells (iPSCs) may allow for the development of new therapies for RDEB. iPSCs can be derived from many cell types, such as fibroblasts, keratinocytes, and blood cells. The isolation of fibroblasts and keratinocytes requires a skin biopsy, an invasive procedure commonly associated with scarring and inflammation, and venipuncture is potentially traumatic for RDEB patients due to the fragility of their skin. The collection of urine is a non-invasive method and can provide exfoliated renal epithelial cells (RECs), which are amenable to reprogramming. Current non-integrating methods of REC reprogramming utilize DNA plasmids or Sendai viral vectors. However, these methods are typically associated with elevated cell death, long and tedious regimens and low efficiency of reprogramming. Here, we report the successful reprogramming of healthy and RDEB RECs into iPSCs using a non-integrating RNA-based approach. This approach was adapted from our previously published high-efficiency RNA-based protocol for reprogramming fibroblasts into iPSCs by modifying cell culture conditions and REC transfection regimens. As a result, we developed a protocol that consistently generates clinically relevant iPSCs from RECs at high efficiency. The generated iPSCs are chromosomally stable, express pluripotency markers, and can be differentiated in vitro and *in vivo*. Thus, our new method provides a safer approach to generate iPSCs from RDEB patients and can accelerate the translation of an iPSC-based therapy for RDEB into the clinic.

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An essential role for CRL complex signaling in epidermal differentiation

M. C. Winge, D. L. Reynolds, L. Ducoli, R. M. Meyers, P. Khavari
Dermatology, Stanford University, Stanford, California, United States

Maintenance of skin homeostasis requires meticulous regulation of the induction, repression and degradation of key signaling factors governing progenitor cell homeostasis and terminal differentiation programs. Deregulation of these pathways is a hallmark of the skin barrier disruption often manifested in skin disease. Timed and controlled orchestration of these networks involves ubiquitin and ubiquitin-like proteins, as abnormal targeted degradation or post-translational modifications are evidenced in conditions harboring mutations in these pathways including xeroderma pigmentosum, autosomal recessive congenital ichthyosis, skin cancer, and dermatomyositis. By combining a CRISPR genetic screen of genes encoding ubiquitin and ubiquitin-like signaling proteins with single-cell RNA-sequencing of primary, differentiated keratinocytes, we demonstrate a cluster of 26 proteins with a marked impact on epidermal differentiation. Through analysis of individual gene targets, gene signatures and the cell state affected by these perturbations, we identify new functions for regulating differentiation and proliferation. We demonstrate specific receptor-adaptor-substrates tethered in Cullin-RING E3 ubiquitin ligase complexes (CRLs), having a dramatic effect on key differentiation pathways. This adaptor-receptor-substrate specific effect of CRLs determines terminal differentiation, which has implications for progenitor cell differentiation and therapeutic interventions of this pathway.

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Hunting the hair cycle clock (HCC): Evidence that mitochondrially localized MPZL3 is a key HCC element in murine and human hair follicles

C. Nicu[1], T. C. Wikramanayake[1], J. Gherardini[1, 2], A. Mello[1], J. Chéret[1], R. Paus[1, 2]
[1]*Dr. Phillip Frost Department of Dermatology & Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States,* [2]*Monasterium Laboratory, Münster, Germany*

Hair follicles (HFs) undergo cycles of growth (anagen), regression (catagen) and relative quiescence (telogen), whose timing and velocity are controlled by the "hair cycle clock (HCC)", an intrinsic and autonomous oscillator system whose molecular nature remains to be elucidated. In this study, we have further explored the hypothesis that Myelin Protein Zero-like 3 (MPZL3), a nuclear-encoded protein localized to the mitochondria, is a key element of the HCC. We have previously shown that a) MPZL3 is expressed in the secondary hair germ, a known epicenter of murine hair cycle regulation, and b) Mpzl3 global knockout mice display strikingly accelerated HF cycling, i.e., a precocious telogen-anagen transition during the second hair cycle, suggesting that MPZL3 functions as a molecular brake on anagen entry. Additionally, keratin 14-Cre-mediated Mpzl3 knockout mice also show accelerated HF cycling, indicating that keratinocyte-derived, MPZL3-dependent, intramitochondrial signals control the core HCC in mice. We now show that MPZL3 mRNA and protein are also expressed in the hair matrix of human anagen scalp HFs, and that MPZL3 knock-down in organ-cultured scalp HFs prolongs anagen duration and retards catagen development, likely via reducing TGF-β2 expression. MPZL3 silencing also reduces mitochondrial-specific VDAC1/PORIN expression. These observations underscore the relevance of MPZL3 to human HF cycling, and suggest that 1) mitochondria are much more actively involved in hair cycle control than previously recognized, 2) MPZL3-dependent signaling in HF keratinocytes plays a central role in the elusive HCC and, 3) HCC-related functions of MPZL3 are conserved between murine and human HFs. Targeting MPZL3-dependent signaling pathways, therefore, may provide innovative and effective strategies for therapeutic hair cycle manipulation. CN and TCW contributed equally.

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Restoration of hair follicle inductive properties by depletion of senescent cells

J. Kim[1], M. Zhang[1], A. Pappalardo[1], H. E. Abaci[1], A. M. Christiano[1, 2]
[1]*Department of Dermatology, Columbia University, New York, New York, United States,* [2]*Department of Genetics and Development, Columbia University, New York, New York, United States*

Under physiological conditions, senescent cells (SCs) attract immune cells through secretion of senescence-associated secretory phenotype (SASP), which facilitates immune-mediated clearance of SCs. If SCs are not efficiently cleared, SASP accumulation leads to pathologic dysfunction of nearby non-senescent cells. Human dermal papilla (DP) cells lose their original inductive properties when expanded in in vitro culture, in which senescent cells are never cleared without specific treatment. We discovered that human DP culture accumulated SCs over passaging, which exhibited enlarged nucleocytoplasmic morphology correlating with β-galactosidase and p21 expression. Protein and RNA-seq analysis revealed a significant accumulation of DP-specific SASP factors including IL-6, IL-8, MCP-1, TIMP-2. We determined SASP-mediated repressive interactions on neighboring cells by detection of IL-6 signaling pathway upregulation in non-senescent cells nearby SCs. To investigate whether the depletion of SCs had a beneficial effect on the hair inductive potential of cultured DP cells, we used combined senolytic treatment of dasatinib and quercetin to deplete SCs. Senolytic depletion reversed SASP accumulation and SASP-mediated repressive interactions, resulting in an increase of LEF1 activity of human DP culture. Senolytic-depleted DP cells in *ex vivo* 3D skin constructs showed restored inductive potential by enhancing hair lineage specific differentiation of keratinocytes. Using *in vivo* hair follicle reconstruction assays, we found that senolytic-depleted DP cells recovered their hair inductive properties by regenerating de novo hair follicles with improved efficiency compared to untreated DP cells. Our study uncovered the senescence-mediated repressive mechanisms hampering the inductive potential of cultured DP cells, and its pharmacologic reversal by the depletion of SCs from cultured human DP cells for HF regenerative approaches.

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TCF-4 negatively regulates IL-17C In human keratinocytes and in a mouse model of psoriasis

R. Singh[1], Y. Jiang[2, 3], L. C. Tsoi[2], M. Sarkar[2], O. Plazyo[2], A. Billi[2], E. Maverakis[4], J. M. Kahlenberg[5], J. E. Gudjonsson[2], N. L. Ward[1]
[1]*Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States,* [2]*Dermatology, University of Michigan, Ann Arbor, Michigan, United States,* [3]*Dermatology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Beijing, China,* [4]*Dermatology, University of California Davis School of Medicine, Sacramento, California, United States,* [5]*Rheumatology, University of Michigan, Ann Arbor, Michigan, United States*

IL-17C is a keratinocyte-derived cytokine that promotes and amplifies innate defense in various inflammatory skin diseases including psoriasis. However, little is known about the mechanisms regulating IL-17C expression or its downstream proinflammatory effects in epithelial cells that promote psoriasis inflammation. Here we identify a critical role for the transcription factor TCF4 in the negative regulation of IL17C expression. RNAseq analyses of psoriasis patient lesional skin (n=99) revealed a negative correlation (r=-0.43, p=6.4E-06) between TCF4 and IL17C, and immunohistochemistry of lesional psoriasis skin confirmed that increases in IL-17C correspond to decreases in nuclear TCF4. Downregulation of TCF4 in human keratinocytes using siRNA caused increases in IL17C (4-fold, n=3, p<0.05) and its target ZC3H12A (1.35-fold, n=3, p<0.001), and IL-17C stimulation of keratinocytes increased ZC3H12A expression (1.6-fold, n=3, p<0.01) in an IL17RA/RE/RC-dependent manner. Using the KC-Tie2 mouse model of psoriasis that has 5-fold increase in skin IL-17C (p<0.001), we demonstrate that genetic elimination of Il7re or Il17ra increases Tcf4 gene expression (1.8-fold and 2.2-fold, respectively, p<0.01), and this corresponds to an improvement in the psoriasis-like skin phenotype. Furthermore, topical application of Tcf4 siRNA onto ear skin of KC-Tie2 mice exacerbates skin inflammation (1.15-fold increase in acanthosis, p<0.01) and increases expression of Il17c (1.3-fold, p<0.05) and Zc3h12a (1.2-fold, p=ns). Together our findings identify a role for TCF4 as a negative regulator of IL-17C and provide new insights into IL-17C-mediated mechanisms of inflammatory skin response.

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Identification of novel loci associated with scalp hair-whorl direction

J. Luo[1], J. Tan[2, 3], H. Huang[2, 1], W. Chen[1], L. Jin[2, 3, 4], S. Wang[1, 5]

[1]CAS Key Laboratory of Computational Biology, Chinese Academy of Sciences Shanghai Institute of Nutrition and Health, Shanghai, Shanghai, China, [2]State Key Laboratory of Genetic Engineering at Fudan University, Shanghai, Shanghai, China, [3]Ministry of Education Key Laboratory of Contemporary Anthropology, Fudan University School of Life Sciences, Shanghai, China, [4]Taizhou Institute of Health Sciences, Fudan University, Shanghai, Shanghai, China, [5]Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences Kunming Branch, Kunming, Yunnan, China

A hair whorl is a circular patch of hair that grows in a clockwise or counterclockwise pattern around a visible central point. Though scalp hair-whorl direction (HWD) is one of the most visible phenotypes in humans, its formation mechanism and genetic contribution remain unclear. Here, we conducted the first genome-wide association study to explore the genetic basis of HWD. We identified a significant association at 7p21.3 (P = 2.72 × 10–8) in the National Survey of Physical Traits cohort (n=2,149) and validated this association in the Taizhou Longitudinal Study cohort (P = 4.61 × 10–7; n=1,998). Meta-analysis of the two cohorts identified three additional genome-wide significant signals at 5q33.2, 7q33, and 14q32.13. The locus at 7p21.3, the most significant signal over the genome, showed the most promising results by fine-mapping and functional annotations. The prioritized SNP is also an eQTL for ARL4A (ADP Ribosylation Factor Like GTPase 4A) in keratinocyte cells. ARL4A is a modifier of the actin cytoskeleton, while actin dynamics are known to be essential in the process of movement and fusion that leads to closure of the top of the head. This study provides new insight into the genetic basis of HWD and gives a clue to its formation mechanism during embryonic development.

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Single-cell transcriptomics reveals lineage trajectory of human scalp hair follicle and informs mechanisms of hair graying

S. Wu[1, 3], Y. Yu[2], C. Liu[4], Z. Xia[3], P. Zhu[3], X. Yan[2], Y. Li[2], P. Hua[2], Q. Li[4], S. Wang[2], L. Zhang[2]

[1]Fudan University, Shanghai, Shanghai, China, [2]Chinese Academy of Sciences Shanghai Institute of Nutrition and Health, Shanghai, Shanghai, China, [3]Chinese Academy of Sciences Shanghai Institute of Nutrition and Health, Shanghai, Shanghai, China, [4]Shanghai Jiao Tong University School of Medicine, Shanghai, China

Hair conditions, such as hair loss and graying, are prevalent human conditions. But they are often poorly controlled due to our insufficient understanding of human scalp hair follicle (hsHF) in health and disease. Here we describe a comprehensive single cell RNAseq analysis on highly purified black and early stage graying hsHFs. A concise single cell atlas for human HF and its early graying changes is generated and verified using samples from multiple independent individuals. These data reveal hsHF's lineage trajectory in unprecedented detail and uncover its multiple unexpected features not found in mouse HFs, including presence of an innerbulge like compartment in growing phase, lack of a discrete companion layer, and enrichment of EMT features in HF stem cells (HFSCs). Moreover, we discover that besides melanocyte depletion, early stage human hair graying is also associated with specific depletion of matrix hair progenitors but not HFSCs. The hair progenitors' depletion is accompanied by their intrinsic transcriptional changes that can be pharmaceutical targeted to ameliorate hair graying in mice, enlightening a promising therapeutic avenue for this prevalent hair condition.

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Genome-wide association analyses identified variants of potassium channel genes associated with sweating phenotypes

W. Chen[1], L. Wang[2], J. Luo[1], G. Chen[2], L. Jin[3], S. Wang[1, 4]

[1]Chinese Academy of Sciences Shanghai Institute of Nutrition and Health, Shanghai, Shanghai, China, [2]Hunan Provincial Key Lab on Bioinformatics, School of Computer Science and Engineering, Central South University, Changsha, Hunan, China, [3]State Key Laboratory of Genetic Engineering at Fudan University, Shanghai, Shanghai, China, [4]Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences Kunming Branch, Kunming, Yunnan, China

Sweating is essential for human thermoregulation and its capacity varies among people. Genetic studies have reported that sweating ability is heritable. However, the genetic effects on the variation of sweating ability have not been well elucidated. Here, we performed genome-wide association studies (GWASs) of 14 thermal sweating and 2 psychological sweating phenotypes in two independent cohorts (Ndiscovery=3,886; Nreplication=2,548). We identified one locus at 3p24.3 - KCNH8 (Pdiscovery=1.84×10-08, beta=0.06) was significantly associated with the scalp sweating. And it was successfully replicated in the replication cohort (Preplication =0.02, beta=0.05). Meta-analysis identified one additional signal at 2p25.1 - KCNF1 (Pmeta=6.28×10-09, beta=-0.06) associated with pressure sweating. Functional annotation showed that pressure sweating variants are eQTLs of KCNF1. Further candidate gene analyses identified 4p15.3 - KCNIP4 (Pmeta=1.66×10-06, beta=0.86) and 12p13.3 - KCNA6 (Pmeta=2.92×10-05, beta=0.80), two statistically significant associations with pressure sweating and scalp sweating, respectively. We also observed a notable enrichment of potassium channel genes (KCN) variants associated with sweating phenotypes at lower GWAS P-value thresholds, suggesting that KCN genes play an important role in the genetic architecture of sweating. Taken together, our findings provide insights into a better understanding of the roles of potassium channel genes in sweating.

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Efficacy of silybum marianum extract, manganese PCA and lespedeza capitata extract on hair growth and anchorage in human dermal papilla cells

D. Bacqueville[1], C. Mas[1], M. Leveque[1], M. Haure[1], V. Mengeaud[2], S. Carrere[1], S. Bessou-Touya[1], H. Duplan[1]

[1]R&D Department, Pierre Fabre Dermo-Cosmetique SAS, Lavaur, Occitanie, France, [2]Laboratoires Dermatologiques Ducray SAS, Lavaur, France

The hair follicle (HF) undergoes cycles of growth (anagen), regression (catagen) and rest (telogen) phases. Both chronic and reactive hair loss are linked to a dysfunction of the HF cycle leading to a premature hair loss. Therefore, the use of specific active ingredients targeting the HF could improve hair loss. The aim of this study was to evaluate the efficacy of 3 active ingredients on hair growth and anchorage in dermal papilla cells isolated from human hair follicle (DPC): a new patented extract from Silybum marianum containing less than 2% silymarin (SME) and stimulating hair shaft anchorage by keratin 75 modulation (Patent WO/2021/023820), Manganese PCA (MnPCA) and a Lespedeza capitata extract (LCE) (Patent WO/2020/020791A1). Antibody array and gene reporter assays allowed to analyse Receptor tyrosine kinase phosphorylation and Wnt/β catenin pathways activation, respectively. ELISA tests were performed to quantify the release of Versican, VEGF and DKK1. Activity of 5α-reductase (5αR) was also measured. SME 30 µg/mL activated growth factor receptor signaling pathways (EGFR x1.9 and PDGFR x2.8 respectively) and their downstream effectors (ERK, GSK3, Akt, STAT x1.2 to 2) after 1h exposure. MnPCA stimulated the Wnt/β catenin pathway (+80% at 0.009%) and induced both Versican production (x33 at 0.01%) and VEGF secretion (x3.3 at 0.009%). LCE reduced DKK1 release (-72% at 0.001%) and 5αR activity (-59.6% at 0.01%). To conclude, SME modulated DPC growth by acting on EGFR/PDGFR signaling pathway. MnPCA and LCE enhanced anagen phase via the Wnt/β catenin pathway. MnPCA also improved HF anchorage and microcirculation by stimulating Versican and VEGF, respectively. Finally, LCE favored dihydrotestosterone decrease. Altogether, the data suggest that a combination of SME + MnPCA + LCE may be useful to improve hair loss treatment by a specific action on both hair growth and anchorage.

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Repressive epigenetic mechanisms mediated by PRC1 safeguards adult hair follicle stem cell quiescence

P. Flora[1], M. Li[1], D. Zheng[2], E. Ezhkova[1]
[1]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Albert Einstein College of Medicine, Bronx, New York, United States

Hair follicle stem cells (HFSCs) have the unique capacity to fuel hair growth throughout the lifetime of an organism. During the onset of new hair growth, HFSCs get activated and proliferate to give rise to transit-amplifying cells that fuel the production of a new hair follicle. This activation is transient and HFSCs quickly return to quiescence. The balance between HFSC activation and quiescence is maintained by a specific transcriptional landscape. Epigenetic mechanisms are critical regulators of gene transcription, however, its role in controlling HFSC function in the adult skin remains largely unexplored. The Polycomb proteins are evolutionarily conserved epigenetic regulators that mediate transcriptional repression. Polycomb group of proteins are classified into two multi-subunit complexes, Polycomb Repressive Complex (PRC) 1 and 2, and our known to facilitate each other's recruitment establish repressive Polycomb domains. Despite prior genome-wide mapping of H3K27me3, a repressive mark catalyzed by PRC2, in HFSCs had implied PRC2's instructive role, we have recently shown that PRC2 and H3K27me3 are dispensable in the adult HFSCs. Notably, our work in the developing epidermis had shown that PRC1 has a more prominent role in gene repression compared to PRC2. Moreover, contrary to the previously established models, studies have reported that the bulk of PRC1-dependent H2AK119ub distribution does not depend on PRC2. Based on these observations, we hypothesized that PRC1 functions independently of PRC2, to regulate transcriptional programs essential for safeguarding HFSC function. We show that ablation of PRC1 in quiescent HFSCs leads to repetitive accelerated hair cycling indicating that PRC1 is critical for maintaining HFSC quiescence. RNA-seq analysis revealed that PRC1-null quiescent HFSCs upregulate a transcriptional program akin to that of activated HFSCs. In conclusion, our functional studies have elucidated the instructive role of epigenetic regulation that mediates proper HFSC function in the adult skin.

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DNA dioxygenases Tet2/3 regulate gene promoter accessibility and three-dimensional chromatin topology in lineage-specific loci to control hair growth

G. Chen[2], Q. Xu[2], M. Fessing[3], A. Mardaryev[3], A. Sharov[1], G. Xu[2], V. Botchkarev[1]
[1]Dermatology, Boston University, Boston, Massachusetts, United States, [2]Shanghai Institute of Biological Sciences, Shanghai, China, [3]University of Bradford, Bradford, West Yorkshire, United Kingdom

Lineage-specific and functionally-related genes frequently form conserved clusters or loci in the mammalian genomes. Execution of lineage-specific differentiation programs requires tight coordination between many regulators including Ten-eleven translocation (TET) family enzymes catalyzing DNA oxidation. However, the role of Tet genes in the control of cell differentiation at the levels of lineage-specific gene loci is unknown. Epithelial keratin genes are organized in the genome into two distinct (Keratin type I and II) loci on mouse chromosomes 11 and 15. Here, by using Keratin 14-driven ablation of Tet genes in skin epithelial cells, we demonstrate that Tet enzymes play essential roles in the control of gene expression in Keratin type I/II gene loci and execution of hair-specific differentiation program. Mice with Keratin 14-driven ablation of Tet2/Tet3 or all three Tet genes exhibit marked alterations of hair shape and length followed by hair loss. We show that through DNA demethylation, Tet2 and Tet3 control chromatin accessibility, Dlx3 binding and promoter activity of the Krt25 and Krt28 genes regulating hair shape, as well as regulate interactions between the Krt28 gene promoter and distal enhancer. However, Tet2/3 also control three-dimensional chromatin topology in Keratin type I/II gene loci via catalytic activity-independent mechanisms. These data demonstrate for the first time the essential roles for Tet2/3 in establishment of lineage-specific gene expression program and control of Dlx3/Krt25/Krt28 axis in hair follicle epithelial cells and implicate modulation of DNA methylation as a novel approach for hair growth control.

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MPZL3 functions as a negative regulator of sebaceous gland size and sebocyte proliferation

T. C. Wikramanayake[1], C. Nicu[1], J. Gherardini[1, 2], A. Mello[1], J. Chéret[1], R. Paus[1, 2]
[1]Dr. Phillip Frost Department of Dermatology & Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, United States, [2]Monasterium Laboratory, Münster, Germany

The sebaceous gland (SG) engages in holocrine secretion to produce lipid-rich sebum critical for epidermal barrier function, skin and hair lubrication, and microbiome management. SG undergoes continuous self-renewal to replenish differentiated sebocytes lost during holocrine secretion, as well as hair cycle-dependent oscillations in size. Previous studies have shown that a mitochondrially localized, nuclear encoded immunoglobulin-like v-type protein, Myelin Protein Zero-like-3 (MPZL3), regulates lipid metabolism and energy expenditure, as well as epidermal keratinocyte differentiation and function. In this study, we investigate the role of MPZL3 in murine SG size and sebocyte proliferation. Mpzl3 global knockout mice display SG hypertrophy and sebocyte hyperplasia throughout the first two hair cycles, with increased Ki-67 staining. These results suggest that MPZL3 primarily controls SG size by negatively regulating sebocyte proliferation. Importantly, SG hypertrophy is also observed in keratin 14 promoter-driven Cre-mediated epithelia-specific Mpzl3 knockout (epiKO -/-) mice, demonstrating that intraepithelial, mitochondrially localized MPZL3 negatively controls murine SG size. We also detect MPZL3 mRNA and protein expression in human SGs. Interestingly, MPZL3 mRNA expression is restricted to the junctional zone where sebocyte progenitors are localized, and the peripheral zone and proliferative zone, while MPZL3 protein is also detected in the maturation zone and degenerative zone, which could result from a much longer half-life of MPZL3 protein compared to transcripts. Given that perturbed SG homeostasis and function are observed in many dermatoses such as acne vulgaris, atopic dermatitis, cicatricial alopecia, psoriasis, seborrheic dermatitis, and benign or malignant SG tumors, understanding MPZL3 regulation of SG homeostasis may have significant clinical implications. TCW and CN contributed equally.

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The role of THY1 in the sex dimorphism of dermal fat

X. Zhang, X. Zhang, L. Sun, L. Zhang
Xiamen University, Xiamen, Fujian, China

Sexual dimorphism exists in human skin and in the regional distribution of fat tissues, including dermal white adipose tissue (dWAT, dermal fat), which has been recently recognized as an important deep skin layer that regulates several non-metabolic functions of the skin. However, the sexual dimorphism in dermal fat has not been fully characterized, and the underlying mechanism remains poorly understood. By analyzing the age and sex-dependent changes in dWAT and the in vitro adipogenic potential of dermal fibroblasts (dFBs), we found that male dWAT volume and the adipogenic potential of male dFBs were rapidly lost by 2 months of age, whereas female dWAT and dFBs were relatively resistant to these age-dependent changes. RNAseq and ScRNAseq analysis identified Thy1, gene encoding a glycoprotein highly expressed on adipocyte progenitors (APs), as a key gene that was selectively lost in male but not in female skin and dFBs during aging. In addition, we found that androgen testosterone downregulated the expression of Thy1 in dFBs in vitro, and testectomy surgery rescued age-related loss of Thy1 expression and dWAT in male mice. Next we generated Thy1 knockout (KO) mice, and found that deletion of Thy1 not only decreased the in vitro adipogenic potential of dFBs but also reduced dWAT volume. Finally, we found that Thy1 deficiency led to a blockage in the regeneration of dWAT layer in a large wound induced skin fibrosis mouse model. Together, our results show that sexual dimorphism of dermal fat is regulated by THY1 and loss of Thy1 inhibits adipogenesis and promotes skin fibrosis. Results from our study may provide new insights into mechanisms underlying sex-related skin diseases, such as systemic sclerosis and systemic lupus erythematosus.

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The Wnt-inhibitor Dkk4 is required for primary hair follicle induction and patterning

H. Khatif[1, 3], H. Bazzi[1, 2]
[1]Dermatology & Venereology, Universitat zu Koln, Koln, Nordrhein-Westfalen, Germany, [2]Exzellenzcluster CECAD in der Universitat zu Koln, Koln, Nordrhein-Westfalen, Germany, [3]Exzellenzcluster CECAD in der Universitat zu Koln, Koln, Nordrhein-Westfalen, Germany

Androgenetic alopecia is a common form of pattern hair loss, characterized by miniaturized hair follicles (HFs) at the front and parietal scalp, while hairs on the occipital scalp are preserved. Moreover, different sites of the human body exhibit distinct types and patterns of HFs. Understanding the molecular basis of this heterogeneity will help to design targeted treatment strategies. The Wnt signalling pathway and its Dickkopf (Dkk) inhibitors have been suggested to regulate HF induction and patterning. We have previously shown that Dkk4 is specifically expressed in HF placodes during mouse skin development. To elucidate the functions of Dkk4 in HF patterning, in this work, we generated Dkk4-knockout mice by using CRISPR/Cas9. Dkk4 mutants showed disrupted HF patterning with an increase of the interplacodal distance. Surprisingly, the lateral back skin of Dkk4 mutants completely lacked the first wave of HFs. In order to address the regional differences in HF formation, we generated a Dkk4-EGFP knockin mouse line, which recapitulated the expression of Dkk4 in HF placodes. Our data revealed intra- and interplacodal differences in the expression of Dkk4-EGFP, suggesting that a WNT-DKK axis underlies the regional specificities in HF induction and patterning. Elucidating the molecular mechanisms of this heterogeneity will shed light on hair loss patterns such as in androgenetic alopecia.

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Basigin controls cell cycle and the secretory phenotype of senescent epidermal keratinocytes.

C. Kremslehner[1, 2], M. Narzt[1], M. Sochorova[1, 2], I. Nagelreiter[1, 3, 2], M. Mildner[1], J. Grillari[4], F. Gruber[1, 2]
[1]Dermatology, Medizinische Universitat Wien, Wien, Wien, Austria, [2]CDL-SKINMAGINE, Christian Doppler Forschungsgesellschaft, Wien, Austria, [3]Center for Brain Research, Medical University of Vienna, Vienna, Austria, [4]Institute for Experimental and Clinical Traumatology, Ludwig Boltzmann Gesellschaft, Wien, Wien, Austria

Using models for pro-aging redox stress and replicative senescence models for epidermal keratinocytes (KC) we identified Basigin (EMMPRIN, CD147) to be induced in KC senescence. Known receptor- and signaling function makes Basigin a prime candidate for a functional mediator of keratinocyte senescence. Basigin regulates matrix metalloproteinases (MMPs) and the cell cycle. It thus may shape an aged proteolytic microenvironment and maintain cell cycle arrest in senescent keratinocytes. Another hypothesis was that it's paracrine signaling that leads to a "bystander" effect of cellular senescence. Studying the role of Basigin in 2D and 3D organotypic models we found that that expression of the Basigin ligand S100A9 increased concomitantly with KC senescence marker expression, making an autocrine or paracrine action scenario feasible in which binding of S100A9/8 dimer to Basigin would initiate, promote or sustain cellular senescence within the cell and its microenvironment. The data indicate that indeed Basigin expression can affect cell cycle relevant gene expression. and that expression levels of Basigin affect collagen contraction, probably by regulating MMP activity. Organotypic cell culture supernatants revealed that knock-down of Basigin led to a global reduction in chemo- and cytokine secretion, whereas Chitinase3like1 (Chi3L1), usually reported a cancer prognostic marker and pro-proliferative factor, was strongly elevated. Chi3L1 and Basigin expression in the BSG deficient organotypic cell cultures were mutually exclusive. Thus we confirmed Basigin as a prime target for a KC proliferation-controlling protein in intrinsic and extrinsic KC senescence, and suggest it is an active contributor to the senescence associated KC secretome or SA(A)SP.

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Primary human sebocytes optimized culture method for the development of acne and seborrhea models

E. Lomonte, E. Coste, P. Markioli, L. Valenti
R&D, Exsymol SAM, Fontvieille, Monaco

Nowadays, most cosmetical and dermatological laboratories use sebocyte cell lines to study physiopathological conditions such as seborrhoea or acne. Immortalized human sebaceous gland cell lines provide large amounts of sebocytes but are not as relevant as primary sebocytes that remain a better and more useful tool for studying the activity and the regulation of the sebaceous glands. However, as part of a holocrine gland, once in culture, primary sebocytes tend to quickly stop proliferating and undergo a differentiation process that will eventually lead to their death. This physiological feature becomes a technical constraint that makes difficult their use in 2D models. Here, we present an optimized method for isolating and cultivating primary sebocytes and how two models were developed for the study of acne and seborrhoea. Sebaceous glands from human skin explants were isolated and cultured in an original optimized proliferative media that allows for several cell amplifications steps and results in a large number of cells. The isolated cells were then characterized by their morphological parameters, their specific expression of keratin 7 and their ability to produce neutral lipids under the differentiation process. Two models of seborrhoea were developed: One was induced by hydrocortisone (HC), a hormone mainly produced after a psychological stress. The other was induced by Insulin Growth Factor-1 (IGF-1), produced in rich diet conditions. The sebocytes were cultured for 3 or 7 days with or without HC or IGF-1 in the presence or in the absence of an inhibitor. The differentiation process and the neutral lipid production were both assessed using Nile Red labelling detected in the red and in the green fluorescence respectively. HC and IGF-1 were able to stimulate sebocyte differentiation and to produce large amounts of lipids while the inhibitors decreased lipogenesis. Using an original and efficient method for amplifying primary sebocytes, we have implemented two validated models that may be useful for screening active ingredients for the treatment of acne and seborrhoea.

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Upregulation of caveolin-1 in lesional biopsies of hidradenitis suppurativa: A case series report

N. Seth, H. Lev-Tov, B. A. Abdo Abujamra, J. Chéret, R. Paus, I. Jozic
Dermatology, University of Miami School of Medicine, Miami, Florida, United States

Hidradenitis suppurativa (HS) is an overlooked and often misdiagnosed inflammatory skin condition characterized by painful recurrent nodules and abscesses that rupture, leading to development of sinus tracts. Because of the associated pain, sensitive locations, drainage, odor and scarring, this condition has a tremendously negative psychosocial impact. Considering that 1) early stages of HS are often mistaken for other conditions, 2) pathophysiology is relatively unknown and 3) there are no established biomarkers of disease, it is unsurprising that an average delay in the correct diagnosis of HS is between 7-12 years. Consequently, there is an urgent and unmet need to accurately and robustly diagnose this debilitating disease early on, which is precisely the goal of this study. We utilized lesional and perilesional biopsies (n=3) from the HS patients (Hurley Stage I) to develop spatial genomic profiles that underly pathogenesis of this disease. We identified an upregulation of caveolin-1, caveolin-2 and cavin-1, all of which are structural components of specialized membrane microdomains known as caveolae. We then performed immunohistochemistry staining and validated upregulation of each component in lesional biopsies in comparison to perilesional biopsies from the same patient. Sex/location matched normal skin undergoing routine reduction surgeries served as control. Our preliminary data will serve as the basis for subsequent studies with a larger recruitment size where we will probe the role of caveolae on development and progression of HS by performing spatial genomic, proteomic and lipidomic profiling using Hurley stages I-III.

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Elevated levels of caveolin-1 in frontal fibrosing alopecia, central centrifugal cicatricial alopecia and lichen planopilaris suggests a conserved pathway in pathogenesis of scarring alopecia

B. A. Abdo Abujamra, M. Miteva, J. Chéret, R. Paus, I. Jozic
Dermatology, University of Miami School of Medicine, Miami, Florida, United States

Cicatricial alopecias (CAs) are a disfiguring and difficult to treat types of hair disorder that are associated with distressing scalp symptoms and significant secondary morbidity, marked psychological impact, and significant loss of quality of life. The purpose of our study was to generate a proof-of-principle for a fundamentally innovative strategy for treating CAs by therapeutically targeting caveolin-1 (Cav1), which is the principal structural component of specialized membrane microdomains, using cholesterol depleting agents (cyclodextrins), in full length human *ex vivo* human hair follicle (HF) organ culture and *in vivo* mouse models. Our preliminary data suggest that Cav1 preferentially localizes to the outer root sheath cells in the bulge of human scalp HFs, which houses the epithelial hair follicle stem cells (eHFSCs) required for HF development, maintenance and cyclic renewal, while Cav1 is overexpressed and mislocalized in lesional frontal fibrosing alopecia (FFA), central centrifugal cicatricial alopecia (CCCA) and lichen planopilaris (LPP) (n=3). Moreover, localized topical application of cyclodextrins (MβCD) downregulates Cav1 expression in the bulge, and similar to Cav1 knockout mice skin (inducible under K15 promoter), exhibits: 1) upregulation of key guardians of HF immune privilege (IP) (CD200 and IL-10), 2) downregulation of HF IP collapse-promoting signals (Substance P, β2MG, MHC Class I and CXCL11), and 3) upregulation of E-cadherin. This strongly suggests a previously unrecognized, clinically important involvement of Cav1 in human eHFSC function and survival and a role for Cav1 overexpression in CA pathobiology and supports therapeutic downregulation of Cav1 as a novel management strategy.

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Generation of a laser capture microdissection and RNAseq-based human anagen hair follicle transcriptome atlas

M. Fehrholz[1], I. Piccini[1], L. Timperi[1], A. Mardaryev[2, 3], D. Pinto[4], F. Rinaldi[4], R. Paus[1, 3, 5], T. Bíró[1, 3], M. Bertolini[1]
[1]*Monasterium Laboratory, Skin & Hair Research Solutions GmbH, Muenster, Germany,* [2]*University of Bradford, Bradford, West Yorkshire, United Kingdom,* [3]*CUTANEON, Hamburg, Germany,* [4]*Giuliani Pharma, Milano, Italy,* [5]*Dermatology, University of Miami School of Medicine, Miami, Florida, United States*

Characterizing the transcriptome of defined hair follicle (HF) compartments and cell populations is a fundamental hair research challenge, which has already been mastered for murine HFs (www.hair-gel.net). Yet, a comprehensive atlas of the human HF transcriptome remains to be generated. As a key step into this direction, we have used laser capture microdissection coupled with RNA sequencing to reveal the gene expression profiles of 8 selected compartments of human anagen VI HFs. Principal component analysis (PCA) identified a high degree of similarity of the distinct transcriptional profile of each examined compartment between biological replicates and a clear separation of the HF compartments. To validate our transcriptomic map, we show that well-known signature genes map to the correct HF compartment. For example, expression for VCAN (versican) and ALPL (alkaline phosphatase) was detected in the dermal papilla, and lower in the dermal cup and proximal connective tissue sheath, while KRT85 (keratin 85) and KRT31 (keratin 31) were almost exclusively detected in the precortical hair matrix, and TCHH (trichohyalin) in the non-cornified inner root sheath. Finally, we present an easily accessible database for analysing the expression of genes of interest and gender-dependent transcriptional differences between human HF compartments. This in situ-human HF transcriptome map and the related interactive database can be instructively compared with single cell RNAseq results. Our atlas also allows to identify novel, HF compartment-specific signature genes and serves as guidance for developing compartment-specific therapeutic interventions for the management of human HF disorders.

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Treatment with cyclohexyl salicylate, an olfactory receptor 2A4/7 agonist, promotes human hair follicle growth and bulge stem cell progeny expansion

J. Edelkamp[1], D. Pinto[2], H. Erdmann[3], T. Purba[4], F. Jimenez[5], M. Bertolini[1], R. Paus[1, 6]
[1]*Monasterium Laboratory Skin & Hair Research Solutions GmbH, Munster, Nordrhein-Westfalen, Germany,* [2]*Giuliani SpA, Milano, Lombardia, Italy,* [3]*Kosmed Klinik, Hamburg, Germany,* [4]*Centre for Dermatology Research, University of Manchester, Manchester, United Kingdom,* [5]*Mediteknia Hair Transplant Clinic, Las Palmas de Gran Canaria, Spain,* [6]*University of Miami School of Medicine, Miami, Florida, United States*

Topically applicable non-drugs that enhance the efficacy of available FDA-licensed drugs would be a welcome therapeutic addition in hair loss management. Since the olfactory receptor (OR) agonist, Sandalore, promotes human hair growth and reduces telogen effluvium, we have explored here whether other cosmetic OR ligands can unfold similar properties. We focused on OR2A4/7, since its stimulation promotes epidermal keratinocyte proliferation and differentiation. In situ hybridization showed that OR2A4/7 mRNA is widely expressed in the hair follicle (HF) epithelium, namely the outer root sheath (ORS) and hair matrix (HM). Yet, in freshly embedded human scalp skin, OR2A4/7 protein was mainly restricted to the infundibulum. However, OR2A4/7 protein became widely expressed in the ORS and HM during HF organ culture (OC), suggesting up-regulation of intrafollicular OR2A4/7 expression under tissue stress conditions. In scalp HF OC, the cosmetic OR2A4/7 agonist, cyclohexyl salicylate (CHS), delayed catagen development and tendentially increased HM proliferation. While CHS did not seem to impact on K15+ bulge stem cells, the % of CD34+ cells, i.e. their immediate progeny, was significantly increased in the suprabulbar ORS, as was the % of CD71+ transit amplifying cells (thought to derive from CD34+ cells) in the HM and suprabulbar ORS. Thus, stimulating OR2A4/7 via the non-drug CHS promotes hair growth and expands the progeny of K15+ stem cells, inviting the use of CHS as a novel cosmetic adjuvant treatment for hair loss disorders characterized by premature catagen development and a reduced capacity of K15+ stem cells to generate progeny, such as androgenetic alopecia.

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G-protein-coupled receptors (GPCRs) in the regulation of keratinocyte proliferation and differentiation

P. Pedro[1, 2], K. Lund[1, 2], R. Iglesias-Bartolome[1, 2]
[1]*Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland, United States,* [2]*National Institutes of Health, Bethesda, Maryland, United States*

G-protein-coupled receptors (GPCRs) and their associated heterotrimeric G protein-signaling cascades regulate epidermal stem cell fate. We have previously demonstrated that G-alpha stimulating (Gs) and inhibitory (Gi) proteins regulate downstream cAMP signaling which controls keratinocyte differentiation and proliferation in vitro and *in vivo*. In particular, Gs inactivation causes an expansion of the stem cell compartment resulting in basal cell carcinoma formation. On the other hand, decreasing cAMP signaling by Gi activation leads to epidermal hyperplasia and unbalanced keratinocyte proliferation and differentiation. To study the specific receptors modulating epithelial stem cell fate upstream of these heterotrimeric G-proteins, we performed RNA sequencing (RNAseq) analysis in primary human keratinocytes (HEK). We identified 53 significantly expressed GPCRs. Using a pooled interference RNA (siRNA) library, we found that individual knockdowns of GPR137, GPR153, HCAR3, and LTB4R lead to reduced proliferation in HEK cells and immortalized keratinocytes (NTERT). Remarkably, HCAR3 and LTB4R couple to Gi, and subsequent RNAseq following knockdown of these receptors confirmed their involvement in cell cycle entry and keratinocyte differentiation. Moreover, overexpressing these GPCRs in NTERT cells reduced differentiation and impaired skin development in a 3D organotypic model. We also found that the differential expression of HCAR3 affects keratinocytes migration in vitro, which might implicate this receptor in skin wound healing. We are currently elucidating the downstream signaling mechanisms responsible for our observed phenotypes. By defining the roles of specific receptors and their downstream effectors in epithelial cell fate, we are defining global mechanistic models for functional skin biology and uncovering attractive targets for therapeutic intervention of unbalanced stem cell activity.

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Development of multicellular organoids for skin injury and disease modeling

A. Gorkun[1, 2, 3], A. M. Jorgensen[1], N. Mahajan[1], D. J. Gironda[1], M. Wu[1, 4], S. Soker[1], A. Atala[1]

[1]*Wake Forest Institute for Regenerative Medicine, Winston-Salem, North Carolina, United States,* [2]*FGBNU Naucno-issledovatel'skij institut obsej patologii i patofiziologii, Moscow, Russian Federation,* [3]*Sechenov University, Institute for Regenerative Medicine, Moscow, Russian Federation,* [4]*Higher Education Institution of Guizhou province, Zunyi Medical university, Zunyi, China*

Modeling a skin complex layered structure provided tissue-specific functionality is one of the actual challenges in Regenerative Medicine. In this study, we have utilized novel skin organoids (SO) containing the key skin cell types as a tool for in vitro disease modeling and chemical skin irritation testing in immersive conditions. Key human skin cells were induced to form SO and further were exposed to UVB, retinol, 1% Triton solution, Isopropanol, Hexyl Salicylate, 5%KOH on d7. Melanoma cells were added on days 0 and 7 for melanoma modeling. Formed skin organoids were analyzed by Live/Dead assay, histology, IHC, Photometry, RT-PCR, etc at 7, 14, & 21 days of cultivation. Statistics were performed by using Graphpad Software Inc. Skin cells self-organized into spherical organoids with skin-like layered microstructure, with the surface zone formed by epidermis cells and the central core formed by dermal and hypodermal cells. As well, SO showed epidermal barrier formation, vasculogenesis, and pigmentation. Generated skin organoids were capable to utilize retinol into retinoic acid and to develop ER-stress reaction in response to UVB exposure, and responded to skin-irritating chemicals accordingly to their irritation index. The melanoma modeling in skin organoids showed that malignant melanoma cells formed rapidly growing tumor spheroid outside of the organoid and small spherical cluster foci of melanoma in the organoid's central core. Therefore, this study of novel multicellular skin organoids is showing that SO are capable of high-throughput analysis in immersive conditions, and able to recapitulate skin structure as well as functionality and physiological response. Ultimately, this technique provides an in vitro model of skin and could be used as a platform for the investigation of dermatopathology.

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Antifibrogenic activities of novel vitamin D3 derivatives are altered in human fibroblasts expressing low vitamin D receptor or CYP27B1 enzyme.

Z. Janjetovic[1], S. B. Reddy[1], G. Scott[1], S. Qyyum[1], E. Podgorska[1], A. Mobley[1], A. Fabisiak[2], P. Brzeminski[1], A. T. Slominski[3]

[1]*Dermatology, The University of Alabama at Birmingham College of Arts and Sciences, Birmingham, Alabama, United States,* [2]*Uniwersytet Warszawski, Warszawa, Poland,* [3]*Dermatology, The University of Alabama at Birmingham College of Arts and Sciences, Birmingham, Alabama, United States*

The vitamin D receptor (VDR) serves as a receptor for active vitamin D hydroxyderivatives. The formation of vitamin D hydroxyderivatives requires the presence of specific cytochrome P450 (CYP) enzymes for vitamin D metabolism. These hydroxyderivatives can be further hydroxylated by CYP27B1 at the C1α-position which increases their affinity for the VDR. However, the role of CYP27B1 in antiproliferative and antifibrotic activities of CYP11A1-derived D3-metabolites is unknown. We tested the action of chemically synthesized vitamin D derivatives: 20,23(OH2D3, 1,20(OH)2D3, or 1,20,23(OH)3D3 and compared to the action of 1,25(OH)2D3 or 20(OH) D3 (non-calcemic compound). Human fibroblasts were isolated freshly form the neonatal skin and used for the treatment with derivatives. Tested vitamin D3 derivatives inhibited proliferation of fibroblasts and decreased collagen production to a similar degree as 1,25(OH)2D3 or 20(OH)D3. Profibrotic and antifibrotic activities are confirmed by determining gene expressions, such as of Serpine 1 and TGFB1, which were downregulated, and stimulating MMP1 gene, respectively. ShRNA or siRNA was used to silence the expression of the VDR and CYP27B1 enzyme in fibroblasts. The results showed that antiproliferative and antifibrotic activities of vitamin D3 hydroxyderivatives were diminished in fibroblasts lacking VDR. These effects were also reduced in CYP27B1 deficient cells treated with 20(OH) D3 or 20,23(OH)2D3. Therefore, we conclude that that antiproliferative and antifibrotic activities of the vitamin D derivatives are dependent on the VDR and CYP27B1 enzyme

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Defining the epigenetic regulation of fibroblast lineages during embryonic development

Q. M. Phan, R. Driskell

School of Molecular Biosciences, Washington State University, Pullman, Washington, United States

The mammalian skin can support hair follicle formation during embryonic development, but this ability is lost as the skin matures. We have previously shown that, in murine skin starting at embryonic day 16.5 (E16.5), the dermal fibroblasts contained two distinct lineages with different functions. The upper papillary fibroblasts population supports the formation of new hair follicles. However, the mechanism by which embryonic fibroblasts lineages are established remained undefined. By performing ChIP-seq for different histone modifications markers comparing early embryonic fibroblasts (E14.5) and late differentiated embryonic fibroblasts (E17.5), we found a significant difference in H3K27me3 peaks at genes associated with fibroblast differentiation. We have performed *in vivo* skin reconstitution assays and multi-omics experiments in conjunction with tissue specific knockout models to define the epigenetic mechanisms that regulate fibroblast heterogeneity in the dermis.

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Deep learning methods identify eyelid laxity as the main feature causing the aging look

F. Wang[1, 2, 3], Z. Li[4], X. Hu[2, 3], R. Ye[2, 3], L. Du[2, 3], S. Wang[1]

[1]*CAS Key Laboratory of Computational Biology,, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China,* [2]*Department of Science, Inertia Shanghai Biotechnology Co., Ltd, Shanghai, China,* [3]*Department of Science, DermaHealth Shanghai Biotechnology Co., Ltd, Shanghai, China,* [4]*Xiamen Meitueve Technology CO., Ltd, Xiamen, China*

The aging look has become a widespread concern which could be potentially related to one's health status and well-being. Previous studies have tried to identify extrinsic and intrinsic risk factors of the aging look. In this study, we focus on the main facial aging signs which caused the aging look. Firstly, by carrying out the eye movement experiment and deep learning visualization in 26 volunteers, we identified orbit is the main region contributing to the aging look. Then, using deep learning methods, we quantified four periorbital aging features (i.e., laxity eyelids, pouch, crow's feet and lacrimal sulcus) in the Eve cohort (n=255,578). We showed that eyelid laxity is the major factor for the aging look, compared with the other periorbital aging signs ($\beta = 0.42$, $P = 0.01$). Moreover, a stratification analysis by age showed that the weight of eyelid laxity is highest in all age groups (0.31-0.42), while the weights of lacrimal sulcus and eyes bag increased after 40 years old (0.14-0.24 and 0.07-0.18, respectively). At last, in a Han Chinese cohort (n=5,032) with data of eyelid laxity as well as the genome and environmental factors, we found eyelid laxity is significantly associated with environmental factors (i.e., sun exposure and air conditioner usage), but not with any genetic locus. This indicates that the periorbital aging features are probably affected by genes with minor effects, while environmental factors play a more prominent role.

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Genome-wide association study of the nasolabial fold identified novel variants associated with facial morphology

F. Wang[1, 2, 3], Y. Zhao[1], X. Hu[2, 3], R. Ye[2, 3], L. Du[2, 3], Z. Li[4], S. Wang[1]

[1]CAS Key Laboratory of Computational Biology, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China, [2]Department of Science, Inertia Shanghai Biotechnology Co., Ltd, Shanghai, China, [3]Department of Science, DermaHealth Shanghai Biotechnology Co., Ltd, Shanghai, China, [4]Xiamen Meitueve Technology CO., Ltd, Xiamen, China

The nasolabial fold (NLF) is among the most notable phenotypes of facial aging for aesthetic physicians. Previous studies have clarified the three-dimensional structures of the NLF and verified their detailed composition. Reportedly the NLF has a reasonable heritability, but related genetic factors have not yet been identified. In this study, we developed a deep learning-based method to measure the NLF, and performed the largest genome-wide association study of the NLF to date in 12,322 Han Chinese. Two novel loci on 2q31.1 and 3p26.1 were significantly associated with the NLF (P=7.18×10-19 and 3.02×10-10, respectively). Interestingly, the genes near these two loci have both been reported to play important roles in the development of facial morphology. By further examining the 3D facial images of the same individuals with the NLF measurement, we could directly demonstrate the association between the NLF and facial morphology. Specifically, the distances between nose and mouth were strongly associated with the NLF (subnasale-labiale inferius: P=0.04; left alare-labiale inferius: P=0.01; left alare-labliale superius: P=0.04), consistent with results from previous anatomical studies. In conclusion, we expanded the knowledge of the NLF development and verified its relationship with facial morphology from a genetic perspective.

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Niche adipocytes activate hair follicle stem cells through metabolic priming

K. Tai[3], C. Chen[1], S. Fan[5], T. Chang[1], M. Plikus[4], S. Lin[1, 2]

[1]Department of Biomedical Engineering, National Taiwan University, Taipei, Taiwan, [2]Department of Dermatology, National Taiwan University Hospital, Taipei, Taiwan, [3]Genome and System Biology Degree Program, National Taiwan University and Academia Sinica, Taipei, Taiwan, [4]University of California Irvine, Irvine, California, United States, [5]Department of Biomedical Research, National Taiwan University Hospital, Taipei, Taiwan

The ability to sense and to respond to the external environment capacitates tissue stem cells to tailor their activity to meet organismal needs. It has long been observed in human that external irritation leads to acquired hypertrichosis, but the mechanism remains unclear. We found that, in mice, hair follicle stem cells (HFSCs) are activated by external irritation with prominent hair regeneration. The external irritation is not directly sensed by HFSCs themselves but by niche adipocytes which undergo lipolysis to activate HFSCs. Taken up by HFSCs, fatty acids released by adipocytes activate HFSCs by priming HFSCs for fatty acid oxidation and increasing mitochondrial oxidative phosphorylation capacity for ATP production. Inhibiting lipolysis, fatty acid uptake or fatty acid oxidation suppresses irritation-induced hair regeneration. Therefore, adipocytes form an injury-sensing niche that enables HFSCs to respond to external irritation to begin a new round of hair growth for skin protection.

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Development and validation of an automated lesion counting method of facial acne based on convolutional neural network

D. Kim[1], S. Sun[3], S. Cho[1], H. Kong[4], J. Lee[1], J. Lee[1, 2], D. Suh[1, 2]

[1]Dermatology, Seoul National University College of Medicine, Seoul, Korea (the Republic of), [2]Acne, Rosacea, Seborrheic Dermatitis and Hidradenitis Suppurativa Research Laboratory, Seoul National University Hospital, Seoul, Korea (the Republic of), [3]Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea (the Republic of), [4]Transdisciplinary Department of Medicine and Advanced Technology, Seoul National University Hospital, Seoul, Korea (the Republic of)

Although lesion counting is an evaluation method that best reflects the severity of facial acne, its usage in clinical practice and research is limited due to laborious and time-consuming nature. Herein, we aimed to develop and validate an automated algorithm that detects and counts acne lesions by type. A total of 1213 images of 398 facial acne photography set (frontal and both lateral views) from 258 patients (male 38.4%, mean age 22.7±5.97) were collected and labeled into 5 classes (white and black comedo, papule, nodule, and pustule). The dataset was manually labeled by two dermatology residents initially, and then was reviewed by a board-certified dermatologist to develop the final dataset. The algorithms based on convolutional neural network were trained with each dataset for classifying 5 classes of acne lesions or 2 classes for inflammatory and noninflammatory lesions. Mean average precision was 15.59 (5 classes) and 20.84 (2 classes) in the algorithm trained by the initial dataset (A-ID), and was 15.03 (5 classes) and 28.48 (2 classes), respectively, in the algorithm trained by the final dataset (A-FD). Pearson's correlation of lesion counts between algorithm and ground-truth increased from 0.85 (inflammatory) and 0.66 (non-inflammatory) in the A-ID, to 0.90 and 0.72 in A-FD, respectively. In conclusion, our algorithm had demonstrated clinically applicable performance in detection and counting facial acne lesions by type, and these results suggest its utility as an assistance tool for evaluating acne severity.

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Structural adaptations of epidermal stem cells to mechanical stress

S. Huang, G. Rice, P. Rompolas

Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States

The skin has a pronounced ability to adapt to physical changes in the environment by exhibiting plasticity at the cellular level. Transient mechanical deformations applied to the skin are accommodated without permanent changes to tissue structure. However, sustained physical stress induces long-lasting alterations in the skin, which are mediated by shifts in keratinocyte activity. To elucidate the cellular mechanism of epidermal stem cell plasticity, we implemented 2-photon intravital imaging to capture the response of keratinocytes in live mouse skin, to varying degrees of mechanical force. We show that mechanical stress induces the formation of intracellular vesicles, specifically in stem cells within the basal layer of the interfollicular epidermis. Under sustained mechanical force, these intracellular vesicles gradually enlarge, leading to the deformation of the cell nucleus. By lineage tracing analysis we demonstrate that the morphological adaptations of individual epidermal stem cells are linked to changes in their cell fate. Calcium signaling is critical for regulating keratinocyte activity and commitment to terminal differentiation. Utilizing a fluorescent *in vivo* reporter, we captured intracellular calcium dynamics in keratinocytes under different mechanical stress conditions. We show that applied mechanical force induces a sustained increase in intracellular calcium within basal epidermal stem cells. To further resolve the mechanism of keratinocyte response to mechanical stress, we hypothesized that the mechanosensitive ion channel, Piezo1 is involved in mediating these observed phenomena. Conditional deletion of Piezo1 in adult epidermal stem cells caused an increase in force-induced intracellular vesicle formation. Moreover, calcium dynamics in epidermal keratinocytes during force loading were disrupted in Piezo1 knockout epidermis compared to wild type. Subsequently, we found that myosin activity is required for intracellular vesicle formation. This study uncovers how adult epidermal stem cell respond to mechanical stress *in vivo*.

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Epithelial cells in human and murine blood and bone marrow

S. M. Holtorf[1], T. Schuster[1], J. Monts[1], D. Gordon[2], R. J. Morris[1]
[1]The Hormel Institute, Regents of the University of Minnesota, Minneapolis, Minnesota, United States, [2]Institute of Human Genetics, Rutgers The State University of New Jersey, New Brunswick, New Jersey, United States

Cytokeratin positive cells are frequently found in the blood (BL) and bone marrow (BM) of patients with epithelial cancers and are attributed to metastasis. We document here the reproducible presence of epithelial cells (ECs) in normal human and murine BL and BM. We used four different methods: immunofluorescence microscopy (IF), Krt1-14;mTmG transgenic mice, rtPCR, and flow cytometry. To this end, we have made several novel and interesting findings. First, we discovered rare but reproducible pan-cytokeratin+ cells the size of small lymphocytes in smears and cytospins of untreated human and murine BL and BM. Second, we found that Epithelial Cell Adhesion Molecule positive (EpCAM+) cells in human BL constituted 0.18% ± 0.0004 (SEM; n=7 biological replicates, 4 experimental replicates), and for BM, 3.53%±0.006 (SEM; n=3 b.r., 4 e.r.) of mononuclear cells. In mice, blood EpCAM+ cells constitute 0.45% ± 0.0006 (SEM; n=2 b.r., 4 e.r.) and in BM, 5.17%± 0.001 (SEM; n=3 b.r., 4 e.r.). In mice, virtually all the EpCAM+ cells were immunoreactive to pan-cytokeratin as determined by IF microscopy. Third, using Krt1-14;mTmG transgenic mice, we found low (8.6 native GFP+ cells per 106 cells analyzed (0.085% of viable cells), but significant numbers ($p<0.0005$) of GFP+ cells in normal murine BM that were not the result of randomness. Fourth, rtPCR detected traces of cytokeratins 14 and 15 in mouse BL and BM. Further, flow cytometric analyses disclosed heterogeneity within the EpCAM+ population of mouse cells when compared with CD45 (0.58% in BM; 0.13% in BL) and CD44 (0.13%) lineage markers. We conclude from these observations that cells expressing cytokeratin proteins and mRNAs are reproducibly detectable among mononuclear cells from human and murine BL and BM. These observations set the stage for determining the functions of these most curious and novel epithelial cells.

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Hair follicles can "taste": Stevioside stimulation of the bitter taste receptor, TAS2R4, inhibits human hair growth *ex vivo*

J. Gherardini[1, 2], T. Rouille[1], M. Fehrholz[1], W. Funk[3], J. Rodriguez-Feliz[4], A. J. Bauman[5], T. Bíró[1], J. Chéret[2, 6], R. Paus[2, 6]
[1]Monasterium Laboratory Skin & Hair Research Solutions GmbH, Munster, Nordrhein-Westfalen, Germany, [2]University of Miami School of Medicine, Miami, Florida, United States, [3]Clinic for Plastic, Aesthetic and Reconstructive Surgery Dr. Dr. Funk, Munich, Germany, [4]Skin & Hair, Plastic Surgery Dr. Rodríguez-Feliz, Coral Gables, Florida, United States, [5]Bauman Medical Group, Boca Raton, Florida, United States, [6]CUTANEON, Hamburg, Germany

Besides taste buds, many other tissues express taste receptors (TR), where they exert various, often ill-explored non-gustatory functions, e.g. in immunity and metabolism. These evolutionary ancient functions of human TRs can be optimally interrogated in human skin, whose keratinocytes express selected TRs, and its dominant appendage, the hair follicle (HF). However, the role of TRs in human skin physiology is unknown. Therefore, we have asked whether TRs are expressed in human scalp HFs and if their specific stimulation or silencing affects hair growth. Here, we show that human scalp HF keratinocytes prominently and hair cycle-dependently express the bitter taste receptor, TAS2R4 (mRNA, protein). When TAS2R4 was stimulated in organ-cultured anagen scalp HFs by the sweet-tasting steviol-glycoside TAS2R4 agonist, rebaudioside A (Reb A), this significantly inhibited hair matrix keratinocyte proliferation and induced premature catagen development *ex vivo*. These RebA effects could be reversed by TAS2R4 knockdown *ex vivo*. Mechanistically, these HF effects are mainly caused by TAS2R4-mediated enhanced expression/ secretion of the key catagen-promoting growth factor, TGFβ2, since TGFß-neutralization counteracted RebA-induced catagen induction and since RNAseq analysis showed upregulation of TGFß-related signaling pathways. Thus, we introduce here TRs as important, ancestral chemosensory regulators of human skin physiology, human (mini-)organ remodeling (=HF cycling), and growth factor production (TGFß). We also identify a novel, drug-free, "gustatory" strategy for the therapeutic inhibition of unwanted hair growth (hirsutism, hypertrichosis) with a natural sweetener.

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Role of epithelial stem cells in meibomian gland development, dysfunction, and dry eye disease

E. J. Tchegnon[1, 2], C. Liao[1, 3], E. Ghotbi[1], L. Q. Le[1, 2, 4]
[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Simmons Comprehensive Cancer Center, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [3]Graduate Institute of Medical Sciences, Taipei Medical University, Taipei, Taiwan, [4]Hamon Center for Regenerative Science and Medicine, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

Dry eye disease (DED), one of the most common disorders of the ocular surface, affects over 16 million adults in the U.S. DED occurs from insufficient lubrication of the cornea, a consequence of destabilization of the tear film. In the majority of DED cases, this destabilization is caused by Meibomian gland dysfunction (MGD). MGD encompasses various conditions characterized by a defect in the production of meibum, the lipid outer layer of the tear film. MGD results from a variety of causes including loss of the Meibomian gland. In this study, we evaluated the importance of the zinc-finger transcription factor KROX20 and KROX20-expressing cells in Meibomian gland morphogenesis and homeostasis. During our characterization of mice lacking KROX20 protein in epithelial cells, we serendipitously observed that these mice developed squamous metaplasia of the ocular surface, a hallmark of DED. Histological analyses revealed that these mice lacked Meibomian glands. Furthermore, expression analyses showed that while KROX20-expressing cells are restricted to the central duct area of the Meibomian gland, Krox20-lineage cells are spread throughout the entire Meibomian gland, indicating that KROX20 marks a stem/progenitor cell population that gives rise to the whole Meibomian gland structure. Similar to the phenotype observed upon depletion of KROX20 protein, depletion of epithelial KROX20-expressing cells resulted in the failure of Meibomian gland formation, indicating that Krox20-lineage cells are critical for Meibomian gland morphogenesis. Taken together, this study identifies Krox20 as an important driver of Meibomian gland development and homeostasis, and provides a robust stem cell-centric model for studying DED secondary to MGD and for therapeutic testing.

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Regional expression of secreted WNT inhibitors dictates formation of hairless and poorly haired skin

A. Ho[1, 2], M. Xu[1, 2], S. Millar[1, 2, 3]
[1]Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Department of Cell, Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, New York, United States, [3]Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, New York, United States

Different regions of mammalian skin vary in characteristics such as thickness, and hair follicle and sweat gland presence, size and density, that are reflected in differential responses to injury and disease. Positional information resides in the upper dermis but the responsible molecular mechanisms are poorly understood. To uncover these, we carried out comparative scRNA-seq of mouse embryonic dorsal, ear and plantar skin. This revealed differential regional expression of several secreted Wnt inhibitors, including Dkk2 and Sostdc1, suggesting regional modulation of Wnt signaling as an important mechanism controlling skin heterogeneity. In line with this, we showed previously that Dkk2-null mice develop regenerative hair follicles in normally hairless plantar skin. However, hair follicles in other regions had normal density and size. Loss of Sostdc1 is known to cause ectopic hair formation in nipple skin, extra vibrissae, and larger hair follicle placodes in trunk skin. We hypothesized that Dkk2 and Sostdc1 function partially redundantly to control additional regional skin features. To test this, we generated mice lacking both inhibitors. Double mutants displayed elevated Wnt signaling in embryonic plantar and ear skin, increased formation of plantar hair compared with Dkk2 single mutants, and growth of abnormally dense, long, thick hair on the external ears. Thus, regional modulation of Wnt signaling controls both the formation of hairless versus hairy skin, and the size and density of hair follicles in haired skin. These data support a new paradigm in which, rather than being dictated by an activating dermal signal as postulated in classical models, hair follicle formation is a default pathway that is actively suppressed by secreted inhibitors in skin regions that lack hair follicles or are poorly haired.

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Transcriptomic profiling of frontal and occipital dermal papilla reveals potential role of TRPS1 in androgenic alopecia

S. Limbu[3], N. Farjo[2], B. Farjo[2], P. Kemp[1], C. Higgins[3]
[1]HairClone, Manchester, United Kingdom, [2]Farjo Hair Institute, Manchester, United Kingdom, [3]Imperial College London, London, London, United Kingdom

In androgenic alopecia (AGA), hair follicles in the balding scalp miniaturize. This is accompanied by shortened anagen, premature catagen and longer telogen. There are no changes to the bulge cells (BC) in AGA but there is a decrease in progenitor cells. We hypothesize the loss of progenitors is due to aberrant signaling from the dermal papilla (DP) in the balding scalp, which is important for BC activation and anagen induction. This could increase telogen phase and may contribute to miniaturization. To determine if differences in anagen initiation ability exists between balding (frontal, FDP) and nonbalding (occipital, ODP) DP, we developed an in vitro double spheres assay that combined FDP or ODP cells with BC. We tested the induction of a hair marker keratin 75 (K75) in double spheres and found that FDP cells induce a 1.23X reduction ($p<0.01$) in K75 expression compared to ODP, suggesting an impaired BC differentiation ability of FDP. We undertook a transcriptomic analysis of intact FDP and ODP to find the differences that could contribute to this impaired ability of FDP. We found upregulation of TRPS1 by 1.7X in ODP compared to FDP. In cultured FDP and ODP spheres, this difference in TRPS1 expression remained significant but reduced to 1.2X. When we used a siRNA to knockdown TRPS1 in ODP, then cultured cells in double spheres with BC, we found a 1.1X ($p<0.05$) reduction in K75. This suggests that TRPS1 in DP facilitates their ability to signal to BC instructing their differentiation and expression of K75. We looked for upstream regulators of TRPS1 and identified the androgen receptor (AR) in prostate cancer. When AR is activated in DP spheres, it led to a 2X decrease ($p<0.01$) in TRPS1, suggesting the loss of TRPS1 in FDP may be mediated by androgens. Our data supports the theory that the loss of TRPS1 in FDP contributes to miniaturization in AGA by impairing the ability of DP to signal to and activate BC in the anagen to telogen transition.

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Human hair follicles functionally respond to growth hormone and growth hormone-releasing hormone, mimicking the central hypothalamic-pituitary-somatotropic axis

E. J. Horesh[1], J. Chéret[1], M. A. Alam[2, 3], R. Paus[1, 4, 5]
[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States, [2]Dermatology, Hamad Medical Corporation, Doha, Ad Dawhah, Qatar, [3]Translational Research Institute, Hamad Medical Corporation, Doha, Ad Dawhah, Qatar, [4]Monasterium Laboratory Skin & Hair Research Solutions GmbH, Munster, Nordrhein-Westfalen, Germany, [5]CUTANEON, Hamburg, Germany

Human scalp hair follicles (HFs) are a major non-classical peripheral site of neurohormone production. However, it is unknown whether human scalp HFs also produce the key hypothalamic-pituitary-somatotropic (HPS) axis neurohormones, its main member growth hormone (GH), and its upstream hypothalamic regulator, GH-releasing hormone (GHRH). To answer this, we treated microdissected human scalp HFs with either human GH (100 or 300 ng/mL), or GHRH (100 or 300 ng/mL), for mRNA by qRT-PCR or protein analysis by quantitative immunohistomorphometry of key HPS axis elements. Our data showed that human scalp HFs transcribed all HPS axis elements (incl. GHRH, GH), the GH inhibitor, somatostatin (SST), and the GH receptor. GHRH stimulation increased both intrafollicular GH and SST mRNA expression. Furthermore, GH stimulation significantly decreased its own intrafollicular transcription and increased SST, IGF-1, TGF-B2, IGF-1 binding protein, and JAK2 mRNA expression while GHRH was decreased. We also showed a significant upregulation of SST protein expression in human outer root sheath and hair matrix keratinocytes as well as in dermal papilla fibroblasts. These results represent the first preliminary evidence that human scalp HFs operate a functional peripheral equivalent system of the central HPS axis, along with the expected negative feedback loops (including SST) and growth factor responses. Since abnormal GH serum cause hair growth abnormalities, this neuroendocrine system, whose role in HF physiology requires systematic exploration, is clinically relevant and invites novel HPS-targeting neuroendocrine hair growth intervention strategies.

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UBE2N acts in keratinocytes to control epidermal homeostasis and skin inflammation

M. Lee[1, 2], M. Ben Hammouda[1], W. Miao[1], Y. Jin[1], Y. Huang[1], H. Sun[1], J. Zhang[1, 3]
[1]Dermatology, Duke University School of Medicine, Durham, North Carolina, United States, [2]Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, North Carolina, United States, [3]Pathology, Duke University School of Medicine, Durham, North Carolina, United States

UBE2N (encoded by Ubc13 in mice) is a K63-polyubiqutination-specific E2 conjugase. UBE2N plays pivotal roles in signal transduction and gene regulation. Dysregulation of UBE2N is associated with many immunological disorders as well as embryonic developmental defects including loss of epidermal integrity. We have previously reported that Rosa-CreER-mediated deletion of UBE2N on adult mouse back skin leads to highly inflamed skin lesions, raising the question whether the skin inflammation is a result of UBE2N loss in keratinocyte or local immune cells. Here, we report our novel findings that 4-hydroxitamoxifen (4-OHT)-induced K5-CreER-mediated deletion of UBE2N in basal keratinocytes of adult mouse back skin resulted in a range of inflammatory skin abnormalities including erythema, dry/scaly skin, thickening of both the epidermis and dermis, and abnormal hair growth. Further, we observed enhanced pigmentation and dryness that appeared not only regionally where 4-OHT was applied but also distally on ears, hind feet, and tail, suggesting an existence of keratinocyte or immune cell-derived signals mediating a distant reaction. To understand the mechanisms underlying these phenotypic changes, we are using immunostaining and single-cell RNA-seq transcriptomic analyses as well as BASU-mediated BioID proteomic interactome analyses to define cell type-specific effects and UBE2N target proteins.

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Transcriptome analysis of dorsal root ganglion in atopic dermatitis-induced mice reveals potential biomarker for atopic dermatitis

J. Ryu[1], H. Lee[2], Y. Jang[4], J. Lee[2], A. Kim[1], B. Mok[4], D. Kim[1], Y. Song[3], J. Shin[1]
[1]Dermatology, CHA University College of Medicine, Seongnam, Korea (the Republic of), [2]CHA University Department of Biomedical Science, Seongnam, Gyeonggi-do, Korea (the Republic of), [3]Internal Medicine, CHA University College of Medicine, Seongnam, Korea (the Republic of), [4]Biochemistry, CHA University College of Medicine, Seongnam, Korea (the Republic of)

Itch is a typical symptom that exacerbates atopic dermatitis (AD) and is associated with both the central and peripheral nervous systems. In a previous study, pruritogen mediators (histamine, serotonin, substance P, interleukin 31, and thymic stromal lymphopoietin) secreted in the skin bind to their receptors located on somatosensory neurons to transmit itch signals to the brain. In this study, we aimed to elucidate the profile of dorsal root ganglion (DRG), which contains the cell bodies of sensory neurons, from AD mice compared to DRG from control mice. We performed RNA seq analyses and used DESeq2 and Caret R packages for the differentially expressed gene (DEG) and random forest analyses, respectively. A total of 142 DEGs between the control and severe AD mice group were identified. These changed genes were mainly enriched in the GO function related to positive regulation of inflammatory response, leukocyte migration involved in the inflammatory response, and cell adhesion. Also, a total of 87 between the mild AD and severe AD mice group were identified. These changed genes were mainly enriched in the GO function related to inflammatory response, positive regulation of inflammatory response, and cellular oxidant detoxification. In particular, brain-derived neurotrophic factor (Bdnf) and stimulator of interferon genes (Sting) were most distinctly upregulated in the severe AD mice group. Moreover, the expressions of these genes gradually increased in the order of control, mild, and severe AD mice group. Collectively, we found a significant difference in DRG gene expression profiles in comparisons of control and AD mice, as well as mild AD and severe AD mice. Furthermore, biomarkers such as Bdnf and Sting could be targetable to suppress the progression of AD and its symptoms.

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Minocycline suppresses lipogenesis via inhibition of p300 histone acetyltransferase activity in human SZ95 sebocytes

H. Shin[1], C. Zouboulis[2], M. Kim[1], D. Lee[3], J. Chung[1]
[1]Department of Dermatology, Seoul National University College of Medicine, Seoul, Korea (the Republic of), [2]Departments of Dermatology, Venereology, Allergology and Immunology, Brandenburg Medical School Theodor Fontane and Faculty of Health Sciences Brandenburg, Dessau, Germany, [3]Department of Dermatology, Seoul National University Hospital, Jongno-gu, Seoul, Korea (the Republic of)

Minocycline is a second-generation tetracycline drug that is widely used to treat a variety of infectious and inflammatory diseases such as acne vulgaris. The effects of minocycline on acne vulgaris have been primarily attributed to its anti-inflammatory effect; however, the relevance between its sebum-regulating effect and epigenetic regulation in human sebaceous glands remains unexplored. Here, we identified a potential underlying epigenetic mechanism of the sebum inhibitory effect of minocycline in human SZ95 sebocytes. The quantity of lipid droplets were analyzed by Oil Red O staining and TG analysis kit in minocycline-treated SZ95 sebocytes. Minocycline reduced the insulin and liver X receptor agonist-induced lipid accumulation and the expression of the key lipogenic transcription factor sterol regulatory element-binding protein 1 (SREBP1) and its downstream genes, fatty acid synthase (FAS) and acetyl-CoA carboxylase α (ACCα). Minocycline inhibited p300 HAT activity in a concentration-dependent manner, but demonstrated no effect on global HDAC activity, resulting in a significant decrease in histone acetylation. p300 knockdown or treatment of a selective p300 HAT inhibitor significantly suppressed SREBP1 expression, histone acetylation, and lipid accumulation, whereas p300 overexpression enhanced these effects. Moreover, p300 overexpression rescued minocycline-inhibited SREBP1 expression and lipid synthesis. Our findings revealed a novel sebum-regulating effect of minocycline. Moreover, as p300 HAT is a key epigenetic regulator of sebaceous lipogenesis, its inhibitors could be used for the treatment of acne vulgaris.

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Thymic stromal lymphopoeitin controls hair growth

J. Shannon[1, 2], D. Corcoran[5], S. Ziegler[4], A. MacLeod[3, 2], J. Zhang[2, 6]
[1]Immunology, Duke University, Durham, North Carolina, United States, [2]Dermatology, Duke University School of Medicine, Durham, North Carolina, United States, [3]Janssen Global Services LLC, La Jolla, California, United States, [4]Benaroya Research Institute, Seattle, Washington, United States, [5]Duke University, Durham, North Carolina, United States, [6]Pathology, Duke University School of Medicine, Durham, North Carolina, United States

Hair follicles (HFs) are a defining feature of mammals and contain diverse stem cells with robust regenerative capabilities. Hair follicle stem cells (HFSCs) respond to extra- and intra-follicular signals responsible for renewal of the hair follicle early-stage wound healing. Much remains unknown about how specific wound-derived factors modulate stem cell contribution to hair growth. We identified the cytokine, thymic stromal lymphopoietin (TSLP), is produced in the skin by both keratinocytes and immune cells after injury and during active hair growth. We show that local delivery of low dose exogenous TSLP promotes expansion of transit amplifying cells (TACs) and resulted in hair growth both in the presence and absence of skin injury without inducing itch. Similarly, neutralization of TSLP receptor (TSLPR) inhibited wound-induced hair growth. Using Lgr5CreER Tslprfl/fl mice, we demonstrate that TSLP acts through TSLPR on LGR5+ keratinocytes to promote proliferation of TACs in the hair follicle both during wound healing and normal tissue homeostasis. Lgr5CreER-mediated deletion of Tslpr in HFSCs results in delayed onset of developmental waves of hair cycling preventing onset of second anagen, and inhibited wound-induced hair growth in adult mice wounded in second telogen. Skin from Lgr5CreER Tslprfl/fl mice were deficient in ITGA6+ CD34+ progenitor cells but retained LGR5+ cells. Our findings delineate TSLP as a novel and locally produced cytokine that directly stimulates hair follicle cell proliferation in the skin during hair growth and during wound healing.

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Endothelial glycocalyx destruction is the main culprit for cutaneous inflammation in psoriasis

Q. Li, S. Shao, Z. Zhu, J. Chen, J. Hao, Y. Bai, B. Li, E. Dang, G. Wang
Department of Dermatology, Xijing Hospital, Xian, Shaanxi, China

The skin vasculature ensures local homeostasis and plays an essential role in inflammatory skin diseases. Tortuous and dilated skin vessels are one of the hallmarks of psoriasis; however, how they participate in psoriatic pathogenesis remains unclear. Here, we profiled the single-cell transcriptomes of vascular endothelial cells (ECs) from healthy and psoriatic skin and discovered heterogeneity of psoriatic ECs in signaling pathways and immune responses. Pseudotime analysis demonstrated that capillary EC subsets in psoriatic skin were highly activated. Among the most functional alterations of capillary ECs in psoriasis, we noticed that genes related to the degradation of endothelial glycocalyx, the critical component of the endothelial barrier, were highly upregulated. Transmission electron microscopy, immunofluorescence, and ELISA further validated the substantial destruction of the endothelial glycocalyx in psoriatic skin vessels. Of note, degrading the endothelial glycocalyx of IMQ-induced psoriatic mice enhanced inflammatory responses and aggravated psoriatic inflammation. Whole-mount immunofluorescence staining revealed that vessel remodeling and T cell infiltration were enhanced by glycocalyx degradation. Our study provides a detailed profile of the skin EC atlas from psoriasis patients and healthy controls, identifies the primary EC subsets mediating the elaborate process of immune cell infiltration, and demonstrates the critical role of the endothelial glycocalyx in modulating skin inflammation.

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Preferential recruitment of immature neutrophils enables robust skin regeneration

E. Labit[1], S. Sinha[1], E. Kutluberk[1], A. Jaffer[1], R. Arora[1], L. Cao[1], W. Shin[1], N. Rosin[1], B. Yipp[2], J. Biernaskie[1]
[1]*Comparative Biology and Experimental Biology, University of Calgary, Calgary, Alberta, Canada,* [2]*Snyder Institute, University of Calgary, Calgary, Alberta, Canada*

After severe injury, adult mammalian wound healing occurs by formation of fibrotic scar and loss of skin appendages resulting in permanent functional impairment. In mice, severe skin injuries exhibit partial regeneration, including formation of new hair follicles (HFs) in the center of the wound, whereas the periphery remains fibrotic. We previously showed that fibroblasts residing within the regenerative zone exhibit activation of unique transcriptional programs reminiscent of early skin development (Abassi et al, Cell Stem Cell). How these programs are engaged within certain subsets of fibroblasts remains unclear. To test the role of microenvironment, we microdissected and compared regenerative (centre) versus fibrotic (peripheral) wound microenvironments using scRNAseq. Unbiased assessment of matched cell states revealed neutrophils as the most transcriptionally discrepant cell type between regenerative and fibrotic domains. Systemic application of aLy6G/C after injury depleted Ly6G+neutrophils into the wound bed, resulted in a 15-fold increase in neogenic HFs within the wound. Single-cell transcriptomics of aLy6G/C-treated wounds revealed the emergence of Ly6G-MPO+neutrophils that generate large quantities of reactive oxygen species, a feature essential for invigorating regenerative programs. Finally, we show that purposeful recruitment of these immature neutrophils repattern immune-stromal crosstalk within the wound, resulting in an expanded pool of regeneration-competent fibroblasts enabling robust regeneration. Together, our work identifies neutrophils as the cellular linchpin connecting innate immunity to fibroblast fate by achieving near-complete skin regeneration.

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Therapeutic TNF inhibitors exhibit differential levels of efficacy in accelerating chronic cutaneous wound healing *in vivo*

Y. Cao, S. Tanriverdi, B. P. Harvey, T. Radstake, Z. Kaymakcalan
Transformational and Translational Immunology Discovery, AbbVie Bioresearch Center, Worcester, Massachusetts, United States

Adalimumab is the only FDA- and EMA-approved treatment for moderate to severe hidradenitis suppurativa (HS), where etanercept and certolizumab-pegol have been shown to be inefficacious, suggesting that the mechanism of action (MOA) of adalimumab is distinct in HS and may contribute to improved wound healing. We have demonstrated that adalimumab, but neither etanercept nor certolizumab-pegol, induces a wound healing profile in vitro by regulating macrophage differentiation and matrix metalloproteinase expression which may underlie the difference in efficacy between various anti-TNF agents.
To examine and compare the efficacy of therapeutic TNF inhibitors in chronic cutaneous wound healing *in vivo*, a murine chronic wound healing model was established by breeding Lepr+/db mice with human-TNFα transgenic mice. Four 6-mm full thickness excisional wounds were created on the back of hTNF+/+Leprdb/db mice followed by the administration of anti-TNF agents (adalimumab, infliximab, golimumab, etanercept or certolizumab-pegol) or vehicle. Wound area measurements and photographs were taken every 2nd day for up to 4 weeks to assess wound size reduction.
The vehicle group exhibited severe impairments in cutaneous wound healing in hTNF+/+Leprdb/db mice. In contrast, adalimumab and infliximab accelerated wound healing, showing significant improvement of wound healing starting from day 7 and lasting throughout the rest of the healing process. Golimumab was less effective, along with etanercept and certolizumab-pegol, showing insignificant improvement of wound healing in the hTNF transgenic-Db/Db mice. In conclusion, our data suggest therapeutic TNF inhibitors exhibit differential levels of efficacy in accelerating chronic cutaneous wound healing *in vivo*. Additional studies are being undertaken to explore and differentiate the MOA of adalimumab to other TNF inhibitors in accelerated chronic wound healing in the current animal model as well as in HS patients.

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Repurposing of DPP4 inhibition to improve hair follicle activation and regeneration

M. Helm[1], J. Louil, G. Cotsarelis[2], J. C. Simon[1], R. A. Ferrer[1]
[1]*Dermatology, Universitatsklinikum Leipzig Klinik und Poliklinik fur Dermatologie Venerologie und Allergologie, Leipzig, Sachsen, Germany,* [2]*Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States*

Injury to the skin results in establishment of fibrosis, which inversely correlates with capacity for regeneration of appendages such as hair follicles (HF). Large wounds on the back of mice can regenerate HF in a central area with reduced expression of fibrosis markers (wound induced hair follicle neogenesis, WIHN), which is facilitated by similar pathways of anagen activation (Wnt, AKT). Identification of modulators of anagen entry and WIHN might aid pinpointing targets for allowing regeneration of skin appendages after trauma or inducing hair growth in individuals affected for example by alopecia. Single cell RNAseq analysis identifies Dpp4 as a factor overrepresented in telogen (HF resting) skin and in non-regenerative wound settings, which we could confirm in situ. Interestingly, DPP4 inhibition in unwounded skin with sitagliptin (Sit) causes an activation of HF with upregulation of Wnt, AKT and downregulation of pro-fibrotic pathways TGFb and Hippo as shown with scRNAseq. We hypothesized that DPP4 inhibition might favor anagen entry and HF activation as well as WIHN. DPP4 inhibition during telogen results indeed in faster anagen induction after depilation of dorsal skin in mice and Sit treatment during wound healing results in increased anagen induction in the periphery of wounds as well as increased WIHN by increasing Wnt signaling. DPP4 is known to potentially cleave Wnt ligands required for Wnt activation. Treatment of Wnt responsive fibroblasts with potential DPP4 target WNT10a in presence of Sit achieves higher Wnt activation seen by Lef1/LEF1 expression compared to fibroblasts without DPP4 inhibition, suggesting that reduced cleavage of Wnt ligands by DPP4 in presence of Sit could be one of the mechanisms by which DPP4 inhibition improves HF activity. Together our observations support the notion of DPP4 inhibition as a mean to favor hair follicle activation and regeneration

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Skin wounding alters the gut microbiome by inducing antimicrobial peptide and mucin production from intestinal epithelia

T. Dokoshi, B. Taylor, K. Cavagnero, R. Knight, R. Gallo
University of California San Diego, La Jolla, California, United States

Intestinal and skin microflora can influence several systemic health behaviors. In this study, we hypothesized that skin and gut tissue microbiota are linked, and that skin health can control the gut microbiota. To test this, we compared intestinal responses in control mice to co-housed littermates with full-thickness incisions on back skin or K14Cre-targeted expression of HYAL-1 in the epidermis. The latter mice represent a skin-specific model of the hyaluronidase activity produced by wounding but without a systemic inflammatory or stress response. Both skin-specific interventions greatly exacerbated intestinal disease when mice were subsequently challenged by oral Dextran sulfate sodium salt(DSS) (60% vs 0% mortality, 11-fold induction of IL6 in survivors, p<.001). The DSS response was dependent on the gut microbiome as Germ-free mice or SPF mice given oral Vancomycin were rescued from the DSS effects exacerbated by skin injury or K14HYAL1. 16S rDNA sequencing of feces confirmed that the skin interventions altered the gut microbiome and showed a relative decrease in gram-positive bacteria including protective bifidobacterium. Mechanistically, this response could be explained by the results of spatial transcriptomics using 10X Visium sequencing that showed the colon of wounded or K14HYAL1 mice had greatly increased expression of Mucin 2(Muc2) and Antimicrobial Peptide Regenerating Family Member 3(Reg3). qPCR and immunochemistry validated this increased Muc2 and Reg3b in the transverse colon. Furthermore, this response was modeled by addition of 6.8kDa hyaluronan fragments to gut epithelial cells (HT-29), showing these HA fragments produced by skin injury or K14HYAL1 expression resulted in a 1.7 fold greater Muc2 and 2 fold greater Reg3a expression (p<.01) in vitro. Since intestinal mucin and Reg3 are known to regulate gram-positive bacteria in the intestine, these findings demonstrate how skin injury alters the gut microbiome and susceptibility to disease.

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Wound healing in aged skin exhibits systems-level alterations in cellular composition and cell-cell communication

R. Vu[1], S. Jin[2], P. Sun[1], Q. Nie[3], X. Dai[1]
[1]Biological Chemistry, University of California Irvine, Irvine, California, United States, [2]School of Mathematics and Statistics, Wuhan University, Wuhan, Hubei, China, [3]Department of Mathematics, University of California Irvine, Irvine, California, United States

Delayed or impaired wound healing in aging skin poses major medical, social, and economic challenges, yet a systematic understanding of the contributing cellular and molecular alterations is lacking. In this work, we use single-cell RNA sequencing to interrogate differences across epithelial, fibroblast, and immune cell types in the skin wounds of young and aged animals. We have identified major changes in their subset compositions and molecular profiles during wound healing. Our data uncover a more inflammatory phenotype in wounds from aged mice, featuring altered immune cell compositions and a previously unknown inflammatory Arg1Hi macrophage subset, compared to young counterparts. Using CellChat algorithm to examine signaling changes in aged skin wounds, we found altered communication between fibroblasts and Arg1Hi macrophages. Particularly, osteospontin (OPN) expression is elevated in macrophages from aged skin wounds during mid- and late-stages and might contribute to delayed healing in aged skin. Our study unveils alterations in cellular composition and cell-cell communication for future functional studies in wound healing.

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Assessing the natural history of recessive dystrophic epidermolysis bullosa wounds using a home photography app

S. Fulchand, N. Harris, S. Li, J. So, J. Nazaroff, J. Tang
Dermatology, Stanford University, Stanford, California, United States

Recessive dystrophic epidermolysis bullosa (RDEB) is a devastating, genetic, blistering condition caused by the absence of type VII collagen (C7) resulting in wounding. One major barrier to clinical trial development is the lack of understanding of the natural history of RDEB wounds, as the measurement of wound change has not been studied prospectively or validated. We conducted a longitudinal observational study of 13 participants with RDEB, that were not currently participating in interventional clinical trials. They used a mobile phone photography application with built-in machine learning to outline and track RDEB wounds autonomously. Participants used this mobile application to capture weekly photographs of chronic or recurrent wounds for up to six months and reported associated pain and itch. In total, 773 photos were collected from 72 wounds, of which 39 were chronic wounds (54.2%). The median time for wound closure (≥90% decrease in size from the first photo) was 249 days for chronic open wounds and 68 days for recurrent wounds. A chronic open wound was defined as a wound that has not healed for more than 12 weeks and a recurrent wound heals in less than 12 weeks, but re-opens. For chronic open wounds, wound size was positively correlated with pain and itch using spearman correlation coefficients (0.76, $p<0.001$ and 0.74, $p<0.001$, respectively). Wound size for recurrent wounds were also positively associated with pain and itch (0.37, $p<0.001$; 0.32, $p<0.005$). The COVID-19 travel restrictions have shown the value of remote wound monitoring to assess the natural history of RDEB wounds. Challenges included patient difficulties in navigating the mobile application, uploading regularly, and limited participation due to enrollment in other clinical trials. The next step of this work is to compare our findings of the closure time for chronic versus recurrent wounds against published wound size data of 60 participants from a recent clinical trial, which will enable our group to test definitions of chronic and recurrent wound duration.

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Mechanical tension mobilizes Lgr6+ epidermal stem cells to drive skin growth

Y. Xue[1], C. Lyu[1, 4], A. Taylor[3], A. van Ee[1, 4], A. Kiemen[2], Y. Choi[2, 4], C. Lee[1], D. Wirtz[2], L. Garza[1], S. Reddy[3, 4]
[1]Dermatology, Johns Hopkins Medicine, Baltimore, Maryland, United States, [2]Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, Maryland, United States, [3]Plastic Surgery, Johns Hopkins Medicine, Baltimore, Maryland, United States, [4]Biomedical Engineering, Johns Hopkins Medicine, Baltimore, Maryland, United States

Uniquely among mammalian organs, skin is capable of dramatic size change in adults, yet the mechanisms underlying this striking capacity are unclear. Here, we utilize a system of controlled tissue expansion in mice to uncover cellular and molecular determinants of tension-induced skin growth. Through machine learning-guided three-dimensional tissue reconstruction, we capture morphometric changes in growing skin. We find that most growth is driven by the proliferation of the epidermis in response to mechanical tension, with more limited changes in dermal and subdermal compartments. Epidermal growth is achieved through preferential activation and differentiation of not Lgr5+, but instead Lgr6+ stem cells (SCs) of the epidermis. By using the gain- and loss-of-function mouse models, we find the tension-stimulated skin growth is driven in part by the Hippo pathway. By single-cell RNA sequencing, we uncover further changes in inflammatory responses and mechanosensitive and metabolic pathways, including glycolysis and hypoxia, underlying growth control in the skin. Pseudotime analysis of Lgr6 lineage cells reveals Lgr6+ SCs adapt early to tension, which is corroborated by pulse labeling the Lgr6+ SCs at early or late stages of tissue expansion. We conclude that preferential activation of Lgr6+ SCs by mechanical tension leads to the creation of new skin. Taken together, these studies point to therapeutic strategies to enhance skin growth and establish a platform for understanding organ size dynamics in adult mammals.

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Cellular landscape of the skin is primed by the oral epithelial regenerative transcription factor Pitx1 to promote wound healing

A. Overmiller, A. Uchiyama, E. Hope, A. Sawaya, S. Nayak, K. Hasneen, S. Dell'Orso, S. Brooks, M. Morasso
NIAMS, National Institutes of Health, Bethesda, Maryland, United States

Cutaneous wounds repair and scar while oral wounds regenerate more rapidly and maintain tissue fidelity. We have shown that the oral keratinocyte transcription factor Pitx1 is part of a regenerative transcriptional program in oral mucosa. We hypothesize that the pleotropic transcriptional program initiated, in part, by Pitx1 can be leveraged to shift cutaneous wound healing to a regenerative state. As rapid repair or regeneration of epithelial wounds requires the concerted interplay of all resident cell types, we employed single-cell RNA-sequencing (scRNA-Seq) of murine skin ectopically expressing Pitx1 (K5-Tet-Pitx1) to assess the pan-cellular response to keratinocyte reprogramming. Histologic and scRNA-Seq analysis revealed that Pitx1 expression in the skin induces sebaceous gland hypertrophy, activates sebocyte differentiation factors including PPARγ and RXRα, and positively regulates cholesterol biosynthetic pathways. Additionally, while the skin barrier was maintained, trajectory analysis of keratinocyte differentiation revealed a deviation towards a quasi-oral progression as defined by expression of oral keratins (Krt4/13) and an oral keratinocyte global transcriptional shift. The immune cell landscape illustrated that Pitx1 expression in keratinocytes induced transcriptional changes in the resident Langerhans cell population and recruited a higher number of macrophages to the healthy skin. Furthermore, there was an overall reduction in the number of melanocytes and Ingenuity Pathway Analysis of dermal fibroblasts predicted inhibition of pro-fibrotic pathways. Finally, Pitx1 expression in the skin enhanced cutaneous wound healing by promoting keratinocyte migration and activating retinoid biosynthesis and retinoic acid-related signaling cascades. This work illustrates the dynamic interplay of Pitx1+ keratinocytes on the cellular neighborhood of the skin and identifies potential pathways that may tip the balance of cutaneous wound healing towards regeneration.

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Contribution of resident immune cells in a human autologous 3D skin model

E. Attiogbe[2, 3], S. Larochelle[2, 3], C. Mainzer[1], B. Closs[1], C. Gilbert[3, 4], V. Moulin[2, 3, 4]
[1]R&D Department, SILAB, Brive, France, [2]Centre de Recherche en Organogenèse Expérimentale de l'Université Laval (LOEX), QC, Quebec, Canada, [3]Centre de Recherche du CHU de Québec - Université Laval, QC, Quebec, Canada, [4]Faculté de Médecine, Université Laval, QC, Quebec, Canada

Skin wound healing results in hemostasis, inflammation, proliferation, and remodeling. The early phases require the recruitment of several immune cells from the blood. However, the role of these cells is not well defined. In addition to monocytes-derived macrophages, known to regulate the outcome of wound healing, resident macrophages in the injured skin may play a distinct role in the healing process. The aim was to develop a 3D autologous skin model containing immune and endothelial cells, to investigate their contribution during wound healing. A cell extraction technique was firstly developed to isolate skin resident cells from the same donor (keratinocytes, fibroblasts, immune and endothelial cells). Analysis by flow cytometry revealed the presence of resident skin macrophages (CD45+ CD14+ CD163+); lymphocytes (CD45+ CD3+); dendritic cells (CD45+ CD14- CD1a+) and endothelial cells (CD45- CD31+) in freshly isolated skin cells. From this isolation, an autologous in vitro 3D human skin model was developed. This approach was applied to 4 donors. Immunofluorescence staining confirmed the presence of previously described immune cells in this model. Endothelial cells self-assembled to form capillary-like networks and immune cells did not exfiltrate after 14 days in culture and are able to synthesize TNFα. Moreover, characterization of the proliferation rate in the epidermal basal layer by Ki67 staining revealed a difference between reconstructed skin supplemented with resident immune and endothelial cells compared to the model containing only fibroblasts and keratinocytes. These results indicate that this innovative skin model provides a great tool to study the interaction between resident immune cells such as macrophages with other skin cells and their contribution to healing in a wounded model.

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Commensal microbiome promotes hair follicle regeneration by inducing keratinocyte HIF-1α signaling and glutamine metabolism

G. Wang[1], E. Sweren[1], W. Andrews[2], M. Kane[2], L. Garza[1]
[1]Dermatology, Johns Hopkins Medicine, Baltimore, Maryland, United States, [2]University of Maryland Baltimore, Baltimore, Maryland, United States

Tissue injury induces metabolic changes in stem cells, which likely modulate regeneration. Using a model of organ regeneration called Wound-Induced Hair Follicle Neogenesis (WIHN), we identify skin-resident bacteria as key modulators of keratinocyte metabolism, demonstrating a positive correlation between bacterial load, glutamine metabolism, and regeneration. Specifically, through comprehensive multi-omic analysis and single-cell RNA sequencing in murine skin, we show that bacterially-induced hypoxia drives increased glutamine metabolism in keratinocytes with attendant enhancement of skin and hair follicle regeneration. In human skin wounds, topical broad-spectrum antibiotics inhibit glutamine production and are partially responsible for reduced healing. These findings reveal a conserved mechanism by which bacterially-induced metabolic changes improve the tolerance of stem cells to damage and enhance regenerative capacity. This surprising, pro-regenerative function of the skin microbiome affords novel approaches to wound healing, and suggests a refinement of the role of cutaneous antimicrobials.

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Radiation injury upregulates miR-196, increases dermal collagen, and triggers a pro-fibrotic genomic response that spreads in a murine model of radiation-induced skin fibrosis

R. Stone[1], J. Burgess[1], N. Balukoff[1], T. C. Wikramanayake[1], S. Elliot[2], G. Azzam[3], S. Samuels[3], D. Wan[4], M. T. Longaker[4], M. Tomic-Canic[1]
[1]Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States, [2]Department of Surgery, University of Miami School of Medicine, Miami, Florida, United States, [3]Department of Radiation Oncology, University of Miami School of Medicine, Miami, Florida, United States, [4]Department of Plastic and Reconstructive Surgery, Stanford University, Stanford, California, United States

Radiation-induced skin fibrosis (RISF) is a devastating result of cutaneous injury by irradiation that is delivered as part of clinical care, causing functional impairment and diminished quality of life for patients. To model RISF, we irradiated C57BL/6 mice (n=6) and collected skin from irradiated and non-irradiated sites after 24 weeks. Fibrosis was quantified by measuring dermal thickness and by machine learning ultrastructure analysis of dermal collagen network in tissue sections. Collagen levels were measured by hydroxyproline assay. Transcriptomic profiles (RNA-sequencing and miRNA qPCR) of skin from irradiated and non-irradiated sites were compared with profiles of mice that had not received radiation. Mice developed alopecia, hypopigmentation, and increased dermal thickness at the site of radiation. However, collagen ultrastructure and hydroxyproline quantifications did not differ significantly between irradiated and non-irradiated areas. Fibrosis-associated genes showed similar expression in RNA-seq profiles from irradiated and non-irradiated skin, yet were distinct from profiles of mice that had no radiation exposure. Similarly, radiation-responsive miR-196 was upregulated at the radiation site, yet pro-fibrotic miR-29 expression did not differ by location in the irradiated mouse. Taken together, the RISF response in this murine model extended beyond the treatment field, as evidenced by parallel increases in indices of fibrosis in skin from irradiated and non-irradiated sites. Interventions that minimize off-target effects of radiotherapy to the surrounding skin are needed to prevent the extension of RISF.

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Targeting dermal fibroblast senescence with senomorphic and epigenetic properties of haritaki fruit extract: Effects on SASP and miR over-expression in senescent fibroblasts' extracellular vesicles.

P. Bogdanowicz[1], N. Roullet[1], P. Bensadoun[2], D. Redoules[3], S. Bessou-Touya1, J. Lemaitre[2], H. Duplan[1]
[1]Pharmaco Clinical Research, Pierre Fabre Dermo-Cosmetique SAS, Toulouse, Occitanie, France, [2]IRMB, UMR1183 INSERM, Montpellier, France, [3]Laboratoires Avène, Pierre Fabre Dermo-Cosmetique SAS, Lavaur, Occitanie, France

Skin aging is the consequence of two biological process: intrinsic genetically programmed factors and extrinsic environmental factors. At the cellular level, accumulation of senescent cells (SC) is also involved in skin aging. SC stop to proliferate but remain metabolically active. The secretome of SC (Senescence Associated Secretory Phenotype: SASP), is a cocktail of mediators like CSF3, CXCL1, IL8, IL1b, IL6, MMP-1 and -3, involved in skin aging. Extracellular vesicles (EV) have also been reported to act as cytokines during intercellular communication. They transport proteins, mRNAs and miRNAs, over short or long distances. miRNAs are a class of short, single-stranded, noncoding RNA molecules. As epigenetic modulators, miRNAs affect the protein levels of the target mRNAs without modifying the gene sequences. Several miRs are known to be involved in senescence and skin aging. The aim of our study was to evaluate, the effect of Haritaki fruit extract (HF extract) on SASP and senescence associated miRs contained in EV. Our results showed that, 14 days after ionizing radiation (7.5 Gy), human dermal fibroblasts presented a senescent phenotype: flattened and irregular shape, senescence associated ß-galactosidase activity, SASP expression (RT-PCR) and miR 24a-3p, miR 186-5p, miR 30a-3p and miR 29a-3p overexpression in EV (RT-PCR). Our results also showed that incubation of senescent fibroblasts with HF extract strongly decreased SASP mRNA level and miR 34a-5p, miR 24a-3p, miR 186-5p, miR 30a-3p and miR 29a-3p over-expression in EV. Taken together these results demonstrated that Haritaki fruit extract is a strong senomorphic agent with epigenetic effect, suggesting that it may be useful for the development of new anti-aging dermo-cosmetic products.

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A new vascularized skin substitute to study angiogenesis

A. Mauroux[1, 2], S. Gofflo[2], Y. Atlas[1], S. Bordes[2], B. Closs[2], F. Ruggiero[3], L. Muller[1]
[1]*Center of Interdisciplinary Research in Biology CIRB, Collège de France, CNRS, INSERM, PSL Research University, Paris, France,* [2]*R&D Department, SILAB, Brive, France,* [3]*Institut de Genomique Fonctionnelle de Lyon, Lyon, Auvergne-Rhône-Alpes , France*

Vasculature plays an essential role in skin physiology and its architecture and function are altered in aged and diseased skin. There is thus a need to develop innovative 3D in vitro models with adjustable vasculature. Several in vitro skin models co-seeding endothelial cells with fibroblasts and keratinocytes have been proposed using scaffolds or bioprinting. However, these models fail in reproducing native skin microenvironment and do not demonstrate a functional vascular network. The aim of this work was to develop a vascularized skin substitute and to demonstrate its functionality in response to angiogenic molecules. To do so, we used a scaffold-free approach to better reproduce the skin microenvironment. We stacked-up skin primary fibroblast cell sheets co-seeded with endothelial cells or keratinocytes to generate the vascularized full-thickness skin substitute. Using immunofluorescence and transmission electron microscopy, we confirmed the presence of a fully differentiated epidermis and a well-structured dermal-epidermal junction. Endothelial cells organized into a dense vascular network throughout the dermis. Capillaries displayed a lumen and were stabilized by a well-organized basement membrane and perivascular cells. Modulating concentration and time of application of Vascular Endothelial Growth Factor (VEGF) differentially regulated angiogenesis in our model, resulting in distinct vascular network length and branching. Interestingly, these variations also impacted epidermis differentiation and proliferation. We have thus developed a novel vascularized skin substitute responding to subtle angiogenic stimuli. This model is of interest to mimic physiological and compromised skin conditions involving the vascular component (aging, rosacea, etc) and to evaluate the capacity of natural active molecules to restore skin vascular homeostasis.

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Co-expression of oral epithelial regenerative factors Sox2 and Pitx1 induces wound activation signature in skin keratinocytes

E. Hope[1], A. Overmiller[1], A. Sawaya[1], A. Uchiyama[1], S. Nayak[1], K. Hasneen[1], S. Brooks[2], M. Morasso[1]
[1]*Laboratory of Skin Biology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, United States,* [2]*National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, United States*

Wounds in the oral mucosa heal more efficiently with little to no scarring in contrast to cutaneous wounds. By elucidating the intrinsic molecular differences distinguishing oral keratinocytes from skin keratinocytes, we can identify potential molecular factors that could be therapeutically targeted to improve re-epithelialization in the skin. We previously demonstrated that endogenous expression of the transcription factors Sox2 and Pitx1 in oral keratinocytes mediates a distinct transcriptional network important for the regenerative capacity of the oral mucosa. However, it is unclear if Sox2 and Pitx1 have overlapping regulatory roles in the endogenous oral regenerative transcriptional program. To determine if concomitant, ectopic expression of Sox2 and Pitx1 can induce wound-activation networks in the skin, we generated mice with conditional, basal expression of both Sox2 and Pitx1 in the skin (K14-Cre/LSL-Sox2 and K5-rtTA/Pitx1-Tet). RT-qPCR and immunofluorescence revealed that together, Sox2 and Pitx1 induce expression of wound activation markers (keratins 6a, 6b, 16, and 17) in healthy, unwounded epidermis. Analysis of RNA-sequencing data reveals that expression of differentially expressed genes correlates more strongly with expression of Sox2 than Pitx1. Gene Ontology analysis reveals that Sox2 expression alters epidermal cell processes such as keratinization, keratinocyte migration, and cornified envelope formation, while Pitx1 expression influences processes related to lipid metabolism. Altogether, these findings indicate that ectopic Sox2 and Pitx1 can induce wound-activation networks in murine skin which may promote a more efficient response to injury. Critically, the downstream pathways modulated by co-expression of Sox2 and Pitx1 may offer several potentially druggable targets to enhance cutaneous wound healing in the clinic.

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A biopsy-sized 3D model of skin vascular plexus and appendages enables monitoring T cell trafficking

L. Sorrells, A. Pappalardo, D. Alvarez-Cespedes, E. Jeon, H. E. Abaci
Columbia University, New York, New York, United States

The human skin vasculature has distinct anatomy with two vascular plexuses and specialized capillary loops near skin appendages and rete ridges, and has diverse functions including thermal regulation and immune cell trafficking. There is currently no engineered 3D skin model to capture this level of complexity. The goal of this study is to generate an anatomically-relevant 3D skin model with five different skin cell types, patterned hair follicles, and a perfusable microvasculature mimicking the superficial vascular plexus of the skin. To achieve such complexity, we combined the digital-light-processing and extrusion-based 3D-bioprinting to create a 6 mm biopsy-sized 3D skin model. The design of the vascular pattern allowed for capturing a gradient of shear-stresses relevant to skin arterioles, venules, and capillaries. The microchannels populated with dermal endothelial cells exhibited selective permeability to 70 and 10 kDa-sized molecules uniformly throughout the vascular pattern. In the bioprinted dermis, dermal papilla cells formed spheroids and interacted with the overlaid keratinocytes to form specific layers of the hair follicle, including the inner and outer root sheaths. The epidermis was formed properly with terminally differentiated layers and stratum corneum. Finally, we introduced fluorescently-tagged naïve T cells in the flow and captured the rolling, tethering, and initial extravasation events. The level of resolution allowed us to monitor T cell diapedesis and detailed T cell morphology, e.g., lamellipodia, in real-time, confirming important vascular function of immune cell recruitment in our 3D skin model. This study brings us closer to generating a truly functional human skin with its tissue-specific vasculature and appendages. In addition, the ability to circulate immune cells and monitor immune-EC interactions in the context of the skin microenvironment provides the opportunity to better understand the immune cell trafficking to the skin in healthy and inflammatory conditions as well as during wound healing using human cells.

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Dermal fibroblast expression of lef1 is critical to normal skin and hair development and regenerative wound healing in mice

S. M. Thompson, Q. M. Phan, G. Fine, I. Busch, Y. Du, S. Winuthayanon, I. Driskell, R. Driskell
School of Molecular Biosciences, Washington State University College of Veterinary Medicine, Pullman, Washington, United States

Fibroblasts are the main cell type in the dermis and are major contributors to the wound healing process. During skin development, papillary fibroblasts from the upper dermis uniquely give rise to specialized hair follicle-supporting fibroblasts like the dermal papillae, which controls hair development and cycling, as well as the arrector pili muscles that grant hair its contractile functions. Neonatal papillary fibroblasts are also the only fibroblasts which are supportive of hair follicle regeneration, which we have previously shown to occur naturally in neonatal wound healing and in chamber grafting experiments in adult wounds. We recently investigated neonatal papillary fibroblasts' chromatin architecture and have also shown that expression of the transcription factor Lef1 is sufficient to permit hair follicle regeneration in adult mouse wounds. However, the specific role that Lef1 plays in regulating neonatal papillary chromatin architecture is unknown. We hypothesized that the role of Lef1 during skin development, skin aging, and wound healing could identify the key genes and pathways that are required for functional skin development and repair. We have used a single-cell multi-omics approach to identify how the loss of Lef1 alters the epigenetic landscape and gene expression in papillary fibroblasts to identify the genes and pathways controlled by Lef1 whose alteration denies their regenerative potential. This approach identified the key genes and pathways that are controlled by Lef1 and are essential to normal skin and hair development and wound healing. Additionally, histological assessments of young and aging wild-type and Lef1 KO tissue confirmed the importance of Lef1 to skin and hair's structural development and aging. Finally, wound healing assays identified the importance of embryonic and neonatal Lef1 expression in priming the papillary lineage to contribute positively to wound healing outcomes in neonatal and aging skin.

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Wearable human skin constructs with region-specific properties

A. Pappalardo[1], D. Alvarez-Cespedes[1], S. Fang[2], A. R. Herschman[2], E. Jeon[1], K. Myers[2], J. Kysar[2], H. E. Abaci[1]
[1]*Columbia University Irving Medical Center, New York, New York, United States,* [2]*Columbia University, New York, New York, United States*

The human skin is a complex organ to bioengineer because of its cellular diversity and regional properties. Recreating this complexity in vitro has substantial implications on personalized skin replacement therapy and drug screening. The current paradigm in reconstructing 3D skin generates planar patches with open boundaries on all sides and disregards the fact that human skin is, in fact, a fully-enclosed organ with complex geometries. In this study, we challenge this paradigm by reimagining 3D skin constructs as fully-enclosed continuous tissues which can be seamlessly transplanted as a biological clothing, e.g., skin gloves, on any part of the body. We established a 3D printing-based method that allows for producing skin in any desired and continuous geometry and further integrated endothelial cells for vascularization. We demonstrated the feasibility of generating a human-scale 3D skin glove completely covered by the epidermis expressing specific barrier markers. We showed that the fully-enclosed geometry of the wearable skin (WESC) significantly enhanced the secretion and organization of extracellular matrix and basement membrane proteins, conferring improved mechanical properties, such as rupture stress and tangent modulus, compared to the conventional method. Remarkably, WESC had region-specific and shape-dependent extracellular organization, resembling the Langer lines and leading to anisotropic mechanical properties that are found in human skin. To our knowledge, this level of organization is the first of its kind in any bioengineered skin tissue. Finally, we tailored WESCs for murine hindlimbs, and seamlessly transplanted them as a wearable graft with full integration onto the hindlimb. This study presents a new compelling technology that may have a transformative impact on the way we envision and produce engineered skin. In addition, our finding highlights the importance of the long-overlooked geometry component and supports the emerging notion that function may follow form.

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Reprogramming of diabetic wounds by SOX2 triggers a pro-healing transcriptional signature

C. O'Neill[1], A. Sawaya[1], S. Mehdizadeh[1], M. Tomic-Canic[2], M. I. Morasso[1]
[1]*National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, United States,* [2]*University of Miami School of Medicine, Miami, Florida, United States*

Diabetic foot ulcers (DFUs) are a severe complication of diabetes and a common cause of lower limb amputations and are associated with a high mortality rate. We previously demonstrated that the transcription factor SOX2 establishes a transcriptional network that primes the oral epithelium for rapid wound repair and reprogrammed cutaneous keratinocytes to present accelerated wound resolution both in vitro and *in vivo*. However, the mechanisms by which SOX2 reverses effects of diabetes during wound healing are not known. We used a combination of genomics of human and mouse datasets and diabetic mouse models to identify molecules and pathways responsible for rapid healing by SOX2 that reverses the effects of diabetes and can be therapeutically targeted in patients with DFUs. Transcriptional profiling revealed SOX2 to be inhibited in DFUs compared to human acute wounds, contributing to inhibition of healing. In addition, we found that SOX2 reversed effects of diabetes on wound healing and significantly enhanced healing in a diabetic mouse model *in vivo*. Single cell analysis revealed increased populations of keratinocytes enriched for genes involved in migration and proliferation processes (Hmgb, Stmn1, Ccnd1 and Cdk1) and stem cell populations (Krt15 and Itga6) in Sox2-overexpressing unwounded samples compared to wild-type control mice. Additionally, keratinocyte differentiation markers loricrin and filaggrin were found downregulated in Sox2-overexpressing mice. Our data indicate Sox2 reprograms diabetic wounds to enhance the stem cell population and activation of keratinocytes to trigger a pro-healing response that can ultimately lead to development of novel therapies that target SOX2-regulated pathways to restore healing in patients with DFUs.

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Novel approach for skin regeneration: mRNA-based telomerase enhancement of autologous skin cell suspension

D. Chang[1], R. Holgate[1], K. Court Pinto[1], E. Olmsted-Davis[1], A. Hopkin[2], L. Mangum[2], K. A. Bush[2], A. Quick[2], B. Godin[1], J. Cooke[1]
[1]*Departments of Cardiovascular Sciences and of Nanomedicine, Houston Methodist, Houston, Texas, United States,* [2]*Avita Medical Americas LLC, Valencia, California, United States*

The RECELL® Autologous Cell Harvesting Device enables point-of-care preparation of autologous skin cell suspension (ASCS). Currently, ASCS is approved for the treatment of acute thermal burn injuries, with additional indications under investigation. We hypothesize the therapeutic potential of ASCS may be enhanced for skin regeneration and primed for skin rejuvenation by improving the cells' ability to divide and differentiate by reverse-aging through telomere extension. A method to safely extend telomeres in fibroblasts and myoblasts has been demonstrated using the delivery of modified mRNA encoding telomerase (TERT). This method increased telomerase activity, delayed expression of senescence markers, and increased proliferative capacity, while avoiding immortalization and insertional mutagenesis. Notably, our previous studies showed TERT mRNA therapy reversed vascular senescence and extended lifespan in progeria mice. The purpose of this work is to demonstrate feasibility of modifying telomerase activity of ASCS through delivery of TERT mRNA using lipid nanoparticles (LNPs). mRNA uptake and downstream protein expression were evaluated in vitro using various LNPs. Data from Incucyte and FACS experiments demonstrated onset of mRNA-LNP uptake with cationic LNPs in 15-30min, yielding transfection efficiency up to 95% within 12h. Telomerase activity was identified in transfected ASCS using a TRAP assay, with a significant increase in activity observed over control. Animal studies using an immunocompromised mouse transplanted with human derived ASCS are ongoing to understand the impact of TERT on skin regeneration. Methods have been developed to efficiently transfect ASCS with TERT mRNA and substantially increase telomerase activity. This novel approach holds promise for skin regeneration and rejuvenation and has the potential to be extended to other RNA-based cellular therapies aimed at cutaneous defects.

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Gene profiling of a 3D psoriatic skin model enriched in T cells: Downregulation of PTPRM promotes keratinocytes proliferation through excessive ERK signaling

G. Rioux[1, 2], F. Turgeon[1, 2], C. Grenier[1, 2], G. Le-Bel[1, 3, 4], S. Guérin[1, 3, 4], R. Pouliot[1, 2]
[1]*Centre de Recherche en organogénèse expérimentale de l'Université Laval/LOEX, Axe Médecine Régénératrice, Centre de recherche du CHU de Québec-Université Laval, Québec, Quebec, Canada,* [2]*Faculté de Pharmacie, Université Laval, Québec, Quebec, Canada,* [3]*Centre Universitaire d'Ophtalmologie-Recherche, Axe Médecine Régénératrice, Centre de recherche du CHU de Québec-Université Laval, Québec, Quebec, Canada,* [4]*Département d'Ophtalmologie, Faculté de Médecine, Université Laval, Québec, Quebec, Canada*

Psoriasis is a complex, immune-mediated skin disease involving a wide range of epithelial and immune cells. In this study, we examined the molecular pathogenesis of psoriasis using transcriptomic approaches. To achieve this purpose, we generated T-cell enriched human 3D skin models using lesional psoriatic skin cells ($PS^{+Tcells}$). The analysis of the $PS^{+Tcells}$ transcriptome identified 1015 differentially expressed genes compared to healthy controls ($HS^{+Tcells}$; fold change〈 2). Ingenuity Pathway Analysis highlighted the downregulation of the PTPRM gene that encodes protein tyrosine phosphatase receptor type M (PTPμ; fold change = -1.819; p-value = 0.046833), a protein that acts as an inhibitor of the extracellular signal-regulated kinase (ERK) pathway. Western blot analysis confirmed the decreased expression of PTPμ in $PS^{+Tcells}$ and showed elevated levels of phosphorylated ERK1/2 in PS+Tcells compared to healthy controls. To validate the involvement of the ERK signaling pathway in keratinocytes proliferation, $PS^{+Tcells}$ were treated with BI-D1780, an inhibitor of the ERK1/2 downstream target ribosomal s6 kinase involved in this pathway. Treated $PS^{+Tcells}$ showed a decrease in epidermal thickness as well as a reduction in Ki67-positive cells. These results suggest, for the first time, a contribution for the product of the PTPRM gene in the pathogenesis of psoriasis. Due to its involvement in the excessive proliferation of pathological keratinocytes, the PTPμ/ERK signaling pathway may hold promise for the treatment of psoriasis.

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Acceleration of diabetic wound closure by rescue of deficient serpin inhibitor protein (SERPIN) in extracellular vesicles

D. Park[1], E. Duggan[2], R. A. Dorschner[3], M. Dobke[1], J. Nolan[2], B. Eliceiri[1]
[1]Surgery, University of California San Diego, San Diego, CA, Afghanistan, [2]Scintillon Institute, San Diego, California, United States, [3]Dermatology, University of California San Diego, San Diego, California, United States

Chronic metabolic diseases such as diabetes are characterized by delayed wound healing and a dysregulation of the inflammatory phase of wound repair. Our study focuses on changes in the payload of extracellular vesicles (EVs) communicating between immune cells and stromal cells in the wound bed, which regulate the rate of wound closure. Adoptive transfer of EVs from genetically defined mouse models are used here to demonstrate a functional and molecular basis for differences in the pro-reparative biological activity of diabetic (db/db) vs. wildtype EVs in wound healing. We identify several members of the Serpin family of serine protease inhibitors that are absent in db/db EVs, then we overexpress Serpin A1, F2 and G1 in EVs to evaluate their effect on wound healing in db/db mice. Serpins have an important role in regulating levels of elastase, plasmin and complement factors that coordinate immune cell signaling in full thickness wounds in a diabetic model. Here, we establish a novel therapeutic approach by engineering the payload of EVs based on proteomic analysis. Serpin-loaded EVs were used to rescue the Serpin deficiency identified by proteomics and promote wound healing in db/db mice, as well as evaluated how EVs affected extracellular matrix remodeling and the resolution of tissue injury. Therefore, we propose that the identification of EV payloads that are downregulated in diabetic wounds can be systematically analyzed for their functional activity and potential as a therapeutic, based on whether their re-expression in engineered EVs restores normal kinetics of tissue repair in chronic wounds.

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TSG-6 and TSP-1 exhibit altered expression in the skin of diabetic patients

J. P. Gallop[1], M. Alipour[3], Y. Wang[3], G. Botek[4], E. Maytin[2, 3]
[1]Lerner College of Medicine, Cleveland Clinic, Cleveland, Ohio, United States, [2]Dermatology and Plastic Surgery Institute, Cleveland Clinic, Cleveland, Ohio, United States, [3]Department of Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio, United States, [4]Orthopedic Surgery and Rheumatological Institute, Cleveland Clinic, Cleveland, Ohio, United States

Diabetes Mellitus and chronic hyperglycemia are associated with many complications including delayed wound healing and diabetic foot ulcers. Mechanisms behind this wound healing failure remain poorly understood but may include differential expression of two molecules, tumor necrosis factor stimulated gene 6 (TSG-6) and thrombospondin 1 (TSP-1). These proteins, which interact with each other, play important roles in wound healing and display altered expression in diabetic mouse models and cell cultures. To our knowledge, the relationship between TSG-6 and TSP1, diabetes, and wound healing has never before been studied in humans. Here, we undertook a prospective study of diabetic and non-diabetic patients with chronic wounds who were recruited from a podiatry clinic. Tissue samples and clinical data (i.e. HbA1c, wound size, demographics, confounding medical history) were collected at each visit. Western blot and qPCR were performed on tissue samples to assess levels of TSG-6, TSP-1, pro- and anti-inflammatory cytokines, and leukocyte markers. Similar clinical and molecular data were also collected from lower extremity amputations in a cross-sectional tissue study. Skin of normoglycemic patients (defined as HbA1c ≤ 6.4) had significantly lower levels of TSP-1 than skin of patients with poor glycemic control ($p=0.02$). Conversely, levels of TSG-6 were lower in the skin of patients with poor glycemic control. We have demonstrated a clear association between glycemic control and levels of TSG-6 and TSP-1 proteins, which regulate inflammation and angiogenesis. This suggests a new mechanism for impaired wound healing in diabetes. Our translation of this conceptual paradigm from mice to humans increases its clinical relevance and offers potential for new predictive biomarkers and biological targets for future treatment of non-healing diabetic wounds.

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Structural and molecular similarities between plantar and wound keratinocytes - is the foot a chronic wound?

C. Fuchs[1, 2], K. J. Stalnaker[1], Y. Wang[1, 2], L. Pham[1], C. L. Dalgard[3, 4], S. Cho[5], R. R. Anderson[1, 2], J. H. Meyerle[5], J. Tam[1, 2]
[1]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Department of Dermatology, Harvard Medical School, Boston, Massachusetts, United States, [3]The American Genome Center, Uniformed Services University of the Health Sciences, Bethesda, Maryland, United States, [4]Department of Anatomy, Physiology and Genetics, Uniformed Services University of the Health Sciences, Bethesda, Maryland, United States, [5]Department of Dermatology, Uniformed Services University of the Health Sciences, Bethesda, Maryland, United States

Plantar skin is known for specializations such as lack of hair, accelerated epidermal turnover, poor barrier function, and expression of cytokeratin 9, but both clinically and scientifically it is often regarded as just thicker versions of “normal” skin. Using the porcine model (which we have found shares many structural and molecular similarities with human plantar skin, more so than rodent models), we recently found that plantar skin is more distinctive than previously recognized, including a unique gene expression profile, and substantial alterations in both tissue calcium distribution and store-operated calcium entry response, with broad implications as calcium is both a key regulator of keratinocyte differentiation and a critical component in second messenger signaling. As calcium is also an upstream injury signal, we developed a porcine plantar wound model to compare the healing response between different skin types, and found that plantar wounds had inherently slower closure rates, little wound contraction, and sustained abnormalities in post-closure epidermal architecture. Notably, while plantar keratinocytes are clearly distinct from their non-plantar counterparts, the former shares many morphologic and molecular similarities with wound keratinocytes from other parts of the body. We posit that plantar keratinocytes may constitutively exhibit certain wound-like phenotypes even in the absence of injury, which could lead to a proclivity towards delayed healing and ulceration that becomes most prominently manifested when combined with other extrinsic stressors, such as diabetes and aging.

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Culturing *ex vivo* skin explants under tension increases viability and tissue integrity

B. Szeder, S. W. Butler, S. K. Hirata Tsutsumi, D. Grussu, M. J. Conneely, R. P. Hickerson
University of Dundee School of Life Sciences, Dundee, United Kingdom

In vivo skin is under tension on the body. However, the gold standard of skin research model systems are *ex vivo* skin explants, prepared from “relaxed” tissue obtained from patients undergoing abdominoplasty surgeries. This tissue maintains almost all properties of native skin, but conventional culturing methods do not maintain *in vivo* tension. In our approach, skin explants are cultured at the air-liquid interface stretched in all directions, thus providing constant defined tension throughout the culture period. Our data suggests that skin cultured at an empirically determined optimal tension increases viability and cohesiveness of the tissue. Viability was assessed by measuring cell metabolic activity in intact tissue using the WST-8 assay. Cell proliferation was evaluated by staining for Ki-67. Finally, tissue integrity was evaluated by measuring intercellular space following toluidine blue staining. Tensioned and non-tensioned explants demonstrated a difference in all three assays. After two weeks in culture, the tensioned tissue showed twice the viability than that of skin cultured without tension (WST-8 assay). Tensioned cultures also exhibited a higher ratio of Ki-67 positive cells than non-tensioned samples. Tension also helped to maintain tissue integrity. Culturing without tension resulted in increased intracellular gaps in the epidermis suggesting decreased cell cohesiveness. When tension is maintained, intercellular spaces are smaller, and the cells show a higher degree of “connectedness”. These data taken together suggest that the application of constant optimized tension on skin can help maintain viability and tissue integrity. We can conclude that tension is an important factor involved in normal epidermal function, therefore culturing explants with tension enables us to mimic *in vivo* conditions with higher fidelity.

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Overexpression of Vgll3 induces cutaneous fibrosis in a mouse model of lupus-like autoimmunity using single-cell RNA analyses

M. Gharaee-Kermani, A. Billi, M. C. Hildebrandt, J. Martens, R. Wasikowski, J. M. Kahlenberg, J. E. Gudjonsson
University of Michigan, Ann Arbor, Michigan, United States

Fibrosis is characterized by collagen deposition, fibro/myofibroblast accumulation, and extracellular matrix remodeling. Fibrosis can be seen in autoimmune diseases where it may be widespread and affect organs beyond the skin, with high morbidity and mortality, and no effective treatment. In cutaneous lupus, scar formation after discoid lesion eruption may evolve from enhanced fibrotic phenotypes. Our research has shown that epidermal-directed overexpression of murine Vgll3 causes severe lupus-like skin lesions suggestive of discoid lupus erythematosus (DLE). Given the apparent fibrotic nature of the skin lesions in transgenic (TG) Vgll3 mice, we wanted to determine if Vgll3 induces fibrosis. We analyzed male and female TG and wild-type (WT) mice aged 2-3 months. Fibrotic biomarkers of human DLE and scleroderma were analyzed. Epidermal Vgll3 overexpression resulted in development of not only cutaneous inflammation, but also severe fibrosis characterized by significant expression of fibrotic biomarkers found in human DLE and scleroderma lesions. Overall, lesional Vgll3 TG skin exhibited higher expression of Col1a1, Col1a2, Tgfb1 and Ctgf. ScRNA-seq of Vgll3 TG lesional skin vs. WT skin demonstrated that the increased expression of these collagen genes was localized to fibroblast (FB) and myofibroblast populations. Four FB subclusters were identified across all samples, with one detected almost exclusively in Vgll3 TG skin. This subcluster was distinguished by higher expression of Col1a1, Col1a2, Tgfb1, Ly6c1 and Eln. The presence of this unique FB subcluster in the skin of Vgll3 TG mice suggests that epidermal overexpression of Vgll3 impacts fibrosis development, and there may be a role for this unique FB subcluster in early fibrosis.

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Regulation of human cutaneous wound healing by the FAAHP1 pseudogene

S. Y. Chen, B. Sun
Dermatology, University of California San Diego, La Jolla, California, United States

The study of individuals with exceptional phenotypes can lead to key scientific insights. Recently, a 69-year-old woman with remarkable tolerance to pain and accelerated cutaneous wound healing was found to have an 8 kb genomic deletion in the fatty acid amide hydrolase pseudogene, FAAHP1. Pseudogenes derive from protein-coding genes and have acquired mutations during evolutionary history that disable full protein-coding potential. They were long thought to be biologically inert. However, recent studies have demonstrated functional roles for pseudogenes in biology and disease, operating through DNA- and RNA-based mechanisms. Here, we aimed to elucidate how FAAHP1 regulates wound healing. Our central hypothesis is that FAAHP1 regulates expression of FAAH, which subsequently controls the production of fatty acid amides that critically moderate wound healing behavior of skin resident cells after injury. Using CRISPR interference and RNAi we found that FAAHP1 depletion leads to repression of FAAH, indicating that it is a positive regulator of its cognate gene. The FAAHP1 transcript contains conserved microRNA binding sites that are present in FAAH, which has raised the hypothesis that FAAHP1 sequesters microRNAs and releases post-transcriptional inhibition of FAAH. We performed FAAHP1 microRNA luciferase reporter assays in primary keratinocytes but observed no repressive activity. An alternative mechanism is that the FAAHP1 genomic locus itself may function as an activating element. Consistent with this possibility, FAAHP1 contains a 1.2 kb cis-regulatory element marked by histone 3-lysine 27 acetylation. Enhancer reporter assays and CRISPR/Cas9 deletion of the candidate enhancer will be performed to assess transactivation potential. Collectively, our data highlight a novel role for a functional pseudogene that regulates key functions of the skin and demonstrates the potential for individuals with exceptional phenotypes to bring new mechanistic understanding to processes such as wound healing.

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Deficiency of the TLR4 inhibitory homolog RP105 exacerbates fibrosis

W. Wang[1], S. Bale[1, 2], P. Verma[2], S. Hasan[2], B. Yalavarthi[2], P. Tsou[2], J. Varga[1, 2], S. Bhattacharyya[1, 2]
[1]Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]University of Michigan, Ann Arbor, Michigan, United States

The pathogenic networks of immune, vascular, and fibrotic processes underlying non-resolving fibrosis in SSc remain poorly understood. Our previous studies demonstrated that while TLR4 and its cognate damage-associated endogenous ligands (DAMPs) in SSc elicited potent profibrotic effects and myofibroblasts activation, genetic targeting of TLR4 or its DAMPs in mice accelerated fibrosis resolution. To prevent aberrant DAMP-TLR4 signaling, a variety of negative regulators evolved to dampen the magnitude and duration of the signaling. Radioprotective 105 KDa (RP105), a TLR4 homolog, competitively inhibits DAMP recognition of TLR4 ,and block TLR4 signaling in immune cells. However, the role of RP105 in TLR4-depedent fibrotic responses in SSc is unknown. Using unbiased transcriptome analysis of skin biopsies, we found that both TLR4 and its adaptor MD2 were elevated in SSc and significantly correlated with each other (r=-0.54, p=0.0062). In contrast, levels of RP105 and its adaptor MD1 failed to show significant elevation and association (r=-0.35, p=0.11). Notably, RP105 expression was negatively associated with the myofibroblasts marker alpha-smooth muscle actin (ASMA) in SSc fibroblasts (r=-0.53). In vitro, exogenous RP105 abrogated DAMP-induced fibrotic responses, while RP105-depleted fibroblasts showed exaggerated responses. Importantly, *in vivo* ablation of RP105 in mice aggravated skin fibrosis in complementary disease models and was associated with augmented TLR4 signaling. Thus, we identify RP105-MD1 as a novel cell-intrinsic negative regulator of TLR4-MD1-driven sustained fibroblast activation, constituting a critical regulatory network governing the fibrotic process. Moreover, we propose that impaired RP105 function in SSc might contribute to progression of the disease.

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Multicellular bioprinted skin directs the formation of human-like epidermal architecture and capillary formation in full-thickness wounds

A. M. Jorgensen[1], A. Gorkun[1], N. Mahajan[1], M. Wu[1], K. Willson[1], C. Clouse[1], C. Ahn[2], S. Lee[1], J. J. Yoo[1], J. Molnar[3], S. Soker[1], A. Atala[1]
[1]Wake Forest Institute for Regenerative Medicine, Winston-Salem, North Carolina, United States, [2]Department of Dermatology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States, [3]Department of Plastic Surgery, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States

Current skin substitutes fail to meet the need for skin replacement in full-thickness wounds. Bioprinting is a promising alternative method to generate skin substitutes, as it can replicate the structural organization of the skin into biomimetic layers. In prior studies, we demonstrated that wounds treated with bioprinted skin had accelerated wound healing driven by epithelialization with reduced contraction and immunofluorescent staining demonstrated a mixed composition of human and mouse cells in the healing wounds. The purpose of this study was to further characterize the human cells in the regenerated wound area, and examine their effect on epidermal maturation and neo-blood vessel formation. 2.5 x 2.5cm full-thickness excisional wounds on mice were treated with a bioprinted tri-layer skin constructs and followed out to 90 days. By day 21, complete epidermal coverage was confirmed by histological analysis. PCR and electrophoresis demonstrated the presence of both human and mouse mitochondrial markers; however, further histological characterization by immunofluorescent staining with human cellular marker Lamin AC was negative in the epidermis and positive in the dermis. This staining pattern was persistent at day 90, and the epidermis of bioprinted skin treated wounds had formed human-like rete ridges. Staining with CD146 and human hCD31 demonstrated the integration of human endothelial cells in dermal capillaries. Taken together, these findings show that bioprinted skin with multiple cell types provides a cellular bridge for epithelialization, directs the formation of epidermal rete ridges, and facilitates neovascularization. This technology may be a preferred treatment for full-thickness wounds in human patients.

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Preclinical assessment of MicroMatrix® + Cytal® and Integra® DRT in a porcine third-degree burn model

A. M. Jorgensen[1], R. Nelson[1], C. Clouse[1], C. L. Scott[1], U. Gandhi[1], J. Molnar[2], A. Atala[1], S. V. Murphy[1]

[1]*Wake Forest Institute for Regenerative Medicine, Winston-Salem, North Carolina, United States,* [2]*Department of Plastic Surgery, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States*

Third-degree burn wounds can cause devastating loss of functionality for patients. As such, platform technologies that allow effective wound management are needed. The purpose of this study is to describe the use of MatriStem™ urinary bladder matrix (UBM)-based products (MicroMatrix® + Cytal®) (MM+C) and a collagen and glycosaminoglycan (GAG) product (Integra® Dermal Regeneration Template (DRT)) (DRT) in a porcine third-degree burn wound model. Full-thickness third-degree burns were created on dorsal porcine skin, debrided using an excisional model, then treated with MM+C or DRT. Importantly, treatment with either MM+C products or DRT, resulted in an extracellular matrix composition and cellular organization similar to healthy skin. MM+C treatment promoted rapid wound closure and neo-epithelialization, while DRT treatment seemed superior in promoting a healthy and pro-regenerative dermal wound bed. Histological analysis demonstrated complete epidermal closure at various levels of maturation and presented mature, thick, and organized collagen bundles in all treated wounds. DRT treated wounds appeared to have more similar histological resemblance to native tissue compared to MM+C and untreated control. MM+C treated wounds appeared to have faster wound closure and epithelialization compared to untreated and DRT treated wounds. Multi-factor wound healing analysis demonstrated that both MM+C and DRT had favorable wound healing based on the metrics of contraction, epithelialization, and wound closure rate. Taken together, both MM+C and DRT resulted in effective wound closure. The synergistic benefits of both DRT (promoting healthy and pro-regenerative dermal wound bed) and MM+C treatment (promoting rapid wound closure and neo-epithelialization) could be explored for use in a combination device to facilitate full-thickness wound closure.

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Characterization of mechano-biological pathways involved in bioprinted skin remodeling

S. Anis, S. Soker, K. Willson, A. Atala

Biomedical Engineering, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States

Reorganization of fibrin is the first stage in the matrix maturation process of bioprinted skin and takes place over a few hours to a few days. Fibrin is a popular component of many bioinks and a provisional component of the extracellular matrix present during wound healing. Cells embedded in fibrin gels attach and exert traction forces to realign and/or degrade the fibrin fibers, and sense and respond to the surrounding substrate in a mechanical feedback loop. Such cellular actions may remodel the printed construct in such a way, rendering it no longer feasible for implantation. The goal of this study is to understand the maturation process of fibrin based bioprinted constructs to better engineer a 3D bioprinted skin. Results show that fibroblasts in fibrin hydrogels change the viscoelastic properties of the fibrin networks at the micron-scale, suggesting that the cells and fibrin network mechanically interact. Rheological analysis revealed a strain-stiffening pattern due to non-linearly elastic cellular traction forces, exhibiting strain-stiffening when subjected to large deformations. mRNA analysis shows that cells express elastin and collagen within the fibrin hydrogels. Phenotypic changes in fibroblasts were also observed where cells became more elongated over time. Overall, it was found that fiber alignment and changes in the mechanical environment regulate cell behavior and phenotype. The addition of a mechanical constraint, such as a plastic frame around the printed construct, significantly impacts the remodeling and maturation process of fibrin based 3D bioprinted skin constructs when compared to those that do not have any mechanical constraints. Fibrin is a widely used component of many bio-inks, therefore, information and data obtained from these experiments can be used to better understand the mechanobiology of cell-matrix interactions which has important implications for understanding the maturation process of fibrin in a printed construct to better engineer 3D bioprinted tissues.

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Deconstructing the dermal adipogenesis program during skin development and regeneration

L. Sun, X. Zhang, S. Wu, W. Liu, Y. Liu, Y. Liao, R. Wu, T. Xia, X. Zhang, M. Yin, Y. Yang, L. Zhang

School of Pharmaceutical Sciences, The State Key Lab of Cellular Stress Biology, Xiamen University, Xiamen, Fujian, China

Dermal white adipose tissue (dWAT), a unique layer which is enriched with adipocytes, preadipocytes and adipocyte progenitors, is indispensable for maintaining skin homeostasis and tissue regeneration during skin wound healing. However, the origin, developmental path and the cellular complexity and plasticity of dWAT lineage cells remain poorly understood. Here, by single-cell RNA-seq (scRNA-seq) analyses of dermal cell populations isolated from mice at various post-developmental ages or at indicated post-wounding days, we have carried out in-depth analysis to define how dermal adipocytes are differentiated from their progenitors and to identify key signaling pathways that regulate dermal adipogenesis. We have identified a dFB population of hypodermal interstitial adipose derived stem cell (HI-ADSC) in mouse skin, which is poised to differentiate into preadipocyte and lipid-laden adipocyte and is an important cellular source for dermal extracellular matrix during development and adult homeostasis. Pseudotime analysis of scRNA-seq and immunofluorescence analysis suggested that HI-ADSCs are highly plastic cells and these cells rapidly migrated into the wound center and may give rise to adipocyte lineage cells and myofibroblasts during wound healing, contributing to dermal regeneration. We further show that dermal adipogenesis during development and wound regeneration was dynamically regulated by an interplay between WNT and inflammatory signaling pathways, dysregulation of which was associated with abnormal wound responses in keloid. Together, our study has defined how dWAT lineage cells are regulated during skin development and regeneration and these results suggest that targeting adipogenesis maybe novel therapeutic approach for abnormal wound healing, such as chronic wounds or keloid.

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Metabolic crosstalk in the wound bed: How adipocytes and immune cells communicate during wound healing

M. Forni[1], T. Xu[1], W. Krause[1], R. Pannone[1], R. Kibbey[2], M. Rudolph[3], V. Horsley[1]

[1]*Molecular Cellular and Developmental Biology, Yale University, New Haven, Connecticut, United States,* [2]*School of Medicie, Yale University, New Haven, Connecticut, United States,* [3]*Harold Hamm Diabetes Center, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, United States*

After an injury, a timely inflammatory response that involves the recruitment of blood-derived circulating monocytes is essential for tissue repair. Once in the wound bed, these cells differentiate into macrophages during the inflammatory phase, a process that goes awry during aging in chronic non-healing wounds. Cell differentiation is a highly energy-demanding process, and metabolic substrates present in the wound bed niche are important for the wound healing outcome. We previously demonstrated that dermal adipocytes release fatty acids into wound beds after injury to promote inflammation, yet the function of adipocyte-derived fatty acids in the initiation of the inflammatory response after the injury is not known. To unveil the role of adipocytes as providers of fatty acids used to fuel the metabolic requirements of immune cell differentiation, we set out to evaluate their role on monocyte to macrophage differentiation in the skin wound bed. Here, we utilize *in vivo* mouse models of skin injury and metabolic assays to reveal that monocytes utilize fatty acids to fuel metabolic programs that induce macrophage differentiation. We show that extracellular vesicles (EVs) loaded with lipids are actively taken up by monocytes in vitro and in mice *in vivo*. Using real-time respirometry, we show that these fatty acids activate the b-oxidation metabolic pathway in monocytes and its inhibition leads to the abrogation of macrophage differentiation. Furthermore, we show that with age, not only adipocyte-derived EV production was impaired, but more importantly, these particles were less effective in rewiring monocyte metabolism towards oxidative phosphorylation. In summary, our findings reveal an essential adipocyte-monocyte metabolic axis that controls inflammation in the wound bed niche.

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Finding new therapeutical strategies for systemic sclerosis: SARA as a novel key molecule in myofibroblast transdifferentiation during fibrogenesis

K. Corano Scheri[1, 2], X. Liang[2], V. Dalal[1, 2], I. Le Poole[3], J. Varga[4], T. Hayashida[1, 2]
[1]Pediatric Nephrology, Ann and Robert H Lurie Children's Hospital of Chicago, Chicago, Illinois, United States, [2]Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [3]Dermatology, Microbiology and Immunology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [4]Internal Medicine, University of Michigan Michigan Medicine, Ann Arbor, Michigan, United States

Fibrosis is the hallmark of systemic sclerosis (SSc), an autoimmune disorder that affects 300,000 people in the US. Myofibroblasts are the cells responsible for the unbalanced deposition of the extracellular matrix and preventing their transdifferentiation might significantly improve the current therapeutical approaches. Smad Anchor for Receptor Activation (SARA) plays a critical role in maintaining epithelial cell phenotype. Mice overexpressing SARA specifically in PDGFRb+ pericytes and pan-leukocytes (SARATg) developed significantly less skin fibrosis in response to bleomycin injection compared to wild-type littermates (SARAWT). Single cell RNASeq analysis of skin PDGFRb+ cells identified a cluster of pericytes that assumes myofibroblast features upon bleomycin treatment. In addition, after fibrotic stimuli, a significant enrichment in a cell cluster expressing Retnla, a target of Th2-specific IL-31 pathway and macrophage activation, was observed in skin of SARAWT but not in SARATgmouse. IL-31 expression was also increased in SARAWT mouse skin treated with bleomycin, as well as in SSc patient skin samples. Inter-cluster interaction analysis revealed that signals from the lymphocytes in SARAWT mice regulate pericytetransdifferentiation, whereas with SARA overexpression the fibrogenic signals in the pericytes were suppressed. Moreover, a pericyte cluster was enriched with Lgals1, which encodes galectin 1, that strongly induces lymphocyte apoptosis. These data suggest a critical role for SARA in a novel crosstalk between myofibroblast precursors and immune cells in the pathogenesis of SSc.

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Development of a wound healing model produced from diabetic patient cells

M. M. Lemarchand[1, 2], T. De Serres-Bérard[1, 2], S. Bellenfant[1, 2], T. Galbraith[1, 2], Y. Douville[1, 2], F. Berthod[1, 2]
[1]CHU de Quebec-Universite Laval, Quebec, Quebec, Canada, [2]Surgery, Universite Laval, Quebec, Quebec, Canada

Diabetic patients suffer from frequent formation of sores on the foot, called diabetic ulcers (DU), that are struggling to heal and subject to frequent infections that may require amputation. Our main hypothesis is that the lack of innervation in the lower limb of diabetic patient is partly responsible for the impairment of the wound closure, as sensory neurons are involved in the regulation of the inflammation and reepithelialization through the release of neuropeptides (SP,CGRP). In order to advance on a treatment for DU, we propose to replicate a complete human skin in the laboratory using skin cells extracted from diabetic patients. Our first aim is to assess the ability of our diabetic wound healing model (dWHM) to recapitulate the characteristic of a DU and then develop a new strategy to improve reepithelialization. Methodology: Populations of fibroblasts and keratinocytes were isolated from the diabetic patient skin harvested after a foot amputation. The dWHM is obtained by seeding these diabetic population of keratinocytes and fibroblasts and healthy endothelial cells on chitosan-collagen sponges. Once the epidermal layer is mature, a wound is performed with au biopsy punch on the sponge. The reepithelialization is then monitored during 8 days, with or without the addition of neuropeptides in the culture medium. Controls are produced the same way using age-matched healthy fibroblasts and keratinocytes. Results: Diabetic cells were able to form mature epidermis. dWHM were unable to close without treatment in contrast to healthy skin model. The addition of neuropeptides in the culture medium successfully improve the reepithelialization of the dWHM. The formation of capillary network, inhibited in the dWHM, is stimulated with the addition of the neuropeptides. Conclusion: We successfully produce a wound healing model able to recreate the condition of a diabetic ulcer, which is suitable for the development of a treatment for DU. We demonstrate that the be a good strategy to advance on a cure for DU.

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Endosomal GLUT3 is essential for macrophage signaling, polarization, and function in wound healing and atopic dermatitis

D. Yu[1], J. Zhao[2], E. Lee[1], E. Kolitz[1], R. Mahapatra[1], R. C. Wang[1]
[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Harvard Medical School, Boston, Massachusetts, United States

Macrophages are immune cells that play critical roles in both inflammation and tissue homeostasis. Classically activated (M1) macrophages promote antimicrobial and tumoricidal activity, while alternatively activated (M2) macrophages promote phagocytosis and tissue homeostasis. The facilitative GLUT1 and GLUT3 hexose transporters are expressed abundantly in different hematopoietic lineages, but their specific roles in macrophages, and leukocytes in general is poorly understood. We discovered that GLUT3 expression was increased after M2-activation stimuli in macrophages. Notably, GLUT3 KO BMDM (bone marrow-derived macrophages) showed marked defects in M2, but not M1, polarization. Consistent with defects in M2 polarization, GLUT3 KO macrophages showed impaired wound healing and decreased inflammation in calcipotriol-induced, atopic dermatitis-like inflammation. Mechanistically, we found that the IL-4-STAT6 axis, the main signaling pathway for M2 polarization, was strongly impaired by GLUT3 deficiency. Unlike GLUT1, which localized to the plasma membrane, GLUT3, along with components of the IL-4 signaling pathway, localized to endosomes. The depletion of GLUT3 in a variety of cancer cell lines revealed an essential role for GLUT3 in STAT3 signaling, suggesting broader roles for GLUT3 in the regulation of signaling endosomes. Thus, our studies reveal that GLUT3 is essential for macrophage polarization and function and suggest a more broadly conserved role in the regulation of endosomal signaling.

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Baicalin accelerates wound healing in a murine model of ischemic pressure ulcer lesions

E. Kim[1], J. Kim[1, 2], J. Lee[1, 2]
[1]Dermatology and Cutaneous Biology Research Institute, Severance Hospital, Yonsei University College of Medicine, Seodaemungu, Seoul, Korea (the Republic of), [2]Scar Plastic Surgery and Laser Center, Yonsei Cancer Center, Seodaemun-gu, Seoul, Korea (the Republic of)

Pressure ulcers are a common skin disease that affects the elderly and patients with perceptual or mobility disorders. A pressure ulcer is an ischemic skin necrosis or ulcer that occurs when pressure is applied to a region of the body for an extended period of time, producing poor blood circulation and a lack of oxygen and nutrients. Natural origin pharmaceuticals account for more than half of all modern drugs, and they play an essential role in drug development programs. Baicalin is one of the most important flavonoids in plants, and it is responsible for their pharmacologic effects. This type of natural ingredients provide a wide range of pharmacologic benefits, including anti-inflammatory and anti-tumor properties. The two key processes linked with wound development and effecting the wound healing process with baicalin antioxidant in treating pressure ulcers are oxidative stress and inflammation. However, the effect of baicalin on wound healing in pressure ulcers has not been thoroughly investigated. The wound healing efficacy of baicalin in treating pressure ulcers in a murine pressure ulcer model is investigated in this study. First, the development of 1, 2, 3, 4 stage pressure ulcers after ischemia-reperfusion(I/R) cycle in a murine mouse model. In this model, we investigated the effectiveness of a baicalin in the wound healing of pressure ulcers. We found that baicalin promotes granulation tissue formation, accelerated wound closure, reduced proinflammatory cytokines production and inducing the differentiation of keratinocytes were observed. We expect that this study will contribute to the potential wound therapeutic agent for a pressure ulcer patient.

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Topical thyroid hormones as novel endocrine wound healing promoters in human skin *ex vivo*

J. Gherardini[1, 2], F. D. Scala[2], E. J. Horesh[2], G. Epstein-Kuka[3], J. Rodriguez-Feliz[4], J. Chéret[2], R. Paus[1, 2, 5]
[1]*Monasterium Laboratory Skin & Hair Research Solutions GmbH, Munster, Nordrhein-Westfalen, Germany,* [2]*Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States,* [3]*Foundation for Hair Restoration, Miami, Florida, United States,* [4]*Skin & Hair, Plastic Surgery, Coral Gables, Florida, United States,* [5]*CUTANEON, Hamburg, Germany*

Skin ulcers (SU) severely impact the quality of life of millions of patients worldwide, steadily increase in prevalence, and are associated with major secondary morbidity and increased mortality. Therefore, more effective, safe, and affordable SU therapies are urgently required. Triiodothyronine [T3] and/or thyroxine [T4]) are inexpensive, clinically widely used thyroid hormones with well-characterized toxicological profiles that easily penetrate human skin. Topical T3 promotes skin wound healing (WH) in mice, and T4 human skin *ex vivo* when added to the culture medium. While this makes T3/T4 attractive candidate WH promoters, it remains to be tested how topical application of 100pM of T3 or 100nM of T4 (in a vehicle that can be used for SU therapy) impacts on the different mechanisms of human skin WH (i.e. angiogenesis, re-epithelialization). To do so, we have topically applied a thermodynamic gel (solidifying at room temperature) constituted of Poloxamer 407, Glycerol 85% and Polihexanid 20%) containing either 100pM of T3 or 100nM T4 for 5 days to organ-cultured, experimentally wounded human skin. Our preliminary data show that both T3 and T4 significantly promote epithelial tongue (ET) length and area. This was confirmed by the significant increase of proliferation within the ET and within 100µm next to it while apoptosis was not affected. Protein expression of cytokeratin (CK) 6, a marker of hyperproliferation, and the epithelial stem cell marker, CK15, was also significantly increased by 100pM of T3. In addition, 100nM of T4 stimulated angiogenesis *ex vivo* in the dermal skin fragment. Thus, short-term pulse monotherapy with inexpensive and safe topical T3/T4 targets multiple key WH processes and might become a game-changer in clinical SU management.

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Chronic skin wounds of genetically modified (db/db) and diet-induced diabetic mice have altered levels of TSG-6 and TSP-1

M. Alipour[1, 2], J. A. Mack[1], E. Maytin[1, 2]
[1]*Cleveland Clinic Lerner Research Institute, Cleveland, Ohio, United States,* [2]*Cleveland State University, Cleveland, Ohio, United States*

Chronic non-healing wounds are a major complication of diabetes. Effective communication between immune cells and the extracellular matrix (ECM) is crucial for regulating wound healing. Tumor necrosis factor-stimulated gene-6 (TSG-6) modifies the extracellular matrix (ECM) and regulates inflammation; Thrombospondin 1 (TSP-1) regulates angiogenesis and binds to TSG-6. In both TSG-6 and TSP-1 knockout mice, wound closure is delayed. We hypothesize that hyperglycemia causes dysregulation of TSG-6 and TSP-1, which together results in fewer heavy chain-hyaluronan complexes (HC-HA) in the ECM, an acceleration of leukocyte infiltration, a pro-inflammatory milieu, and wound healing delay. In this study, dysregulation of TSG-6 and TSP-1 was examined in multiple diabetic models. Our diet-induced diabetic wound model is novel and resembles human diabetes in terms of endocrine dysfunction and delayed healing. Feeding mice a high-fat diet (HFD) for 8 months failed to generate hyperglycemia or cause wound closure delay. The high-fat diet was then modified by adding low dose streptozotocin (STZ) injections, testing various factors to arrive at a successful HFD+STZ protocol that rendered animals diabetic but still able to survive the stress of wounding. HFD+STZ mice showed higher body weights and higher serum glucose levels than mice on a normal diet (p= 0.015, p=0.004 respectively; n=6). Wound closure was delayed in the HFD+STZ group at day 5 post wounding (p=0.0018).Tissues were analyzed for levels of TSG-6, TSP-1, anti-inflammatory cytokines, and leukocyte markers using western blot and qPCR. Both db/db and HFD+STZ models showed lower levels of TSG-6 and higher levels of TSP-1. Understanding the link between hyperglycemia-induced alterations of TSG-6 and TSP-1 and persistent inflammation may help to direct development of a therapeutic agent to prevent and manage non-healing diabetic wounds.

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Dendritic cells express the receptor Axl in wound healing

O. Justynski[1], V. Horsley[1, 2]
[1]*MCDB, Yale University, New Haven, Connecticut, United States,* [2]*Dermatology, Yale School of Medicine, New Haven, Connecticut, United States*

The skin has an amazing capacity for repair, which requires the coordination of many different cell types and cellular signals. Insufficient repair leads to chronic or non-healing wounds that cause high healthcare costs and decreased quality of life for patients. Inflammation is essential for proper healing and requires the infiltration and activation of many immune cells, some of which are activated by detecting cellular damage and death via damage-associated molecular patterns, which activate toll-like receptor (TLR) signaling. Interestingly, TLRs also induce the expression of the receptor tyrosine kinase Axl, which binds apoptotic cells and alters immune cell activation. Thus, I hypothesize that the detection of dead and dying cells in skin wounds may be crucial for the inflammatory phase of repair. I find that skin wounds in mice display elevated mRNA and protein expression of the receptor Axl from 3 to 24 hours after injury in CD11b+CD11c+ dendritic cells. Consistent with activation of Axl signaling, I find that mRNA expression for the Axl-responsive proteins SOCS1/3 are upregulated at 24 hours after injury and later. Additionally, Axl+ cells are often adjacent to macrophages in early wound beds and I find that macrophages express Gas6, the ligand for Axl. Interestingly, the canonical TLR3 signaling pathway known to upregulate Axl expression can be induced in the skin via injection of the TLR3 agonist Poly(I:C), which stimulates upregulation of the type 1 interferon Ifnb. However, a TLR3-independent mechanism induces Axl upregulation during skin repair in the absence of TLR3, indicating the potential for a complex and redundant pathway upstream of Axl expression. Based on these data, I hypothesize that Axl primes the early wound environment for proper inflammation to coordinate healing. In future work I will determine if Axl is necessary for wound repair to define the molecular mechanisms by which cell death controls tissue repair.

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Complete wound healing utilizing procine urinary bladder matrix in a serise of traumatic wounds with significant soft tissue loss

J. Rahesh, K. Holder
Texas Tech University System, Lubbock, Texas, United States

PUBM is a non-synthetic, completely resorbable xenograft product with a myriad of uses including management of burns, acute and chronic wounds, soft tissue reinforcement, and hernia repair. The material is available in both powder and sheet form allowing for excellent coverage of irregularly shaped wounds. We present three patients with significant soft tissue loss that underwent xenograft implantation utilizing PUBM. Despite challenging locations and severity of wounds, 100% healing and wound closure with excellent cosmetic results was achieved. Data and information were collected via retrospective chart review from the initial emergency room visit, otolaryngology consultation, initial acute care, surgical management, and clinic follow up for a minimum of one year. PUBM appeared to be an excellent alternative to skin grafting in patients with large, traumatic wounds on cosmetically sensitive areas.

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The "T" in cutaneous wound healing

U. Onay[1], D. Xu[2], D. Biyashev[1], M. Demczuk[1], S. Evans[1], J. Podojil[2], S. Miller[2], K. Lu[1]
[1]Dermatology, Northwestern University, Chicago, Illinois, United States, [2]Microbiology-Immunology, Northwestern University, Chicago, Illinois, United States

Immune-modifying particles (IMPs) composed of biodegradable poly(lactic-co-glycolic acid) (PLGA-IMP) are taken up by circulating inflammatory monocytes via scavenger receptor MARCO and lead them to traffic to the spleen where they undergo apoptosis. PLGA-IMPs has been demonstrated to improve immune-mediated pathology in diverse tissues and inflammatory conditions. Here we report using intravenous PLGA-IMPs for severe acute skin inflammation in a C57/BL6 model of injury induced by topical nitrogen mustard (NM) exposure. PLGA-IMPs significantly reduced skin edema and delayed eschar formation in NM-insulted mouse skin. Here we show that daily intravenous administration of PLGA-IMP post NM cutaneous insult not only significantly decreased infiltration and activation of inflammatory monocytes into the wound area ($p<0.025$, n=5), but also resulted in enrichment of natural FoxP3+ regulatory T cells (nTreg) in wound tissue as early as day2 post NM insult ($p=0.022$, n=5). To understand the potential role of nTreg enrichment on reduction of inflammatory monocytes and inflammatory cytokines in the eschar, as well as resolution of the inflammation, we examined the effects of nTreg depletion/inhibition following anti-CD25 depletion. While significantly reducing nTregs ($p=0.0003$, n=5), CD25 depletion also reduced the expression of immune inflammatory and regulatory markers such as ST2, IL10-Tgfb, and PD-1 ($p<0.001$, n=5). In addition, reduction of nTregs following CD25 depletion reduced the numbers of IL10-Tgfb producing macrophages ($p=0.0015$, n=5). Infiltration of nTregs into skin wounds has been previously reported at days 5-7 during wound resolution. Importantly, our data shows the PLGA-IMP treatment accelerates accumulation of Tregs in the skin to as early as day2 post NM insult indicating that it may serve as a novel disease modulatory treatment. We are confident that PLGA-IMPs are a novel therapeutic that protects mice from severe injury following toxic exposure to a chemical insult.

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Topical suppression of miR-193b-3p promotes diabetic wound healing

J. Marjanovic, I. Jozic, R. Stone, B. A. Abdo Abujamra, R. Kirsner, H. Lev-Tov, I. Pastar, M. Tomic-Canic
Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States

Negative outcomes associated with diabetic foot ulcers (DFUs) are devastating, ranging from lower limb amputations to death. One of the major contributing factors of impairment in DFUs healing is inefficient re-epithelialization marked by keratinocyte hyperproliferation and poor migration. Thus, we focused on the role of master-regulators, microRNAs (miRs), in regulation of re-epithelialization in DFUs. We collected tissue samples from DFUs (n=15), control skin samples (n=15), and human acute wounds (n=3) and performed LCM and transcriptomic analyses using Ingenuity Pathway Analysis (IPA). qPCR was used to analyze miR-193b-3p and its target genes. We performed gain- and loss-of function experiments. Wound re-epithelialization was evaluated using human *ex vivo*, diabetic murine *in vivo*, and human organotypic wound models. Formation of stress fibers was studied using immunocytochemistry and RhoA pull-down assay. miR-193b-3p was found significantly induced in the epidermis of DFUs, but not in acute wounds. Moreover, knockdown of miR-193b-3p promoted migration of human keratinocytes. The inhibition of miR-193b-3p expression in *ex vivo* human wounds and diabetic murine *in vivo* wounds accelerated re-epithelialization. Conversely, overexpression of this miR impaired re-epithelialization in a 3D organotypic wounds. The underlying mechanism of miR-193b-3p anti-migratory activity is through a disruption of stress fiber formation and a reduction of RhoA activity. Further in-depth genomic analysis revealed the target network of miR-193b-3p contributes to poor healing in DFUs. We confirmed suppression of downstream targets of miR-193b-3p in DFUs and further showed that this transcriptomic landscape found in non-healing DFUs can be reversed upon miR-193b-3p suppression. Together, we identified miR-193b-3p as an important regulator that represents a target for promotion of wound healing in DFUs.

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Senescent adipocytes accumulate in the dermis of obese mice deficient in leptin signaling

E. Y. Lee[1, 2], R. Perez-Lorenzo[1], S. Youssef[1], A. M. Christiano[1, 3]
[1]Dermatology, Columbia University Irving Medical Center, New York, New York, United States, [2]Medical Scientist Training Program, Columbia University Irving Medical Center, New York, New York, United States, [3]Genetics and Development, Columbia University, New York, New York, United States

The hair follicle is a complex mini-organ that undergoes continuous cycles of growth and regeneration. Hair cycling depends on tightly coordinated crosstalk between epithelial cells, fibroblasts, immune cells, and adipocytes. Recent studies demonstrated that in addition to metabolic dysregulation and neurocognitive impairment, mice that are genetically deficient in leptin (ob/ob) or its receptor (db/db) exhibit a prolonged telogen, suggesting a role for obesity and/or leptin signaling in the control of hair cycling. Cellular senescence is a state of irreversible cell cycle arrest in which cells also secrete pro-inflammatory cytokines (known as the senescence-associated secretory phenotype; SASP) that promote aging and age-related disorders. Previous studies showed that adipocytes in obese ob/ob and db/db mice are senescent, and that their SASP drives pathology by recruiting macrophages. Our laboratory previously established that Trem2+ macrophages promote hair follicle stem cell quiescence (HFSC) during telogen. However, whether these macrophages are responsible for the prolonged telogen phenotype observed in leptin signaling-deficient mice, and whether their recruitment depends on the presence of senescent dermal adipocytes in these mice, remain unknown. Here, we show that the dermal adipocyte layer is expanded in db/db mice, and is characterized by an accumulation of senescent adipocytes that showed upregulated SASP expression. Furthermore, db/db skin was defined by an increased infiltration of Trem2+ macrophages. We postulate that the prolonged telogen phenotype in db/db mice is driven by the presence of senescent adipocytes, which in turn recruit HFSC quiescence-promoting Trem2+ macrophages via SASP. Our studies suggest a physiological role for senescent adipocytes in hair follicle biology, and further underscore the importance of adipocyte and immune cell crosstalk in hair cycle control.

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ECRG4 regulates neutrophil responsiveness to proinflammatory signals during recruitment to infection

K. D. Pool[1], B. Eliceiri[2], R. A. Dorschner[1]
[1]Dermatology, University of California San Diego, La Jolla, California, United States, [2]Surgery, University of California San Diego, La Jolla, California, United States

Rapid neutrophil recruitment to cutaneous injury is critical for controlling local infection and preventing systemic dissemination. Neutrophils are mobilized from bone marrow reserves and home to vasculature near the source of inflammation, where they extravasate and migrate to the site of injury. We found that Esophageal Cancer Related Gene 4 (ECRG4), a leukocyte surface protein, amplifies early neutrophil recruitment to injury and hypothesized that ECRG4 may be important for the inflammatory response to infection through its ability to regulate neutrophil recruitment. Using an intradermal Methicillin Resistant Staphylococcus Aureus (MRSA) infection model, we found that ECRG4 KO mice developed 2-fold larger lesions that persisted longer ($P<0.001$), had a 25% decrease in neutrophil recruitment at 24 hours ($P<0.01$) and had increased bacteria in their lesions, lung and spleen. Infected skin had similar expression of TNFa and 10-fold higher IL-1b in KO mice ($P<0.01$), suggesting that local production of chemotactic signals is intact. Ex-vivo, KO neutrophils demonstrated a 50% decrease in migration to C5a ($P<0.01$) and LPS, but not fMLP. In-vivo, KO mice had decreased neutrophil mobilization from the bone marrow in response to systemic LPS ($P<0.05$). Evaluation of adhesion molecules on KO mouse neutrophils demonstrated a 35% greater induction of CD11b following activation with LPS ($P<0.01$). These data show that loss of ECRG4 results in decreased neutrophil recruitment to cutaneous infection with decreased responsiveness of KO neutrophils to chemoattractants, such as C5a and LPS, as well as increased expression of adhesion molecules that may retain neutrophils in the bone marrow. These results support the hypothesis that ECRG4 amplifies neutrophil recruitment to cutaneous infection by regulating their responsiveness to chemoatractants and mobilization from bone marrow reserves, which positions it as a therapeutic target for antimicrobial therapies.

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Optimal tension facilitates wound healing in a full-thickness *ex vivo* human skin model

M. J. Conneely[1], D. Grussu[1], S. K. Hirata Tsutsumi[1], S. Roque[1], P. A. Campbell[2], R. P. Hickerson[1]

[1]*Biological Chemistry and Drug Discovery, University of Dundee School of Life Sciences, Dundee, United Kingdom,* [2]*School of Science and Engineering, University of Dundee, Dundee, United Kingdom*

The use of human skin models is a widely accepted approach for studying skin biology. These models include organotypic systems reconstructed from human cells as well as models based on discarded surgical tissue. Cell-based systems are time-consuming to set up and maintain, are unable to fully recapitulate the differentiated architecture of the skin, and typically lack minor skin cell populations. Traditional full-thickness skin models prepared from excised skin tissue collected during surgery quickly lose viability and the ability to respond to stimuli once off the body. Traction-force balance is essential for maintaining skin homeostasis and is, therefore, an important factor in regulating both tissue structure and physiological function. Here we describe a tension-based skin explant model to address the unmet need for a human skin model that reliably mimics *in vivo* skin even in complex biological processes such as wound response and healing. This tension-based model mimics an *in vivo*-like response to laser ablative wounding, with keratin 17 observed throughout the wounded skin sample, whereas only minimal expression is observed in skin cultured without tension. Additionally, observation of the wound sites for up to 3 weeks shows that reformation of the basement membrane is highly dependent on the presence of tension. qPCR analysis of a variety of wound healing markers also showed a delayed and diminished response to wounding in non-tensioned skin compared to skin cultured at optimal tension. Application of optimized tension can therefore restore skin's inherent mechanobiology, enabling a more *in vivo*-like behavior, greatly increasing the utility of full-thickness *ex vivo* skin models. Conflict of interest: MJC and RPH are founders and directors of Ten Bio Ltd, a company focused on development of *ex vivo* skin models.

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Interleukin-17 pathway activation in radiation dermatitis

Y. Kost[1], A. Muskat[1], K. Mieczkowska[1], A. Deutsch[1], K. Shinoda[2], B. McLellan[1]
[1]Dermatology, Albert Einstein College of Medicine, Bronx, New York, United States, [2]Medicine, Albert Einstein College of Medicine, Bronx, New York, United States

Each year, 10 million cancer patients are treated with radiotherapy (RT) worldwide. RT is paramount in cancer treatment, however up to 95% of patients develop radiation dermatitis (RD). Patients with RD develop painful skin breakdown, which can be therapy limiting and detrimental to quality of life. No evidence-based standard for the management of RD exists, which highlights the inadequate understanding of its pathogenesis. The pro-inflammatory cytokine interleukin-17 (IL-17) is known to play a role in numerous inflammatory skin conditions. As preclinical studies suggest that IL-17 is upregulated in RD murine models, we examined IL-17 pathway activation in RD. Using a murine RD model receiving a single dose of 25 Gy, we demonstrated strong correlation between IL-17 target gene upregulation and increased severity of RD in irradiated skin via qRT-PCR ($p < 0.001$). Additionally, we utilized single cell RNA-sequencing (scRNA-seq) to profile cells from sham and irradiated murine skin and identified a novel keratinocyte subtype exclusive to the irradiated group with abundant IL-17 Receptor Type C (IL-17RC) expression. Both genetic knockout of IL-17RC and blocking IL-17A, the ligand of IL-17RC, with neutralizing antibody independently prevented severe RD in irradiated murine models. Finally, we confirmed IL-17A target gene upregulation in human stratum corneum of patients treated with RT using tape strip gene expression profiling, a non-invasive skin sample collection method for gene expression analysis. With IRB approval, we collected tape strip samples from patients (n=6) receiving ≥15 fractions of RT for breast cancer before and after RT from the patient arm and breast. Tape-strip gene-expression profiling was conducted via qRT-PCR, which revealed induction of an IL-17 pathway target by 12.18-fold on average in irradiated skin ($p< 0.05$ by one sided paired t-test). These results support the role of IL-17 in RD development and provide a basis for future clinical trials investigating anti-IL17 therapies for RD.

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Cutaneous leukocyte-endothelial interactions predict outcomes after hematopoietic cell transplantation

I. Saknite[1, 2], J. R. Patrinely[1, 3], Z. Zhao[1, 3], H. Chen[4], T. Kim[5, 6], M. Jagasia[6], M. Byrne[5, 6], E. R. Tkaczyk[3, 1, 6]
[1]Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States, [2]Biophotonics Laboratory, Latvijas Universitate, Riga, Latvia, [3]US Department of Veterans Affairs, Nashville, Tennessee, United States, [4]Biostatistics, Vanderbilt University Medical Center, Nashville, Tennessee, United States, [5]Hematology/Oncology, Vanderbilt University Medical Center, Nashville, Tennessee, United States, [6]Vanderbilt-Ingram Cancer Center, Nashville, Tennessee, United States

Hematopoietic cell transplantation(HCT) is a potential cure for hematologic malignancy, but is associated with a risk of relapse and death. Dynamic biomarkers to inform treatment decisions after HCT are a major unmet clinical need. We quantified cutaneous leukocyte-endothelial interactions and tested their associations with patient outcomes. METHODS: In this prospective cohort study, patients underwent noninvasive skin videomicroscopy. In videos of dermal microvascular flow, blinded observers counted leukocytes adherent to and rolling along the vessel wall per hour(A&R). RESULTS: Among 56 patients(median age, 59 years; 38 male, 18 female) imaged a median of 40 days after HCT, 21 had high and 35 had low A&R. After correcting for the revised disease risk index (rDRI), patients with high A&R had greater rates of relapse (HR, 5.40 [95% CI: 1.45–20.08]; p=0.01), reduced relapse-free survival(RFS; HR, 3.71 [1.36–10.09]; p=0.01), and reduced overall survival (OS; HR, 3.04 (1.01–9.13); p=0.05). In the prognostic adequacy calculation by using Cox models, our new imaging biomarker (A&R) accounted for 78% - 92% of the prognostic information to predict each outcome. By contrast, the best existing clinical predictor of rDRI accounted for 13% to 31% of the prognostic information in the same model. CONCLUSIONS: Leukocyte-endothelial interactions, visualized directly in patients' skin via live videomicroscopy, are strongly associated with outcomes. Whereas pre-HCT features are static, this dynamic marker could be assessed and monitored noninvasively and in real-time at the bedside. Directly quantifying leukocyte-endothelial interactions may become a method to develop biomarkers for a wide range of dermatologic applications.

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Dermoscopic findings and histopathological correlation in large cell acanthoma

J. Park, S. Jeong, J. Jung, D. Kwon, S. Seong, J. Kim, M. Jang
Dermatology, Kosin University College of Medicine, Busan, Korea (the Republic of)

Large cell acanthoma (LCA) is a benign epidermal tumor that presents with pigmented hyperkeratotic patch with well-demarcated boundaries in sun-exposed areas. Since various epidermal pigmented tumors present overlapping clinical and histopathological features, additional methods to differentiate them would be of great clinical significance. Although there are numerous data on the dermoscopic findings of other comparable epidermal pigmented tumors, there is a lack of data on the dermoscopic features of LCA. Therefore, this study aimed to identify distinct dermoscopic findings of LCA and to describe dermoscopic-histopathological correlation. Dermoscopic and histopathological evaluation was conducted on 33 lesions that were histopathologically diagnosed with LCA. Common dermoscopic findings included yellow opaque homogeneous background (97.0%), absence of vascular structures (87.9%), brown dots (60.6%), moth-eaten border (57.6%), and follicular openings (45.5%). Other observed findings included prominent skin markings (42.4%), short white streaks (39.4%), white-to-yellow surface scales (27.3%), negative pigment network (18.2%), pseudonetwork (18.2%), and linear irregular vessels (12.1%). Regarding dermoscopic-histopathological correlation, yellow opaque homogeneous background corresponded to compact and uniform hyperkeratosis, brown dots to small aggregates of pigments in the basal layer, and follicular openings to structural invagination above the follicular infundibulum without follicular keratotic plugs. Dermoscopy significantly aids in the distinction of LCA. Yellow opaque homogeneous background, brown dots, and moth-eaten border are common findings in LCA. Although less common, prominent skin markings and short white streaks are additional distinguishing features that are rare in other comparable epidermal tumors.

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Experience of advanced dermatology and cosmetic surgery clinics providers with teledermatology amidst the coronavirus disease-19 lockdown

C. M. Infante[1], J. Jueng[1], A. Su[1], B. Maner[2], T. Harding[1, 2], S. Eubanks[2], J. Solomon[2]
[1]University of Central Florida College of Medicine, Orlando, Florida, United States, [2]Ameriderm Research, Maitland, Florida, United States

Advanced Dermatology and Cosmetic Surgery (ADCS) is a practice with over 4.7 million dermatology patients, 350 providers, and 140 offices nationwide. During the coronavirus disease-19 (COVID-19) lockdown all but 16 offices were closed, and the electronic medical record was integrated with video teledermatology. An anonymous Qualtrics survey was distributed to ADCS providers and there were 92 respondents with an estimated response rate of 26%. The survey had up to 52 questions that evaluated provider utilization and sentiment of various technologies involved in teledermatology both during and after the COVID-19 lockdown. Simple descriptive statistics were performed to analyze the results. Of the 92 respondents, 13 were excluded from data analysis because of failure of 100% completion. 61 of the remaining 79 providers participated in teledermatology during the lockdown, and 38 providers participated after the lockdown. The participation rates were 77% and 48%, respectively. Providers most utilized live video, using Doxy.me most frequently followed by FaceTime and the EMR's video call platform. Poor video quality was the most experienced limitation to teledermatology during the lockdown. Patients' ability to connect to the platform was the most common limitation after the lockdown. More than 90% of providers ranked visualization of the patient's skin and technology reliability as "very important" to the success of a visit. During the lockdown providers were most satisfied with the ability to have real-time patient interaction and least satisfied with technology reliability. After the lockdown providers were most satisfied with patient security and least satisfied with technology reliability. This study can be used to evaluate and improve teledermatology services. The expansion of telehealth is dependent on the acceptance of both patients and providers and if utilized optimally, can provide quality care while minimizing COVID-19 transmission.

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Network analysis suggests Th1, Th2 and Th17 mechanisms to be linked together in pathogenesis of cutaneous lupus erythematosus

F. Chin[1, 2], T. Vazquez[1, 2], J. Patel[1, 2], R. Feng[3], V. Werth[1, 2]
[1]University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]Dermatology, VA Medical Center Corporal Michael J Crescenz, Philadelphia, Pennsylvania, United States, [3]University of Pennsylvania Department of Biostatistics and Epidemiology, Philadelphia, Pennsylvania, United States

The immunopathogenesis of cutaneous lupus erythematous (CLE) is highly diverse and involves activity of many different cell types and pathways. Here we profiled 44 CLE biopsies using multiplexed imaging mass cytometry to characterize the cell and pathway composition of CLE infiltrate. Biopsies were stained with two different panels of 37-metal conjugated antibodies that served as markers for different cell types, cytokines and pathway proteins. The relationships among cell and cytokine immune markers were modeled using Gaussian graphical model. The values were log-transformed and standardized prior to the analysis. The GGM algorithm identifies and displays only significant correlations between markers while leaving out non-significant ones. Of our network consisting of 11 T-cell subtypes, GGM identified Th1, Th2 and Th17 to be inter-related with each other, forming a triangle. No other positive findings were found between any other cells, including our main cell network consisting of 11 common immune cell types. Of our cytokine/pathway network consisting of 16 different markers, GGM identified IL4, IL17 and IFNγ to be among the strongest related markers in the network. Correlation testing confirmed remarkably strong associations between IL4, IL17 and IFNγ (all 3 pairs were p<1x10-13) as well as significant correlations (p<1x10-6) amongst all possible permutations of the aforementioned GGM-significant markers. These findings suggest that Th1, Th2 and Th17 co-occur together in the pathogenesis of CLE. Furthermore, heatmap visualization of T-cell K-means clustering was also performed and revealed the majority of patients (n=27) to be Tem dominant (83% CD4 and 76% CD8), with a smaller subgroup (n=14) to being Tcm dominant (57% CD4 and 50% CD8).

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Epidermal inflammatory activity is an important driver of hidradenitis suppurativa lesions

S. L. Schell[1], Z. Cong[1], M. L. Sennett[1], S. L. Gettle[1], A. L. Longenecker[1], S. R. Goldberg[2], J. S. Kirby[1], M. Helm[1], A. M. Nelson[1]
[1]Dermatology, Penn State College of Medicine, Hershey, Pennsylvania, United States, [2]General Surgery and Trauma, Mary Washington Hospital, Fredericksburg, Virginia, United States

Hidradenitis suppurativa (HS) is an inflammatory skin disorder of the pilosebaceous unit defined by painful nodules, tunnels, and scarring. Previous studies documented dermal cell infiltrate and the abundance of inflammatory cytokines in HS lesions, including TNFα, IL-17, and IL-1β. However, not much is currently known about the contribution of keratinocytes and immune cell activity within the epidermis in HS. We found that the degree of epidermal inflammation in HS lesions positively correlated with Hurley stage of disease (r =0.746; p=0), indicating the evolution of epidermal responses with disease severity. Keratinocytes were the primary producers of TNFα (p<0.05) and IL-6 (p<0.05) in HS lesions at all Hurley stages. Increased expression of CXCL3 (5-fold; p<0.01) in keratinocytes of stage III lesions positively correlated with increased recruitment of neutrophils to stage III lesional epidermis. Compared to healthy keratinocytes, enhanced production of chemokines from HS keratinocytes, including CCL22 (stage II 4-fold; p=0.09) and CCL3 (stages II and III p<0.05; 30-fold and 10-fold respectively), was associated with CD8 T cell infiltration and corresponding chemokine receptor expression in the epidermis. These recruited immune cells strongly produce or enable the production of IFNγ (>50 fold), IL-17A (500-fold), and IL-1β (5 to 10-fold), as well as IFNβ1, CXCL10, and STAT1 (HS II/III lesions; p<0.05 for all) by multiple cell types locally in the epidermis, likely generating positive feedback for increased inflammation. As epidermal inflammation evolves during disease progression and contributes to immune cell recruitment, our data suggest that topical therapeutics that block cytokine and chemokine production from the epidermis may be efficacious in HS. In all, our results collectively indicate that keratinocytes are not simply bystanders in HS inflammatory events, but rather actively contribute to HS pathogenesis.

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Fibroblast subpopulations orchestrate chronic inflammation in hidradenitis suppurativa

K. R. van Straalen[1], F. Ma[2], O. Plazyo[1], M. Calbet[4], X. Xing1, M. Gharaee-Kermani[1], [2], P. W. Harms[1, 3], R. Wasikowski[1], L. Nahlawi[1], A. Billi[1], J. M. Kahlenberg[2], E. Maverakis[5], L. C. Tsoi[1], J. E. Gudjonsson[1]
[1]Dept of Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, United States, [2]Div of Rheumatology, Dept of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan, United States, [3]Dept of Pathology, University of Michigan Medical School, Ann Arbor, Michigan, United States, [4]R&D Center, Almirall SA, Sant Feliu de Llobregat, Barcelona, Spain, [5]Dept of Dermatology, University of California, Sacramento, California, United States

Hidradenitis suppurativa (HS) is a chronic, inflammatory skin disease characterized by a massive immune cell infiltrate, extensive fibrosis, and tissue destruction. We aimed to elucidate the role of fibroblasts in the pathophysiology of HS. Single-cell RNA-sequencing was performed on chronic lesional skin samples of 6 HS patients and skin samples of 7 controls. Spatial sequencing was performed on 4 HS skin samples. Fibroblast subclustering revealed 6 subsets (SFRP2+, COL11A+, SFRP4+, LSP1+, RAMP1+, and CXCL13+). Two of these, SFRP4+ and CXCL13+, were specifically derived from HS samples and displayed enhanced pro-fibrotic characteristics (increased COL1A1 and COL3A1 expression). Development and activation of these subsets were found to be driven by key HS-associated inflammatory cytokines (IFNγ, IL-1β, and TNF). IHC and spatial sequencing revealed that their location demarcates the inflammatory area in HS skin. Activated SFRP4+ and CXCL13+ FB subsets were shown to help coordinate the characteristic chronic infiltrate by attracting neutrophils and Th17 cells through the expression of chemokines including CXCL5, CXCL6, and CCL20. Furthermore, increased CXCL13 expression by the CXCL13+ subset likely facilitates migration of B cells to the inflammatory environment and formation of tertiary lymphoid structures located within the fibrotic zone surrounding the infiltrate. These findings demonstrate the role of distinct FB subsets, activated by key HS-associated cytokines, in shaping the immune cell composition and spatial architecture of HS.

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Segmentation of cutaneous chronic graft-versus-host disease by a deep learning neural network

A. J. McNeil[1, 2, 3], K. Parks[2, 3], X. Liu[1, 2, 3], I. Saknite[2], F. Chen[2, 3], T. Reasat[1], L. Wheless[2, 3], B. Dawant[1], E. R. Tkaczyk[3, 2]
[1]Electrical and Computer Engineering, Vanderbilt University, Nashville, Tennessee, United States, [2]Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States, [3]Dermatology Service and Research Service, VA Tennessee Valley Healthcare System, Nashville, Tennessee, United States

Surface area involvement is a key measure for staging and tracking cutaneous chronic graft-versus-host disease (cGVHD) but is limited by availability of expert dermatologist visual evaluation. We developed a deep learning algorithm to segment (mark out) skin affected by cGVHD and tested its performance on unseen patients. We took 179 affected and 181 unaffected skin 3D photos from 36 cGVHD patients. As ground truth, a human annotator marked each region affected by cGVHD. A U-Net algorithm was trained and tested in a leave-one-patient-out validation experiment. Without knowing ground truth, a board-certified dermatologist assessed each segmentation for clinical relevance. The Dice coefficient was used to quantify spatial overlap. Surface area error was used to quantitatively estimate clinical error. In the unseen patient, the algorithm identified affected cGVHD skin photos with overall accuracy, positive predictive value, and negative predictive values exceeding 90%. 77% of segmentations were rated clinically acceptable to excellent. At the pixel-level, relative to the ground truth, the algorithm achieved a median Dice coefficient of 0.74 (interquartile range: 0.40 – 0.89), and median surface area error of 8.89% (22.10 – 3.69%). A significant difference in performance was observed between Fitzpatrick skin types I-III compared to IV-VI (median surface area error 7.17% vs 24.06% respectively). No significant difference was found between patients with erythematous or sclerotic disease. The developed algorithm provided clinically acceptable segmentations for cGVHD affected skin for the majority cGVHD patients in this study. Automated segmentation algorithms could provide a consistent method to score and track surface area involvement.

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Histopathology features of cutaneous acute graft-versus-host disease can be reliably detected by noninvasive reflectance confocal microscopy

I. Saknite[8, 1], M. Gill[2, 3], C. Alessi-Fox[4], J. Zwerner[8], J. Lehman[5], M. Shinohara[6], R. Novoa[7], H. Chen[8], M. Byrne[8], S. Gonzalez[3], M. Ardigo[9], E. R. Tkaczyk[8]
[1]*Biophotonics Laboratory, Latvijas Universitate, Riga, Latvia,* [2]*Skin Medical Research & Diagnostics, Dobbs Ferry, New York, United States,* [3]*Universidad de Alcala, Alcala de Henares, Comunidad de Madrid, Spain,* [4]*Caliber Imaging and Diagnostics, Rochester, New York, United States,* [5]*Mayo Clinic Minnesota, Rochester, Minnesota, United States,* [6]*University of Washington, Seattle, Washington, United States,* [7]*Stanford University, Stanford, California, United States,* [8]*Vanderbilt University Medical Center, Nashville, Tennessee, United States,* [9]*Istituto Dermatologico San Gallicano Dipartimento Clinica e Ricerca Dermatologica, Roma, Lazio, Italy*

BACKGROUND: The reliability to noninvasively identify features of inflammatory dermatoses by reflectance confocal microscopy (RCM) remains unknown. Specific consensus terminology with representative images is necessary to ensure consistent feature-level interpretation among RCM readers. METHODS: Through an iterative process of refinement and discussion among five international RCM experts, we developed a glossary with representative images of RCM features of aGVHD. In 17 lesions from 12 patients with cutaneous aGVHD, four dermatopathologists and four RCM readers independently evaluated the presence of aGVHD features in scanned histopathology slides and 1.5x1.5 mm RCM submosaics at 4 depths, respectively. Interobserver reproducibility was calculated by mean pairwise difference U statistic. Concordance between modalities was determined by fraction agreement. RESULTS: We present a glossary with representative images of 18 aGVHD features by RCM. The average interobserver reproducibility among RCM readers (75%, confidence interval 71%−79%) did not differ significantly from dermatopathologists (80%, 76%−85%). The concordance between RCM and histopathology was 59%. CONCLUSIONS: By using the validated glossary, the implementation of RCM can now be advanced in a variety of inflammatory conditions with a validated glossary and representative image set.

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Hidradenitis suppurativa type 17 T-cell transcriptome is different from psoriasis

J. Kim[1, 2, 3], A. Moreno[1], J. Lee[1], H. Lee[1], X. Li[1], W. Zhou[4], J. Cao[4], J. G. Krueger[1]
[1]Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York, United States, [2]Department of Dermatology, University of California Davis, Davis, California, United States, [3]Dermatology Section, Sacramento VA Medical Center, Mather, California, United States, [4]Laboratory of Single-cell Genomics and Population Dynamics, The Rockefeller University, New York, New York, United States

Hidradenitis suppurativa (HS) is a chronic systemic and cutaneous inflammatory disease driven by a multitude of immune cells. Recent studies have demonstrated that IL17A/IL17F isoform signal blocking psoriasis (PsO) drugs can effectively treat HS, suggesting Type 17 T-cells (T17 cells) play a critical role in HS lesion development. We sought to define and compare the single-cell transcriptomes of HS T17 cells to PsO T17 cells and further compare their respective ligand receptor interactions with neighboring immune cell subsets using single cell transcriptome data. Applying single-cell transcriptomics to emigrating cells from human skin, which we recently established, we compared cutaneous immune cells from HS skin samples (5,922 cells) to PsO skin samples (18,561 cells) and control skin samples (12,497 cells). Across HS and PsO skin samples, we discovered CD161+ T cells as the major IL17A/IL17F expressing T cells. In comparing HS to PsO CD161+ T-cell cluster cells, HS cells expressed significantly higher levels of IL17A, IL17F, IL1R1, more IL17F than IL17A (88.4% in HS versus 67.7% in PsO) and lower levels of IL23R ($p < 0.01$). IL1B, the primary ligand interacting with IL1R1 receptors of T17 cells, was mainly expressed by semimature dendritic cells with significantly lower IL23A expression than PsO dendritic cells. IL1A, an ancillary ligand interacting with IL1R1 receptors, was primarily expressed by stratum corneum keratinocytes of HS dermal tunnels. Our human skin single-cell data suggests HS T17 cells may be activated by a mechanism independent of IL23A, most likely IL1 interacting with semimature DCs, dermal tunnel keratinocytes and fibroblasts.

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***In vitro* and Clinical Evaluation of Cannabigerol (CBG) Produced via Yeast Biosynthesis: A Cannabinoid with a Broad Range of Anti-inflammatory and Skin Health Boosting Properties**

E. Perez[1], J. Fernandez[1], C. Fitzgerald[1], K. Rouzard[1], M. Tamura[1], C. Savile[2]
[1]Signum Biosciences, Monmouth Junction, New Jersey, United States, [2]Willow Biosciences, Mountain View, California, United States

Cannabigerol (CBG) is a minor, non-psychoactive cannabinoid typically extracted from the plant Cannabis sativa (C. sativa). Utilizing our novel yeast fermentation technology platform, minor cannabinoids such as CBG can be produced in a more sustainable, cost-effective, and timely process as compared to plant-based production. Our aim was to characterize and compare the in vitro activity profile of CBG and the more widely studied Cannabidiol (CBD) in skin and be the first group to test CBG clinically on human skin. Gene microarray analysis conducted using 3D human skin equivalents demonstrates that CBG regulates more genes than CBD, including several key skin targets. Human dermal fibroblasts (HDFs) and normal human epidermal keratinocytes (NHEKs) were exposed in culture to pro-inflammatory inducers to trigger cytokine production and oxidative stress. Results demonstrate that CBG and CBD reduce reactive oxygen species levels in HDFs better than Vitamin C. Moreover, CBG inhibits pro-inflammatory cytokine (Interleukin- -1β, -6, -8, tumor necrosis factor alpha) release from several inflammatory inducers such as ultraviolet A (UVA), ultraviolet B (UVB), chemical, C. acnes and in several instances does so more potently than CBD. A 20-subject vehicle-controlled clinical study was performed with 0.1% CBG serum and placebo applied topically for 2 weeks after Sodium Lauryl Sulfate (SLS)-induced irritation. CBG serum showed statistically significant improvement above placebo for transepidermal water loss (TEWL) and reduction in the appearance of redness. Altogether, CBG's broad range of in vitro and clinical skin health promoting activity demonstrates its strong potential as a safe, effective ingredient for topical use and suggests there are areas where it may be more effective than CBD.

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Proteomic and cytokine profiling in prurigo nodularis features increased IL-13 and circulating blood mediators of systemic inflammation

V. Parthasarathy[1], J. Deng[1], Z. Sun[2], S. Engle[2], A. Auxier[2], N. Hahn[2], J. Sims[2], A. Okragly[2], M. P. Alphonse[1], S. G. Kwatra[1]
[1]*Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States,* [2]*Eli Lilly and Company, Indianapolis, Indiana, United States*

Prurigo nodularis (PN) is a chronic inflammatory skin disease characterized by persistent, severe itch and firm nodules on the extremities and trunk. The immunophenotype of PN is thought to involve multiple immune axes, with varying cutaneous and blood involvement of T-helper (Th) responses, including Th1, Th2, Th17, and Th22 cytokines. Here, we investigated the role of the Th2-related cytokines IL-13, IL-4, and IL-5 along with a panel of proteomic mediators of systemic inflammation in PN. Ultra-sensitive immunoassays measured circulating levels of IL-13, IL-4, and IL-5 cytokines in 29 plasma samples of PN patients and 18 control samples from healthy patients. All patients were recruited from the Johns Hopkins Itch Center. Additionally, periostin and immunoglobulin (Ig)E immunoassays and a 92 inflammation O-link panel assay were used to assess changes in additional protein biomarkers. IL-13 was significantly elevated in PN patient plasma samples with a 4-fold increase ($p=0.0008$). IgE, IL-4, and IL-5 levels were not significantly elevated. Several markers of systemic inflammation were significantly elevated in the plasma samples of PN patients relative to healthy controls including periostin (1.47, $p=0.012$), CUB domain-containing protein 1 (CDCP1; 1.68, $p=0.0002$), chemokine ligand 7 (CCL7; 1.57, $p=0.0009$), tumor necrosis factor (TNF; 1.44, $p=0.02$), and chemokine ligand 9 (CXCL9; 1.48, $p=0.03$). In conclusion, IL-13 levels are significantly increased in plasma samples from PN patients. Targeting IL-13 may be an appropriate therapeutic approach in PN.

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FIBI (Fluorescence Imitating Brightfield Imaging) for rapid, slide-free dermatopathology

T. Engel[1], T. M. Abraham[2], T. Morningstar[2], M. A. Fung[3, 2], A. Rangchi[2], M. Kiuru[3, 2], F. Fereidouni[2], R. M. Levenson[2]
[1]University of California Davis School of Medicine, Sacramento, California, United States, [2]Pathology and Laboratory Medicine, University of California Davis Health System, Sacramento, California, United States, [3]Dermatology, University of California Davis Health System, Sacramento, California, United States

Background: Fluorescence Imitating Brightfield Imaging (FIBI) is a novel alternative microscopy method that can image freshly excised, non-sectioned tissue.We examine its potential utility in dermatopathology. Methods: Five skin samples embedded in paraffin blocks were superficially deparaffinized using xylene and ethanol and then briefly stained with hematoxylin and eosin (H&E). FIBI captured tissue surface histology images using simple microscope optics and a color camera. Subsequently, we applied deep learning-based models to improve resemblance to standard H&E coloration and contrast. FIBI images were compared to corresponding standard H&E slides and concordance was assessed by two dermatopathologists who numerically scored epidermal and dermal structure appearance and overall diagnostic utility. Results:The mean dermatopathologist scores indicate that FIBI images are equivalent to standard H&E slides for visualizing structures such as epidermal layers, sweat glands, and collagen. In addition, FIBI images were of diagnostic quality for most specimens including non-melanoma skin cancer and cysts. Conclusion: Images acquired with FIBI are comparable to traditional H&E-stained slides, suggesting that this rapid, inexpensive, and non-destructive microscopy technique, applicable to fresh tissue specimens, is a conceivable alternative to standard histology processes. Applications include time-sensitive procedures and settings with limited histology resources.

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UVB initiates skin inflammation by promoting keratinocyte ferroptosis

K. Vats[1], O. Kruglov[1], A. Mizes[2], S. N. Samovich[3], A. A. Amoscato[3], V. A. Tyurin[3], Y. Y. Tyurina[3], V. E. Kagan[3], Y. L. Bunimovich[1, 4]
[1]Dermatology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [2]School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [3]Center for Free Radical and Antioxidant Health, Department of Environmental Health and Occupational Health, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [4]Hillman Cancer Institute, UPMC, Pittsburgh, Pennsylvania, United States

Epidermis, composed primarily of the keratinocytes, is subjected to ultraviolet radiation (UVR)-induced oxidative and genotoxic stresses. That the keratinocytes are the initial source of pro-inflammatory mediators in the skin after UVR exposure has been well established. Ultraviolet B radiation (UVB) is a strong initiator of cutaneous inflammation, and contributes to the etiology and the exacerbation of several cutaneous diseases, such as lupus erythematosus. However, the mechanism of UVB-induced inflammation in the skin remains unclear. Utilizing primary human keratinocytes and human epidermal explants, we found that ferroptosis, a type of non-apoptotic programmed cell death associated with an excessive phospholipid peroxidation, is activated in the keratinocytes after UVB exposure. We further found that keratinocyte susceptibility to UVB-induced ferroptosis is dictated by the extent of lipid peroxidation and the dysregulation of glutathione system. Ferroptosis inhibition prevented HMGB1 release from the keratinocytes and protected UVB-irradiated skin from inflammation. While apoptosis and pyroptosis were also detectable in the keratinocytes after UVB exposure, we determined that ferroptosis of the keratinocytes plays a dominant role in initiating UVB-induced cutaneous inflammation. Our findings have significant implications for the prevention and treatment of a wide range of skin diseases fostered by UVB-induced inflammation.

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Indolium 1 exerts activity against vemurafenib-resistant melanoma *in vivo*

R. Radi[1], J. Elsey[1], Y. Jung[2], C. Huang[1], V. Corces[2], J. Arbiser[1, 3]
[1]Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States, [2]Genetics, Emory University School of Medicine, Atlanta, Georgia, United States, [3]Dermatology, Veterans Affairs Medical Center, Decatur, Georgia, United States

The development of targeted therapies (Braf/MEK inhibitors) and immunotherapy have had a major impact on the treatment of melanoma. However, the majority of patients with advanced melanoma succumb to their disease. The mechanisms of resistance to both targeted therapies and immunotherapies are numerous and have been well described. These include alternative activation of Braf/MEK signaling, novel compensating mutations in additional oncogenes, and loss of neoantigens. Thus, there is an urgent need for novel therapies for advanced melanomas utilizing additional mechanisms of action. We have previously identified triphenylmethanes as a class which shows activity against a wide variety of tumors. We have synthesized a novel triphenylmethane, indolium 1, and demonstrated its efficacy against an aggressive vemurafenib-resistant melanoma *in vivo*. ATAC Seq analysis of tumors treated with Indolium 1 vs vehicle revealed a novel mechanism of action against melanoma. Indolium 1 induces the tumor suppressor EphA3 *in vivo*. Surprisingly, indolium 1 also downregulates expression of the Rb gene both in vitro and *in vivo*, providing a biomarker of activity. While Rb has long been thought to be a tumor suppressor gene, its expression is amplified in advanced human melanoma, thus suggesting that it may have oncogenic activity as well. Moreover, Rb is required for Ras-mediated transformation. Our findings in an aggressive melanoma may extend the requirement for Rb for transformation to Braf as well. We thus provide the initial description of a novel small molecule with a mechanism completely different than existing therapies. We believe that pre-IND studies are warranted for this novel compound given its mechanism of action and ability to inhibit the growth of vemurafenib-resistant melanoma *in vivo*.

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Insulin-like growth factor-binding protein 5 alleviates skin inflammation in psoriasis mice model

G. Peng[1, 2], S. Yoshiba[2], S. Tsukamoto[1, 2], K. Okumura[2], H. Ogawa[2], S. Ikeda[1, 2], F. Niyonsaba[2, 3]
[1]Department of Dermatology and Allergology, Juntendo Daigaku Igakubu Daigakuin Igaku Kenkyuka, Bunkyo-ku, Tokyo, Japan, [2]Atopy (Allergy) Research Center, Juntendo Daigaku Igakubu Daigakuin Igaku Kenkyuka, Bunkyo-ku, Tokyo, Japan, [3]Faculty of International Liberal Arts, Juntendo Daigaku, Bunkyo-ku, Tokyo, Japan

Antimicrobial peptide derived from insulin-like growth factor-binding protein 5 (AMP-IBP5) is expressed in keratinocytes and displays both antimicrobial and immunomodulatory properties. In contrast to many antimicrobial peptides (AMPs), insulin-like growth factor-binging protein 5, the parent protein of AMP-IBP5, is downregulated in psoriasis, an inflammatory skin disease characterized by epidermal hyperplasia, erythematous plaques, abnormal epidermal differentiation, neutrophil infiltration and interleukin (IL)-17 overproduction into the psoriatic lesions. Although several AMPs have been implicated in the pathogenesis of psoriasis, the role of AMP-IBP5 remains unknown. This study aimed to investigate the effects of AMP-IBP5 in psoriasis using an imiquimod-induced psoriastic mouse model. Following subcutaneous administration of AMP-IBP5 into the psoriatic mice, AMP-IBP5 reduced the presence of dry scales and plaques, epidermal thickness, hyperkeratosis, parakeratosis, hyperplasia of dermal vessels and neutrophil and IL-17+ T cell infiltration, compared with normal mice. Consistently, AMP-IBP5 diminished the expression of inflammatory cytokine TNF-α, AMPs such as cathelicidin CRAMP and S100A proteins, and angiogenesis factors, including vascular endothelial growth factor and platelet-derived growth factor from the psoriatic lesional skin. Interestingly, administration of receptor-associated protein (RAP), an antagonist of low-density lipoprotein receptor-related protein 1 (LRP1), exacerbated psoriasis and abolished AMP-IBP5-mediated improvement of psoriasis. This indicates that LRP1 may play a crucial in the pathogenesis of psoriasis and that AMP-IBP5 may improve psoriasis via LRP1 signaling pathway. Collectively, we provide evidence that AMP-IBP5 might be a novel potential therapeutic target for the treatment of psoriasis.

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Correlation between skin cytokine profile and response to dupilumab in atopic dermatitis

K. Singh, K. Valido, M. Swallow, J. M. Cohen, W. Damsky
Dermatology, Yale School of Medicine, New Haven, Connecticut, United States

Dupilumab, a monoclonal antibody that inhibits IL4 and IL13, has significantly advanced the treatment of atopic dermatitis (AD). However, as few as 1/3 of patients with AD treated with dupilumab achieve clear or almost clear skin. Although the reasons for this are not fully understood, it has been proposed that suboptimal response in some patients may be related to immunologic heterogeneity in AD. Here, we evaluate whether expression of IL4, IL13, or other cytokines correlate with responsiveness to dupilumab in AD. We conducted a retrospective cohort study of 60 patients with AD treated with dupilumab at our institution. Levels of IL4, IL13 and other cytokines were determined using RNA in situ hybridization (RISH) on archival diagnostic skin biopsies for each patient. Cytokine profiles were compared among complete/near complete responders (n=16), partial responders (n=37), and those with no response or clinical worsening (n=7). We found that IL13 expression correlated most closely with dupilumab responsiveness. Patients achieving clear or almost clear skin with dupilumab had a median of 7.64 strongly positive (4+) cells per mm2 compared to a median of 0.225 in 4+ cells per mm2 for patients whose skin worsened or failed to improve (p=0.033). Patients achieving clear/almost clear skin also had greater 4+ IL13 expressing cells compared with patients who were partial responders, who had a median of 0.675 per mm2 (p<0.001). We found that expression patterns of other cytokines provided additional information on the pattern of clinical response. These findings suggest that molecular heterogeneity may play a role in response to dupilumab and can be evaluated using a histochemical staining approach on diagnostic specimens. This study is limited by the retrospective nature of the analysis. Prospective evaluation of this approach is ongoing. As the therapeutic armamentarium for AD continues to increase, methods to help choose the optimal agent will be helpful. Cytokine RNA in situ hybridization appears to hold promise in personalizing therapy in AD.

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T helper 2 cell immunity eliminates pre-cancerous skin lesions

T. Oka[1], M. Azin[1], T. Cunningham[1], M. Tabacchi[2], L. Cornelius[2], S. Demehri[1]
[1]Center for Cancer Immunology and Cutaneous Biology Research Center, Department of Dermatology and Cancer Center, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Division of Dermatology, Washington University in St Louis School of Medicine, St Louis, Missouri, United States

Actinic keratosis (AK) is a precursor to cutaneous squamous cell carcinoma (SCC). Effective therapeutics to eliminate AKs and prevent SCC are lacking. Long treatment durations and severe side effects have limited the efficacy of cytotoxic AK treatments including 5-fluorouracil (5-FU) chemotherapy and photodynamic therapy. Previously, we have shown that thymic stromal lymphopoietin (TSLP), an epithelium-derived cytokine, induces a robust antitumor immunity in the skin. Since this discovery, we have demonstrated the high efficacy of calcipotriol, a topical TSLP inducer, in combination with 5-fluorouracil (5-FU) as a novel immunotherapy for AK treatment with potential for SCC prevention. Here, we investigated the underlying mechanism that resulted in the synergism between calcipotriol and 5-FU. In conjunction with TSLP induction, we identified several Damage-associated molecular patterns (DAMPs) and HLA class II to be highly induced in premalignant keratinocytes upon calcipotriol plus 5-FU treatment, which led to a massive infiltration of AKs by T helper 2 (Th2)-polarized CD4+ T cells. Using Il4ra knockout mice and adoptive T cell transfer models, we showed that Th2 polarization was required and CD4+ T cells were sufficient for TSLP-mediated antitumor immunity in the skin. RNA sequencing and multiplex immunostaining revealed that interleukin (IL)-24 was upregulated specifically in AK keratinocytes after calcipotriol plus 5-FU Immunotherapy. As an effector molecule, IL-24 is known to induce apoptosis and toxic autophagy in a cancer-selective manner. Th2 cytokines induced IL-24 expression in SCC cells in vitro. Importantly, IL-24 overexpression caused cytotoxicity in SCC cells. These findings reveal a novel mode of immunity against premalignancy with broad implications for cancer immunoprevention.

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Circulating monocyte biomarkers are predictive and responsive in psoriasis subjects treated with apremilast

E. L. Larson[1, 2], D. DeMeo[1, 2], A. Johnson[1, 2], A. Young[2], S. Margevicius[2], J. Rutter[1, 2], A. Davies[1, 2], N. Korman[1, 2], J. B. Travers[3], C. A. Rohan[3], T. McCormick[1, 2], K. Cooper[1, 2]
[1]University Hospitals, Cleveland, Ohio, United States, [2]Case Western Reserve University, Cleveland, Ohio, United States, [3]Wright State University, Dayton, Ohio, United States

We asked how psoriasis patients characterized by a hyperadhesive aberrant monocyte (AM endotype) respond to cAMP phosphodiesterase (PDE)-4 inhibition (apremilast) at the clinical and monocyte subset level. Subjects aged 18-65 with moderate-to-severe psoriasis enrolled into a 16-week clinical trial with apremilast standard dosing. Entry criteria included elevated levels of AM-endotype, defined as hyperadhesive monocyte doublets, monocyte platelet aggregates (MPAs), or intermediate monocytes > 150% of healthy control values. Clinical laboratory values, patient reported outcome (PRO) measures, flow cytometric monocyte biomarker profiles and transcriptomic profiles of negatively-selected blood monocytes were evaluated longitudinally. At week 16, 61% of subjects achieved a PASI 50 response, 30% of subjects achieved PASI 75, and PROs significantly improved (p< 0.001). The absolute number of circulating doublets and MPAs are significantly reduced in subjects at 8 and 16 weeks (p= 0.042, 0.0014 & 0.034, 0.0014, respectively), suggesting an effect of apremilast on the hyperadhesiveness of blood monocytes. Reduction in intermediate monocytes did not achieve significance. A more robust clinical response was observed in subjects whose baseline percentage of circulating doublets was > 0.4% vs < 0.4% (82% vs. 46% PASI 50 respectively). Monocyte differentially expressed genes (DEGs) in responders (PASI≥50) vs non-responders (PASI<50) at baseline included TNF superfamily members, SERPIN12 and ADAMTS5. Pathway analysis of the monocytes pre/post therapy revealed DEGs that participate in thrombosis, lectin signaling/adhesion, and transcription factors associated with proliferation and inflammation. In conclusion, we demonstrate a relationship between hyperadhesive circulating doublets and MPAs and cAMP PDE-4 inhibition with implications for biomarking clinical responses and comorbidities.

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Encapsulated activated-grape seed extract (E-AGSE): A novel liposome-based formulation that promotes anti-aging, brightening and hydration in human skin

J. Fernandez[1], C. Fitzgerald[1], K. Rouzard[1], M. Tamura[1], J. Healy[1], K. Tao[2], L. L. Guo[2], X. Hu[2], M. Stock[1], J. B. Stock[1, 3], E. Perez[1]
[1]Signum Biosciences, Monmouth Junction, New Jersey, United States, [2]Shanghai Chicmax Cosmetic Co., Ltd, Shanghai, China, [3]Molecular Biology, Princeton University, Princeton, New Jersey, United States

Protein phosphatase 2A (PP2A) is a critical target to control cumulative oxidative stress and chronic inflammation during skin aging by reversible methylation of the C-terminal leucine of the PP2A catalytic subunit (PP2Ac). A novel, proprietary Activated-Grape Seed Extract (AGSE), enriched for PP2A-activating flavonoids, was recently developed, and has demonstrated potent antioxidant and anti-inflammatory activities. AGSE is a dark, purple-colored powder with limited solubility, which restricts its use in a broad range of formulations. To combat these restrictive properties, we developed an encapsulation formulation that both reduced the color and increased the solubility of AGSE, making it more amenable to application in a wider array of products. Encapsulation was performed utilizing a liposome and hydroxypropyl-β-cyclodextrin, (HPCD)-based approach to produce Encapsulated AGSE (E-AGSE). Human dermal fibroblasts and epidermal keratinocytes were used to determine expression levels of aging and dermal–epidermal junction (DEJ) markers. EpiDerm™ was UVB-irradiated to measure the effects against cytokine release, DNA damage, apoptosis, and skin barrier. We demonstrated that E-AGSE inhibits PP2A demethylation, increases key anti-aging (collagen I, III, elastin) and DEJ markers, protects against UVB-induced DNA damage and reduces inflammation. Moreover, E-AGSE reduces melanin production via tyrosinase inhibition. Clinical assessment of E-AGSE showed that it reduces the appearance of wrinkles, brightens the skin, and boosts hydration. E-AGSE is a novel grape seed extract formulation enriched for PP2A-activating flavonoids that is clinically effective in human skin, providing several benefits.

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Translational analysis reveals complex interplay of T cell subsets in drug hypersensitivity reactions

P. N. Shah[1], G. A. Romar[1], A. Manukyan[2], W. C. Ko[2], P. Hsieh[1], E. M. Schunkert[1], X. Fu[1], R. T. Bronson[3], A. H. Waldman[1], A. Mostaghimi[1], B. A. Schmidt[4], V. Barrera[6], R. K. Foreman[5], M. Garber[2], S. J. Divito[1]

[1]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Bioinformatics Core, University of Massachusetts, Worcester, Massachusetts, United States, [3]Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, United States, [4]Pathology, Boston Children's Hospital, Boston, Massachusetts, United States, [5]Pathology, Massachusetts General Hospital, Boston, Massachusetts, United States, [6]Bioinformatics Core, Harvard University T H Chan School of Public Health, Boston, Massachusetts, United States

Delayed-type drug hypersensitivity reactions(dtDHR) are significant causes of morbidity and mortality yet the phenotype and function of pathogenic T cells across the spectrum of disease severity is unknown. Herein we investigated the contribution of T cell subsets to disease. Bulk transcriptional profiling and microscopic analysis of clinical skin samples suggested recruitment of T cells from circulation into skin in severe forms of dtDHR, SJS/TEN and DRESS but not milder dtDHR, MDE. Deeper interrogation using single cell RNAseq+CITEseq +TCRseq of prospectively collected skin and blood of SJS/TEN, MDE and healthy patients demonstrated that both skin-resident and recruited cytotoxic populations were present in SJS/TEN but more variably so in MDE. Clonality patterns varied across samples and functional T cells were found in both expanded & non expanded populations. We next identified a cohort of lymphopenic patients who developed MDE with T cell infiltrates comparable to non-lymphopenic patients despite the inability to recruit cells from circulation into skin, intimating that skin-resident T cells were sufficient to mediate mild disease. Parallel findings were observed in a novel mouse model in which HLA-B*57:01 mice containing drug-reactive memory T cells developed dermatitis in response to systemic drug challenge, despite the inability to recruit T cells from secondary lymphoid organs. Together, these data suggest a complex interplay of multiple T cell subsets in dtDHR across disease severity favoring skin resident populations in milder disease and recruited populations in severe disease.

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Bile acid supplementation ameliorates western-diet induced psoriatic dermatitis in mice

J. D. Bloomstein, X. Wu, S. T. Hwang

Dermatology, University of California Davis, Sacramento, California, United States

Diet and microbiome are intricately linked to mucosal gut immunity and the systemic immune response, yet their connection to inflammatory skin diseases, such as psoriatic dermatitis (PsD), has yet to be elucidated. Recent studies have shown bile acids (BA)s are involved in dampening systemic Th17 pathway activity and improving Th17-induced ulcerative colitis. We investigated into whether BA supplementation could downregulate the Th17 response and improve PsD in mice. We fed mice a western diet (WD) to induce PsD, with and without the BAs lithocholic acid (LCA) and deoxycholic acid (DCA). After one month, mice exhibited ear scaling and thickening and an increase in IL-17a mRNA expression in skin and draining cervical lymph nodes, compared to the control diet. Supplementation with LCA or DCA reversed the skin inflammation and Th17 pathway upregulation. LC-MS analysis revealed that WD predominantly reduced serum levels of secondary, as opposed to primary, BAs. Supplementation with secondary BAs (SBA) LCA or DCA in WD-fed mice not only restored serum levels but also led to increased levels of other SBAs, including ursodeoxycholic acid (uDCA). We then found uDCA had a similar anti-inflammatory role when added to WD. Gut microbiome sequencing showed that WD-fed mice had a lower abundance of Lachnospiraceae NK4A136, known to produce SBAs, supporting the use of BA supplementation. To explore the mechanism of BA-induced improvement in skin inflammation, we cultured primary mouse keratinocytes with IL17A, with or without LCA. LCA suppressed transcriptional targets of the IL17A pathway, including S100A8 and S100A9. LCA led to the upregulation of TGR5 and VDR, which code for BA receptors involved in immune homeostasis. Together these findings suggest that BA supplementation leads to improvement in WD-induced, IL17-mediated skin dermatitis, possibly through the TGR5 or VDR receptors. BA supplementation shows promise for the treatment of PsD, and uDCA, already an FDA-approved agent, may be a new candidate for treatment of psoriatic inflammation.

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Have FOXP3, will travel: Human treg preferentially recirculate and suppress the activation of skin resident effector T cells

T. Benson[1], Q. Zhan[1], J. Crouch[1], C. Lian[1], N. Smith[2], T. Kupper[1], A. Villani[2], M. Wells[2], J. Teague[1], A. Gehad[1], N. Gerard[1], R. A. Clark[1]

[1]Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Massachusetts General Hospital, Boston, Massachusetts, United States

Regulatory T cells (Treg) restrain skin inflammation under healthy conditions and after immune challenges. Adult human skin contains 20 billion T cells and 1-3 billion are highly suppressive FOXP3+ Treg. We observed inflammatory skin eruptions in CTCL patients treated with alemtuzumab (αCD52), an antibody that purges recirculating T cells from skin. αCD52-induced eczematous dermatitis had 5-fold lower Treg numbers ($p<0.0001$) and 7-fold lower FOXP3+ Treg frequencies ($p<0.0001$) compared to age matched controls with eczematous dermatitis, suggesting αCD52 may be inducing eruptions by depleting recirculating Treg from skin. Immunostaining of healthy human skin showed that only 18% of FOXP3+ Treg expressed resident memory T cell (TRM) markers (CD69 and/or CD103) compared to 70% of FOXP3- T cells. Flow cytometry studies of T cells from healthy skin confirmed by a second method that only 16% of FOXP3+ Treg expressed TRM markers, compared to 53% and 72% of conventional CD4 and CD8 T cells, respectively. Only 2% of total skin TRM were Treg; 98% were conventional T cells. Single cell RNA sequencing of T cells from healthy human skin showed that Treg were enriched for recirculation associated genes. To functionally study the recirculation of human Treg, we biopsied the skin of CTCL patients before and after treatment with αCD52. In all cases, αCD52 reduced both the absolute number and relative frequencies of FOXP3+ Treg in skin (89% of Treg were depleted), confirming that Treg are preferentially recirculating. In summary, we report that the Treg in human skin are preferentially recirculating and conventional T cells are preferentially skin resident. The spontaneous inflammatory reactions that occur in patients treated with αCD52, a medication that depletes T cells as they enter the bloodstream, demonstrates that the presence of recirculating Treg in skin is required for suppression of inflammation.

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ALK expression in metastatic cutaneous squamous cell carcinoma: A pilot study

M. B. Lobl[1], C. Georgesen[1], J. Black[2], M. Lum[2], S. Lauer[3], M. J. Whitley[1], A. Wysong[1]

[1]Dermatology, University of Nebraska Medical Center, Omaha, Nebraska, United States, [2]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, Nebraska, United States, [3]Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, United States

Background: Cutaneous squamous cell carcinoma (SCC) is a common skin cancer, with over 1,000,000 cases and up to 9,000 deaths annually in the United States. Treatment options for metastatic SCC are limited, although inhibitors of PD-1 and EGFR have shown some success clinically. Our group identified ALK as a potential driver mutation in metastatic SCC through targeted sequencing. Here, we examine the role of ALK overexpression in SCC. Methods: All metastatic SCCs at our institution from 2010-2018 with tissue available were included, which yielded fifteen primary SCCs and fifteen patient-matched lymph node metastases. A tissue microarray from formalin-fixed paraffin-embedded tissues were assessed for protein expression with antibody ALK D5F3 (Cell Signaling). Staining of the normal epidermis and a known ALK+ anaplastic lymphoma was performed as controls. Results: Fifteen lymph node metastases were evaluated and 1/15 (6.7%) was positive for ALK expression. No primary tumors stained positively for ALK. For comparison, a random sample of nine localized SCCs were stained for ALK. One sample, a high-risk T2b SCC was also positive for ALK expression (11.1%). This tumor was characterized by poor differentiation and invasion into the subcutaneous fat. For both positive tumors, the staining pattern for ALK consisted of 1+ and 2+ intensity staining that was granular and cytoplasmic. Discussion: In this sample, ALK overexpression occurred in 6.7% of lymph node metastases, whereas our previous work demonstrated ALK driver mutations in 10% of lymph node metastases. Future studies with larger sample sizes correlating ALK mutations, gene expression, and protein expression would be an important next step to validating ALK as a potential target for metastatic SCC.

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RPT193, a CCR4 inhibitor, improves the inflammatory skin transcriptomic profile in patients with atopic dermatitis

E. Guttman-Yassky[1], A. Pavel[2], P. Facheris[1], J. Correa Da Rosa[1], A. D. Pagan[1], E. Del Duca[1], Y. Estrada[1], R. Bissonnette[3], M. Kumar[4], D. Trujillo[4], J. Rulloda[4], N. Lee[4], S. Ikeda[4], J. Jankicevic[4], D. Wustrow[4], D. Brockstedt[4], W. Ho[4], L. Cheng[4], P. Kassner[4]

[1]Laboratory of Inflammatory Skin Diseases, Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Dermatology, University of Mississippi, University Park, Mississippi, United States, [3]Innovaderm Research, Montreal, Quebec, Canada, [4]RAPT Therapeutics, South San Francisco, California, United States

The chemokine receptor CCR4 mediates CCL17- and CCL22-driven chemotaxis of Th2 cells into the skin of patients with atopic dermatitis (AD). RPT193 is an oral CCR4 inhibitor that has been developed to treat AD and other allergic inflammatory diseases. The safety and efficacy of RPT193 as monotherapy for moderate-to-severe AD was previously described in a placebo-controlled, double-blinded Phase 1 study. Here, we present the skin and peripheral biomarker analysis from this Phase 1 trial. Following 28 days of 400 mg daily RPT193 treatment, RNA-seq and/or RT-PCR demonstrated significant downregulation of general inflammation- (MMP12; p<0.01), innate immunity- (IL8; p<0.01), T-cell/T-cell activation- (IL2, CCL19, CCR7, ICOS; p<0.05), Th1- (CCL2; p<0.05), Th2- (CCL22, CCR4; p<0.05), and Th17/Th22-related markers (IL22, CCL20, CCR6, S100A8, S100A9, S100A12, PI3/Elafin; p<0.05) compared to baseline in RPT193-treated, but not placebo-treated, subjects. Significant modulation was also seen compared to placebo in genes related to general inflammation, T-cell activation, and T helper subsets (MMP12, IL-2, ICOS, CCR6, DEFB4, IL-22, and S100A8, p<0.05). We also observed decreased CCR4 surface expression on circulating Th2 cells but no major changes in abundance of T-cell subsets. In conclusion, these data suggest that RPT193 treatment improves the AD skin transcriptome, consistent with the observed clinical efficacy, as well as decreases CCR4 expression in the skin and on circulating Th2 cells in the periphery. A Phase 2 study is planned to investigate the safety and efficacy of RPT193 in patients with moderate to severe AD.

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Circulating blood metabolite deficiency reveals immunometabolic reprogramming as a therapeutic strategy for the treatment of chronic itch

J. Deng[1], V. Parthasarathy[1], Z. A. Bordeaux[1], N. Sutaria[1], M. Szeto[1], K. Lee[1], T. Pritchard[1], E. A. Cahill[1], A. Alajmi[1], S. Guo[2], C. Zhang[2], J. G. Meyer[3], A. Le[2], S. Kang[1], M. P. Alphonse[1], S. G. Kwatra[1]

[1]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [3]Biochemistry, Medical College of Wisconsin, Milwaukee, Wisconsin, United States

Chronic itch dramatically reduces patient quality of life and has limited therapeutic options. While recent investigations have focused on immune mediators in itch pathogenesis, little is known about the role of altered metabolism. Through untargeted plasma metabolomics, we analyzed two chronic pruritic diseases (CPDs), atopic dermatitis (AD) and chronic pruritus of unknown origin (CPUO). A total of 37 metabolites from 20 AD, 11 CPUO, and 24 healthy control (HC) patients were identified with ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Raw intensities were normalized and compared with ANOVA and Tukey's test using Metaboanalyst 5.0. Compared to HCs, CPUO and AD had >100-fold decreases in isoleucine, creatinine, tyrosine, threonine, carnitine, tryptophan, valine, methionine, acetyl-L-carnitine, and phenylalanine (FDR<10-11). In 3 clinically improved AD patients with dupilumab treatment (≥4 point improvement in WI-NRS), there were increases in plasma tyrosine, methionine, valine, phenylalanine, threonine, and creatinine (all fold changes>1.30). As a confirmatory analysis, multi-center data of 780,706 AD and HC patients revealed AD patients have lower phenylalanine (81.0 vs. 100.4 umol/L, p=.019) and creatinine (0.83 vs. 0.99 mg/dL, p<.0001), and that creatinine levels in AD patients taking dupilumab were higher compared to untreated patients (p=.0005). AD metabolites were Spearman's rank-correlated to AD plasma cytokine levels, which revealed a significant (p<.05) negative correlation of IL-1α with creatinine (rs=-.56) and carnitine (rs=.56) and CCL20 with acetyl-L-carnitine (rs=-.60) and carnitine (rs=-.51). These findings suggest immunometabolic reprogramming as a potential therapeutic approach in managing chronic itch.

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Spatial transcriptomic analysis of HS skin lesions reveals that tunnels are immunologically active and activity correlates with disease severity

C. Dunlap[1], C. Li[1], J. Frew[2], J. Guo[3], I. Charo[1], T. Schall[1], K. Sullivan[1]

[1]ChemoCentryx Inc, San Carlos, California, United States, [2]Dermatology, University of New South Wales, Sydney, New South Wales, Australia, [3]NanoString, Seattle, Washington, United States

Hidradenitis suppurativa (HS) is an inflammatory skin disease characterized by neutrophil-rich inflammatory nodules, abscesses or tunnels (sinus tracts). Tunnels, unique to HS, are deep dermal cavities lined with squamous epithelium that are immunologically active, contributing to disease pathology. The role of tunnels in driving ongoing disease is poorly understood. We used the GeoMX Digital Spatial Profiler to analyze a moderate (Hurley stage II) compared to a severe (Hurley stage III) HS skin lesion to explore biological differences between and within lesions. Four distinct regions per sample were analyzed: tunnel epithelium, immune infiltrate adjacent to a tunnel, immune infiltrate not associated with a tunnel, and sub-epidermis. We observed higher expression of keratinization genes (e.g. K16, S100A7) in the more differentiated tunnel epithelium of the severe lesion compared to the moderate lesion tunnel. Genes for proinflammatory cytokines (e.g. IL36, IL1B), chemokines (e.g. CXCL1, CCL20), neutrophil activation (e.g. ELANE, MPO) and B cells (CD79A, SDC1) had higher expression in the severe compared to moderate HS lesion. We found a spatial gradient in gene expression for IL36 and neutrophil-attracting chemokines (CXCL1, CXCL8), from high expression in the tunnel epithelium to lower expression in the sub-epidermis. Both lesions displayed these gradients, but those in the severe HS lesion were more pronounced. Conversely, spatial gradients of fibroblast-related genes (e.g. PDGFR, FAP) had higher expression in the sub-epidermis and lower expression adjacent to tunnel epithelium. These data suggest a marked increase in dermal inflammation in more severe HS lesions. The spatial gradients of cytokine and chemokine gene expression support the idea that more differentiated tunnels are more immunologically active, potentially contributing to ongoing severe disease, rather than representing remnants of prior disease.

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Assessment of a circulating tumor DNA test for detecting recurrence of Merkel cell carcinoma

T. Akaike[1], N. So[2], D. Hippe[3], L. E. Gunnell[1], C. Doolittle-Amieva[1], K. Lachance[1], E. Hall[1], N. Hook[4], A. Rodriguez[4], A. Ecklund[4], A. Aleshin[4], P. Nghiem[1], L. Zaba[2]

[1]University of Washington School of Medicine, Seattle, Washington, United States, [2]Stanford University School of Medicine, Stanford, California, United States, [3]Fred Hutchinson Cancer Research Center, Seattle, Washington, United States, [4]Natera, Inc., Austin, Texas, United States

Approximately 40% of patients with Merkel cell carcinoma (MCC) develop recurrence or metastasis. Early detection can result in better outcomes, and effective surveillance is critical in MCC management. We assessed whether circulating tumor DNA (ctDNA) can accurately detect clinically evident and/or occult MCC. We used the SignateraTM platform in which tumor-specific mutations are identified from archival tumors, and blood is interrogated for those mutations. Since April 2020, we have collected longitudinal ctDNA samples from 95 MCC patients. Of these, 38 had clinically evident MCC and 57 did not. Of the 38 with clinically evident MCC, all had a positive ctDNA test (sensitivity: 100%, 95% CI: 91-100%). Among these 38, 20 were newly diagnosed with MCC. The median tumor size for these newly diagnosed MCC cases was 2.5 cm (range 1-8.5), and the median ctDNA was 30 mean tumor molecules/mL (range 0.08-1470). Primary tumor size and ctDNA value were strongly correlated (Spearman's r = 0.74, p < 0.001). Among the 57 without clinical evidence of disease, 49 had a negative ctDNA test. Eight patients were positive for ctDNA without current clinical evidence of disease. Four of these 8 developed recurrent MCC at 55-217 days later. The specificity of ctDNA for current or subsequently evident MCC was 92% (95% CI: 82-98%). Of the remaining 4 patients with a positive ctDNA test, 2 had independent evidence of early recurrent disease based on marked elevation of Merkel cell polyomavirus oncoprotein antibody titers (an established surveillance test for MCC recurrence in the half of MCC patients who produce such antibodies). We conclude that ctDNA quantitation may be more sensitive than imaging to detect MCC minimal residual disease, and this test can perform well regardless of tumor viral status.

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Recurrence risk in seropositive merkel cell carcinoma: A web-based calculator to interpret merkel cell polyomavirus antibody test results

L. E. Gunnell[1], K. Lachance[1], D. Hippe[2], M. Bierma[1], K. Cahill[1], T. Akaike[1], P. Nghiem1
[1]*Dermatology, University of Washington Department of Medicine, Seattle, Washington, United States,* [2]*Fred Hutchinson Cancer Research Center, Seattle, Washington, United States*

Merkel cell carcinoma (MCC) is a rare skin cancer that recurs in ~40% of cases and is linked to the Merkel cell polyomavirus (MCPyV) in ~80% of cases in the US. Antibodies to MCPyV oncoproteins are detected in ~50% of MCC patients at diagnosis. Seropositive patients can be tracked with a clinically available MCPyV antibody test which correlates with tumor burden and is more sensitive and specific than imaging studies. The antibody test is incorporated in the NCCN guidelines which notes MCPyV serologies can be performed at diagnosis and sequentially for surveillance in seropositive patients. Although this test is widely used, interpretation of results can be challenging. We developed a web-based calculator that uses sequential antibody results to determine individualized MCC recurrence risk. Our cohort consisted of 245 seropositive patients with 1,517 antibody tests. Median follow-up was 4.3 years and 61 patients had a recurrence. A Cox regression model with time-varying covariates that updated with each test was trained with recurrence as the outcome. Predictive performance was summarized using the concordance index (c-index) with an optimism adjustment. The best fitting model included the baseline titer (HR: 1.23 per 2-fold increase; p<0.001) and ratio of current titer to baseline titer (HR: 1.75 per 2-fold increase, assuming current titer is stable; p<0.001). The ratio of current titer to previous titer (p < 0.001) and interaction between ratios also improved the model (p = 0.018). These terms had continuously variable HR ranging from 2.12 per 2-fold increase when current titer was 90% of baseline to 4.77 when current titer was 10% of baseline. The risk calculator was more strongly predictive of recurrence within 180 days after each antibody test than stage alone (c-index: 0.83 vs. 0.68, p < 0.001). This web-based calculator will improve clinicians' use of antibody results to inform appropriate surveillance.

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Psoriasis exacerbation by obesity reflects reduced adiponectin regulation of PPAR-γ/Th17 pathway activation and is reversal by adiponectin receptor agonism

S. Kim, H. Liu, K. Kwan, J. Im, H. Soltani, N. Kaplan, A. Paller
Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Although obesity-related deficiency of adipokines is known to contribute to increased psoriasis severity and decreased clinical response to interventions, the mechanism by which it exacerbates psoriasis is poorly defined. We hypothesized that adipokines suppress the activation of innate immune responses in keratinocytes and skin-resident Th17/γδ T cells. In preliminary studies, we confirmed that diet-induced obese (DIO) mice had significantly lower serum adiponectin levels than lean mice when subjected to topical imiquimod (IMQ) treatments to induce the psoriasis-like disease, supporting the use of this model for studies. We, then demonstrated that DIO mice receiving daily injections of adiponectin mimetic peptide (ADP355) showed clinical, histological, and transcriptional improvement only in the obese, not the lean mice. ADP355 administration led to a 2.1-fold increase in cutaneous mRNA expression of Adipoq (encoding adiponectin), suggesting that ADP355 not only directly agonizes the receptor but also promotes endogenous adiponectin expression. Flow cytometry revealed that ADP355-administered DIO mice had a reduced frequency of Th17/γδ T cells in lesional skin and draining lymph nodes (51 % reduction; p<0.01), suggesting that ADP355 suppresses Il17a-secreting cells. Our mechanistic studies using ChIP-assays in human keratinocytes showed that ADP355 enhanced PPAR-γ binding to regulatory regions in psoriatic genes (5.4-, 3.4-, and 2.7-fold increase, respectively, in IL23, IL17RA, and LOR binding; all p<0.005) to inhibit expression of IL23 (63 % reduction; p=0.0164), PI3 (51 % reduction; p=0.0002), and IL17RA (46 % reduction; p=0.0151) and stimulate LOR expression (2-fold increase, p=0.014). Our findings suggest that adiponectin limits psoriasis severity by activating the PPAR-γ pathway and thereby suppressing the Th17 activation. Small molecule adiponectin mimetic therapy may be an effective adjunctive therapy for psoriasis in obese individuals.

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Targeted proteomics and spectral flow cytometry analysis of cutaneous lupus erythematosus

N. Haddadi, H. S. Raef, K. Afshari, M. Ahmed Refat, E. Kim, J. Galindo de Laflin, J. Harris, M. Rashighi, J. M. Richmond
Dermatology, University of Massachusetts Chan Medical School, Worcester, Massachusetts, United States

Cutaneous Lupus Erythematosus (CLE) is an inflammatory skin disease characterized by perivascular and periadnexal lymphohistiocytic infiltrate and interface dermatitis. Using suction blister biopsies of lesional and nonlesional skin from four CLE patients and three healthy donors, we did spectral flow cytometry to study the immune cells infiltrating the skin and inflammatory cytokines in interstitial skin fluid. We identified a significant increase in HLA-DR+antigen-presenting cells in lesional (P<0.0001) and nonlesional (P<0.0001) skin compared to healthy controls. We detected the presence of T-cells, B-cells, natural killer cells, and plasmacytoid dendritic cells, key cell populations thought to drive disease immunopathogenesis. We found different clusters of cells expressing CXCR3 in the skin, while the total frequency and MFI of CXCR3+cells were not significantly different in healthy skin compared to CLE. Further, we measured 184 protein analytes in interstitial skin fluid using targeted proteomics. Our data confirmed the elevation of chemokine ligands previously reported to increase in highly inflamed CLE skin, CXCL9 (P<0.0001) and CXCL11 (P=0.0007), validating the blister biopsy as a minimally invasive method to monitor disease activity. Further, we identified potential novel biomarkers, including CASP8 (P=0.002), CTF1 (P=0.01), HGF (P=0.0005), Flt3L (P<0.0001), IFNL1 (P=0.02), CXCL6 (P<0.0001), CCL25 (P=0.005), and CCL28 (P=0.03) that are increased in the lesional skin compared to healthy. We uncovered proteins with increased concentration in clinically normal skin of CLE patients, which may define the preclinical stage of the disease. These differentially expressed proteins (DEPs) between nonlesional skin and healthy controls included increased CXCL6 (P=0.002), HGF (0.01), Flt3L (P<0.0001), and CCL25 (P=0.003), and decreased CEACAM3 (P=0.0002). We hypothesize that these proteins may serve as early disease activity biomarkers or predictors of disease progression.

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Cytotoxic T lymphocytes target henle's layer in alopecia areata

J. Kim[1], E. Chang[1], E. Y. Lee[1], E. Wang[1], A. M. Christiano[1,2]
[1]*Department of Dermatology, Columbia University, New York, New York, United States,* [2]*Department of Genetics and Development, Columbia University, New York, New York, United States*

Autoimmune disease occurs when a specific adaptive immune response is mounted against self-antigen(s) and immune-mediated tissue damage is sustained until the self-antigen is eliminated. We recently identified immunogenic cell death via necroptosis in the hair follicle (HF) epithelium as the primary initiating mechanism in alopecia areata (AA). Here, we aimed to identify the major target cells of T cell-mediated cytotoxicity in AA, after the autoimmune response was initiated. Using the C3H/HeJ mouse model of AA, we first established a spatiotemporal map of caspase-8-driven immune-mediated apoptosis in HF epithelial cells during premature HF regression in AA. We found that c-caspase-8+ apoptotic cells were selectively localized within Henle's layer, the outermost Krt71+ layer of the inner root sheath (IRS), but not in the outer root sheath or the hair matrix region. We previously reported that Krt71+ Henle's layer also showed high expression of H60, a mouse ligand for the NKG2D receptor expressed on CD8+ T cells. Similarly, in lesional scalp of AA patients, we found that c-caspase-8+ apoptotic cells were also located within the KRT71+ IRS layer, which showed high expression of ULBP2/5/6, human ligands for the NKG2D receptor. The expression of Krt71 in Henle's layer of the IRS extended up the lower half of the HF during anagen, which therefore defined the anatomical zone of CD8+ T cell infiltration and limited the area of immune-mediated tissue damage to the bulb region of HF. Interestingly, we occasionally found residual CD8+ T cells expressing tissue-resident memory T cell (Trm) markers in the infundibulum or isthmus regions of telogen HFs. The persistence of a Trm population within HFs suggests these cells may participate in disease re-initiation in the subsequent hair cycle. Our study identified Henle's layer as the major target of autoreactive CD8+ T cell attack in AA, and provides a mechanistic explanation for why the infiltration of CD8+ T cells in AA is limited to the bulb region of anagen HFs.

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Topical ivermectin modulates the skin microbiome and improves symptoms of rosacea

A. M. Butcher[1], J. Cheng[2], F. Shafiq[2], R. Gallo[2], T. Nakatsuji[2], T. Hata[2]
[1]University of Toledo College of Medicine and Life Sciences, Toledo, Ohio, United States, [2]Department of Dermatology, University of California San Diego, La Jolla, California, United States

Rosacea is a chronic inflammatory skin condition that affects 16 million people in the United States, with worldwide prevalence reported as high as 5%. The pathophysiology of rosacea involves an abnormal innate immune response that may include a response to dysbiosis of the skin microbiome, a condition that contributes to inappropriate inflammation in several disease states. Here, we hypothesized that the clinical improvement in rosacea symptoms with topical ivermectin is due not only to a decrease in the Demodex mite population, but also to a change in the population of commensal skin microbes such as coagulase-negative Staphylococcus (CoNS) that contribute to skin health. Thirteen subjects with papulopustular rosacea received topical ivermectin (Soolantra) treatment once daily for three months (UCSD IRB#160765). Analysis of facial skin swabs pre- and post-treatment with ivermectin by qPCR with species-specific primers revealed an increase after treatment in the absolute abundance of the CoNS bacteria S. epidermidis and the commensal facultative anaerobe C. acnes, ($p=0.004$ and $p=0.0056$). This increase in commensal bacteria correlated with clinical improvement in the subjects as measured by the level of erythema (CEA) and papule count. The increase in S. epidermidis also correlated with decreases in CEA score ($r=-0.4264$, $p=0.0298$) and total number of papules ($r=-0.4786$, $p=0.0134$). A decrease in Demodex mites ($p<0.001$) measured by qPCR also correlated with a decrease in total number of papules ($r=0.5559$, $p=0.0039$) but did not correlate with CEA score. 16S rRNA sequencing showed Shannon and Chao diversity increased after treatment, albeit only the change in Shannon diversity reached significance. These observations suggest that treatment with topical ivermectin may play a role in improving the symptoms of patients with papulopustular rosacea by modulation of skin microbiome beyond decreasing Demodex.

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Proteasome inhibitor functional profiling in CTCL

S. Xu[1], J. Lewis[1], A. King[1], S. Umlauf[2], K. Carlson[1], F. Foss[3], M. Girardi[1]
[1]Dermatology, Yale School of Medicine, New Haven, Connecticut, United States, [2]Yale Center for Molecular Discovery, Yale University, New Haven, Connecticut, United States, 3Internal Medicine, Yale School of Medicine, New Haven, Connecticut, United States

Cutaneous T-cell lymphoma (CTCL) is a non-Hodgkin T-cell lymphoma that presents with skin manifestations and is often incurable at advanced stages with blood involvement. Genomic profiling of malignant cells from CTCL patients has revealed a diverse range of genetic mutations underlying the disease, including single nucleotide mutations and gene copy number alterations involving the JAK/STAT and NF-κB signaling pathways. Synergistic drug combination treatment in CTCL may allow for the targeting of multiple aberrant pathways while minimizing toxicity and single-agent resistance. Proteasome inhibitors have been used to treat other hematologic malignancies and act through multiple mechanisms to induce cancer cell death, including suppression of NF-κB activation. We previously reported that JAK1/2 inhibitor ruxolitinib synergistically potentiates the cytotoxic effect of bortezomib, a first-generation reversible proteasome inhibitor, in CTCL primary cells and cell lines. We are currently investigating first and second-generation proteasome inhibitors with different proteasome subunit selectivity for their therapeutic potential in CTCL as single agents and in combination with BCL2, HDAC, BET, or JAK inhibitors that we previously identified as having therapeutic potential in CTCL. Our in vitro viability assays have shown similar sensitivity of CTCL primary cells and cell lines to bortezomib as well as second-generation proteasome inhibitors such as oprozomib with IC50 values in the single and double-digit nanomolar range. Preliminary data using proteasome inhibitors in combination with BCL2, HDAC, BET, or JAK inhibitors show varying degrees of synergy using the Chou-Talalay method when tested in patient-derived CTCL cells. These screenings provide a methodology for strategic combination drug assessment as well as a useful reference for the exploration of proteasome inhibitors as therapeutic agents in CTCL.

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3D Bioprinted vascularized human skin models can be used to screen modulators of angiogenesis for medical and dermo-cosmetic applications

C. McGuckin[1], M. Frechet[2], M. Lègues[1], C. Gonindard[2], C. Milet[1], R. Boisseau[1], R. Besseyre[1], W. Ferrier[1], S. Delaunois[2], N. Forraz[1], H. Chajra[2]
[1]CTIBiotech, Meyzieu, France, [2]Clariant active ingredients, Toulouse, France

Lack of skin vascularization or in contrast its hypervascularization are aggravating factors of some medical pathologies (ulcers and cancers) and dermo-cosmetics troubles (rosacea and dark circles). Thus, developing a suitable in vitro model to screen pharmaceutical and cosmetics ingredients able to stimulate or mitigate the angiogenesis process without changing the normal skin physiology are not easy and hampered by the existence of unreliable in vitro models. Traditional models of skin angiogenesis concentrate on analyzing the co-culture growth of endothelial cells with or without added fibroblasts or keratinocytes. However, these models do not work for topical applications and top-down layering analysis required for complex studies and do not translate well to clinical analysis. Here we describe the creation of a 3D bioprinted vascularized skin model, designed for screening those type of molecules. Human Dermal Microvascular Endothelial Cells, dermal fibroblasts and epidermal keratinocytes were harvested and expanded from juvenile foreskins. Cells were selected for optimal growth and mixed with a bioink into which adhesion proteins were added and cartridged into a CELLINK pneumatic 3D bioprinting system allowing the generation of 3D vascularized printed full thickness skins. Resulting skin models had a vascularization bed created in the lower quadrant of the dermal structure, which was confocal microscopy imaged with anti-CD31 fluorescent labelling. Reduction of epidermal growth factor and pituitary supplement resulted in the sequential reduction and cell death of the vascular bed over 14 days, whilst continual supplementation maintains the model and cellular interconnections. With 3D Bioprinting we can produce hundreds of models in one afternoon giving the potential to screen many combinations of molecules before moving to more expensive clinical experimentations.

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Reintroduction of caveolin-1 scaffolding domain improves pathogenesis of psoriasiform dermatitis

D. A. Lin, S. A. Revah, B. A. Abdo Abujamra, P. Romanelli, I. Jozic
Dermatology, University of Miami School of Medicine, Miami, Florida, United States

Psoriasis is a chronic, inflammatory skin disease characterized by well-defined, erythematous plaques with silvery scale secondary to aberrant keratinocyte proliferation. We have previously identified that a structural membrane protein caveolin-1 (Cav1) is involved in regulation of aberrant keratinocyte proliferation and differentiation, and thus the aim of this study was to elucidate the role of Cav1 in pathogenesis of psoriasis vulgaris. We utilized spatial genomic & proteomic analyses combined with immunohistochemistry (IHC) with patient samples and mouse models. Omics analyses identified caveolae and their structural components (Cav1, -2 and cavin-1) as downregulated in imiquimod (IMQ)-treated mouse model of psoriasis. These observations were validated in patient samples of multiple types of psoriasis stratified by PASI score (plaque, inverse, guttate and nail) by IHC staining and quantified by QuPath image analysis software. Although all biopsy samples exhibited an inverse relationship with levels of Cav1, plaque psoriasis samples were the most pronounced. To assess whether reintroduction of Cav1 can alleviate some of the features associated with IMQ-induced model of psoriasis (increased skin thickness/scaling and upregulation of psoriasis associated markers), C57BL6 and K14cre-Cav1 knockout mice were treated with soluble caveolin scaffolding domain (CSD) peptide, in presence or absence of IMQ. We observed that Cav1ko mice exhibited a trend in increased skin thickness and expression of CD11b, CD14, IL-6, IL-12, IL-17a and TNFa, although to a lesser than IMQ-treated C57BL6 mice (Cav1 wild type). Moreover, topical CSD treatment decreased both skin thickness and scaling, as well as dampened upregulation of aforementioned inflammatory markers. Together, these data support the hypothesis that Cav1 is major pathophysiological target in psoriasis and introduce different approach to localized topical treatment of psoriasis by targeting structural components of the specialized membrane microdomains, such as caveolae.

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In vivo phenotyping of the tumor-immune microenvironment in skin cancers

A. Sahu[1], L. Kraehenbuehl[1], A. Holland[1], M. Cordova[1], M. Gill[4], C. Alessi-Fox[2], S. Gonzalez[4], N. Kurtansky[1], A. Rossi[1], A. Marghoob1, P. Guitera[3], M. Pulitzer[1], C. Jason Chen[1], T. Merghoub[1], M. Rajadhyaksha[1]

[1]Memorial Sloan Kettering Cancer Center, New York, New York, United States, [2]University of Rochester Medical Center, Rochester, New York, United States, [3]Melanoma Institute Australia, North Sydney, New South Wales, Australia, [4]Alcala University, Madrid, Spain

Current phenotyping of tumors into hot or cold based on T-cell provides suboptimal response prediction to immunotherapies. We investigated a novel *in vivo* approach for improved phenotyping combining inflammation and vasculature, through high-resolution reflectance confocal microscopy (RCM). Using skin cancers as a model, 53 patients (13 melanoma, 40 basal cell carcinoma or BCC) were imaged to characterize density, spatial distribution of inflammation, vasculature and leukocyte trafficking. Unsupervised clustering on graded TiME features was performed to explore phenotypes, which were correlated with immune cells, tertiary lymphoid structures (TLS), and gene expression. Three main phenotypes were found: InflamHIGHVascHIGH, InflamHIGHVascLOW and InflamLOWVascHIGH. Overall, the InflamHIGH phenotype correlated with high immune signatures, VascHIGH phenotype with immune exclusion and altered vasculature. In BCC, the InflamHIGHVascLOW demonstrated a 2-4-fold increase in CD3+ T-cells, CD8+ and CD8+ PD-1+ T-cells as compared to InflamHIGHVascHIGH ($p<0.01$) and InflamLOWVascHIGH ($p<0.01$), respectively, confirming inflamed and exhausted features. Differential gene expression and gene ontology analysis demonstrated upregulated pro-inflammatory genes in the InflamHIGH phenotype. In melanoma, 3-fold higher presence of CD3+ T-cells ($p<0.001$) was found in the InflamHIGH phenotype. No phenotypic correlation with TLS was found in either cancer. Most responders (5 out of 7) to topical imiquimod therapy belonged to InflamHIGHVascLOW. Preliminary results support presence of unique TiME phenotypes, these will be correlated with response to checkpoint therapies in future.

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Evaluation of differences in c acnes, s epidermidis and demodex between rosacea subjects and normal controls

O. C. Osuoji[1], J. Cheng[1], A. M. Butcher[2], F. Shafiq[1], R. Gallo[1], T. Nakatsuji[1], T. Hata[1]

[1]Dermatology, University of California San Diego, La Jolla, California, United States, [2]University of Toledo College of Medicine and Life Sciences, Toledo, Ohio, United States

The role of the microbiome in the etiology and exacerbation of rosacea has been highlighted in recent studies. Increased Demodex mite density is frequently reported in association with rosacea, but multiple studies have failed to correlate Demodex and rosacea severity. S. epidermidis has been isolated from rosacea pustules, and decreased abundance of C. acnes, dominant on healthy facial skin, has been demonstrated in rosacea subjects. We sought to better understand these players in the rosacea microbiome and to elucidate the differences between rosacea and normal control subjects. In our study, 13 subjects with papulopustular rosacea were compared to 3 normal subjects. Rosacea subjects had at least one papule and mild erythema. Lesional (L) and nonlesional (NL) facial swabs of rosacea subjects and swabs of normal subjects were obtained and analyzed for abundance of bacterial and Demodex DNA by qPCR. C. acnes rCFU showed no significant difference between normal subjects and L and NL rosacea skin. Demodex copy number also exhibited no difference between rosacea subjects L and NL skin, but there was a slight trend ($p=0.0622$) toward lower copy number in NL rosacea subjects when compared with normal. S. epidermidis exhibited no difference between normal subjects and rosacea subjects L and NL skin, but there was a slight trend toward a significant difference between S. epidermidis L and NL skin ($p=0.0594$). The results of our study show no significant difference between normal subjects and rosacea subjects at baseline in their L and NL skin. The trend toward significance between normal skin and NL rosacea subjects; Demodex copy number, as well as the increase in S. epidermidis in L verses NL skin, will need to be further explored. Expansion of our healthy control subjects and improved uniformity in swab sites are currently underway to further explore these differences.

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Elucidating the cellular composition of lipomas to develop targeted treatment

A. Muskat, Y. Kost, A. Deutsch, M. Pirtle, B. McLellan, K. Shinoda

Albert Einstein College of Medicine, Bronx, New York, United States

Lipomas are benign tumors that consist of mature adipocytes. Most lipomas are sporadic, but some familial conditions predispose affected individuals to the development of up to thousands of lipomas. Currently, no targeted treatments exist. To develop such an agent, the cellular composition of lipomas must be elucidated. Using single-cell resolution techniques, we have successfully begun to characterize the transcriptomic landscape of lipomas. By determining the transcriptome of lipoma-derived adipocytes, we confirmed that lipomas consist of white adipocytes (WAT), not brown or beige types. We also compared lipomatous tissue to physiological WAT by single-cell RNA-sequencing and found that cellular composition was strictly conserved between the two. 4 major cell types were identified in lipomas: fibroblasts, immune, mural, and adipocyte progenitor cells. We conducted receptor expression profiling with 6 lipomas from different anatomic locations using a TaqMan array assay. The goal was to identify adrenergic and muscarinic receptors as potential therapeutic targets since modulators of these receptors are widely used in medical settings. 9 adrenergic and 3 muscarinic genes were selected, and the results were largely consistent between the samples. Alpha-1A, followed by beta-2, were the most highly expressed receptors. Stimulation of alpha-1 receptors in human adipose tissue has been shown to increase rates of lipolysis supporting its potential therapeutic capacity in lipomas. Surprisingly, the beta-3 receptor was minimally expressed or undetectable in all samples. In physiological WAT, the beta-3 receptor plays a vital role in the remodeling of WAT via lipolysis and fatty acid oxidation suggesting that these metabolic activities may be induced by different receptor-signaling pathways in lipomas than physiological WAT. To our knowledge, this is the first study to identify specific receptors types amongst several lipoma samples. These conclusions suggest that targeting alpha-1A and beta-2 receptors in lipomas may represent a novel medical treatment for their reduction.

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Characterization of cutaneous hormone production through analysis of skin secretions

J. L. Pineider[1], K. Eckert[3], J. McDonald[3, 4], T. A. Harris-Tryon[1, 2]

[1]Dermatology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Immunology, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [3]Center for Human Nutrition, The University of Texas Southwestern Medical Center, Dallas, Texas, United States, [4]Molecular Genetics, The University of Texas Southwestern Medical Center, Dallas, Texas, United States

The skin acts as an endocrine organ capable of hormone production and response. Moreover, skin conditions such as acne, hirsutism, androgenetic alopecia, and hidradenitis suppurativa clinically improve with anti-androgen therapies. Despite their importance, we have an incomplete understanding of the composition of hormones produced by the skin. In our current study, we have characterized the hormonal landscape of the skin across anatomical sites and between the sexes through analysis of skin secretions. In this observational pilot study, we collected skin secretions from the antecubital fossa, forehead, back, and axilla of twelve male and ten female subjects using commercially available, Sebutape. We then developed a method to extract and quantify hormones from these secretions through liquid chromatography tandem mass spectrometry. We were able to detect seven hormones and observed anatomical site differences in glucocorticoids, cortisone and 11-deoxycorticosterone. Most notably we observed marked elevations in dehydroepiandrosterone in the axilla ($p<0.0001$) and androstenedione on the forehead ($p<0.001$). We also detected differences in several sex steroid hormones between male and female subjects consistent with known systemic hormone differences between the sexes. In conclusion, we have developed a reliable method to quantify locally produced hormones in skin secretions using Sebutape. We have also found that hormonal composition varies based on sex and anatomical site. Through this approach, we can determine how hormonal composition of skin secretions may be altered in skin diseases.

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Rete ridges improve epidermal adhesion in engineered skin substitutes containing recessive dystrophic epidermolysis bullosa (RDEB) keratinocytes and normal or RDEB fibroblasts

D. M. Supp[2, 3], B. N. Blackstone[1], J. M. Hahn[2], H. M. Powell[1, 3]
[1]The Ohio State University, Columbus, Ohio, United States, [2]University of Cincinnati College of Medicine, Cincinnati, Ohio, United States, [3]Shriners Children's Ohio, Dayton, Ohio, United States

Recessive dystrophic epidermolysis bullosa (RDEB) is a severe, hereditary blistering disease due to mutations in collagen VII (COL7A1 gene), which forms anchoring fibrils that attach the epidermis to the dermis. Engineered epidermal grafts containing COL7A1-expressing keratinocytes have shown promise in clinical trials, but preclinical studies suggest that engineered skin substitutes (ESS) containing both COL7A1-expressing fibroblasts and keratinocytes might provide greater benefit. Rete ridges are interdigitations of the epidermis and dermis that enhance tissue mechanics, increase epidermal adhesion, and improve shear resistance. Hypothetically, incorporation of rete ridges in ESS will increase epidermal adhesion. To test this, ESS were prepared with RDEB patient-derived keratinocytes (mutated COL7A1) in the epidermis (all groups), and either RDEB or normal (COL7A1-expressing) fibroblasts in the dermis (N=12-14/group). Half of ESS in each group were laser-ablated to create rete ridges; the other half had flat dermal-epidermal junctions (DEJs). After culturing in vitro for 14 days, mechanical peel testing of ESS was used to measure epidermal adhesion. Force required to remove the epidermis from dermis was recorded in Newtons. Statistical analyses utilized SigmaPlot 14.5 and $p<0.05$ was considered significant. The results showed significant differences among groups, with the greatest dermal-epidermal adhesion in ESS prepared with normal fibroblasts and rete ridges. Compared to ESS with flat DEJs, the presence of rete ridges significantly increased epidermal adhesive strength, whether the dermis contained normal or RDEB fibroblasts. The results suggest that bilayer ESS containing rete ridges can provide improved epidermal adhesion and resistance to blistering compared with skin substitutes containing flat DEJs, and may represent an attractive therapy for treatment of wounds in RDEB patients.

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Early biomarker identification for immune sensitization and prevention with oral vitamin D3

M. Ernst[1], S. Evans[1], J. Techner[1], R. M. Rothbaum[1], L. Christensen[2], U. Onay[1], D. Biyashev[1], M. Demczuk[1], K. Cooper[2], K. Lu[1]
[1]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]UH Cleveland Medical Center, Cleveland, Ohio, United States

Reaction to chemical irritants is a common cause of dermatology consultation and a challenge for topical medication utility. Cholecalciferol (D3) mitigates inflammation from experimental sunburn in humans and chemical injury in mice. To investigate the clinical utility of D3 for chemical injury in humans, we conducted a double-blinded, placebo-controlled trial. 28 healthy subjects had 3x8mm2 of arm skin exposed to 0.0016% topical nitrogen mustard (NM) under occlusion. 2 weeks later, subjects were randomized to receive 200,000 IU oral D3 or placebo after repeat NM exposure on the other arm. By proteomic analysis of skin biopsies, placebo subjects have 22% more differentially expressed inflammatory proteins (DEP) than D3 subjects after 3 days and 650% after 6 weeks. PCA of RNAseq separates samples by exposure yet shows 2 intervention-independent subclusters in NM exposure 2. One shows a normal skin profile (NS) and the other shows immune memory cell enrichment, suggesting immune sensitization (S). S subjects show more inflammation than NS by skin erythema ($p<0.01$), edema ($p=0.05$), and histopathologic score ($p<1e-4$). S and NS D3 subjects express fewer DEPs than placebo at 3 days (S:41%;NS:82%) and 6 weeks (S:86%;NS:100%). Subgroup analysis of exposure 1 identifies early S-associated biomarkers. After exposure 2, NS subjects express no S markers if given D3 vs 75% if given placebo. Results identify biomarkers of sensitization that may have predictive clinical utility and demonstrate that oral D3 mitigates chemically-induced inflammation for up to 6-weeks. Thus, D3 may have preventative properties against chemical sensitization and serve as a low-risk treatment and prevention in those undergoing repetitive chemical exposure.

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Unsupervised learning reveals different degrees of heterogeneity as well as cell involvement in cutaneous lupus erythematous antimalarial treatment response subgroups

F. Chin[1, 2], T. Vazquez[1, 2], J. Patel[1, 2], R. Feng[3], V. Werth[1, 2]
[1]University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [2]Dermatology, VA Medical Center Corporal Michael J Crescenz, Philadelphia, Pennsylvania, United States, [3]University of Pennsylvania Department of Biostatistics and Epidemiology, Philadelphia, Pennsylvania, United States

First-line treatment for cutaneous lupus erythematosus involves the use of antimalarials. Treatment response is highly variable with some patients responding well to hydroxychloroquine (HCQ), some necessitating the addition of quinacrine (QC) and others refractory to treatment from both (NR). Here we used unsupervised learning to highlight differences between the immune infiltrate composition of the three treatment groups. Infiltrate composition was characterized using imaging mass cytometry, where we stained biopsies (12 HCQ, 11 QC, 20 NR) with two separate panels of 37-metal conjugated antibodies, which enabled the measurement of different cell types, cytokines and pathway proteins within the tissue sample. Correlation matrices of cytokines/pathways were constructed for each of the three treatment groups to visualize correlation strength for all markers simultaneously. Hierarchical clustering was performed on top of matrices, which revealed a single large correlation cluster in HCQ, two medium clusters in QC and many clusters in NR. This indicates relatively homogenous cytokine/pathway activity in HCQ, two separate mechanisms in QC and significant heterogeneity in NR. Cell measurements were scaled within each cell type and then subjected to K-means clustering. Clustering results were then visualized on heatmaps which demonstrated heavy CD4/CD8 dominance in HCQ, two noteworthy QC subgroups, one being dominated by CD8 cells (61% vs. 7%, $p=0.1$) and the other by cDC (62% vs. 15%, $p=0.1$), and three noteworthy NR subgroups being CD8-dominant (48% vs. 9%, $p<0.00001$), cDC-dominant (38% vs. 10%, $p<0.001$) and CD14+CD16+ dominant (45% vs. 4%, $p<0.00001$). This analysis was repeated for SCLE and DLE clinical subtypes which demonstrated remarkably similar cell population heterogeneity between the two groups.

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EGFR signalling contributes to pachyonychia congenita pathogenesis and its inhibition improves patients' quality of life

J. Basset[1], L. Marchal[1], M. McGrath[1], A. Hovnanian[1, 2]
[1]Dermatology, Institut Imagine Institut des Maladies Genetiques, Paris, Île-de-France, France, [2]Necker Hospital, Paris, France

Pachyonychia congenita (PC) is a rare keratinizing disorder characterized by thickened nails, painful and inflammatory palmoplantar keratoderma (PPK) and blistering for which no standard treatment is currently available. PC is caused by dominant mutations in keratin (KRT) 6A, 6B, 6C, 16 and 17 genes which are involved in wound healing and epidermal barrier formation. Previous reports pointed to mTOR activation and oxidative stress with dysfunctional NRF2 as contributors to PPK. However, the relationship between KRT mutations and pathogenesis of pain and PPK in PC, remains elusive. Recent studies have shown that epidermal growth factor receptor (EGFR) is involved in the regulation of oxidative stress (OS) and that its inhibition prevents reactive oxygen species generation. We confirm that NRF2, a key regulator of OS, is hypophosphorylated in lesional skin from 3 PC patients with KRT6 or KRT16 mutations, indicating defective OS regulation. We describe a strong increase in transglutamisase-1 activity which expression is directly induced by EGFR signaling in differentiating keratinocytes. Additionally, mTOR pathway was upregulated in all PC patients, consistent with EGFR activation. EGFR forms a signaling complex with the transient receptor potential vanilloid-3 (TRPV3) and is involved in the modulation of sensitivity and pain. TRPV3 transcript levels were significantly increased in hyperkeratotic lesions from the 3 patients suggesting that TRPV3 channels might connect EGFR with nociception in PC. We inferred that pharmacological inhibition of EGFR might be an effective approach for treating PC. Our preliminary results showed that oral Erlotinib treatment of 3 PC patients was well tolerated and led to an early, drastic and sustained reduction of pain with major improvement of quality of life after a few weeks of treatment. Together, our results suggest that EGFR activation contributes to PC pathogenesis and provide evidence that pharmacological inhibition of EGFR is a powerful strategy in PC.

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Novel human skin organ culture models for the identification and characterization of anti-aging actives *ex vivo*

M. van Lessen[1], A. Mardaryev[1, 2], C. Mauri[1], D. Broadley[1], M. Bertolini[1], J. Edelkamp[1], R. Paus[1, 3], T. Bíró[1]

[1]*Monasterium Laboratory Skin & Hair Research Solutions GmbH, Munster, Nordrhein-Westfalen, Germany,* [2]*Centre for Skin Sciences, University of Bradford, Bradford, West Yorkshire, United Kingdom,* [3]*Dermatology, University of Miami School of Medicine, Miami, Florida, United States*

Research of chronological (intrinsic) aging in human skin and the development of potent anti-aging and rejuvenating agents are impeded by the limited availability of clinically relevant models. As current cell culture approaches cannot satisfactorily recapitulate normal human skin physiology and aging, even under 3D conditions, human full-thickness skin organ culture (hSOC) remains an excellent, but under-used alternative for pre-clinical aging research and the testing of candidate senolytics. Here, we present three distinct, complementary novel hSOC approaches to interrogating intrinsic, UV-free human skin aging. In our spontaneous aging model (SPAM), we show that hSOCs from healthy subjects rapidly develop aging over a 3-day culture in a defined, serum-free medium, as demonstrated by significantly decreased epidermal keratinocyte proliferation and mitochondrial activity, reduced collagen 17A and sirtuin-1 as well as increased epidermal γH2AX levels, along with decreased dermal hyaluronan and collagen I levels. This assay is complemented by two distinct "speed-aging" hSOC models (SpeedAM1/2) where certain aspects of the spontaneous *ex vivo* skin aging phenotype can be further accelerated by BrdU, a known cell senescence inducer, or a well-defined cocktail of agents that engage aging-associated signaling pathways (e.g. autophagy, protein glycation, ROS production), using a similar battery of aging read-outs. Supplementing the hSOC media with established human skin anti-aging compounds (e.g. caffeine, tretinoin) "normalized" the spontaneous or induced aging phenotypes, thus validating the SPAM and SpeedAM1/2 models and indicating that key aging phenomena are reversible in these models. Leads from these instructive, cost-effective, accessible, and clinically relevant new hSOC aging research assays can be followed up *in vivo* in aged human skin xenografted to SCID mice.

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Gene-environment interaction effects of AKR1C3 and particulate matter exposure in atopic eczema

C. Vogeley[1], S. Kress[1], A. Rossi[1], M. Nakamura[1, 2], D. Lang[3], J. Krutmann[1], T. Schikowski[1], T. Haarmann-Stemmann[1]

[1]*IUF - Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany,* [2]*Department of Environmental and Geriatric Dermatology, Nagoya City University, Nagoya, Japan,* [3]*Bayer AG, Wuppertal, Germany*

The etiology of atopic eczema (AE) is complex and involves genetic as well as environmental factors. Chronic exposure to airborne particulate matter (PM) contributes to the development and worsening of AE. Biological effects of PM are mainly initiated by surface-bound polycyclic aromatic hydrocarbons (PAH). Previous data of our laboratory indicate that by stimulating cutaneous aryl hydrocarbon receptor activity and downstream expression of aldo-keto reductase (AKR) 1C3, PAH may enforce the 11-ketoreduction of mast cell-derived prostaglandin (PG) D2, a metabolically unstable stimulator of Th2 cells, to 9α,11β-PGF2, a stable inducer of proatopic Th2 responses. To assess the clinical relevance of AKR1C3 for PM-related AE, we focused on a single nucleotide polymorphism (SNP) in the coding region of the AKR1C3 gene and conducted a gene-environment interaction study. Specifically, by using data from a cohort study, we tested the interaction between the single SNP rs12529 and PM with an aerodynamic diameter of ≤2.5, ≤10, and 2.5-10µm from land-use regression models on AE by adjusted logistic regression analyses. We observed a significant interaction effect between PM exposures and the AKR1C3 SNP on AE in adolescents. The SNP interacts with the exposure to PM2.5 (p-valueint=0.037), PM10 (p-valueint=0.019) and PMcoarse (p-valueint=0.036). To elucidate whether the SNP affects AKR1C3 enzyme activity, we next generated AKR1C3-deficient keratinocytes and transiently transfected them with expression constructs for wild-type and polymorph (point-mutated) AKR1C3. PGD2 treatment and subsequent LC-MS based analyses of 9α,11β-PGF2 formation revealed that the AKR1C3 SNP enhances enzyme activity. Our data strongly indicate that exposure to airborne PM may worsen AE symptoms by enforcing the AKR1C3-mediated production of Th2-stimulatory 9α,11β-PGF2.

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IgE in the pathobiology of itch in epidermolysis bullosa pruriginosa and beneficial clinical response to omalizumab

M. Papanikolaou[1], J. Mellerio[1], M. Tesfamicael[2], L. A. Nattkemper[3], G. Yosipovitch[3], H. Gould[1], J. McGrath[1]

[1]*School of Basic and Medical Biosciences, King's College London, London, United Kingdom,* [2]*Viapath Analytics LLP, King's College Hospital, London, United Kingdom,* [3]*Miami Itch Centre, University of Miami School of Medicine, Miami, Florida, United States*

Dystrophic epidermolysis bullosa pruriginosa (DEB-P) is an extremely itchy variant of DEB. The cause for the itch is unknown and there are no effective treatments. We hypothesised a role for IgE in DEB-P itch. To explore this, we measured total IgE in the blood and skin of 15 non-atopic DEB-P subjects, and two matched control groups of 15 non-itchy DEB subjects (DEB-NP) and 15 healthy volunteers (HV), via ImmunoCAPTM Total IgE and tissue immunofluorescence respectively. Serum total IgE (normal range 0-81kU/L) was higher in DEB-P (median 263kU/L, range 2–6431kU/L) than in DEB-NP (median 24kU/L, range 2–225kU/L) and HV (median 18kU/L, range 3-296kU/L), p 0.0008 and 0.0005 respectively. Serum IgE was raised in 13 out of 15 DEB-P but only in 4 DEB-NP and 3 HV subjects. Nine DEB-P subjects had serum IgE levels more than three times the upper normal limit. There was weak positive correlation between serum IgE and circulating eosinophils (r 0.35, p 0.0162). Serum IgE did not correlate with IL-4, IL-5, IL-6, IL-13 and IL-31 levels, as measured with the Luminex bead-based immunoassay. IgE expression in DEB-P skin, which was mostly interstitial and detected in the upper dermis, was increased compared with DEB-NP (ns) and HV (p 0.0318). This did not correlate with skin expression of IL-13 and IL-31. Use of omalizumab in one DEB-P subject led to reduction in itch scores (itch NRS from 9 to 4 out of 10, Leuven Itch Scale from 85% to 50% and 5D scale from 23 to 17 out of 25, at 39 weeks into treatment), with associated improvement in wound healing (EBDASI activity score from 31 to 19 out of 276) and skin erythema. Our data implicate a role for IgE in DEB itch pathobiology and highlight anti-IgE therapy as a potential treatment for this.

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HDL composition, particle number and size is associated with non-calcified coronary plaque in psoriasis

Á. Gonzalez-Cantero[2], N. Patel[1], C. Hong[1], C. Abbad-Jaime de Aragón[2], E. Berna-Rico[2], J. Solis[3], A. Ballester[2], A. Sorokin[1], H. Teague[1], M. Playford[1], M. Barderas[4], L. Fernandez-Friera[5], N. Mehta[1]

[1]*National Heart, Lung, and Blood Institute, Bethesda, Maryland, United States,* [2]*Hospital Universitario Ramon y Cajal, Madrid, Spain,* [3]*Hospital Universitario 12 de Octubre Centro de Investigacion Biomedica, Madrid, Comunidad de Madrid, Spain,* [4]*Fundacion del Hospital Nacional de Paraplejicos para la Investigacion y la Integracion, Toledo, Castilla-La Mancha, Spain,* [5]*Hospital Universitario HM Sanchinarro, Madrid, Spain*

There is an evolving appreciation that high-density lipoproteins (HDL) is highly heterogeneous in both composition and function. With the advent of advanced lipid-testing techniques that allow for the quantitation and recovery of individual particle populations, we are beginning to connect the functionality of HDL subspecies with chronic inflammatory diseases. Psoriasis (PSO), provides a reliable human model to study how HDL composition may relate to non-calcified coronary plaque burden (NCB). We hypothesized that HDL composition would directly associate with NCB beyond traditional cardiovascular risk factors. Methods: Patients with severe PSO (45) without known cardiovascular disease who were candidates for biologic therapy underwent CCTA (320 detector, Toshiba) for coronary plaque burden quantification using QAngio (Medis). All patients had fasting blood draws for the measurement of HDL composition. Results: Psoriasis subjects were middle aged 46.8 (±SD 11.2) and predominantly men(80%). NCB was negatively associated with multiple HDL parameters including HDL-concentration (β=-.55; p=.001), HDL particle number (β=-.41;p=.007), HDL particle size (β=-.44; p=.009) and large HDL particle number (β=-.54;p=.001) after adjusting for age, sex, hypertension, hyperlipidemia, diabetes mellitus and statin use. Furthermore, patients with higher than median NCB had a worse cardiometabolic profile and lower HDL concentration, particle number and size. Conclusions: HDL composition is associated with non-calcified coronary burden in psoriasis and HDL particle number and size may serve as a biomarker for subclinical atherosclerosis.

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A new case series of olmsted syndrome subjects confirms EGFR activation and long term efficacity of oral erlotinib with acceptable tolerance

J. Basset[1], Y. Diab[2], F. Santiago[3], L. Azulay[4], K. Cordoro[5], A. Zhang[6], F. Watanabe[7], A. Kirkorian[2], F. Frascari[5], D. Siegel[8], E. Bourrat[9], R. Howard[5], A. Hovnanian[1,10]

[1]Institut Imagine Institut des Maladies Genetiques, Paris, Île-de-France, France, [2]George Washington University, Washington, District of Columbia, United States, [3]Santo André Hospital, Leira, Portugal, [4]Instituto de Dermatologia Prof Rubem David Azulay, Rio de Janeiro, Brazil, [5]UCSF, San Francisco, California, United States, [6]Medical College of Wisconsin, Milwaukee, Wisconsin, United States, [7]Hospital Infantil Pequeno Principe, Curibita, Brazil, [8]Stanford Hospital and Clinics, Stanford, California, United States, [9]Saint Louis Hospital, Paris, France, [10]Necker Hospital, Paris, France

Olmsted syndrome (OS) is a rare, painful and severe form of palmoplantar keratoderma (PPK). It is most often caused by dominant mutations in the transient receptor potential vanilloid-3 (TRPV3) gene, a heat-sensitive ion channel that forms a signaling complex with the epidermal growth factor receptor (EGFR). TRPV3 mutations are thought to activate EGFR causing abnormal keratinocyte proliferation and differentiation. We previously reported that blocking EGFR with oral Erlotinib resulted in remarkable improvement in 4 young patients. Here, we describe EGFR activation in situ and evaluate the long-term efficacy and tolerance of oral Erlotinib in 9 OS patients (3-to 58-year-old) up to 39 months. Active EGFR homodimers were directly assessed by proximity ligation assay and revealed a significant increase in hyperkeratotic lesions. mTOR pathway and transglutaminase-1 activity were upregulated consistent with EGFR activation. Treatment with Erlotinib improved the quality of life of the 9 OS patients. Specifically, 4 patients showed remarkable improvement with near complete resolution of PPK, pain, pruritus, and few side effects; 2 patients had comparable efficacy but poorer tolerance; and 3 young patients showed partial efficacy but a significant reduction in pain and good tolerance. Our results confirm EGFR activation in OS and reveal long term efficacy and acceptable tolerance of oral Erlotinib with inter-individual differences in OS patients.

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Modeling basal cell carcinoma in 3D organotypic raft cultures

M. W. Dukes[1], B. E. Perez White[2], T. J. Meade[1]

[1]Chemistry, Northwestern University, Evanston, Illinois, United States, [2]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Basal cell carcinoma (BCC) is the most-commonly diagnosed cancer worldwide with limited availability for non-surgical treatments. The Hedgehog signaling cascade drives tumorigenesis, but efforts to develop effective chemotherapeutics have been thwarted by chemoresistant tumor recurrence. A contributing factor to this is an overall lack of human-based preclinical models. We endeavored to develop 3D organotypic raft models that mimic characteristics of human BCC by combining primary normal human epidermal keratinocytes (NHEK) with established murine BCC cells. . We hypothesize that the co-culture of BCC cells with NHEK will elicit pro-tumorigenic, pre-cancerous changes in the NHEK. NHEK and the ASZ BCC cell line were mixed at ratios of 90:10, 75:25, and 50:50 NHEK to ASZ with 100% NHEK and 100% ASZ controls and were seeded onto dermis mimics. Samples were harvested after 12 days of culture and analyzed by hemotoxylin and eosin (H&E) staining, qPCR, western blot, and immunostaining. H&E staining supports successful incorporation of cancer cells with phenotypes that display features of early-stage BCCs. Species-specific immunostaining and qPCR analyses of the human cell populations revealed abnormal development of cytoskeletal structure, cell adhesion, and barrier function that mimic the pathology of patient BCC. Specifically, keratin 10, desmoglein, desmocollin, and filaggrin proteins and transcripts are down-regulated specifically in human cells in the ASZ-containing cultures. As the percent of BCC cells incorporation increased in the 3D cultures, these components decrease. These results suggest that BCC cells can influence neighboring non-cancerous keratinocytes and prevent their proper differentiation. These results are promising for evaluating pre-existing and new chemotherapeutics designed for treating BCC.

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Inhibition of ATR augments immunogenicity in merkel cell carcinoma: A promising approach to address resistance to anti-PD-1 immunotherapy

R. Bhakuni[1], P. Goff[2], J. H. Lee[1], T. Pulliam[1], S. Tabachnick-Cherny[1], C. D. Morningstar[1], P. Nghiem[1]

[1]Medicine, University of Washington, Seattle, Washington, United States, [2]Radiation Oncology, University of Washington, Seattle, Washington, United States

ATR (ataxia telangiectasia and Rad3-related kinase) ensures completion of DNA replication prior to mitosis. It mediates a key survival mechanism for cells with high replication stress (e.g., tumors with high Ki-67 positivity). Surprisingly, after the recent development of potent, selective ATR inhibitors (ATRi), preclinical and clinical studies have shown that ATR inhibition can also reinvigorate anti-tumor immunity and augment immune checkpoint inhibitor (ICI) efficacy. Merkel cell carcinoma (MCC), an immunogenic cancer with Ki-67 positivity in ~70% of MCC cells, may serve as an appropriate tumor model to test whether ATR inhibition can overcome resistance to PD-1 therapy. To test this hypothesis, a Merkel cell polyomavirus-positive MCC cell line (WaGa) was treated with an ATRi, alone or in combination with radiation (4 Gy RT), and then co-cultured with human monocyte-derived dendritic cells (moDCs). 48 hours post ATRi ± RT treatment of MCC cells, ATRi was washed out and these treated MCC cells were co-cultured with moDCs for another 48 hours. Next, moDCs were assayed for markers of dendritic cell activation (CD80, CD86) and immune markers (PD-L1, MHC-I) via flow cytometry. ATRi and radiation synergized to induce activation and immunogenic markers on moDCs when compared to ATRi or RT treatment alone; enhancing MCC tumor cells' ability to activate dendritic cells in vitro. Simultaneously, single-cell RNA sequencing and quantitative RT-PCR were performed on ATRi ± 4 Gy RT treated WaGa cells at 72 hours to evaluate the possible mechanisms of action for augmented tumor cell immunogenicity. ATRi synergized with RT to induce upregulation of NF-κB transcriptional activity, inflammatory cytokine production and p53-related transcripts, indicating augmented immunogenicity. In aggregate, these data provide support for a clinical trial (in development) of ATR inhibition for patients with PD-1-refractory MCC.

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Optimization of intravenous gentamicin to restore functional laminin 332 in junctional epidermolysis bullosa patients harboring nonsense mutations

B. A. Levian[1], D. Mosallaei[1], R. Antaya[2], D. Woodley[1], M. Chen[1]

[1]University of Southern California, Los Angeles, California, United States, [2]Yale School of Medicine, New Haven, Connecticut, United States

Junctional epidermolysis bullosa (JEB) is an incurable and fatal inherited blistering skin disease most commonly caused by nonsense mutations in LAMA3, LAMB3, or LAMC2 genes. These mutations impair the ability to produce functional laminin 332, needed for epidermal-dermal adherence. Previously, we demonstrated that intravenous (IV) gentamicin daily at 7.5 mg/kg for two weeks promoted readthrough of nonsense mutations and produced functional laminin 332. Here, we determined if treating the patients with higher gentamicin doses and more prolonged treatment would induce more functional laminin 332, and provide more long-term clinical improvement. We administered IV gentamicin to two JEB patients with nonsense mutations in either LAMB3 or LAMA3. At day 0, multiple Test Sites from open wounds and intact skin were selected for measuring wound closure. Both patients received biweekly infusions of 10 mg/kg of gentamicin for 3 months. Skin biopsies were examined for the expression of laminin 332, and wounds were evaluated using standardized photographs before and at one and three months after treatment. We also evaluated the patients' overall clinical improvement using EB disease activity scores. Test Sites after IV gentamicin displayed newly created, properly localized laminin 332 at the dermal-epidermal junction of the patients' skin. In addition, IV gentamicin promoted wound closure and improved the patients' clinical scores. Most interestingly, we also observed improvement of airway symptoms in JEB patients. Lastly, increasing the dosage and duration of infusions resulted in more laminin 332 expression and greater clinical improvement. We did not detect any adverse effects or auto-antibodies against the new laminin 332. An optimized dose of IV gentamicin may be a readily available, safe and potentially efficacious therapy that reduces disease severity for this population of JEB patients.

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Potential role of skin in SARS-CoV-2 infection

D. Chudakova[1], A. Klopot[1], B. Shi[1], P. Bhalla[1, 2], L. C. Tsoi[3], B. E. Perez White[1], I. Budunova[1, 2]
[1]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]SBDRC, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [3]University of Michigan Medical School, Ann Arbor, Michigan, United States

It is well accepted that the main route of the SARS-CoV-2 entry to the body is via respiratory epithelial cells. However, skin cells also express ACE2 and TMPRSS2, the major factors that control SARS-CoV-2 host cell entrance. The dermatological lesions including chilblain-like acral lesions (COVID toes), vasculitis-like, urticaria-like lesions and maculopapular eruptions were reported in 1%–20% of COVID-19 patients, and SARS CoV-2 RNA was detected in skin biopsy and autopsy of some of COVID-19 patients. This might be explained by direct viral entry via the skin or deposition of circulating virus during conditions such as viremia usually associated with cytokine storm. Among the most important pro-inflammatory cytokines involved in cytokine storm are TNFa, IL-6, IL-1b and INFg. Their expression is also increased in skin of patients with atopic dermatitis and psoriasis reported to have an increased risk of COVID-19. To assess the potential role of skin in SARS-CoV-2 infection, we used 3D human skin equivalents (HSE) made from primary human epidermal keratinocytes treated with TNFa, IL-6, IL-1b and INFg. The co-treatment with all cytokines drastically increased ACE2 and TMPRSS2 mRNA/protein levels in 3D-HSEs. Interestingly, there was a positive crosstalk between individual cytokines as they induced each other expression. We also found the significant, ~ 30% overlap between published molecular signature of SARS-CoV-2 in lungs of COVID-19 patients and transcriptome changes induced by cytokine combination in 3D-HSE. Finally, we infected 3D-HSEs with Spike-pseudotyped lentiviral tdTomato reporter (encoding Spike-protein from wt Wuhan-Hu-1 strain), and revealed fluorescence tdTomato signal and presence of tdTomato mRNA in epidermis of infected 3D-HSE cultures. These experiments provide proof of principle that skin could be an additional entry port for SARS-CoV-2 or serve as a viral reservoir if infected by circulating SARS-CoV-2 virus.

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Cancer associated fibroblasts in different T-stage lesions of cutaneous T-cell lymphoma

W. Han, M. Goswami, M. Duvic, X. Ni
The University of Texas MD Anderson Cancer Center, Houston, Texas, United States

The tumor microenvironment (TME) has been shown to play an important role in tumor initiation, development, and metastasis. Among all TME factors, cancer associated fibroblasts (CAF) have been suggested to play a key role. Two studies have reported that fibroblasts from cutaneous T-cell lymphoma (CTCL) lesions, mycosis fungoides (MF) and/or Sézary syndrome (SS), were hyper-activated and exerted a pro-tumorigenic activity on CTCL cells in vitro. But much regarding CAFs in development of CTCL remains to be understood. In this study, we assessed CAFs in different T-stage CTCL lesions (n=31) by immunohistochemistry using six CAF markers (FAP, PDGFRβ, S100A4, Vimentin, PDGFRα, and α-SMA) in comparison to psoriatic lesions (n=7) and normal skins (n=4). FAP was expressed in various fibroblasts, and FAP+ cells were increased in psoriatic and CTCL lesions compared to normal skins. The strongest staining FAP+ cells were seen in T1 and T4 lesions. Like FAP, PDGFRβ was also expressed in various fibroblasts, but the strongest staining PDGFRβ+ cells were seen in T2, T3, and T4 lesions. In contrast, S100A4 and Vimentin were not only expressed in fibroblasts, but also on others including langerhans cells, macrophages, and endothelial cells. S100A4+ cells and Vimentin+ cells were increased in CTCL lesions, and a subset of lymphocytes with atypical nuclei were also positive. The strongest staining S100A4+ cells and Vimentin+ cells were seen in T3 and T4 lesions. PDGFRα was mainly expressed in round large fibroblasts in all skins/lesions, and some of keratinocytes were also positive. The expression of α-SMA was exclusively found in vascular smooth muscle cells and arrector muscle cells, and fibroblasts were mostly negative. There was no difference in the numbers and staining intensities of α-SMA+ cells between CTCL and psoriatic lesions or normal skins. Our results suggest that FAP and PDGFRβ may be useful CAF markers in CTCL lesions, and S100A4 and Vimentin are not only expressed in fibroblasts, but also in lymphoma cells.

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Effect of etrasimod on circulating lymphocyte subsets in atopic dermatitis patients

R. Ryan, F. Kuo, K. Liu, G. Ahluwalia, C. Crosby
Arena Pharmaceuticals Inc, San Diego, California, United States

Background: Etrasimod, a selective sphingosine 1-phosphate receptor 1,4,5 modulator that reduces peripheral lymphocytes and subsequent infiltration inflammation sites, is in development for chronic immune-mediated diseases. In the Phase 2 ADVISE trial(NCT04162769), etrasimod demonstrated efficacy in key secondary outcomes in patients with atopic dermatitis(AD). Previous data support differential effects of etrasimod on immune cell subsets in healthy volunteers; however, the impact is unknown in patients with AD. This immunophenotyping biomarker analysis of ADVISE was designed to further evaluate the effect of etrasimod on circulating immune subsets. Methods: 140 subjects were treated once-daily with etrasimod 1 mg, 2 mg, or matching placebo for 12 weeks, followed by a 4-week washout(Week 16). In a subset (n=104), immune cells were assayed by flow cytometry from isolated peripheral blood mononuclear cells, and by EpiontisID, an epigenetic immunophenotyping method, from whole blood collected pre-dose on Day 0, Week 4, Week 12, and Week 16. Significant immune cell subset modulations were evaluated in etrasimod-treated groups versus placebo at Weeks 4 and 12. Results: Significant dose-dependent reductions were seen with total CD4 and CD8 T cells, including naïve, central memory, and Th2 subsets, and B cells($P < 0.05$, etrasimod vs placebo at Weeks 4 and 12). Notably, there were also trends of reductions of skin-homing T cell subsets, including CLA+CD4+ central memory T cells. Monocytes, conventional dendritic cells(DC), and natural killer cells were increased($P<0.05$ at Weeks 4 and 12). Affected subsets recovered at Week 16. No meaningful treatment effects were observed on effector memory T cells(Tem), Th1, Th17, natural killer T, and plasmacytoid DCs. Conclusions: Etrasimod treatment lowered select lymphocyte subsets, including Th2 and skin-infiltrating T cell subsets. In contrast, cells involved with maintaining immune surveillance, such as Tem cells, were relatively preserved. These data suggest that etrasimod may act as a selective immunomodulator that warrants further investigation.

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A novel expression based, non-invasive method to differentiate atopic dermatitis and psoriasis

R. Ratnappan, J. Whitaker, T. Allen, J. Rock, M. D. Howell
DermTech Inc, La Jolla, California, United States

Psoriasis and atopic dermatitis (AD) are two of the most prevalent chronic inflammatory skin diseases in the world. Currently, diagnosis of psoriasis and AD is conferred based on the combination of a visual exam and a review of medical history. In some instances, the overlapping clinical characteristics and disease manifestations make it difficult to distinguish between psoriasis and AD, so a skin biopsy is collected for pathological analysis. While effective, skin biopsies are invasive and have the potential for complications in dermatological diseases already characterized by abnormalities in the skin barrier. Here, we describe a non-invasive method to differentiate AD and psoriasis by comparing the expression of key genes involved in disease pathogenesis in AD and psoriasis. Epidermal skin samples were non-invasively collected from the skin of the patients with moderate to severe AD (n=20) or moderate to severe psoriasis (n=20) using the DermTech Smart Sticker™. RNA was isolated and analyzed by quantitative real-time PCR for the expression of IL-13, IL-23, IL-17A, S100A8, S100A9, CXCL9, CXCL10, CCL17, CCL18, CCL27, TLSP, and NOS2. Upregulation of IL-13, CCL17, IL-17A, and NOS2 exhibited the greatest differences between psoriasis and AD. When combined, Receiver Operating Characteristic (ROC) Curve analysis of the data set generated an AUC of 0.94 that can be used to differentiate the two disease conditions. Overall, this study demonstrates the potential utility of non-invasive skin sampling to differentiate AD and psoriasis patients based on a molecular signature from a set of four genes. The ability to distinguish the two disease conditions provides a valuable asset in the hands of physicians for clinical decision-making and can be utilized for the personalized treatment of AD and psoriasis patients.

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Bermekimab, anti-IL-1α antibody, inhibits skin injury induced response in healthy subjects

M. W. Leung[1], J. Angsana[1], K. Chen1, B. E. Keyes[1], Y. Zhuang[2], E. G. Ghorayeb[3]
[1]*Immunology Translational Sciences and Medicine, Janssen Research and Development La Jolla, San Diego, California, United States,* [2]*Clinical Pharmacology & Pharmacometrics, Janssen Research and Development Spring House, Spring House, Pennsylvania, United States,* [3]*Immunology Global Medical Affairs, Janssen Research and Development Horsham, Horsham, Pennsylvania, United States*

Interleukin-1 alpha (IL-1α) is constitutively expressed in epithelial cells located at barrier sites, such as skin, lung and gut. Upon cellular death, injury or infection, IL-1α is released. Serum levels of IL-1α are largely undetectable in healthy volunteers (HV), or atopic dermatitis (AD) and hidradenitis suppurativa (HS) patients, while levels in the skin, especially in the epidermis and exudates from HS tunnels, are highly concentrated. Bermekimab (BMK) is a first-in-class fully human anti-IL-1α monoclonal antibody that has been tested in Ph2A AD & HS studies. To support understanding of PK/PD relationships, we developed a human skin explant model to assess proteomic & transcriptomic effects of IL-1α blockade on injury-induced inflammation. After 24-hours culture, IL-1α was detected in the media. *Ex vivo* IL-1α blockade resulted in significant decreases of CXCL1, IL-8, GCSF & IL-6 levels compared to untreated 24-hours samples ($p<0.05$ for all analytes), and consistent reduction of 73 genes (N=10 donors). Next, we utilized this model to measure post-treatment skin PD effects in HV receiving a single dose of BMK in a Ph1 study (NCT04544813). BMK exhibited linear PK following a single IV (400-1200 mg) or SC (200-800 mg) administration. Consistent with *ex vivo* blockade, CXCL1, IL-8, GCSF & IL-6 were reduced in culture media from post-dose versus pre-dose skin explants with significantly higher % reduction of IL-8 & IL-6 observed in the 800mg compared to the 200mg SC cohorts ($p<0.05$ for both analytes). Down-regulation of the same 73 genes in skin explants were also observed. These data support the relevance of IL-1α as a key skin alarmin driving tissue injury inflammation, and BMK reduces the downstream skin injury responses. Clinical research evaluating BMK in inflammatory skin diseases is ongoing.

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Cutaneous transcriptomics identifies fibroproliferative and neurovascular gene dysregulation in prurigo nodularis compared to psoriasis and atopic dermatitis

Z. A. Bordeaux[1], N. Sutaria[1], Y. Roh[1], J. Choi[1], V. Parthasarathy[1], J. Deng[1], M. Taylor[1], A. Alajmi[1], T. Pritchard[1], Y. R. Semenov[2], M. P. Alphonse[1], S. G. Kwatra[1]
[1]*Dermatology, Johns Hopkins Medicine, Baltimore, Maryland, United States,* [2]*Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States*

Prurigo nodularis (PN) is a chronic inflammatory skin disease that is associated with several systemic comorbidities, suggesting dysregulation of cutaneous and systemic inflammatory processes. Th2, 17, and 22 immune signatures have been identified in PN lesions, highlighting shared cutaneous gene signatures between PN, psoriasis (PsO), and atopic dermatitis (AD). We aimed to identify pathways specific to PN by comparing the skin transcriptomes of PN, PsO, and AD. RNA-seq was performed on lesional and nonlesional biopsies from PN patients without a history of atopy or PsO. Lesional and nonlesional PsO and AD transcriptomes were obtained from the Gene Expression Omnibus. Lesional samples were compared to their respective nonlesional sample. Differentially expressed genes (DEGs) were calculated using DESeq2 for R. Gene ontology (GO) enrichment analysis was performed using GOseq for R, and specific fibroproliferative and neural pathways were explored using Gene Set Variation Analysis (GSVA). We analyzed 26 PN, 26 PsO, and 26 AD samples. PN and PsO shared 3,775 DEGs, PN and AD shared 1,551 DEGs, PsO and AD shared 1,565 DEGs, and all three diseases shared 1,082 DEGs. Top GO categories for PN included epithelium development and cell adhesion, top categories for PsO included cytokine-mediated and Fc receptor signaling and top categories of AD included response to interferon gamma and myeloid differentiation. GSVA revealed upregulation of transforming growth factor beta-induced epithelial to mesenchyme transition (logFC [fold change] 0.44, P=0.001), epidermal acanthosis (logFC 0.61, P<0.001), axon regeneration (logFC 0.46, P=0.011), and vascular endothelial growth factor activity (logFC 0.23, P=0.014) in PN, but not PsO or AD lesions. In conclusion, PN is characterized by distinct fibroproliferative, neuropathic, and angiopathic signatures.

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Peripheral neuropathic changes in prurigo nodularis

M. Marani[1], B. Pan[2], J. Deng[1], V. Parthasarathy[1], M. P. Alphonse[1], M. Polydefkis[2], S. G. Kwatra[1]
[1]*Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States,* [2]*Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States*

Prurigo nodularis (PN) is a chronic inflammatory skin disease characterized by intensely pruritic and hyperkeratotic nodules of unknown etiology. PN is strongly associated with peripheral neuropathies, suggesting a role for neural dysregulation in its pathogenesis. Here we study components of the cutaneous peripheral neural system, including intraepidermal nerve fiber density (IENFD), mechanoreceptors, and blood vessels in lesional and nonlesional skin of 10 patients with PN and 10 matched healthy controls. The mean age of PN patients was 57.0±16.7 years and 60% were female, with identical distributions for matched controls. No patients had an associated peripheral neuropathy. PN patients had a mean Worst Itch Numeric Rating of 8.8±1.6. Paired t-tests were used to compare lesional and nonlesional samples, while ANOVA was used to compare lesional and control samples. Tissue was processed to analyze IENFD (PGP9.5), Merkel cells (cytokeratin 20), blood vessels (CD31), and mast cell density (tryptase) by immunohistochemistry. Lesional PN skin had significantly lower IENFD compared to nonlesional ($P<0.05$) and control ($P<0.001$) skin. Nonlesional skin also had lower IENFD compared to healthy skin ($P<0.01$). Lesional skin had higher Merkel cell density ($P<0.05$), blood vessel density (13.6±0.6 vs 4.7±0.8 blood vessels/mm, $P<0.001$), and mast cell counts in the epidermis compared to nonlesional skin. Blood vessels in the upper dermis of lesional skin had an elongated pattern that paralleled the deeper dermal papillae. These findings highlight the role of neural dysregulation in the pathogenesis of PN by showing alterations in peripheral nerve structures in PN skin. These findings broaden the perception of PN from a dermatological condition to a multisystem disorder with neuropathic changes as a prominent feature. Therapeutics for PN should involve neuromodulation to address underlying cutaneous neural dysregulation.

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Racial differences in dysregulation of the renin-angiotensin-aldosterone system in patients with prurigo nodularis

K. K. Lee[1], N. Sutaria[1], M. Marani[1], J. Choi1, Y. Roh[1], V. Parthasarathy[1], J. Deng[1], Z. A. Bordeaux[1], M. Taylor[1], T. Pritchard[1], A. Alajmi[1], W. Adawi[1], Y. R. Semenov[2], M. P. Alphonse[1], S. G. Kwatra[1]
[1]*Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States,* [2]*Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States*

There is an unclear association between chronic kidney disease and prurigo nodularis (PN). We hypothesized that global dysregulation of the renin-angiotensin-aldosterone system (RAAS) may contribute to both the development of PN skin lesions, renal disease, and help explain observed racial disparities in PN. We thus conducted a cross-sectional analysis of renal comorbidities in PN patients using TriNetX, a global health research network providing access to medical records from approximately 69 million patients. PN patients were matched to control patients by age and sex using 1:1 propensity score matching. Epidemiological findings from this analysis provided the basis for immunoassays on blood plasma and RNA sequencing of skin biopsy samples from PN patients and healthy controls. Lesional and non-lesional skin biopsies were taken from PN patients, and from site matched locations in healthy controls. PN was associated with stages 1-5 of chronic kidney disease (CKD), end-stage renal disease, nephritic syndrome, nephrotic syndrome, glomerular disease, and tubulointerstitial disease, and the associations were significantly stronger in black patients ($P<.05$). Compared to controls, CKD progression was faster (HR 2.88, 95% CI: 1.01 – 8.26) only in black PN patients (10-year survival: 63.5% black vs. 85.5% white). Circulating plasma angiotensinogen levels were also dysregulated ($P<0.001$) only in black PN patients. Cutaneous transcriptomic analysis of genes related to RAAS also revealed dysregulation in PN lesions with greater dysregulation in black patients. Significant dysregulation of the cutaneous and systemic RAAS in black PN patients may explain the increased incidence and severity of renal disease.

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Computational identification of neutrophil specific therapeutic targets in psoriatic arthritis using a single cell transcriptomic approach

B. Tamilselvan[1], R. Martin[1], H. B. Lindley[2], B. Richardson[2], J. Ismail[2], W. Lin[2], A. Young[4], J. Rutter[4], N. L. Ward[3], T. McCormick[4], K. Cooper[4], C. A. Cameron[1]
[1]Nutrition, Case Western Reserve University, Cleveland, Ohio, United States, [2]Population and Quantitative Health Sciences, Case Western Reserve University, Cleveland, Ohio, United States, [3]Dermatology, Vanderbilt University, Nashville, Tennessee, United States, [4]Dermatology, Case Western Reserve University, Cleveland, Ohio, United States

Psoriatic arthritis (PsA) is a debilitating immune-mediated inflammatory disease that affects approximately 20% of patients with plaque psoriasis (PsO). Neutrophil subsets are elevated in psoriatic disease, however their mechanistic role in driving chronic inflammation and synovio-entheseal inflammation has yet to be fully elucidated. We previously reported that single cell RNASeq of peripheral blood neutrophils from PsA and PsO participants revealed unexpected heterogeneity within the neutrophil compartment, and identified specific enriched clusters, key pathways and genes that are differentially enriched in neutrophils from patients suffering PsA vs. PsO. We now report the validation of multiple differentially expressed targets regulating neutrophil effector functions and differentiation state in an independent cohort, and specific small molecule perturbation of these pathways to reverse dysfunction in the neutrophil subset. We have expanded our cohort to include healthy donors in order to identify commonly enriched neutrophil subsets in both PsA and PsO, as well as differentially enriched pathways and genes that uniquely distinguish neutrophils from PsA vs. healthy donors from PsO vs. healthy neutrophils to identify novel potential therapeutic targets that will selectively reverse inflammation in each disease state. We have applied our algorithm for objective computational drug repurposing using a transcriptomic approach, drug perturbation gene set enrichment analysis (dpGSEA), in conjunction with our single cell transcriptomic analyses of the neutrophil compartment in PsA and PsO disease, to significantly advance identification of novel therapeutic strategies specific to psoriatic disease states.

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Skin tape strip proteomics in mycosis fungoides identifies tumor associated biomarkers

J. Techner, S. Evans, M. Hooper, T. LeWitt, K. Lu, J. Guitart, X. Zhou
Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Mycosis fungoides (MF), the most common type of cutaneous T-cell lymphoma (CTCL), is characterized by malignant T-cell skin infiltration that leads to inflammation and skin barrier defects. Identifying reproducible biomarkers in MF skin is important for monitoring disease activity. Skin tape-stripping is gaining recognition as a non-invasive, easy method for tracking disease biomarkers. Tape strips from lesional (L) and non-lesional (NL) skin of 42 MF patients and matched sites of 21 healthy controls (HC) were analyzed with a proximity extension assay of 183 proteins relevant to oncology and inflammation. 33 differentially expressed proteins (DEP) were identified to be upregulated in MF-L compared to MF-NL and HC skin (FC>1.5, FDR<0.05). These DEPs spanned pathways of tumor-associated macrophage (TAM) polarization/activation (CSF-1, HO-1, VEGFA, PGF, CCL3, IL-6RA, Gal-3, IL-8), immune cell migration (CXCL9, CXCL10, CXCL1, CCL3), malignant T-cell survival/inflammation (VEGFA, PGF, TNFRSF9, TNF-R1, HGF), apoptosis (caspase-8, FAS), and skin inflammation (KLK6, CSTB, U-PAR). TAM factors PI3 and ARG1 were upregulated in MF-L versus MF-NL and MF-NL versus HC skin (FC>1.5, FDR<0.05). Th1-associated chemokines CXCL9/10 decreased with higher skin disease severity and stage, as previously described (p<0.05). Skin inflammation markers KLK6, CSTB, and U-PAR were associated with increased skin burden (p<0.05). Known to co-associate in CTCL to aid tumor progression, VEGFA and PGF were upregulated in MF-L compared to MF-NL (p<0.05). Higher pruritis scores were positively associated with KLK6 and CSTB expression and negatively associated with CXCL10 and PGF expression (R2=0.63, p<0.001). Stage IB disease harbored more TAM DEPs compared to stage IA. Skin tape strips were able to detect proteins associated with the MF microenvironment as well as certain MF clinical characteristics. These data support the use of tape strip proteomics for tracking skin disease activity and therapeutic response in MF.

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Increased epigenetic age acceleration in hidradenitis suppurativa

D. Lukac[1], K. Pagani[1], P. A. Collender[2], R. P. Fadadu[2], A. Cardenas[2], J. S. McGee[1]
[1]Department of Dermatology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [2]Division of Environmental Health Sciences, School of Public Health, University of California Berkeley, Berkeley, California, United States

DNA methylation is an epigenetic modification that regulates gene expression without altering the DNA sequence. It commonly occurs at cytosine-guanine (CpG) repeats on chromosomes under the influence of genetic and environmental factors. Studies have demonstrated that epigenetic age, as calculated based on methylation of certain CpG sites, can accurately estimate chronologic age. Moreover, epigenetic age acceleration (EAA) is highly predictive of age-associated disease burden and mortality risk. Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease with significant systemic disease burden. To date, EAA has not been evaluated in HS. In this study, we calculated four measures of EAA from formalin-fixed paraffin-embedded skin samples (11 control and 11 HS) using Illumina 850 methylation BeadChip arrays: intrinsic EAA (IEAA), extrinsic EAA (EEAA), PhenoAge acceleration (PhenoAA), and GrimAge acceleration (GrimAA). Our results demonstrated no significant difference in IEAA among HS compared to control patients (-1.00 years; permutation p value 0.52), significant increases in both EEAA (13.72 years; p value < 0.001) and PhenoAA (7.72 years; p value 0.003), and significant decrease in GrimAA (-5.14 years; p value < 0.001). Our findings suggest that the acceleration of epigenetic age in HS is driven by extrinsic factors, such as changes in the inflammatory and immune cell infiltrates in the skin, rather than non-immune intrinsic pathways of aging. Moreover, PhenoAA indicates an increased all-cause mortality risk in HS. The negative association of GrimAA with HS may be due to the average chronologic age of HS specimens at the time of the collection being significantly younger than those of controls. Future studies should be directed at understanding if appropriate interventions can halt and/or reverse EAA, which can potentially become a powerful biomarker in the management of HS.

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EGFR/MEK inhibitor therapy induces partial hair follicle immune privilege collapse *in vivo* and *ex vivo*

D. Rutkowski[1], R. Warren[1], C. Griffiths[1], R. Paus[1, 2, 3]
[1]Centre for Dermatology Research, University of Manchester, Manchester, United Kingdom, [2]University of Miami Miller School of Medicine, Miami, Florida, United States, [3]Monasterium Laboratory, Münster, Germany

Epidermal growth factor receptor inhibitors(EGFRi) and Mitogen Activated Kinase inhibitors(MEKi) induce a folliculitis in 65-80% of patients, but it is unclear how they impact on the human hair follicle(HF). We have asked whether EGFRi/MEKi-induced folliculitis is associated with damage of the HFs immune privilege(IP) and the excessive HF secretion of pro-inflammatory mediators. Scalp biopsies were taken from EGFRi-treated patients exhibiting a folliculitis and compared to normal scalp skin(n=9). *Ex vivo*, organ-cultured human HFs were exposed to EGFRi(5μM Erlotinib) or MEKi(Cobimetinib 1μM)(n=5 patients, each). Our results showed that, *in vivo*, EGFRi significantly induce:(1) a folliculitis characterized, by dense HF infiltrates of T cells(CD4+, CD8+), macrophages(CD68+), neutrophils and mast cells around the bulge and infundibulum (Immunohistochemistry(IHC));(2) upregulation of MHC class I and II, ß2-microglobulin, and downregulation of TGF-ß1, while CD200 expression was unaffected and PD-L1 was even upregulated (Immunoflourescence (IF)) and (3) increased IL-33 and decreased IL-37 transcriptional expression (RNAseq and in-situ hybridization (ISH)). *Ex vivo*, inhibition of the EGFR-MEK-ERK pathway results in a significant:(1) dysregulation, at 24hrs, of IP-related transcripts including an increase in MHC class I and II, and decrease in TGFβ-1 mRNA(RNAseq);(2) increased expression of MHC class I protein (72 h), decrease in TGF-ß1 and attenuated the expression of PD-L1 protein (IF) and (3) increase in the expression/secretion of IL-33 and decrease of IL-37 (Luminex analysis, ISH and RNAseq) (All results p<0.05). Thus, EGFRi/MEKi induces partial HF IP collapse and IL-33 secretion *in vivo* and *ex vivo*. Future management of EGFRi/MEKi-induced folliculitis should focus on restoring HF IP, inhibiting excessive IL-33 secretion or restoring the anti-inflammatory interlukin IL-37.

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RashX: Immune single-cell transcriptional classification of human chronic inflammatory skin disease

A. Hailer[1, 4], Y. Liu[1, 2], H. Wang[3], M. Taylor[1], C. Cook[1], J. North[1], T. Mauro[4, 1], E. Purdom[3], J. Cheng[1, 4], R. Cho[1]

[1]Dermatology, University of California San Francisco, San Francisco, California, United States, [2]Dermatology, Xi'an Jiaotong University, Xi'an, Shaanxi, China, [3]Statistics, University of California Berkeley, Berkeley, California, United States, [4]San Francisco VA Health Care System, San Francisco, California, United States

We have developed one of the largest single-cell transcriptomic datasets of CD45+ immune cells in human inflammatory skin disease, including 13 psoriasis and 11 atopic dermatitis samples. Our analyses identify skin-resident memory T cells as containing the primary molecular signature differentiating psoriasis and atopic dermatitis, composed of 78 transcripts with disease specificity at a p value < 0.001, only 13 of which have been previously reported. Recomposition into pseudo-bulk transcriptomes diminishes discriminative significance of all but 2 of these genes, demonstrating the critical role of single-cell approaches in discriminating disease states. Based on these discoveries, we have further refined a single-cell RNA sequencing profile database initially designed to help compare atypical chronic rash cases to psoriasis and atopic dermatitis, in order to direct therapeutic choice. To make this resource readily accessible, we present a digital interface by which any research group can submit a 10X Genomics scRNA-seq dataset and visualize the sample in the context of psoriasis and atopic dermatitis samples (accessible at https://rashX.ucsf.edu) and validate its utility on 15 cutaneous inflammatory samples from 4 external studies.

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Blocking the LFA-1 signaling pathway reverses alopecia areata in C3H/HeJ mice

Z. Dai, Y. Chang, A. M. Christiano

Columbia University, New York, New York, United States

Alopecia areata (AA) is caused by T cell-mediated autoimmune attack of the hair follicle. Therefore, inhibition of T lymphocyte migration and pathogenic activity represents an attractive therapeutic approach in the treatment of T lymphocyte-mediated autoimmune diseases, including AA. The lymphocyte function antigen-1 (LFA-1) receptor signaling pathway plays a crucial role in T cell activation, migration of T cells to target tissues and formation of the classical cytolytic immune synapse. We previously showed that the intercellular adhesion molecule 1(ICAM-1), one of the major ligands for LFA-1, is upregulated in lesional skin of both human patients and C3H/HeJ mice with AA. We observed that CD44+CD8+effector/memory T cell expressed high levels of LFA-1 within skin draining lymph nodes as well as within AA lesional skin. Here, we investigated the role of LFA-1 signaling pathway in AA using C3H/HeJ mice, and found that blockade of the LFA-1 signaling pathway using either anti-LFA-1 or anti-ICAM-1 mAbs prevented the development of AA in C3H/HeJ skin grafted mice. Immunohistochemical staining of skin biopsies show that anti-LFA-1- or anti-ICAM-1-treated mice have a striking decrease in skin T cell infiltration together with reduced expression of AA-associated markers (MHC-I and MHC-II). Furthermore, we observed that LFA-1 signaling blockade not only reduced CD44+CD8 +effector/memory T cell migration into the skin, but also led to enrichment of Tregs in skin draining lymph nodes. To limit systemic exposure, we topically treated C3H/HeJ AA mice with lifitegrast, a small molecule antagonist of LFA-1, and found that lifitegrast was effective in reversal of AA. Taken together, we demonstrated that LFA-1 plays a crucial role in T cell migration and activation in AA, and that reversal of AA was achieved by local LFA-1 signaling blockade using topical delivery. Our results invite further clinical investigation of LFA-1 pathway inhibition as a therapeutic target in AA treatment.

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Profiling the gut microbiome of acne patients from different racial and ethnic backgrounds

K. Pagani[1], D. Lukac[1], J. Z. Yi[2], J. S. McGee[1]

[1]Department of Dermatology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [2]Eastern Virginia Medical School, Norfolk, Virginia, United States

There is increasing evidence that the gut microbiome may play a role in the pathogenesis of acne vulgaris (AV). Indeed, recent studies have demonstrated differences in abundance and composition of the gut microbiome between healthy controls and AV patients. Given that the microbiome is known to regulate our immune system, it can be hypothesized that dysbiosis in the gut microbiome can alter the host immune response and promote the inflammatory skin response observed in AV. To date, studies have been limited to profiling the gut microbiome in AV cohorts of similar racial and ethnic backgrounds. In this study, we performed 16S rRNA gene sequencing from fecal samples of 9 control and 7 AV patients (9 white, 2 black, 1 Hispanic, 2 Asian, and 2 unknown). Our results showed that alpha diversity, as measured in three separate metrics (Chao1, Shannon, and observed OTUs), was lower in AV compared to control. This observation was in agreement with prior studies. Beta diversity, the measure of microbial diversity between samples, was estimated using a matrix of the Bray-Curtis distances across all samples, then visualized using principal coordinate analysis. 4 samples clustered together, and they were all a significant carrier of the gut microbe, Prevotella copri (66.0% abundance on average). 3 out of these 4 samples were from acne patients (2 Asian and 1 black), while the remaining sample was from a control patient (1 white). In contrast, all other white controls (a total of 7) and 1 white acne patient were not a significant carrier of Prevotella copri (0.2% abundance on average). Our study was limited by a small, unmatched cohort from a single institution. A larger study is necessary to validate our findings and to further investigate if AV therapeutics can potentially benefit from targeting the racial and ethnic differences of the gut microbiome.

865

Single-cell protein activity inference analysis of full-thickness skin uncovers novel pathways and a rare Arg1+ macrophage population in AA

E. Y. Lee[1, 2], A. Obradovic[2], E. Wang[1], A. M. Christiano[1, 3]

[1]Dermatology, Columbia University Irving Medical Center, New York, New York, United States, [2]Medical Scientist Training Program, Columbia University Irving Medical Center, New York, New York, United States, [3]Genetics and Development, Columbia University, New York, New York, United States

Alopecia Areata (AA) is an autoimmune disease in which T cells attack the hair follicle (HF) and lead to non-scarring hair loss. The healthy HF is a site of immune privilege, in which allows the HF to evade immune recognition. Meanwhile, HFs in AA exhibit strong upregulation of MHC Class I and II, and this breakdown in HF immune privilege has been proposed as one of the key drivers of AA. Although we and others previously demonstrated the role of T cells in AA, the contributions of the HF itself and other cell types in the surrounding skin microenvironment are not well-defined. To investigate the cellular landscape of AA skin, we performed single-cell RNA sequencing (scRNAseq) of full-thickness dorsal skin from graft-induced C3H/HeJ mice with AA as well as ungrafted controls. To uncover rare cell populations as well as address gene dropout issues that are commonly associated with scRNAseq, we also implemented the recently published ARACNE and VIPER computational algorithms to infer single-cell protein activity from scRNAseq datasets. Our analyses identified a prominent keratinocyte-specific MHC Class II signature, as well as a role for necroptosis as a HF-intrinsic immunogenic mechanism of cell death in AA. Interestingly, we also uncovered a novel population of disease-associated Arg1+ macrophages in AA skin, which exhibited upregulation of numerous arginine metabolism genes as well as pro-inflammatory cytokines. To test the translational potential of these findings, we administered the arginase inhibitor nor-NOHA to grafted C3H/HeJ mice, and found delayed onset of AA, suggesting a pathogenic role for Arg1+ macrophages in AA. Our studies illustrate the power of inferred single-cell protein activity to discover novel therapeutic candidates and druggable pathways and targets in AA.

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OCT2Hist: Non-invasive virtual biopsy using optical coherence tomography and machine learning

Y. Winetraub, S. Aasi, K. Y. Sarin, A. de la Zerda
Stanford University, Stanford, California, United States

Histological hematoxylin and eosin–stained (H&E) tissue sections are used as the gold standard for pathologic detection of cancer, tumor margin detection, and disease diagnosis. Producing H&E sections, however, is invasive and time-consuming. Non-invasive optical imaging modalities, such as optical coherence tomography (OCT), permit label-free, micron-scale 3D imaging of biological tissue microstructure with significant depth (up to 1mm) and large fields-of-view, but are difficult to interpret and correlate with clinical ground truth without specialized training. Here we introduce the concept of a virtual biopsy, using generative neural networks to synthesize virtual H&E sections from OCT images. To do so we have developed a novel technique, "optical barcoding", which has allowed us to repeatedly extract the 2D OCT slice from a 3D OCT volume that corresponds to a given H&E tissue section, with very high alignment precision down to 25 microns. Using 1,005 prospectively collected human skin sections from Mohs surgery operations of 71 patients, we constructed the largest dataset of H&E images and their corresponding precisely aligned OCT images, and trained a conditional generative adversarial network on these image pairs. Our results demonstrate the ability to use OCT images to generate high-fidelity virtual H&E sections and entire 3D H&E volumes. Applying this trained neural network to *in vivo* OCT images should enable physicians to readily incorporate OCT imaging into their clinical practice, reducing the number of unnecessary biopsy procedures.

867

Epigenomic and gene expression defects in sezary syndrome

Y. Lu[3], Y. Rahmatallah[1], P. Hsu[2], A. Davis[3], A. Moerman-Herzog[3], S. Mehdi[3], H. K. Wong[3]
[1]*Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States,* [2]*Occupational Health, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States,* [3]*Dermatology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States*

Sezary syndrome (SS) is an aggressive cutaneous T-cell lymphoma with significant dysregulated gene expression. To understand the role of epigenetic alterations in SS, we interrogated the DNA methylome in SS and normal T cells using Illumina 850k Methylation Beadchip for differential DNA methylation which affects the transcriptome. The study identified significant differential methylated positions (DMP)(10%) with beta-values that differ between the SS cohort compared to normal cohort were identified. The SS methylome showed greater hypomethylation compared to the methylome of controls. By gene ontology classification, DMPs showed enrichment to GO for neurosystem development. In analysis of DMPs of highly expressed biomarker genes, PLS3, DNM3, TOX, TIGIT and FCRL3, significant differential methylation were detected between SS and normal. In analysis of relationship of transcriptome of SS T cells to methylome, gene sets with DMP overlapped with gene sets that showed differential gene expression. DNA hypomethylation in SS was strongly associated with increased in gene expression: for example PLS3, DNM3, TIGIT and FCRL3. Hypermethylation was associated with both decreased expression of DPP4 and CD7. We showed SS exhibit reduced induction of IFNG, a Th1 cytokine, compared to normal T cells. Public ATAC-seq data from SS revealed open chromatin for genes with DMPs and increased expression. To validate the role of demethylation in gene regulation in SS, treatment of SS cells and normal T cells with demethylating drug 5-azacytidine increased biomarker gene expression. These findings indicate that SS is associated with characteristic alteration of the epigenetic landscape revealed in the methylome and transcriptome data. Understanding the mechanistic basis of these changes in SS may shed insight into the pathogenesis.

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LB868

Th2 skewing promotes the expression of skin-homing molecules on T cells and is required for the induction of skin lesions in lupus-prone mice

N. Haddadi[1], P. Mande[2], T. Brodeur[2], K. Hao[2], G. Ryan[1], S. Moses[2], S. Subramanian[2], X. Picari[2], K. Afshari[1], A. Marshak-Rothstein[2], J. M. Richmond[1]
[1]Dermatology, University of Massachusetts Chan Medical School, Worcester, Massachusetts, United States, [2]Medicine, University of Massachusetts Chan Medical School, Worcester, Massachusetts, United States

Cutaneous lupus erythematosus (CLE) is an autoimmune skin disease characterized by high IFN expression. Recent advances have challenged the Th1-Th2 paradigm in CLE, as there are reports of expression of both Th1 and Th2 cytokines in lesional skin. We assessed the role of T cell skewing in the initiation of skin disease using a lupus-prone mouse model of CLE that depends on recognizing cognate antigen by infiltrating T cells in an immune environment perturbed by a TLR7-driven type I IFN response. Th2-skewed cells promoted the development of skin lesions; however, Th1-skewed cells could not induce skin manifestations. Importantly, we found that Th2 cells gained the capacity to express and secrete IFN-γ *in vivo*. This capability potentiates over time, as the Th cells isolated from the skin during a flare expressed more IFN-γ as compared to Th cells that were taken during primary disease. The injected Th2 cells preferentially expressed CXCR6 and CD103, consistent with the notion that Th2 utilize these receptors to home to the skin where the ensuing production of type I IFN by pDCs induces a shift in the functional phenotype of the Th cells. Gene expression analysis with NanoString identified changes in Th2 cells after injection *in vivo*, with higher expression of interferon-stimulated genes (ISGs) including CXCL9, IRF5, IFIH1, and MX1. We concluded that Th2 cells trigger skin lesion formation in CLE, and these cells switch toward a Th1 phenotype that is stable within the T cell memory compartment.

LB869

Dermcidin derived polypeptides: DCD(86-103) induced inflammatory reaction in skin by activation mast cells via ST2

T. Jia[1], D. Che1, L. Zhang[2], X. Du[1], Y. Zheng[1], T. Zhou[1], X. Song[1], S. Geng[1]
[1]Department of Dermatology, Northwest Hospital, The Second Hospital Affiliated to Xi'an Jiaotong University, Xi'an Jiaotong University, Xi'an, Shaanxi, China, [2]Xi'an Jiaotong University School of Medicine, Xi'an, Shaanxi, China

Psoriasis is a kind of chronic inflammatory skin disease. Mast cells significantly increase and activate in the lesions of psoriasis patients, which involves in psoriatic inflammation. Dermcidin (DCD) is a natural antibacterial peptide secreted by sweat glands and usually transported to the epidermal surface by sweat. Whether DCD is involved in mast cell activation is unclear. However, the mechanisms that DCD involved in skin inflammatory reaction needs to be investigated. To investigate whether Dermcidin derived polypeptides: DCD(86-103) activates MCs and induces skin inflammatory reaction contributing to psoriasis. Wild-type mice were treated with DCD(86-103) to observe inflammatory reaction in skin and cytokines release *in vivo*. Release of inflammatory mediators by mouse primary mast cells and LAD2 cells were measured in vitro. Molecular docking analysis, molecular dynamics simulation and siRNA transfection were used to prove the receptor of DCD(86-103). DCD(86-103) caused skin inflammatory reaction in WT mice by cytokines release. Moreover, DCD(86-103) directly activated mast cells and induced cytokines release in vitro. ST2 might be a key receptor to mediate the activation effect of DCD(86-103) on mast cells and lead to cytokines release. DCD(86-103) might induce inflammatory reaction and participate in the occurrence and development of psoriasis.

LB870

Transcriptomic profiling of pemphigus lesion infiltrating mononuclear cells reveals a distinct local immune microenvironment and novel lncRNA regulators.

Z. Huang, P. Qu, K. Wang, J. Zheng, M. Pan, H. Zhu
Rui Jin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

Pemphigus is an autoimmune skin disease. Ectopic lymphoid-like structures (ELSs) were found to be commonly present in the pemphigus lesions, presumably supporting in situ desmoglein (Dsg) -specific antibody production. Yet functional phenotypes and the regulators of Lymphoid aggregates in pemphigus lesions remain largely unknown. Herein, we used microarray technology to profile the gene expression in skin lesion infiltrating mononuclear cells (SIMC) from pemphigus patients. On top of that, we compared SIMC dataset to peripheral blood mononuclear cells (PBMC) dataset to characterize the unique role of SIMC. Functional enrichment results showed that mononuclear cells in skin lesions and peripheral blood both had over-represented IL-17 signaling pathways while neither was characterized by an activation of type I Interferon signaling pathways. Cell-type identification with relative subsets of known RNA transcripts (CIBERSORT) results showed that naïve natural killer cells (NK cells) were significantly more abundant in pemphigus lesions, and their relative abundance positively correlated with B cells abundance. Meanwhile, plasma cells population highly correlated with type 1 macrophages (M1) abundance. In addition, we also identify a lncRNA LINC01588 which might epigenetically regulate T helper 17 cells (Th17)/ regulatory T cells (Treg) balance via the PPAR signaling pathway. Here, we provide the first transcriptomic characterization of lesion infiltrating immune cells which illustrates a distinct interplay network between adaptive and innate immune cells. It helps discover new regulators of local immune response, which potentially will provide a novel path forward to further uncover pemphigus pathological mechanisms and develop targeted therapy.

LB871

β-Tryptase promotes inflammatory response in psoriasis by activating keratinocytes

D. Che, S. Geng
Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, Shaanxi, China

Mast cells (MCs) is an effector cells in inflammatory skin diseases by secreting potent mediators. It has been proved that MCs significantly increase and activate in the lesions of psoriasis patients, which can be activated by external factors and a variety of endogenous mediators to participate in the pathological changes and the formation of inflammatory microenvironment of psoriasis. Current studies suggest that MCs participate in the inflammatory axis activation by releasing factors, such as TNF-α and IL-17. MCs also play an important role in T cells differentiation and inflammatory cell recruitment. However, as the largest specific inflammatory mediators releases by MCs, whether β-tryptase show a pathological role in psoriasis has not been reported. In our present study, we detected the level of β-tryptase and inflammatory mediator in skin lesions of psoriasis patients and normal skin by immunohistochemical staining, immunofluorescent staining and ELISA. The results showed that β-tryptase was significant upregulation in psoriasis patients, which was positively correlated with epidermis thickening and inflammatory mediators level. Moreover, β-tryptase analysis in imiquimod mouse model showed the same results. In addition, MCs and keratinocytes (KCs) co-culture in vitro study was executed and the results showed that activated MCs could promote KCs proliferation and induced inflammatory mediators release. Intervention with β-tryptase antagonist can significantly inhibit KCs activation. Our results provides evidence that β-tryptase which is release by MCs might be a key inflammatory medium induces KCs proliferation and activation, which shows a role in pathology and inflammatory response of psoriasis.

LB872

Suprabasin derived polypeptides: SBSN(50-63) induced inflammatory reaction by activation mast cells via TLR4

T. Zhou[1], X. Du[1], L. Zhang[2], Y. Zheng[1], T. Jia[1], X. Song[1], D. Che[1], S. Geng[1]
[1]Department of Dermatology, Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, Shaanxi, China, [2]School of Pharmac, Xi'an Jiaotong University, Xi'an, Shaanxi, China

Psoriasis is an immune-mediated inflammatory skin disease that affects large proportions of populations worldwide. As the key sentinel immune cells, mast cell activation is involved in the early onset of psoriasis. SBSN, a secretory protein processed by stratum spinous of stratified epithelium, regulates epidermal differentiation and skin barrier under physiological conditions. The expression of SBSN is significantly increased in the psoriatic lesions. However, whether it could play a pro-inflammatory role in psoriasis by activating mast cells is unclear. To investigate whether suprabasin derived polypeptides: SBSN(50-63) activates MCs and causes skin inflammatory responses participating in psoriasis. Wild-type mice were subcutaneously injected with SBSN(50-63) to observe inflammatory cells infiltrated in skin and cytokines release *in vivo*. Inflammatory mediators released by mouse primary mast cells and LAD2 cells were measured in vitro. Molecular docking analysis, molecular dynamics simulation and siRNA transfection were used to find and demonstrate SBSN receptor(50-63). SBSN(50-63) induced cytokines release from mast cells, thus participating in skin inflammation. In addition, toll like receptor 4 (TLR4) may be a crucial receptor to mediate the effect of SBSN50-63) on mast cell activation. SBSN(50-63) might be induced skin inflammation and contributed to the pathogenesis of psoriasis.

LB873

Neuroblast differentiation-associated protein derived polypeptides: AHNAK(5758-5775) induced inflammatory reaction by activation mast cells via ST2

X. Song, L. Zhang, X. Du, Y. Zheng, T. Jia, T. Zhou, D. Che, S. Geng
Dermatology, Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, Shaanxi, China

Mast cells significantly increase and activate in psoriasis lesions and are involved in psoriatic inflammation. Neuroblast differentiation-associated protein (AHNAK) mainly express in skin, esophagus, kidney and other organs, which participates in the differentiation of neurons, the formation of cytoskeletal structure muscular regeneration, and the calcium homeostasis process. Whether AHNAK is involved in mast cell activation is unclear, and the mechanisms of AHNAK-induced skin inflammation also need investigation. To investigate whether Neuroblast differentiation-associated protein derived polypeptides: AHNAK(5758-5775) activates mast cells and induces skin inflammation contributing to psoriasis. Wild-type mice were treated with AHNAK(5758-5775) to observe inflammatory cells infiltration in skin and cytokines release *in vivo*. Release of inflammatory mediators by mouse primary mast cells and LAD2 cells were measured in vitro. The receptor of AHNAK(5758-5775) was demonstrated by molecular docking analysis, molecular dynamics simulation and siRNA transfection. AHNAK(5758-5775) could cause skin inflammatory reaction and induce cytokines release in WT mice, and could activate mast cells in vitro. ST2 may be a key receptor mediating the activation effect of AHNAK(5758-5775) on mast cells and leading to cytokine release. AHNAK(5758-5775) might induce inflammatory reaction and participate in the onset and development of psoriasis.

LB874

History of autoimmune disease associated with an increased risk of cutaneous immune related adverse events among patients with advanced cancer

T. V. Jacoby[1, 2], N. Shah[2, 3], M. S. Asdourian[2, 4], N. LeBoeuf[4, 5], Y. Semenov[2, 4], L. L. Thompson[2, 4, 6], K. Reynolds[4, 7], S. Chen[2, 4]
[1]University of Hawai'i at Manoa, Honolulu, Hawaii, United States, [2]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [3]Virginia Commonwealth University School of Medicine, Richmond, Virginia, United States, [4]Harvard Medical School, Boston, Massachusetts, United States, [5]Center for Cutaneous Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States, [6]Medicine, Brigham and Women's Hospital, Boston, Massachusetts, United States, [7]Hematology/Oncology, Massachusetts General Hospital Cancer Center, Boston, Massachusetts, United States

Cutaneous immune related adverse events (cirAEs), are the most common adverse effect of immune checkpoint inhibitors (ICI). The relationship between history of autoimmune disease and the future development of cirAE is unclear; we sought to evaluate this possible association. In this single institution retrospective case-control study, we extracted demographic and clinical information from electronic medical records among patients who developed a cirAE (n=648) and a control group of 504 patients who did not develop a cirAE between 1/1/16-6/29/21. History of autoimmune disease prior to ICI initiation based on predefined criteria was used as the independent variable. Multivariate logistic regression, controlling for relevant co-variates (age, sex, ICI subtype, cancer type/stage, and ECOG score), was used to determine the association between autoimmune disease and the future development of cirAE development, and severity. Among the 648 patients who developed a cirAE between 1/1/16-6/29/21, 103 (15.9%) had a autoimmune disease diagnosis prior to ICI initiation, and 43 (8.6%) among the control population. Patients who had a prior history of autoimmune disease had an elevated odds of developing a cirAE (OR:1.97; p<0.001;CI: 1.319-2.946); there was no association with increased severity among these patients. These results may provide clinicians the ability to better counsel ICI patients that although an autoimmune disease history is associated with increased odds of cirAE, they appear to be similar in severity to those patients who develop them without a history of autoimmune disease.

LB875

Oil Paint Induced Necrotizing Granulomatous Hand Dermatitis

A. Sood[1], D. Barrett[1], J. Cheeley[1, 2]
[1]Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States, [2]Division of General Medicine and Geriatrics, Emory University School of Medicine, Atlanta, Georgia, United States

A woman in her 80s presented with an enlarging, tender, red nodule on the palmar aspect of the left hand. A hand surgeon ordered magnetic resonance imaging without contrast, which demonstrated a multilobulated cystic mass that was subsequently excised. Histology showed extensive necrotizing granulomatous inflammation and exhibited bizarre-appearing hair fibers at the periphery of the inflammation on polarized light microscopy. Despite excision, the mass persisted and enlarged for 6 months, with intermittent purulent drainage. Doxycycline 100 mg and trimethoprim-sulfamethoxazole 800 mg-160 mg, both prescribed twice daily for 7 days yielded no improvement. On presentation to dermatology, the patient denied fevers, chills, malaise, proximal erythema, or tender lymphadenopathy. She reported she is an avid oil painter and used her left hand to clean brushes. She provided a list of all oil materials used for painting, including 15 oil colors and four paint brush bristles. Two punch biopsies showed findings similar to initial excisional histology. Grocott-Gomori methenamine silver, acid fast, and Gram stains were negative for infectious organisms. Tissue cultures and polymerase chain reaction assays performed on fresh tissue were negative. The negative infectious studies, lack of comorbid rheumatoid arthritis, and location of polarizable hair fibers suggested foreign body necrotizing granulomatous dermatitis due to oil paint or solvents. A two-month course of minocycline 100 mg twice daily resolved the patient's nodule and overlying erythema. While foreign materials, and particularly hair particles, have been reported to induce granulomas, the peripheral location of the bristles relative to the granulomatous infiltrate make the bristle fibers an unlikely etiology. The oils used to prepare the pigments are nonpolarizable and represent the most compelling cause for the necrotizing granulomatous inflammation. Physicians should consider oil paint as a possible foreign body capable of inducing granulomatous dermatitis.

LB876

Acute inflammatory edema: A challenging diagnosis in a critically ill patient

S. McGaugh, T. Chakrala, R. O. Prakash, K. Motaparthi
University of Florida College of Medicine, Gainesville, Florida, United States

A 55-year-old man with a prior history of fibrotic lung disease was admitted to the hospital for bilateral lung transplantation. His post-operational course was complicated by severe volume overload requiring initiation of continuous veno-venous hemodialysis (CVVHD). During this hospitalization, he had developed erythematous plaques over his bilateral thighs. Examination revealed erythematous and edematous plaques with a peau d'orange appearance extending over his bilateral thighs and abdominal pannus. Importantly, the inguinal and infra-abdominal folds were spared. The clinical differential diagnosis included acute inflammatory edema (AIE), early lipodermatosclerosis (LDS), and stasis change. Punch biopsy of a thigh lesion revealed prominent dermal edema, a sparse perivascular and interstitial lymphohistiocytic infiltrate, ectactic vessels, and fibrosis: findings consistent with a diagnosis of AIE. Improvement of the patient's volume status, increased mobility, and frequent position changes were recommended. AIE is a variant of pseudocellulitis characterized by erythematous and edematous plaques primarily found on the thighs and abdomen that spare areas of increased pressure, such as the inguinal folds. AIE is an acute lymphedema that predominantly affects critically ill patients with hypoalbuminemia, increased body mass index, renal impairment, and evidence of fluid overload. LDS and stasis dermatitis can also present on the thighs and pannus; however, these conditions also commonly manifest on the distal lower extremities and do not spare skin folds. Additionally, LDS and stasis are typically chronic rather than acute. LDS is a lobular panniculitis with fat necrosis, lipomembranous change, and septal fibrosis. In stasis, there are an increased number of thickened capillaries, red blood cell extravasation, and siderophages.

LB877

2019 EULAR/ACR criteria for systemic lupus may differentially affect classification of different cutaneous lupus subtypes

S. Shah[2], F. S. Esaa[2], L. Baughman[1], M. Gadarowski[3], C. T. Richardson[1]
[1]Dermatology, University of Rochester Medical Center, Rochester, New York, United States, [2]University of Rochester School of Medicine and Dentistry, Rochester, New York, United States, [3]SUNY Upstate Medical University, Syracuse, New York, United States

The European League Against Rheumatism and American College of Rheumatology jointly developed new classification criteria for systemic lupus erythematosus (SLE) in 2019 (EULAR/ACR 2019). In this retrospective pilot study, we evaluated individual subjects with cutaneous lupus (CLE) whose disease classification changed based on the new criteria. We found that EULAR/ACR 2019 criteria may affect systemic disease classification in subjects with DLE and SCLE differently. SLE classification of 50 subjects with CLE was determined using both the ACR 1997 and EULAR/ACR 2019 criteria. Out of 50 subjects with CLE, 24 (48%) had concurrent SLE based on ACR 1997 criteria, while 23 (46%) had SLE per EULAR/ACR 2019 criteria. Despite the apparent similarity in SLE frequency between the two criteria, in reality a substantial number of subjects (9/50; 18%) were classified differently. Of these 9 subjects, 5 met ACR 1997 criteria but failed to meet EULAR/ACR 2019 criteria; 4/5 (80%) of these subjects had DLE. In contrast, among the 4 subjects that met EULAR/ACR 2019 criteria but failed to meet ACR 1997 criteria, 3/4 (75%) had SCLE. These data suggest that DLE patients are less likely to meet EULAR/ACR 2019 criteria for SLE, while SCLE patents are more likely to do so, as compared to the prior ACR 1997 criteria. We then evaluated the specific clinical parameters of each classification criteria to determine which factored into the change in classification status among these 9 subjects. One of the most notable changes from prior criteria was a new entry requirement of a positive antinuclear antibody (ANA) titer of 1:80. As expected, ANA status significantly affected SLE classification among DLE and SCLE subjects. We next examined whether greater severity of skin disease, as measured by the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI), correlated with increased likelihood of SLE classification, but found no association.

LB878

The NINJA mouse develops peripheral tolerance in the skin and is useful as a model for the study of lichenoid immune-related adverse events.

N. I. Hornick[1, 2], M. Damo[1], N. Joshi[1]
[1]Immunobiology, Yale University, New Haven, Connecticut, United States, [2]Dermatology, Yale University, New Haven, Connecticut, United States

The development and proliferation of immune checkpoint inhibitors (CPI) as cancer therapies has represented a transformative shift for cancer treatment. The significant benefits accrued by patients receiving these drugs, however, often come at the cost of immune-related adverse events (irAE), which may occur in any organ system but are most commonly cutaneous (c-irAE). While the majority of these c-irAE can be successfully treated, they incur significant morbidity, and may cause the interruption or discontinuation of CPI treatment. Research into the origins of these reactions, and the development of more targeted therapies for their management, have been hindered by a lack of suitable animal models. Leveraging our lab's iNversion-INduced Joined neoAntigen (NINJA) mouse model, we are able to induce an endogenous immune response to neoantigen expression that bypasses central tolerance. Expressing neoantigen in the skin in these mice produces peripheral tolerance and minimal skin disease, while concomitant CPI treatment yields a more robust dermatitis by gross pathologic scoring ($p<0.01$). Histologic examination of this inflamed skin reveals many features of lichenoid irAE as seen in patients. Comparing single cell transcriptomic analysis of these mice to patient (lesional and nonlesional) and healthy control human skin reveals parallels between the response to self-antigen both in the tolerogenic (healthy) state and in the inflamed (c-irAE) state. These changes suggest the establishment of self-tolerance driven by interferon γ-driven changes in myeloid antigen-presenting cells, which is broken by CPI administration. Based on these features, we present the NINJA mouse as an appropriate platform for further investigation into the mechanisms underlying c-irAE as well as for the identification of treatments that will ameliorate c-irAE without impacting CPI-driven tumor response.

LB879

Friend or foe? Identification of MCPyV-specific B cells in Merkel cell carcinomas suggests a functional role in tumor immunity

H. J. Rodriguez Chevez[2, 1], T. Pulliam[2], A. Remington[2], P. Nghiem[2], J. Taylor[1]
[1]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States, [2]Division of Dermatology, University of Washington, Seattle, Washington, United States

Merkel cell carcinoma (MCC) is a rare and aggressive cancer of the skin with ~30% mortality. Around 80% of MCC tumors originate from integration of the Merkel cell polyomavirus (MCPyV) DNA into the host genome, leading to expression of the viral T-antigen oncoprotein and ultimately, tumorigenesis. Though PD-1 pathway blockade has significantly improved outcomes for advanced MCC, many patients do not benefit from or develop resistance to this immunotherapy. In recent years, tumor-infiltrating B cells have been generally associated with better outcomes in MCC. B cell immunity against cancer is presumed to originate within in situ B lymphocyte maturation aggregates. These 'tertiary lymphoid structures' harbor germinal center B cells that promote anti-tumor CD8 T cell responses in collaboration with CD4 T follicular helper cells. However, the mechanisms by which tumor antigen-specific B cells promote or sometimes antagonize cancer immunity have been difficult to elucidate in human tumors. The obligate expression of the viral T-antigen oncoprotein in all MCPyV-positive MCC tumors allows for cancer-specific B cell responses to be studied across multiple patients. Using a barcoded and fluorescently labeled T-antigen protein tetramer, we analyzed the transcriptome, proteome, and antibody sequences of MCPyV-specific B cells in 13 MCC tumor digest samples using single-cell technologies. MCPyV-specific B cells were identified in both tumor-infiltrated lymph nodes and tumors. Protein and mRNA analyses revealed the presence of MCPyV-specific B cells with hallmarks of germinal center or antibody-secreting cells, indicating the presence of an ongoing B cell response targeting this virus-derived tumor antigen. These data suggest that targeting tumor-specific B cell responses could be a novel approach to improve the efficacy of cancer immunotherapy.

LB880

Identifying common and disease-specific pathways through comparative scRNA-Seq profiling of four skin autoimmune diseases

Y. Wang, M. Frisoli, K. Afshari, N. Haddadi, J. Harris, M. Rashighi, M. Garber
University of Massachusetts Chan Medical School, Worcester, Massachusetts, United States

Motivated by the observation that the risk of developing a second autoimmune disorder increases for patients that are afflicted by one condition and by the large number of shared genetic variants associated with diverse autoimmune diseases, we began a study to uncover both the shared and disease-specific markers of skin autoimmune disorders. Here we present a preliminary analysis of four conditions that differ drastically in their main type of autoimmune response. We collected skin samples from 10 controls and 39 individuals affected with vitiligo (Th1), psoriasis (Th17), dermatomyositis (Th1), and cutaneous lupus erythematosus (complex). Our initial analysis recapitulates well-established differences between these diseases and already provides a surprising observation. CXCL13, a chemokine associated with CXCR5+ B cell recruitment, is upregulated in all four diseases, including vitiligo and psoriasis which are thought to be predominantly T cell-mediated conditions. CXCL13 is upregulated the most in dermatomyositis, in which B cells have not been shown to be recruited and function in the skin. The increased CXCL13 expression is not correlated with B cell population size. Moreover, expression of CXCR5, the cognate CXCL13 receptor, is low throughout the epidermis suggesting that CXCL13 may have an alternative mode of action that does not involve B cells and that could operate through a different receptor.

LB881

Clinical utility of dermoscopy and 31-gene expression profiling by dermatology providers in melanoma management care

A. Witkowski[1], C. Lee[1], E. Latour[1], J. Vetto[1], J. Ludzik[2]
[1]*Oregon Health & Science University, Portland, Oregon, United States,* [2]*Uniwersytet Jagiellonski w Krakowie, Krakow, Małopolska, Poland*

A 31-gene expression profile (31-GEP) test that predicts metastatic risk in patients with cutaneous malignant melanoma (CMM) has previously been validated and is available for clinical use. The test dichotomizes patients into lower risk and higher risk groups based on differences that correspond to unique genetic expression pattern. Although the impact of such a test on dermatology provider's clinical decision-making has been studied, little is known about whether there exists an association between certain clinical features, such as dermoscopy, and 31-GEP results. In this retrospective analysis of 31-GEP test results ordered by dermatologists, we evaluated the frequency of dermoscopic features, using a modified dermoscopy three-point checklist, in 17 cases (n=17) and compared these findings to other key clinicopathologic features including tumor thickness, ulceration, and mitotic rate to 31-GEP results. Additionally, we evaluated the dermatologist's perspective and incorporation of GEP testing as part of patient discussion on melanoma management. 31-GEP stratified patients into 4 groups; groups 1A and 1B are considered low risk of metastasis or recurrence, while 2A and 2B are considered high risk. Of the 17 cases we had fifteen group 1A (88.23%), one 1B (5.88%), and one 2B (5.88%) result. Overall frequency of dermoscopic features is as follows; 100% of lesions presented with asymmetry, 47% with round structures and 70.6% with blue-white color. The average time providers spent explaining and ordering the test was 15 minutes, with a range of 10 to 20 minutes. This study represents our experience and understanding of the dermatologist's role ordering 31-GEP in the care pathway of melanoma patients and we recommend that dermatology providers consider ordering the test for newly diagnosed CMM patients

LB882

Dissecting mechanisms of responsiveness to the combination therapy of radiation and anti-PD-L1/anti-TGFb treatment in murine squamous cell carcinoma models

H. T. Lind, S. Hall, A. Strait, C. Young, P. Owens, X. Wang
University of Colorado - Anschutz Medical Campus, Aurora, Colorado, United States

Squamous cell carcinoma (SCC) is often marked by an immunosuppressive tumor microenvironment, particularly in metastatic/recurrent disease. The purpose of this study is to evaluate the anti-tumor efficacy of and mechanisms of response to the combination therapy of radiotherapy (RT) and anti-PD-L1/anti-TGFb treatment using SCC models. We transplanted tumor cells derived from either carcinogen-induced SCCs or spontaneous SCCs of K15.Kras12D/Smad4-/- mice to syngeneic mouse recipients and subjected them to RT and anti-PD-L1/anti-TGFb therapy. Responders to this combination therapy exhibited complete tumor eradiation while non-responders displayed rapid tumor progression. Response-status was not predicated upon driver mutation. When re-challenged with same SCC cells, responders rejected tumor cells while naïve recipients rapidly developed SCCs, demonstrating that tumor eradiation is associated with anti-tumor immunity and long-term memory T cells. Responder tumor cells exhibited greater levels of MHC Class-I (MHCcI) mRNA and protein-presentation than non-responders. When treated with RT, responder SCC cells induce MHCcI expression and presentation greater non-responder cells. Additionally, knocking out Beta-2-Microglobulin, a subunit of MHC Class I, by CRISPR abrogated the anti-tumor immune response driven by anti-PD-L1/anti-TGFb treatment in mice inoculated with responder SCC cells. Single cell TCR sequencing of splenic T cells reveals that responders, but not non-responders exhibit multi-clonal CD8+ T cell expansion. Our data suggest that 1) elevated MHCcI by SCC tumor cells presentation facilitate a systemic, clonal expansion of long-term memory CD8+ T cells which drives anti-tumor immunity and 2) MHCcI expression at baseline or when induced by RT may serve as a predictive marker for therapeutic response to RT and anti-PD-L1/anti-TGFb combination therapy in SCCs.

LB883

Primary cutaneous aggressive epidermotropic cytotoxic T cell lymphoma: Pitfalls leading to misdiagnosis as mycosis fungoides

T. Chakrala, R. O. Prakash, S. McGaugh, K. Motaparthi
University of Florida, Gainesville, Florida, United States

A 51-year-old woman presented to the ED for severe, intractable pain due to ulcerated skin lesions. She reported an initial presentation of patches and thin plaques, and an earlier biopsy was interpreted as consistent with early mycosis fungoides(MF). Within the past year, widespread ulcerations developed. On exam, there were confluent ulcers with necrosis, surrounded by erythematous patches distributed across the chest, back, axillae, breasts, and lower extremities. Given the clinical evolution of ulceronecrotic lesions from thin patches and plaques, cytotoxic T cell lymphoma(CTCLs), including primary cutaneous aggressive epidermotropic cytotoxic T cell lymphoma(PC-AECTCL), γδ lymphoma, and NK-like lymphoma were considered. Histopathology demonstrated an epidermotropic and syringotropic T cell neoplasm with ulceration and keratinocyte necrosis. The cytotoxic immunophenotype was confirmed by expression of CD8 and granzyme, perforin, and TIA-1. Consistently observed in PC-AECTCL, the neoplastic lymphocytes were negative for CD45RO, CD2, and CD5. Importantly, expression of beta F1(TCR beta) was observed, but T cells were negative for TCR delta and CD56 immunostains, permitting exclusion of γδ and NK-like lymphomas, respectively. In contrast to advanced MF, CD7 expression was retained. Given the guarded prognosis of this CTCL, prompt follow-up with oncology was recommended. While PC-AECTCL can present with ulceronecrotic leisons, some patients present with clinicopathologic features indistinguishable from early MF, albeit with CD8 positivity. Subsequent rapid progression with evolution of ulceronecrotic lesions directly from patches or thin plaques, along with prominent epitheliotropism and retained expression of CD7 on repeat biopsy, should prompt consideration of this rare CTCL. Ulcers typically develop in tumor stage MF or MF with large cell transformation, and loss of CD7 is typical in advanced MF. IHC is required for exclusion of γδ and NK-like lymphomas which also demonstrate a cytotoxic immunophenotype and aggressive clinical course.

LB884

Integrated transcriptome and trajectory analysis of cutaneous T-cell lymphoma identifies putative precancer populations

J. Ren, R. Qu, N. Rahman, J. Lewis, A. King, X. Liao
Yale School of Medicine, New Haven, Connecticut, United States

Cutaneous T-cell lymphoma (CTCL) incidence increases with age, and blood involvement portends a worse prognosis. To advance our understanding of CTCL development and identify potential therapeutic targets, we performed integrative analyses of paired single-cell RNA and TCR sequencing of peripheral blood CD4+ T-cells from CTCL patients to reveal disease unifying features. The malignant CD4+ T-cells of CTCL show highly diverse transcriptomic profiles across patients, with most displaying a mature Th2 differentiation and T-cell exhaustion phenotype. TCR-CDR3 peptide prediction analysis showed limited diversity between CTCL samples, suggesting a role for a common antigenic stimulus. PHATE affinity-based transition analysis identified putative precancerous circulating populations characterized by an intermediate stage of gene expression and mutation level between the normal CD4+ T-cells and malignant CTCL cells. We further revealed the therapeutic potential of targeting CD82 and JAK2 that endow the malignant CTCL cells with survival and proliferation advantages. We found that CD82 deficiency markedly reduced the proliferative capacity of the activated CTCL cells and that apoptotic cells largely increased within the CD82-knockout CTCL cultures. Both CD82 affected downstream signaling pathways and differential gene enrichment highlighted JAK/STAT activation pathways in CTCL cells. To determine if increased target specificity could improve JAK inhibitor activity across CTCL patients, we performed CTCL patient-derived cell viability assays against a panel of FDA-approved agents exhibiting different JAK family member selectivity profiles. We found that CTCL cells exhibited marked preferential sensitivity to JAK2-specific agents, relative to JAK1/2-non-specific and pan-JAK inhibitors, which suggested that therapeutic strategies targeting JAK-2 may allow for more generalized cytotoxic effects against the malignant cells from patients with CTCL.

LB885

Sex-associated differences in genomic profiles of melanoma

D. Y. Kim[1], E. I. Buchbinder[3], R. Hartman[2]
[1]Harvard Medical School, Boston, Massachusetts, United States, [2]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, 3Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States

Sex differences in melanoma incidence and survival are well-documented with females having a decreased risk of metastasis and overall better survival outcomes than males, even after adjusting for tumor stage. There are numerous factors that could contribute to these disparities, including differences in behavior, host immunity, and tumoral factors. Here, we seek to examine sex-based tumor mutational differences, which may impact both melanoma prognosis and treatment outcomes. We assessed the genomic profiles of 2468 melanoma patients in the American Association for Cancer Research Project Genomics Evidence Neoplasia Information Exchange (v10.0). Multivariate linear and logistic regressions were used to estimate the associations between total mutation count and gene-specific mutational frequencies with sex, adjusting for race and ethnicity, sample type, tumor site, and age. In fully adjusted analyses, males had significantly higher total mutation counts than females in both primary (Coefficient = 6.1; 95% CI, 2.4, 9.8; P = 0.005) and metastatic disease (Coefficient = 4.7; 95% CI, 1.9, 7.5; P = 0.002). Similarly, males with primary or metastatic tumors were significantly more likely than comparable females to have mutations in DNA repair genes (Primary: OR, 1.6; 95% CI , 1.1, 2.2; P = 0.01; Metastatic: OR, 1.4; 95% CI, 1.1, 1.7; P = 0.01). To our knowledge, this is the first study to assess sex-based differences in tumor mutational profiles of melanoma. Despite poorer male survival outcomes, we observed that males had significantly more mutations than females and were more likely to have mutations in DNA repair genes, potentially influencing melanoma prognosis as tumor mutational burden and DNA repair mutations have been independently shown to be predictors of immunotherapy response. Our results provide insight into how treatment response and clinical outcomes could differ based on sex.

LB886

Cancer-associated fibroblast activation predicts progression, metastasis and prognosis of cutaneous squamous cell carcinoma

J. Knuutila[1], P. Riihilä[1], L. Nissinen[1], R. Kallionpää[2], T. Pellinen[3], V. Kähäri[1]
[1]Dermatology, Turun yliopisto Laaketieteellinen tiedekunta, Turku, Finland, [2]Auria Biobank, TYKS Turu yliopistollinen keskussairaala, Turku, Varsinais-Suomi, Finland, [3]FIMM, Helsingin yliopisto Suomen molekyylilaaketieteen instituutti, Helsinki, Uusimaa, Finland

Cutaneous squamous cell carcinoma (cSCC) possesses metastatic potential and in metastatic cases the prognosis is poor. At present, there are no established metastasis risk-associated or prognostic biomarkers for cSCC. Cancer-associated fibroblasts (CAFs) promote the progression of cancer, but there is limited evidence for their role in cSCC. We have examined the potential of CAF-associated biomarkers in the assessment of metastasis risk and prognosis of cSCC. We used multiplex immunohistochemistry to profile CAF landscape in tissue microarrays containing metastatic (n=33) and non-metastatic (n=146) primary human cSCCs, metastases (n=13), cSCC in situ (cSCCIS) (n=59), actinic keratoses (AK) (n=67), seborrheic keratoses (n=17) and normal skin (n=73). Quantitative high-resolution image analysis was performed with two separate panels of antibodies for CAF-associated markers and the results were correlated to clinical and histopathological parameters including disease-specific mortality. Increased stromal expression of fibroblast activating protein (FAP), α-smooth muscle actin (α-SMA), and secreted protein acidic and rich in cysteine (SPARC) was associated with progression to invasive cSCC. Elevation of FAP (p=0.026) and platelet-derived growth factor receptor-β (PDGFRβ) (p=0.004) expression in tumor stroma was associated with the progression of primary cSCC to metastatic stage. High stromal expression of PDGFRβ and periostin correlated with poor prognosis. In multivariate Cox regression analysis PDGFRα-/PDGFRβ+/FAP+ CAF subset independently predicted poor cSCC-specific survival (p<0.001). The results show, that fibroblast activation evolves during progression of cSCC. High PDGFRβ expression alone and PDGFRα-/PDGFRβ+/FAP+ CAF phenotype appear as potential biomarkers for metastasis of primary cSCC and for poor prognosis.

LB887

Direct effects of zinc in proliferation and migration of human squamous cell carcinoma cell lines *in vitro*

H. Mitsui, T. Sunaga, T. Kawamura
Yamanashi Daigaku Igakubu Daigakuin Sogo Kenkyubu Igakuiki, Chuo, Yamanashi, Japan

Zinc is a trace element that requires for more than 2000 transcription factors. Thus, zinc could have functions in development, differentiation, and cell growth of many cell types including cancer cells. Prior studies reported that zinc induced apoptosis in cancer cells, such as glioma, bladder, prostate, breast, and melanoma. Topical application of high concentration zinc containing Mohs paste is effective to shrink skin cancers including squamous cell carcinoma (SCC), breast cancer. However, the evidences of direct effect of zinc in growth and cell migration of cutaneous SCC cells are scarce. We aimed to study the functions of zinc in SCC cell growth and migration in vitro. Two cutaneous SCC cell lines (A431 and HSC-5) were cultured in serum free medium with or without different concentration of zinc. TPEN was used to chelate the zinc when the tumor cells were cultured in serum containing medium in some experiments. Zinc (1, 10, 100 μg/dl) significantly inhibits the proliferation of SCC cell lines at 48 hours and 72 hours in a dose dependent manner (p<0.05). Scratch assay was performed to test the effect of zinc in SCC cell migration. Chelation of serum zinc by TPEN (2μM) delayed the wound closure of A431 cells cultured in serum containing medium (p<0.05). On the other hand, addition of zinc to the serum free medium accelerates wound closure of HSC-5 cells compared to control serum free condition (p<0.05). These results demonstrate the suppressive function of zinc against SCC cells by reducing cell proliferation and inhibiting cell migration.

LB888

Role of MFN2 gene as prognostic biomarker for cutaneous squamous cell carcinoma recurrence

M. Cho[1], S. Lee[2], M. Roh[2]
[1]Dermatology, Yongin severance hospital, Yongin, Korea (the Republic of), [2]Gangnam Severance Hospital, Seoul, Korea (the Republic of)

Although vast amount of research has been done on genes related to cancers, it remains unclear whether Mitofusin2 (MFN2) functions as a tumor suppressor or oncoprotein. The impact of MFN2 on carcinogenesis is likely to be more complicated than expected, so further research is needed. We aimed to investigate the effect of MFN2 on the pathogenesis of cutaneous squamous cell carcinoma (cSCC) and analyzed the expression of MFN2 in cSCC tissue and investigated the influence of MFN2 expression on the biological behavior of cSCC. In this retrospective study, 111 cSCC patients treated with Mohs micrographic surgery (MMS) at the Department of Dermatology in Severance hospital from 2000 to 2017 were reviewed. MFN2 expression was examined by immunohistochemistry in 111 specimens, 93 from primary cSCC patients with no recurrence and 18 cSCC patients with recurrence. Correlation between various clinicopathologic factors including MFN2 expression and recurrence were analyzed. Moreover, the influence of MFN2 on biological behavior of cSCC cells was investigated in vitro. In the 111 surgical samples, immunoreactivity against MFN2 was low in 65 (58.6%) cSCC tissues and high in 46 (41.4%) cSCC tissues. Recurrence-free survival was significantly related to tumor size, differentiation status, and expression of MFN2, according to the Kaplan-Meier analysis. In multivariate analysis using age, gender, lesion site, level of MFN2 expression as cofactors, MFN2 expression was an independent risk factor for recurrence of cSCC with a hazard ratio of 8.262 (95% CI, 2.070-32.974; p=0.003). The proliferative, migratory, and invasive abilities of the cells were significantly decreased after MFN2-knockdown than control cells (all p<0.001). In conclusion, it was found that MFN2 expression is a significant indicator of poor prognosis among patients with cSCC and MFN2 has a strong influence on the behavior of cSCC cells in vitro. This study suggests that MFN2 may act as an oncogene by affecting the aggressiveness of cSCC and can be used as a biomarker to predict the prognosis of cSCC.

LB889

Risk stratification of squamous cell carcinoma using weakly supervised multitask learning of whole slide images

A. Choudhary[1], B. Boudreaux[2], P. Bhullar[2], S. Nelson[2], A. R. Mangold[2], R. Iyer[1]
[1]Electrical and Computer Engineering, University of Illinois Urbana-Champaign, Urbana, Illinois, United States, [2]Dermatology, Mayo Clinic Arizona, Scottsdale, Arizona, United States

Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer with rising incidence and mortality. Accurate risk stratification essential to identify high-risk patients and determine appropriate treatment. Histological evaluation of tissue whole slide images (WSI) is the gold-standard for cSCC risk assessment. However, manual tumor localization is tedious due to the large size of WSI and involves human-assessment related variability. We propose an AI-based assistive risk stratification tool for cSCC which performs tumor localization and risk stratification in a reproducible manner ensuring faster turnaround time. By leveraging deep learning, our tool captures complex pathophysiology patterns beyond visual perception and can support pathologist's analysis thus improving diagnostic accuracy. We rely on quantifying two most common high-risk features for cSCC: 1) depth of tumor invasion, 2)histological differentiation; and use multitask classification to model the correlation between risk factors. Our framework uses attention-based multiple instance learning and is trained with minimal supervision (subject-level diagnostic labels). We use graph networks and self-supervised learning to derive context-aware tissue features for classification. We validate our approach on a cohort of 446 patients enriched for intermediate to high-risk cSCC. The tissue WSIs are stained using hematoxylin and eosin staining and analyzed at 10X magnification. Our tool achieves AUC scores of 0.903 (F1 score = 0.799) and 0.937 (F1 score = 0.815) on differentiation and depth-based risk category classification of cancer patients. Our risk factor-based approach achieves more accurate stratification compared to existing cSCC staging frameworks and ensures that the attention-heatmaps align well with pathologist's interpretation by exploiting the correlation between risk factors.

LB890

Cyclic hair growth requires spatiotemporal heterogeneity in hair follicle signaling network

C. F. Guerrero-Juarez[1, 2, 4], Q. Wang[3], Q. Nie[1, 2, 4], M. Plikus[2, 1]
[1]NSF-Simons Center for Multiscale Cell Fate Research, University of California Irvine, Irvine, California, United States, [2]Department of Developmental & Cell Biology, University of California Irvine, Irvine, California, United States, [3]Department of Mathematics, University of California Riverside, Riverside, California, United States, [4]Department of Mathematics, University of California Irvine, Irvine, California, United States

Hair follicles (HFs) are cyclically growing skin mini-organs, that oscillate between small, telogen and large, anagen sizes and form hair fibers of variable length. Although cellular lineages and spatial distribution of HF cell types are largely defined, the dynamic molecular events controlling HF size and hair fiber length remain elusive. We developed a multi-scale mathematical model of HF, that permits computational testing of its growth behavior. Our model predicts that epithelial outer root sheath (ORS) cells need to develop signaling heterogeneity to one or more paracrine pathways for a HF to achieve and maintain its steady-state size in anagen and to not become excessively long, snake-like. Furthermore, for a HF to grow long hair fiber, signaling heterogeneity is required in epithelial matrix cells to allow for their constant replenishment by migrating ORS progenitors. We find proof of such modeling predictions with single-cell RNA-sequencing and genetic lineage tracing experiments. Our study shows how mathematical modeling can drive biological discovery and reveals previously unappreciated dynamic, multi-pathway signaling network of hair growth.

LB891

MANTIS: An integrated digital process for 3-D deconstruction of human skin immune landscape

R. Houmadi[1], M. Scholaert[1, 2], J. Martin[1], N. Serhan[1], M. Tauber[1, 3], E. Braun[2], L. Basso[1], E. Merle[4], P. Descargues[4], C. Paul[3], L. Lamant[3], E. Pages[2], N. Gaudenzio[1, 2]
[1]INSERM UMR 1291, Institute for Infectious and Inflammatory Diseases (Infinity), Toulouse, France, [2]Genoskin SAS, Toulouse, Occitanie, France, [3]Toulouse University and Centre Hospitalier Universitaire, Toulouse, France, [4]Genoskin Inc., Salem, Massachusetts, United States

The human skin is a tightly-organized ecosystem composed of a dense network of thick structural appendices surrounding naturally-resident and recruited immune cells. Routine clinical assays, such as conventional immunohistochemistry, fail to resolve the regional heterogeneity and immune topology of inflammatory skin conditions, which can lead to false interpretation. Here we introduce MANTIS (Multiplexed Annotated Tissue Imaging System), a flexible analytical system specifically designed for spatially-resolved immune-phenotyping of the skin in thick experimental samples or large clinical cohorts. Combining multiplexed 3-D imaging, machine learning and unsupervised bioinformatics, MANTIS automatically projects a representative digital immune landscape, while enabling interactive gating of anatomical regions and concomitant single-cell data quantification of biomarkers. We leverage these properties to analyze skin biopsies from healthy and pathological conditions.

LB892

Distinct endothelial behaviors orchestrate developing versus adult skin vascular responses

C. Kam[1], I. Singh[1], P. Sola[2], G. Solanas[2], J. Bonjoch[2], C. Matte-Martone[1], D. Gonzalez[1], E. Marsh[1], K. Hirschi[3], V. Greco[1]
[1]Genetics, Yale School of Medicine, New Haven, Connecticut, United States, [2]Institut de Recerca Biomedica, Barcelona, Spain, [3]Cell Biology, University of Virginia, Charlottesville, Virginia, United States

A functional network of blood vessels is essential for organ growth and homeostasis. While we possess substantial knowledge of the cellular mechanisms that dictate skin development and homeostasis, much is still not known concerning the cellular and tissue morphogenic events that dictate cutaneous vascular remodeling. To shed light on these fundamental biological processes, we performed longitudinal imaging in the skin of live mice using 2-photon microscopy. We systematically evaluated the same capillaries and endothelial cells (ECs) in neonatal mice, tracking their behaviors as the skin plexus expanded and reached adulthood. We found that capillary plexus expansion is driven by network-wide vessel regression and transient angiogenesis during neonatal stages. These processes involve migratory ECs that relocate from regressed segments to neighboring vessels. Furthermore, we observed that all ECs dynamically rearrange their positions within maturing capillaries, independent of their remodeling status. These rearrangements regulate EC density by ensuring an even cellular distribution during plexus expansion. As the skin approaches adulthood, plexus regression rates decrease, and ECs become positionally stable. However, upon injury, neighboring ECs do not proliferate but collectively elongate or migrate towards damaged sites to repair vessels. Remarkably, we found that injured adult ECs, rather than dying, survive through the activation of a plasmalemmal self-repair response. In contrast, neonatal ECs are disposed to die in response to injury, causing the regression of the vessel segment in which they reside. Our work defines fundamental cellular and tissue principles that underlie skin capillary maturation and maintenance of adult vascular homeostasis *in vivo*.

LB893

Macrophage recruitment after dermal pigmentation removal by 1064 nm laser is mediated by Fn14 upregulation of skin fibroblast

X. Du[1], Z. Wang[1], B. Chen[2], W. Zeng[1]
[1]Department of Dermatology, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China, [2]State Key Laboratory of Multiphase Flow in Power Engineering, Xi'an Jiaotong University, Xi'an, China

There is a high demand to remove undesirable dermal pigmentation by either pathological (e.g. nevus of Ota) or artificial (tattooing). Laser-based dermal pigmentation removal is the primary choice, and 1064 nm laser is regarded as the most effective modality. However, the direct pigment eruption can only partially disrupt the pigment particles, the mechanism underlying the remaining pigmentation clearance is largely unknown. Macrophage is assumed as an important player in post-laser pigmentation clearance. Activation of Fn14 has been reported to induce macrophage infiltration under multiple scenarios. Thus, the current study aimed to investigate the role of Fn14 in pigment clearance post-laser therapy. We established a dermal pigmentation rat model by ink injection, the lesion was treated by 1064nm laser. Then the CD68+ cells and Fn14 expression were quantified at different time points within 2 weeks. CD68+ macrophages infiltration ascended 1 day after the laser therapy and peaked 3 days post laser therapy. Fn14 expression responded more rapidly than macrophages infiltration, which elevated drastically after 1 day and decreased thereafter. To further clarify the role of fibroblast post-laser therapy, human primary fibroblasts were isolated and treated with 1064nm laser. Fn14 mRNA expression was upregulated in a time-dependent manner, which peaked at 12h after laser treatment and remained until 24h. Fn14 activated by its ligand TWEAK elicited fibroblast to produce CCL2 and CCL5, which promoted macrophage recruitment. This study indicated that 1064nm laser-induced Fn14 upregulation might be the cause of elevated macrophage infiltration under the condition of dermal pigmentation, which facilitates pigmentation clearance post-laser therapy.

LB894

Psoriatic inflammation modulates intercellular adhesion and mechanotransduction in human epidermis via ROCK2

M. Shutova[1, 2], J. Borowczyk[2], B. Russo[1, 2], S. Sellami[2], J. Drukala[3], M. Wolnicki[4], N. C. Brembilla[1, 2], G. Kaya[1], A. Ivanov[5], W. Boehncke[1, 2]

[1]Dermatolgoy, Hopitaux Universitaires Geneve, Geneve, Genève, Switzerland, [2]Pathology and Immunology, Universite de Geneve Faculte de Medecine, Geneve, GE, Switzerland, [3]Department of Cell Biology, Uniwersytet Jagiellonski w Krakowie, Krakow, Małopolska, Poland, [4]Pediatric Urology, Uniwersytet Jagiellonski w Krakowie Collegium Medicum, Krakow, Małopolskie, Poland, [5]Inflammation and Immunity, Cleveland Clinic Lerner Research Institute, Cleveland, Ohio, United States

Aberrant mechanotransduction and compromised epithelial barrier function are associated with numerous human pathologies including inflammatory skin disorders. However, the involved molecular mechanisms are not well understood. We investigated the alterations in cell-cell adhesions, mechanosensitive effector molecules and mechanosignaling in human epidermis during inflammation using psoriasis as a model disease. Psoriatic phenotype in human keratinocytes and reconstructed human epidermis was induced using a cytokine stimulation model ("M5" cytokine cocktail comprising of IL-17A, IL-22, Oncostatin M, TNFa, and IL-1a). We show that the inflammation upregulates the Rho-myosin II pathway and destabilizes adherens junctions promoting YAP nuclear entry. We show that the integrity of cell-cell adhesion but not the myosin II contractility per se is the determinative factor for the YAP regulation and barrier function in epidermal keratinocytes. The inflammation-induced disruption of adherens junctions, increased paracellular permeability and YAP nuclear translocation are regulated by ROCK2, independently from myosin II activation. Using a specific inhibitor KD025, we show that ROCK2 executes its effects via cytoskeletal and transcription-dependent mechanisms to shape the inflammatory response in the epidermis. ROCK2 inhibition, therefore, may be a promising strategy for the topical treatment of cutaneous inflammation in general and psoriasis in particular.

LB895

Readability, Understandability, and Actionability of Online Patient Education Materials for Sunscreen

S. Shareef[1], R. Rehman[2], M. Haque[1], J. I. Silverberg[3]
[1]Michigan State University College of Human Medicine, East Lansing, Michigan, United States, [2]Oakland University William Beaumont School of Medicine, Rochester, Michigan, United States, [3]Department of Dermatology, The George Washington University Columbian College of Arts and Sciences, Washington, District of Columbia, United States

Most adults in the United States obtain health information online. It is crucial that online patient education materials (PEMs) are easily interpreted in order to successfully impact patients' health behaviors. This is especially important for sunscreen PEMs, as proper use is associated with decreased risks of melanoma, non-melanoma skin cancers, and photoaging. American Medical Association recommends that PEMs be written at the sixth grade or lower reading level. We analyzed the readability of PEMs for sunscreen in order to identify opportunities to optimize PEMs. A Google search was performed using "sunscreen patient education." Reading level of PEMs was assessed via 10 validated formulas. Descriptive statistics were performed. Sixteen PEMs were identified. The average reading level was 8.2 (range: 6.4-10.9), which was above the recommended sixth grade reading level. Actionability domains that PEMs scored lowest on were lack of clear steps (n=3, 19%), visual aids (n=2, 13%), and tangible tools such as checklists (n=2, 13%). These results indicate that many online PEMs about sunscreens are not easily interpreted. Sunscreen PEMs should be improved by presenting the information at a lower reading level and taking steps to improve actionability.

LB896

Mogamulizumab multimodality therapy with systemic retinoids, interferon, or extracorporeal photopheresis for advanced cutaneous t-cell lymphoma

D. Weiner[1], S. Rastogi[1], D. J. Lewis[1], L. Cohen[1], R. Bhansali[2], J. Villasenor-Park[1], P. Haun[1], S. Samimi[1], C. Vittorio[1], E. Kim[1], A. Rook[1], S. Barta[2]
[1]Dermatology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Hematology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, United States

The purpose of this study was to assess the clinical efficacy of mogamulizumab in combination with systemic interferon, systemic retinoids, and/or extracorporeal photopheresis for patients with advanced cutaneous T-cell lymphoma. There are limited treatment options for patients with CTCL refractory to previous systemic therapies. At our center, mogamulizumab has been combined with other immunotherapies for an enhanced synergistic treatment effect in these patients. This was a retrospective case series on 11 patients (6 female; mean age 73 years) with refractory stage IIIB-IVB CTCL at the University of Pennsylvania CTCL Clinic who were treated with a multimodality regimen of mogamulizumab added to systemic interferon and/or retinoids, with or without extracorporeal photopheresis. 9 patients achieved a global response, 4 of which were a complete response. Response rates by compartment were 90% in skin, 91% in blood, and 50% in lymph nodes. At best response, skin involvement and the blood clonal cell count had decreased by an average of 85% and 74%, respectively. 8 patients remain without progression after a median follow-up of 12.2 months. The most common toxicities were mogamulizumab-associated rash (n=6, 55%; grade 3: n=2, 18%), neutropenia (n=6, 55%; grade 3: n=2, 18%), and lymphopenia (n=6, 55%; grade 3: n=3, 27%). The mogamulizumab-associated rash was managed based on severity by either observation, topical steroids, systemic steroids, or holding of mogamulizumab. In conclusion, these findings demonstrate that multimodality therapy with mogamulizumab offers a highly effective treatment approach for patients with refractory CTCL. Further study of combination immunotherapy for CTCL is warranted.

LB897

The risk of COVID-19 infection in patients with alopecia areata

A. Oulee[1,2], S. Ahn[1,3], S. Shahsavari[1,4], A. Martin[1,2], J. J. Wu[1]
[1]Dermatology Research and Education Foundation, Irvine, California, United States, [2]University of California Riverside School of Medicine, Riverside, California, United States, [3]University of California San Diego School of Medicine, La Jolla, California, United States, [4]Dartmouth College Geisel School of Medicine, Hanover, New Hampshire, United States

Alopecia areata (AA) is an immune-mediated non-scarring hair loss disorder associated with a predominant TH1cytokine profile.1 The risk of individuals with AA contracting COVID-19 is of concern to physicians as well as the entire community affected by AA. We performed a large-retrospective cohort study to determine the risk of contracting COVID-19 in individuals with AA compared to individuals without AA. We queried the Symphony Health-derived data from the COVID-19 Research Database,2 and individuals with a diagnosis of AA were identified. Subjects with no record of AA diagnosis were randomly placed in the control group in a 4:1 size ratio compared with the AA group and matched by age and sex. PCR-confirmed cases of COVID-19 between January 1, 2020, and September 1, 2021, were identified. The COVID-19 incidence rate ratio (IRR) for adults with AA was 0.72 (95% CI 0.68, 0.76) compared with adults without AA (p-value < 0.001). When controlling for comorbidities previously identified as COVID-19 risk factors, the IRR remained significant but increased to 0.86 (95% CI 0.82, 0.91). Individuals with AA have a slightly decreased risk of contracting COVID-19 compared to individuals without AA. It has been demonstrated that interferon-gamma (IFN- γ) leads to the downregulation of the angiotensin-converting enzyme 2 (ACE2), the SARS-CoV receptor.3 Thus, it is possible that increased levels of IFN- γ seen in individuals with AA confer some protection against this viral infection. References: 1. Islam N, Leung PS, Huntley AC , Gershwin ME. The autoimmune basis of alopecia areata: a comprehensive review. Autoimmune Rev 2015;14:81-9. 2. COVID-19 Research Database. COVID-19 Research Database Consortium. 3. de Lang A, Osterhaus AD , Haagmans BL. Interferon-gamma and interleukin-4 downregulate expression of the SARS coronavirus receptor ACE2 in Vero E6 cells. Virology 2006;353:474-81.

LB898

Validation of the paindetect questionnaire in hidradenitis suppurativa using quantitative sensory testing

P. Speck, A. Alsouhibani, D. Mustin, M. Siira, H. Li, D. Harper, L. Orenstein
Emory University School of Medicine, Atlanta, Georgia, United States

Hidradenitis suppurativa (HS) is a painful, inflammatory skin condition that causes nociceptive, neuropathic, and nociplastic pain types. The PainDETECT Questionnaire (PD-Q) is a nine-item survey that has been used to assess the likelihood of neuropathic pain character. However, it has not been validated in HS. Quantitative Sensory Testing (QST) is a standardized series of sensory exams considered the gold standard for evaluation of sensory dysfunction including neuropathic pain. Evaluating the performance of the PD-Q in HS may enable clinicians to screen for neuropathic pain in HS and tailor pain management. In this observational, cross-sectional study, 20 participants with HS underwent QST at a control site on the dorsal hand and a painful HS lesion to evaluate pain phenotype including the presence of neuropathic pain. Participants also completed the PD-Q. Participants were of young age (median 36 years, IQR 30-47) and mostly female (75%) and of Black race (55%). 14 participants (70%) were Hurley stage 2, and 5 (20%) were Hurley stage 3. Participants had substantial inflammatory activity per IHS4 (15, 10-42). 17 HS lesions (85%) were draining tunnels and 3 (15%) were inflammatory nodules. 13 HS lesions (65%) were in the axilla, 6 (30%) were in the groin, and 1 (5%) was on the chest. 10 participants (50%) exhibited neuropathic pain on QST, defined by the presence of dynamic mechanical allodynia at the HS lesion. On the PD-Q, 7 participants (35%) were classified as unlikely having neuropathic pain, 7 (35%) as indeterminate for neuropathic pain, and 6 (30%) as likely having neuropathic pain. QST and the PD-Q demonstrated 75% agreement for pooled likely or indeterminate presence of neuropathic pain versus absence of neuropathic pain with a Cohen's k of 0.5 (p = 0.01) demonstrating moderate agreement. Compared to the gold standard of QST, the PD-Q has modest agreement in screening for neuropathic pain character in HS. Because pain phenotype strongly informs management of chronic pain, the PD-Q may be a useful clinical tool for directing management of HS pain.

LB899

Prevalence of comorbidities in patients with acne vulgaris: a claims database analysis

A. Grada[1,2], P. O. Perche[3], S. R. Feldman[3]
[1]Former Head of R&D and Medical Affairs, Almirall US, Malvern, Pennsylvania, United States, [2]Grada Dermatology Research LLC., Chesterbrook, Pennsylvania, United States, [3]Dermatology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States

Background: Given how common acne vulgaris is, some patients may have comorbidities. Understanding the frequency of these comorbidities may be particularly important for optimizing the care of patients. Objective: The assess the prevalence of comorbidities in patients with acne. Methods: The Truven Health MarketScan® Databases were used to identify patients with acne ≥ 12 years old in 2012. The prevalence of several comorbidities (anxiety, depression, dermatitis, lupus, and rosacea) were assessed via ICD codes. Analysis was performed separately for males and females with acne ≥ 12 years old and females with acne ≥ 25 years old. Results: 329,053 patients with acne and 1,316,212 controls were included in the analysis. In males and females ages ≥ 12 with acne, dermatitis was the most common comorbidity of those assessed, followed by anxiety, depression, rosacea, and lupus (12.4%, 7.8%, 7.2%, 2.6%, and 0.3%, respectively). In females ages ≥ 25 with acne, dermatitis was also the most common comorbidity assessed, followed by anxiety, depression, rosacea, and lupus (16.1%, 11.8%, 11.0%, 5.7%, and 0.6%, respectively). Conclusion: Dermatitis was the most common comorbidity in both groups analyzed and also the comorbidity with the greatest difference in prevalence from controls. Anxiety and depression were also more prevalent in acne patients than in controls. Psychiatric comorbidities not only affect patient well-being but are a strong predictor of poor adherence to treatment and a barrier to improving treatment outcomes. Rosacea and lupus were more prevalent in acne patients versus controls and cutaneous comorbidities are associated with worse quality of life. Both cutaneous and non-cutaneous comorbidities frequently affect acne patients and incorporating them into discussions of care may improve treatment outcomes and patient satisfaction.

LB900

Role of p38β in cutaneous T cell lymphoma

X. Zhang, S. Nam, J. Hsiang, S. Rosen
Beckman Research Institute, City of Hope, Duarte, California, United States

Cutaneous T cell lymphoma (CTCL) is an incurable cancer; understanding its underlying molecular mechanisms may unlock a cure. In patient samples of CTCL, we observed a significant increase in gene expression of p38β while that of p38α decreased, compared to normal healthy CD4+ T cells. This prompted us to further dissect the role of p38β in CTCL to inform the application of small molecule inhibitors that specifically target p38β. Current well-developed small molecule p38 inhibitors target both p38α as well as p38β, as they share ~80% structural similarity. However, multiple clinical trials have shown adverse effects and development of drug resistance when patients with cancer are treated with potent p38 inhibitors alone. Such side effects likely occur because p38α is an essential protein in many cell types; indeed, p38α gene knock-out mice exhibit embryonic lethality. Therefore, any prolonged treatment using p38α inhibition may cause adverse effects. Using Hut78 CTCL cells in which we silenced p38β using lentiviral siRNA, we tested for possible mechanisms of drug resistance that could explain why patients who participated in p38α/β inhibitor clinical trials experience adverse effects. Gene silencing of p38β in Hut78 cells did not decrease cell proliferation; instead, proliferation slightly increased compared to that of WT cells. This aligned with increased IL-17 RA and p38γ which is a driver for cell proliferation in Hut78 cells. Our hypothesis is that p38β-depleted CTCL cells increase survival by elevating the MAPK12-NFAT-IL17 signaling pathway axis, which increases proliferation and propagandas inflammation in the surrounding regions resulting in drug resistance and adverse effects. We used confocal immuno-fluorescence microscopy analyses of p38β-depleted Hut78 cells to reveal a novel molecular mechanism, in which depleting p38β offset cytoskeleton formation in the cytosol. This suggests p38β is important for maintaining the shape or frame of CTCL cells, and may explain why CTCL, a malignant T cell, infiltrate skin, from which novel revenues of drug development may be invented that are complementary to p38β inhibition.

LB901

Inflammaging in human skin: Early onset of senescence and imbalanced epidermal homeostasis across the decades.

J. E. Oblong[1], B. B. Jarrold[1], C. Yan Ru Tan[2], C. Ho[2], A. Soon[2], T. T. Lam[3], X. Yang[4], C. Nguyen[4], W. Guo[4], Y. Chew[4], Y. M. DeAngelis[1], L. Costello[5], P. De Los Santos Gomez[5], S. Przyborski[5], S. Bellanger[2], O. Dreesen[2], A.B Kimball[6]
*[1]The Procter & Gamble Company, Cincinnati, Ohio, United States, [2]A*STAR Skin Research Labs, Singapore, Singapore, [3]Keck MS & Proteomics Resource, Yale School of Medicine, New Haven, Connecticut, United States, [4]Zymo Research Corporation, Irvine, California, United States, [5]Durham University, Durham, Durham, United Kingdom, [6]Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, United States*

Inflammaging is a theory which purports that chronic inflammation leads to cellular dysfunction and premature aging of tissue. Skin is susceptible to inflammaging because it is the first line of defense from the environment and can be heightened in photoexposed skin. To better understand the impact of aging and photoexposure on epidermal biology we performed a systems biology-based analysis of photoexposed and protected sites from women between ages of 20's to 70's. Biopsies were analyzed by histology, transcriptomics, proteomics, and biomarkers from tape strips. We measured with age morphological changes including, thinning of the epidermis, loss of rete ridge pathlength, and thickening of stratum corneum. SASP components IL-8 and IL-1RA/IL1-a and photosensitive metabolite cis-urocanic acid were consistently elevated in photoexposed face across age. Staining for the DNA damage marker 53BP1 showed higher puncti numbers in the basal layer of older photoexposed arms. Expression of genes associated with differentiation and senescence show an increase starting in the 30's and genes associated with hypoxia and glycolysis increase in the 50's. Proteomics comparing 60's vs 20's arms confirmed elevated levels of differentiation and glycolytic proteins. Immunostaining for markers of differentiation, DNA damage, senescence, and hypoxia show similar relationships. This systems biology-based analysis provides a body of evidence that photoexposed skin is undergoing inflammaging. We propose the presence of chronic inflammation and SASP in young skin leads to an imbalance of epidermal homeostasis and prematurely aged appearance of skin.

LB902

Treatment outcomes for silicone granuloma: A systematic review

A. Gangal[1], D. Mustin[1], D. Barrett[1], H. Yeung[1,2]
[1]Emory University School of Medicine, Atlanta, Georgia, United States, [2]Clinical Resource Hub, VA VISN 7, Decatur, Georgia, United States

Injectable silicone has been used for body contouring and enhancement, particularly but not exclusively in transgender women. Large-volume silicone injections are often complicated by the development of silicone granulomas that form over months to years, resulting in pain and disfiguration. To our knowledge, no evidence-based guidelines exist for the treatment of silicone granulomas. This systematic review aimed to characterize treatments and associated outcomes for silicone granuloma. The review protocol was registered with PROSPERO (#CRD42021260380) and was reported in accordance with PRISMA guidelines. We searched PubMed/MEDLINE, Cochrane Library, EMBASE, and Web of Science on 6/4/2021 for observational studies or clinical trials published in English reporting treatment outcomes for silicone injected-based cosmetic foreign-body reactions. We included 98 studies (95 case reports/series, 3 cross-sectional), with 239 total patients (28.5% male, 67.4% female, and 4.2% unknown gender). Patients underwent surgical removal (n=148), systemic therapy (41), multiple modalities (37), laser (6), injection (5), and topical treatments (1). Surgical removal led to complete response in 57/148 (39%) and partial response in 87/148 (59%). Outcomes for systemic steroids led to complete response in 4/19 (21%), partial response in 10/19 (53%), and stable disease in 4/19 (21%). Intralesional triamcinolone or 5-fluorouracil injections led to partial response in 3/5 (60%). Multiple modalities, including combinations of systemic, surgical, and injection options, led to complete response in 9/37 (24%) and partial response in 22/37 (59%). Study limitations include nonuniform outcome definitions and lack of patient-reported outcomes. Current treatment strategies based on limited reported data largely resulted in partial response. There is a paucity of high-quality studies on treatment outcomes for silicone granuloma. Future studies should compare treatment efficacies through randomized controlled trials and outcomes should be followed using long-term cohort studies.

LB903

Association of atopic dermatitis and behavioral problems in childhood

E. Ma[1], S. Hooper[2], J. Wan[3]

[1]University of Maryland School of Medicine, Baltimore, Maryland, United States, [2]University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina, United States, [3]Johns Hopkins Medicine, Baltimore, Maryland, United States

Atopic dermatitis (AD) in children has been linked to neuropsychiatric disorders, such as depression, anxiety and attention deficit hyperactivity disorder, as well as impairments in conduct, emotions and learning. However, the direct impact of AD on behavior has not been rigorously examined to date. Using a birth cohort of U.S. children enrolled in the Study of Early Child Care and Youth Development from 1991 to 2007, we conducted a longitudinal cohort study comparing 116 children with AD and 1,104 children without AD with respect to behavior outcomes measured by the caregiver-reported Child Behavior Checklist (CBCL), a widely-used behavior rating scale, at 9 timepoints between 24 months and 15 years old. AD was assessed at 36 months of age, 54 months, and 1st grade, and caregiver report of AD at ≥2 points was used to define the presence of AD. Using generalized estimating equations adjusted for sex, race/ethnicity, gestational age, parental education, and family income, AD was significantly associated with clinically concerning behavioral problems (t-score ≥70) for internalizing (OR 2.07 [95% CI 1.03-4.16]), externalizing (OR 3.26 [1.20-8.84]), and total problems (OR 2.92 [1.38-6.20]). Among individual CBCL scales, AD was most highly associated with aggressive behavior (OR 2.72 [1.08-6.86]), somatic complaints (OR 2.65 [1.53-4.56]), anxious/depressed symptoms (OR 2.45 [0.89-6.75]), and delinquent behavior (OR 2.17 [0.89-5.31]). Sensitivity analyses of alternative AD definitions led to similar results. Our findings suggest that AD is associated with higher odds of clinically significant internalizing and externalizing behavioral problems during childhood, after adjustment for key sociodemographic characteristics, underscoring the psychosocial burden of AD on children and families. Further work is needed to screen and characterize those children with AD at greatest risk of behavioral problems and to implement age-appropriate interventions to address their needs and those of their families.

LB904

Utilization of indoor tanning: A cross-sectional study using mobile device data

M. Wehner[1], Y. Li[1], A. Sethi[5], C. Hinkston[1], N. Khalfe[2], C. Stender[6], M. K. Nowakowska[2], O. Cohen[4], S. Giordano[1], E. Linos[3]

[1]The University of Texas MD Anderson Cancer Center, Houston, Texas, United States, [2]Baylor College of Medicine, Houston, Texas, United States, [3]Stanford University School of Medicine, Stanford, California, United States, [4]University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [5]Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana, United States, [6]The University of Texas McGovern Medical School, Houston, Texas, United States

Indoor tanning using ultraviolet (UV) radiation increases skin cancer risk. However, there is little objective information on when or where indoor tanning is used. We aimed to evaluate tanning salon geography and patterns of use using objective data rather than self-report. We used data from SafeGraph, a company that combines smartphone location data and proprietary geographic data. Our dataset included aggregate, anonymous data from January 1, 2018-December 31, 2020. We developed and validated an algorithm (positive predictive value 92.6%) to identify businesses offering UV indoor tanning. We evaluated tanning salon locations, number of tanning salons per state population, and foot traffic patterns by visits per month, per day of the week, and per hour of the day. Our algorithm identified 7412 businesses as tanning salons. Of those, 2795 (37.7%) had foot traffic data available. The highest concentrations of tanning salons were in Midwestern states. We found peaks in the spring (April) of 2018 and 2019, a slightly later peak (June) in 2020, and a short-term decrease in tanning salon visits during the early phases of the COVID-19 pandemic (March-May 2020). Visits were most frequent during weekdays (Monday-Friday). Peak times of day were 12pm-3pm. Our study has limitations: it includes only a small portion of the US population (approximately 10% of mobile devices) and we could not account for indoor tanning outside of tanning salons. Indoor tanning is a known carcinogen, but the majority of information on use is based on cross-sectional surveys. Our study represents new information for public health strategies to decrease exposure to this carcinogen.

LB905

Differences in the demographics, incidence, and survival of palmar and plantar acral melanoma

K. M. Daftary[1], L. Feissinger[2], B. Nardone[1], W. Liszewski[1, 3]

[1]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]Dermatology, University of Minnesota Medical School Twin Cities, Minneapolis, Minnesota, United States, [3]Preventative Medicine- Division of Cancer Epidemiology and Prevention, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Objectives: The purpose of this study is to define differences in the demographics, incidence, and survival between palmar and plantar acral melanoma (AM) using two large national datasets. Methods: Data from the 2004-2016 National Cancer Database (NCDB) and 2000-2018 Surveillance, Epidemiology, and End Results (SEER) database were analyzed as they assessed different outcome measures. For NCDB data, inclusion criteria were a known diagnosis of an acral melanoma and a known Breslow depth. The final sample size was 5002 cases. Differences in demographics were assessed by the chi-square test, Fisher's exact, or T-test. Results: Among all individuals, a greater percentage of acral tumors occurred on the plantar surface (82.0%) than the palmar surface (18.0%). Compared to the plantar surface, palmar melanoma were more likely to occur in whites (84.6% vs 76.8%) and were more likely to be treated with amputation (28.1% vs 12.9%, p<0.001). Hispanics had an earlier age of onset of palmar melanoma than whites (56.6 vs 64.4, p<0.001). Asians had a greater Breslow depth than whites for palmar (3.3mm vs 2.1mm, p=0.008) and plantar (2.9mm vs 2.3mm, p=0.006) tumors. The estimated rate of plantar tumors for all races is four times more common than palmar tumors (1.7 vs 0.4 casers per 1,000,000 people per year). Disease-specific five-year survival was similar for all palmar and plantar tumors (80.8% and 78.2%). Hispanics (75.8%) and Blacks (70.3%) had the lowest five-year survival rates for palmar and plantar tumors, respectively. Conclusions: The majority of AMs occur on the lower limb. Between palmar and plantar tumors, differences were observed in age of onset, location, and treatment type by race/ethnicity. Disease specific five-year survival was similar for palmar and plantar tumors.

LB906

Characterization of cutaneous adverse events to enfortumab vedotin

A. Shahin[1], M. Janeczek[2], R. Butterfield[3], P. Bhullar[2, 1], B. Boudreaux[2], T. Ho[4], A. R. Mangold[2]

[1]Mayo Clinic School of Medicine - Scottsdale Campus, Scottsdale, Arizona, United States, [2]Dermatology, Mayo Clinic Arizona, Scottsdale, Arizona, United States, [3]Quantitative Health Sciences, Mayo Clinic Arizona, Scottsdale, Arizona, United States, [4]Medical Oncology, Mayo Clinic Arizona, Scottsdale, Arizona, United States

Enfortumab vedotin (EV) is an antibody drug conjugate for patients with locally advanced or metastatic urothelial carcinoma. Clinical trials evaluating the efficacy of EV have reported treatment-related rash in 43.9% of patients. There is insufficient data characterizing cutaneous adverse events (CAEs) associated with this medication. We sought to characterize CAEs from EV including type of reaction, patient demographics, EV dose regimen, treatments, and disease outcomes. We compared these variables with a control group of patients who did not develop rashes to identify risk factors for CAEs. A retrospective review was performed at Mayo Clinic Health System from 2010-2021 to identify adult patients treated with EV. Patient demographics, EV treatment details, previous treatments, and tumor responses were compared between patients with CAEs and those without using Fisher's Exact and Kruskal-Wallis tests. A total of 59 patients were identified. Fifteen (25.4%) were reported to have a CAE to EV including maculopapular rash (46.7%), macular rash (20.0%), bullous dermatoses (6.7%), generalized pruritus (6.7%), and symmetric drug-related intertriginous and flexural exanthema (6.7%) with grade severity of 1-2. Mean time of onset from first infusion to CAE was 19.9 days (SD=14.7). Mean number of treatment cycles before AEs was 1.1 (SD=0.5). 9/16 patients (60%) did not require change in EV regimen. Treatments for CAEs included topical or systemic corticosteroids and anti-histamine medications with 80% response. Previous cisplatin (RR 4.61) or radiation therapy (RR 4.76) were associated with development of CAEs. Most CAEs to EV are low grade and occur after first cycle of infusion. Previous cisplatin or radiation therapy is associated with development of CAEs. Treatment response for CAEs is high and EV dose regimen may often remain unchanged.

LB907

Sequelae of pediatric allogeneic hematopoietic stem cell transplantation

C. Diamond[1], K. Oeffinger[3], R. Hall[2], T. Driscoll[3], A. Cardones[2]
[1]Duke University School of Medicine, Durham, North Carolina, United States, [2]Dermatology, Duke University Health System, Durham, North Carolina, United States, [3]Duke University Health System, Durham, North Carolina, United States

Pediatric patients are at risk for adverse drug reactions, and oncologic patients are at an even higher risk. (1) This study aims to describe and quantify the long-term dermatologic sequelae and drug reactions in patients who received allogeneic hematopoietic stem cell transplantation (allo-HSCT) as children. 273 patients who received allo-HSCT as children from the period 01/27/2002- 01/27/2022 at Duke University Hospital were identified. All patient visits from the period of 01/1996-01/2020 were obtained via the Duke Enterprise Data Unified Content Explorer (DEDUCE). From the 273 patients, 532 total allergens were identified in 191 unique patients. 149 patients had reactions to medications, blood product infusions, or vaccines. 80/273 (29.3%) patients had a drug reaction to vancomycin. 46/80 (57.5%) patients developed Red Man's Syndrome, 8/80 (10%) developed rash, and 15/80 (18.8%) had an itching reaction. 15/80 (18.8%) patients had dermatologic conditions diagnosed in the period after their allo-HSCT. None of the 8 patients who developed a rash were referred to the Dermatology service for their cutaneous drug reaction. Including outpatient visits over 17 years, 15 patients were seen by Dermatology for a total of 72 dermatologic diagnoses. Red Man Syndrome has previously been documented as an adverse drug reaction to vancomycin, with rates ranging from 5-50% in hospitalized subjects and up to 90% in controls. (2,3) RMS development has been reported in up to 14% of pediatric patients receiving vancomycin. (4) Our data is in line with previously documented studies that vancomycin drug reaction is frequent. There is no data regarding the long-term implications of adverse drug reactions to vancomycin. These results highlight the importance of further research in long-term sequelae of pediatric transplantation, especially in the field of dermatology and that this topic merits further research. Dermatologic conditions may be underreported given the low rate of Dermatology referrals.

LB908

Prevalence and mortality of cancer in patients with dermatomyositis and dermatopolymyositis compared to the general population

A. Hollis[1], A. Allenzara[3], S. Maczuga[2], M. Helm[2], G. Foulke[1]
[1]University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina, United States, [2]Penn State Health Milton S Hershey Medical Center, Hershey, Pennsylvania, United States, [3]University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina, United States

Dermatomyositis (DM) is a rare idiopathic inflammatory myopathy with complications that include cancer. Dermatopolymyositis (DPM) is an ICD-10 code encompassing dermatomyositis and polymyositis. We investigated mortality in patients with an ICD-9/10 code for malignancy and DM/DPM and compared this to cancers in the general population. This retrospective analysis uses raw data from TriNetX database, a national federated network of deidentified data. Patients were identified by entry of DM or DPM ICD coding entered twice separated by 6 months with 3 years continuous follow up. Cancers were identified by ICD coding with secondary malignancies and non-melanoma skin cancer excluded. Of patients with DM (n=5,021), 17% had cancer and 11.6% of those patients died within the study window. Five cancers associated with the most deaths in the DM population were breast, lung, ovary, prostate, lymphoma respectively. Of patients with DPM (n=13,711), 18% had cancer and of those patients 14% died. Five cancers associated with the most deaths in the DPM population were lung, breast, ovary, colon, and prostate. Of patients less than age 50 with DM who died with a diagnosis of cancer, the most frequent ICD code was breast cancer at 38.5%, similarly in patients less than age 50 with DPM who died, breast cancer was the most frequent ICD code at 28.6%. USPSTF guidelines recommend screening for breast cancer at age 50. Our data corroborates the need for individualized screening guidelines adjusted for DM/DPM. We found that the rate of dying with a concomitant malignancy in the general population was 0.4% (n=212,777,108). Given the frequency of death from cancers in patients with DM or DPM, particularly in populations in which we do not generally screen for these cancers, it is important to have a high index of suspicion and low threshold for screening tests or exams.

LB909

Understanding the motivating factors and barriers to participation in eczema clinical trials among patients and caregivers

W. Baghoomian[1], M. Jacobson[1], A. Kastala[1], I. Thibau[2], E. Simpson[1], W. Smith Begolka[2]
[1]Dermatology, Oregon Health & Science University, Portland, Oregon, United States, [2]National Eczema Association, San Rafael, California, United States

Despite the demand for improved eczema therapies and the advent of new eczema clinical trials (eCTs), overall enrollment and participation in eCTs remain low. The purpose of this study is to assess and understand the motivating factors and barriers in participating in eCTs for adult eczema patients and caregivers of pediatric eczema patients in the United States. A 46-question online survey collected information from 1,285 respondents on experience with eCTs, drive for and barriers to participate in eCTs, and likelihood to participate in future eCTs. 71.9% of respondents completed responses about their eCTs experience and were included in the qualitative analysis. Open-text responses were blind coded and analyzed for themes. Major themes for motivation to participate in eCTs included a desire to address the burden of their disease (58.1% of coded responses), being out of treatment options/feeling out of control (23.3%), and expressing altruism to help others or contribute to science (14.5%). When asked why they never participated in eCTs, 5 major themes were identified: issues in awareness of eCTs (44.0% of coded responses), lack of interest (15.4%), fear/risks/unknowns of eCTs (15.2%), eligibility concerns (14.7%) and accessibility issues (5.5%). 74.5% of respondents were somewhat/extremely likely to consider eCTs in the future (n=688). When asked to elaborate on their likelihood rating of exploring eCTs in the future, the most common major theme was a desire to address the burden of their disease (31.8% of all coded responses). Results in this study highlight the importance of designing eCTs that aim to reduce barriers in participation and setting realistic expectations in eCT treatment outcomes. Addressing eCT knowledge and awareness amongst participants are also important factors in increasing eCT enrollment and ultimately developing improved eczema therapies.

LB910

Racial and ethnic disparities in Merkel cell carcinoma

N. Mohsin[1,3], M. Martin[1,3], D. Reed[1], S. Vilasi[1], N. Hill[1], P. Juneau[2], I. Brownell[1]
[1]Dermatology Branch, NIAMS, Bethesda, Maryland, United States, [2]Division of Library Services, ORS, National Library of Medicine, Bethesda, Maryland, United States, [3]Co-first authors, Bethesda, Maryland, United States

Merkel cell carcinoma (MCC) is a rare, aggressive neuroendocrine skin cancer that more often occurs in White patients; however, the proportion of minority patients with MCC grew significantly from 2000 to 2013. We sought to characterize health disparities in MCC with the goal of instituting more equitable management. We used SEER-18 to identify U.S. patients with MCC diagnosed between 2000 and 2018. We fit a Kaplan-Meier survival curve and univariable and multivariable Cox regression models to estimate the effects of race and ethnicity on MCC-specific survival. A total of 9,557 MCC patients were identified. Patients with MCC were predominantly White (89.9%) with Hispanic (5.7%), Asian American/Pacific Islander (AAPI, 2.3%), Black (1.5%), and Other (0.6%) remaining. On average, Black MCC patients presented with more advanced stage disease. Other differences were observed in age, sex, site of MCC, and household income. Overall, White patients demonstrated a 67.0% five-year MCC-specific survival. Accounting for all covariates, Hispanic patients had improved survival (77.0%) relative to White patients, whereas AAPI (69.0%) and Black (62.5%) patient outcomes were not significantly different. The observation that Hispanic ethnicity was associated with improved MCC survival compared to White patients is both novel and interesting. This may reflect the Hispanic health paradox, an epidemiological finding that foreign-born Hispanic Americans have better health outcomes than predicted by their socioeconomic status. Although their survival was not statistically different from White patients, Black patients had the worst MCC outcomes of all racial groups. Having more advanced disease, increased wait times to treatment, and differential access to care may be contributing factors. Increased representation of minorities in cancer registries and clinical trials is needed so that we can begin to build equity among MCC patients. *Authors NM and MM contributed equally to this work.

LB911

Incidence and treatments of actinic keratosis in the Medicare population: A cohort study

L. Navsaria[1], Y. Li[1], M. K. Nowakowska[2], C. Hinkston[1], L. Wheless[3], S. Giordano[1], M. Wehner[4]

[1]Health Services Research, The University of Texas MD Anderson Cancer Center, Houston, Texas, United States, [2]Baylor College of Medicine, Houston, Texas, United States, [3]Dermatology, Vanderbilt University Medical Center, Vanderbilt University Medical Center, Nashville, TN, US, academic/hospital, Nashville, Tennessee, United States, [4]Dermatology, The University of Texas MD Anderson Cancer Center, Houston, Texas, United States

Actinic keratoses (AKs) are common but have not been well-studied on a population level. We investigated AK incidences and treatments in a cohort of 4,999,999 randomly selected, de-identified Medicare beneficiaries aged ≥65 from 2009-2018. We included all patients who had outpatient encounters with AK diagnoses. The main outcomes were encounter characteristics (providers and treatments) and incidences. Out of 4,999,999 eligible patients with a mean of 5.7 years of follow-up (SD 3.6), we identified 1,462,985 (29.3%) with ≥1 AK diagnosis encounter. We identified a total of 8,178,543 AK diagnosis encounters (mean of 5.6 [6.8 SD] per patient). Most of the encounters were with dermatologists (78.6%), followed by physician assistants and nurse practitioners (10.8%), family physicians (4.5%). The majority of encounters had a destruction treatment (81.5%), while a minority had a potential field treatment (topical medications [2.9%], photodynamic therapy [1.5%]). The incidence rate of AK diagnosis encounters was 28,656 per 100,000 person-years (42,970 for men; 20,492 for women; 28,788 age-adjusted). In total, 3 in 10 Medicare beneficiaries had ≥1 encounter with an AK diagnosis over 5 years of follow-up. We report strikingly high incidence rates using individual-level data that have not previously been available. We also report low utilization of potential field treatments and may highlight an opportunity for increased squamous cell carcinoma prevention through topicals such as fluorouracil. Our findings support the need for increased awareness and preparedness for the burden of AKs in dermatology.

LB912

Racial/ethnic differences in biologic treatment patterns among patients in the CorEvitas Psoriasis Registry

C. W. Enos[1], J. Z. Yi[1], R. R. McLean[2], A. P. Sima[2], E. A. Kohl[2], T. Eckmann[2], A. S. Van Voorhees[1]

[1]Dermatology, Eastern Virginia Medical School, Norfolk, Virginia, United States, [2]CorEvitas LLC, Waltham, Massachusetts, United States

Racial disparities impacting management of psoriasis patients are incompletely understood. The objective of this study was to assess biologic discontinuation and switching rates after 6-months of therapy among psoriasis patients of different racial/ethnic backgrounds in the US/Canadian CorEvitas Psoriasis Registry. This study included patients who initiated a biologic between April 2015-March 2021 and had a known treatment status at 6-months follow-up. Patient characteristics at initiation were compared by race/ethnicity. Frequencies of discontinuations (any biologic stop) and switches (stop initial biologic and start another or add non-biologic systemic therapy) by 6-months were reported. Poisson regression was used to calculate relative risks (RR) for discontinuations and switches in each racial/ethnic group relative to Whites. There were 5,500 biologic initiations originating from 4,128 (75%) White, 193 (4%) Black, 460 (8%) Asian, 535 (10%) Hispanic, and 184 (3%) Other patients. Demographics, comorbidities, psoriasis severity, and treatment history were similar across groups. About 33% of patients were biologic-naïve. At 6-months, unadjusted discontinuation rates did not differ across groups. Black patients had lower unadjusted rates of drug switching (6.7%) than White (16.0%), Asian (13.7%), and Hispanic (12.0%) patients. Among switchers, Black patients had the lowest proportion of patients starting another biologic (53.8% vs 69.8-82.8%) and the highest adding a non-biologic therapy (38.5% vs 7.8-23.8%). In adjusted analyses, Black patients were 57% less likely to switch therapy relative to Whites (RR=0.43, 95% CI 0.26, 0.73). Using data from the CorEvitas Psoriasis Registry, our study found that Black patients were less likely than Whites to switch biologic therapy by 6-months, and among switchers, Black patients had the lowest frequency of starting a different biologic. Further research is needed to uncover the determinants of these disparities to inform patient management.

LB913

Low grade cutaneous immune related adverse events leading to immune checkpoint inhibitor discontinuation

T. V. Jacoby[2, 1], M. S. Asdourian[2, 3], N. Shah[2, 4], L. L. Thompson[2, 3, 5], K. Reynolds[3, 6], S. Chen[2, 3]

[1]University of Hawai'i at Manoa, Honolulu, Hawaii, United States, [2]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [3]Harvard Medical School, Boston, Massachusetts, United States, [4]Virginia Commonwealth University School of Medicine, Richmond, Virginia, United States, [5]Medicine, Brigham and Women's Hospital, Boston, Massachusetts, United States, [6]Hematology/Oncology, Massachusetts General Hospital, Boston, Massachusetts, United States

Cutaneous immune related adverse events (cirAEs) are a common toxicity from immune checkpoint inhibitor therapy (ICI). Severe cases frequently lead to treatment discontinuation, in line with guidelines published by the National Comprehensive Cancer Network (NCCN). Mild (grade 1-2) can also lead to ICI discontinuation, seemingly unnecessarily. We evaluated this discrepancy among patients with grade 1-2 cirAEs who had their ICI discontinued due to their cirAE in hopes of elucidating potential areas for intervention to help retain patients on anti-cancer therapy. We retrospectively identified patients (n=9,529) who initiated ICI using billing data. Patients were screened for a cirAE and confirmed through review of the electronic medical record (n=628). Demographic and clinical data were recorded. Ten cases (1.6%) had both mild (grade 1–2) and had their ICI regimen discontinued without retrial due to their cirAE. "Rash not otherwise specified" was the most prevalent cirAE morphological subtype (50%). ICI discontinuation was recommended in all cases by their oncologist, and 80% of cases underwent dermatology evaluation. No dermatologist recommended or reassured ICI reinitiation. Improved dermatology/oncology collaboration may lead to retrial of ICI among the patients. This may have played a role in permanent ICI discontinuation in these cases of low grade cirAEs. Furthermore, with increased attention paid to morphology, providers may be able to subcategorize these eruptions and provide more specialized medical management of these patients, reducing discontinuation. Oncologists should be encouraged to safely rechallenge ICI therapy after the cirAE has been adequately controlled, as this may allow for continued administration of potentially life extending/saving therapy.

LB914

Dermatology evaluation of cutaneous immune related adverse event associated with higher checkpoint inhibitor retrial rates and improved survival

T. V. Jacoby[1, 2], N. Shah[1, 3], M. S. Asdourian[1, 4], L. L. Thompson[1, 4, 5], N. LeBoeuf[4, 6], Y. Semenov[1, 4], E. C. Dee[7], K. Reynolds[4, 8], S. Chen[1, 4]

[1]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]University of Hawai'i at Manoa, Honolulu, Hawaii, United States, [3]Virginia Commonwealth University School of Medicine, Richmond, Virginia, United States, [4]Harvard Medical School, Boston, Massachusetts, United States, [5]Medicine, Brigham and Women's Hospital, Boston, Massachusetts, United States, [6]Center for Cutaneous Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States, [7]Memorial Sloan Kettering Cancer Center, New York, New York, United States, [8]Hematology/Oncology, Massachusetts General Hospital Cancer Center, Boston, Massachusetts, United States

Cutaneous immune related adverse events (cirAEs) are a common adverse effect of immune checkpoint inhibitors (ICI), at times severe enough to require discontinuation of ICI therapy. It is unclear whether the involvement of a board-certified dermatologist (BCD) in the care of cirAEs might affect ICI retrial or survival outcomes. As such, we sought to evaluate these potential associations. In this single institution cohort analysis of patients who developed a cirAE, we extracted demographic and clinical data from electronic medical records. Multivariable logistic regression analysis was used to assess for significance between BCD evaluation and ICI retrial. In order to evaluate possible effects of BCD evaluation on survival while making sure to account for possible guarantee time bias, time varying cox proportional hazards models were used. Potential cofounders, including age, sex, cancer type, cancer stage, cirAE severity, and ICI subtype, as well as time to BCD evaluation as the time varying component, were included in the model. Among the 628 patients who developed a cirAE between 1/1/16-6/29/21, BCD evaluation (n=229) of a cirAE was associated with an increased odds for ICI retrial following discontinuation (OR: 10.52; p<0.001). BCD evaluation was also associated with improved progression-free survival (HR: 0.72; p<0.002), and overall survival (HR: 0.62; p< 0.002). These findings support the critical role BCDs play in the care of the oncologic patient, potentially impacting survival by allowing for higher rates of ICI retrial.

LB915

Occupational and environmental triggers of atopic dermatitis

K. D'Aguanno[1], C. Jack[2], A. Muntyanu[2], E. Netchiporouk[2]
[1]*McGill University Faculty of Medicine and Health Sciences, Montreal, Quebec, Canada,* [2]*Dermatology, McGill University Health Centre, Montreal, Quebec, Canada*

Atopic dermatitis (AD) prevalence varies greatly across nations, particularly Northern/Southern hemispheres, wealthier/developing countries. While genetics undoubtfully play an important role, this geographic variation may also be influenced by the living environment and socioeconomic factors.We aimed to assess the association between AD, environmental and sociodemographic characteristics in Canada. A cross-sectional case control study was performed using CanPath data(Canadian Partnership for Tomorrow's Health), the largest prospective Canadian populational cohort. A total of 32,087 patients with self-reported diagnosis of AD and 1:1 age-sex matched controls were included.Patients were linked to CANUE (Canadian Urban Environmental Health Research Consortium) database based on place of residence (6-digit postal codes)to assess environmental and socioeconomic factors of interest including air pollutants(NO2, PM2.5, O3, SO2), annual average temperature, total precipitation,walkability index (dwelling and intersection density), and nighttime light brightness. Social stratifiers included material and social deprivation indices, education level and annual income. Student's t-test was performed for continuous variables and Chi-Square for proportions. AD patients were significantly more likely to be exposed to higher annual concentrations of air pollutants, NO2 (p= 0.0014), PM2.5 (p= 0.0038), SO2 (p=0.0114), O3 (p <0.0001). Higher nighttime light brightness (p <0.0001) and lower walkability index (measured by dwelling and intersection densities(p<0.0001))were seen in AD. AD was associated with higher socioeconomic class (lower deprivation indices, higher education level, but not annual income), and lower annual temperature (p< 0.0001).Climatic factors (e.g. colder climate), air pollution, neighborhood characteristics and social determinants of health may predispose to development of AD. Better understanding of external risk factors is important to reduce health inequity and improve disease prevention and management.

LB916

The war on melanomaTM: A statewide public health initiative

C. Lee[1], J. Ludzik[1,2], E. Stoos[1], A. Witkowski[1], S. Leachman[1]
[1]*Dermatology, Oregon Health & Science University, Portland, Oregon, United States,* [2]*Bioinformatics and Technology, Uniwersytet Jagiellonski w Krakowie, Krakow, Małopolska, Poland*

Melanomas are visible on the skin, making melanoma a good model to test the effectiveness of educational strategies on early detection. The War on MelanomaTM is a statewide, education-based, prospective, controlled, public health study. It tests the hypothesis that a broad education campaign, coupled with risk-stratified screening recommendations, will lower statewide melanoma mortality rates and stage of disease at diagnosis, and improve the public's melanoma-specific knowledge, attitudes, and self-skin examination behaviors relative to control states (primary outcomes). There is little direct data to show that skin screening reduces death due to melanoma. To address this data gap, we have targeted the lay population, patients, skin service professionals, primary care providers, and melanoma experts throughout the state using education and technology. Our experimental design tailors outreach based on each population's varying knowledge and risk levels. Outreach to the lay community includes mass print, social media, radio and television public service announcements, billboard advertisements, high school curricula, and screening education of rural and Indian reservation communities. Outreach to over 40,000 licensed skin care professionals (e.g., hair, massage, aesthetician industries, etc.) incorporates an educational toolkit with pre- and post-education assessments and is supplemented with home dermoscopy devices (SKLIP) and client access to store-and-forward e-visits with dermatology. A separate educational toolkit for over 4000 primary care providers has been promoted and is supplemented with provision of SKLIP devices, virtual dermoscopy webinars, access to e-consults with dermatology, and electronic medical record tools. A Skin Imaging and Technology Center has been created for expert care of the highest-risk patients. This proof-of-principle public health experiment will provide data that may support a shift of health care resources away from late-stage heroic measures into early detection.

LB917

Treatment patterns and unmet needs of generalized pustular psoriasis (GPP) patients with flares

L. Lavasani[1], J. Weiss[2], B. Krebs[2], J. L. Rhoads[3]
[1]*Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, Connecticut, United States,* [2]*Optum Inc, Eden Prairie, Minnesota, United States,* [3]*Department of Dermatology, University of Utah Health, Salt Lake City, Utah, United States*

Background:Generalized pustular psoriasis (GPP) is a rare disease with little known about the treatment of flares. Objective:To characterize treatment patterns for GPP flares documented in their medical record. Methods:This retrospective descriptive study included adult GPP patients (ICD-10 codes L40.1) identified in the Optum® de-identified electronic health record (EHR) data from 7 July 2015-30 June 2020. The index GPP diagnosis was the first occurrence in the EHR with no history of GPP for at six months prior. Flare episodes were identified using an algorithm based on diagnosis coding, setting of care, type of provider, GPP disease terms, and flare terms and attributes found in the EHR. Treatment patterns were characterized as: pre-flare-90 days before index (first day of flare episode), flare period-index plus 90 days, and post-flare-91+ days after index. Results: Of the 1,535 GPP patients identified, 271 had flares with 78% having 1 episode documented during the study. Although drug utilization increased among drug classes, 17% of patients with only 1 flare documented received no dermatologic treatment during the episode. Trends in drug utilization among patients with multiple episodes were less discernable over time, but there was a steady increase in the proportion of patients with non-biologic systemic treatment from the pre-flare (19%) to during flare (41%) periods. Of patients with 1 documented flare, 41% had the addition of an agent during the flare period, and 39% had the removal of an agent in the post-flare period. Oral steroids were most often added (49%) and removed (65%) followed by non-biologic systemic treatment (36% added, 32% removed). Biologics were less likely to be removed (13%) once started. Conclusions: There remains a large unmet need as 17% of patients with 1 documented flare received no treatment. Oral steroids and systemic non-biologics were more likely to be added during flare episodes and removed in the post-flare flare period.

LB918

The dark side of hydroquinone for skin lightening: 3-fold increased risk of skin cancer - a cohort study.

B. Miles[1], M. Wilkerson[2]
[1]*The University of Texas Medical Branch at Galveston School of Medicine, Galveston, Texas, United States,* [2]*Dermatology, The University of Texas Medical Branch at Galveston, Galveston, Texas, United States*

Purpose: For years, concern has persisted that hydroquinone exposure in humans may be associated with increased risk of cancer but proving this has been elusive. The purpose of this study is to use the TriNetX database to quantify the cancer risk associated with hydroquinone use in humans. Methods: We used the TriNetX platform to create 2 cohorts of patients, both without any prior diagnosis of skin cancer. One cohort had received prior hydroquinone exposure which was determined by medication code 5509 in the TriNetX system, while the other was prohibited from having such exposure. They were then evaluated for development of melanoma and non-melanoma skin cancers via ICD-10 codes C43.x and C44.x, respectively. Type of Study: Cohort: Results: Hydroquinone exposure was associated with a relative risk of 3 for melanoma (95% CI (1.704,5.281), p<0.0001), 3.6 for non-melanoma skin cancer (95% CI (2.815,4.561), p<0.0001), and 3.4 for all reported skin cancers combined (95% CI (2.731,4.268), p<0.0001). Conclusion: Our study shows that hydroquinone use in humans increases the risk of melanoma and non-melanoma skin cancers by more than 3-fold. This information will be useful in the informed consent process for patients who desire treatment and encourage risk mitigation with avoidance of sun exposure and compliance with the use of sunscreen.

LB919

Vigxbsa in a pediatric population: a novel tool for evaluating atopic dermatitis severity and responsiveness

M. M. Appiah[2, 1], L. Loop[2, 1], B. Geng[2, 1], L. F. Eichenfield[2, 1]
[1]University of California San Diego, La Jolla, California, United States, [2]Rady Children's Hospital San Diego, San Diego, California, United States

Assessing severity of atopic dermatitis(AD) plays a key role in determining treatment choice and therapy escalation, but there are currently few severity assessments practical for clinical use. The EASI is a comprehensive severity assessment utilized in clinical trials but is considered too cumbersome for clinical use. The vIGA is a quick severity assessment for AD but fails to incorporate disease extent. A vIGAxBSA calculation has shown promise as a quick assessment which incorporates extent but has not been evaluated in a "real-world" non-clinical trial setting over time. We hypothesized that over time the vIGAxBSA would correlate better with the EASI score than vIGA or BSA alone. We performed a pediatric observational study. We collected vIGA, BSA, and EASI scores of 56 children with historically moderate to severe AD evaluated in a Multidisciplinary AD Clinic from July 2019 to January 2022. A Pearson's r was performed to determine the strength of correlation of each outcome measure at individual visits, and the correlation of change in outcome measures between initial and follow-up visits. At individual visits, EASI correlated more strongly with vIGAxBSA (r= 0.939, p<0.001, n=148) than BSA (r=0.929, p<.0001, n=147) or vIGA (r=0.808, p<0.001, n=164). Comparing change in scores between initial and follow-up visits showed change in EASI correlated most strongly with the change in vIGAxBSA (r=0.901, p<.001, n=83) compared to change in BSA (r=0.891, p<.001, n=84) or change in vIGA (r=0.721, p<.001, n=108). Both correlations between EASI and vIGAxBSA met the threshold for "very strong" correlation. Our results show that vIGAxBSA correlates strongly with EASI at initial visit and over time. This presents a relatively simple method for clinicians to monitor AD disease severity over time and a practice friendly alternative to the EASI score.

LB920

Alcohol consumption and melanoma risk: A prospective analysis from the nih-aarp diet and health study

B. Roberts[1], Y. Li[1], L. Liao[4], A. Qureshi[1, 2], E. Cho[1, 2, 3]
[1]Epidemiology, Brown University School of Public Health, Providence, Rhode Island, United States, [2]Dermatology, Brown University Warren Alpert Medical School, Providence, Rhode Island, United States, [3]Medicine, Brigham and Women's Hospital, Boston, Massachusetts, United States, [4]Health and Human Services, National Cancer Institute Division of Cancer Epidemiology and Genetics, Bethesda, Maryland, United States

There is a lack of consistent epidemiological research observing the associations between alcohol consumption and melanoma. Additionally, some observational studies have found significant differences in melanoma risk based on alcoholic beverage type. In this prospective analysis, we investigated the associations of total alcohol consumption and different types of alcoholic beverages with risk of melanoma among 469,828 adults aged 50-71 who participated in the NIH-AARP Diet and Health Study. Alcohol consumption in the past year was assessed at baseline by questionnaire and defined as a categorical variable: non-drinker, >0-1 drinks/day, >1-3 drinks/day, and >3 drinks/day. Multivariable-adjusted Cox proportional hazards regressions were used to calculate the hazard ratios (HR) and 95% confidence intervals (CI) to observe the associations between alcohol intake and risk of melanoma. We found that alcohol consumption of >1-3 drinks/day had the largest association with melanoma in situ risk (HR: 1.21, 95% CI: 1.07 - 1.36), while intakes of >3 drinks/day had the largest association with malignant melanoma risk (HR: 1.19, 95% CI: 1.05 - 1.35). Although there was no evidence of linearity between total alcohol consumption and melanoma (Ptrend = 0.08), we did observe a significant positive relationship among those who consumed wine and liquor (Ptrend <0.05). Lastly, we found an increased risk of malignant melanoma and melanoma in situ among participants who consumed >0-1 drinks/day of beer. Future investigations are needed to evaluate the association between duration of alcohol consumption on melanoma risk and observe the interactions of alcohol consumption with melanoma risk factors.

LB921

Role of VivaScope ® 2500 in skin pathology: Advantages, limitations, and future prospects

S. Razi[1], K. Oh[2], S. Ouellette[1, 3], B. Rao[1, 3]
[1]Rao Dermatology, New York, New York, United States, [2]Pathology, Mount Sinai Medical Center, Miami Beach, Florida, United States, [3]Dermatology, Rutgers University New Brunswick, New Brunswick, New Jersey, United States

The purpose is to highlight the advantages, limitations and future prospects of a novel cutaneous imaging device (VIvaScope 2500) that was used in a clinical setting for 6 months. This device was used as an adjunct device in a dermatology clinic. This device was used to view margins of excised cutaneous cancers while performing Mohs surgeries; this device was also used to image many excisions that were performed in the clinic before sending them to the histology lab. The staining protocol that we used for freshly excised tissues included 1)Dipping in 20 % Acetic Acid for 30 secs , followed by 2) Dipping in 0.5 mM Acridine Orange solution for 30 secs, followed by 3) Dipping in Normal saline to remove excess dye. *Ex Vivo* Microscopy (EVM) expedites the process to view skin pathology. It takes approximately 5 minutes to view the excised tissue under VivaScope as compared to frozen sectioning which takes 20 minutes on average. EVM is an ideal device to view margins of cutaneous tumors. This feature allows it to be an excellent adjunct tool to be used clinically to expedite Mohs surgeries. EVM can serve as an adjunct tool to confirm the diagnosis of clinically benign lesions. The use of *ex vivo* microscopy is not without limitation , compared to traditional formalin-fixed paraffin-embedded (FFPE) tissue sections, the image's resolution is still incomparable. Despite good nuclear contrast, there is still a considerable gap in resolution for *ex vivo* imaging. In diagnostic pathology, a subtle change in the nucleus can be interpreted as mild dysplasia, which is evident in FFPE slides, not *ex vivo* microscopic (EVM) images. There is great potential in this new *ex vivo* imaging tool. It allows real time quasi histologic microscopic examination of freshly excised cutaneous tissue and does not require traditional tissue embedding, processing and sectioning.

LB922

Sun protection and vitamin D deficiency among racial groups

A. Ahmad[1, 2], R. Chen[5, 2], D. Y. Kim[4], R. Hartman[2, 3]
[1]The University of Texas Health Science Center at Houston John P and Katherine G McGovern Medical School, Houston, Texas, United States, [2]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [3]Dermatology, VA Integrated Service Network 1 (VISN-1), Jamaica Plain, Massachusetts, United States, [4]Harvard Medical School, Boston, Massachusetts, United States, [5]Emory University School of Medicine, Atlanta, Georgia, United States

Introduction: Sun protection plays a role in skin cancer prevention, but there are concerns it increases the risk of vitamin D (VD) deficiency. Here, we examine sun protection dependent differences in VD deficiency among racial groups. Methods: Serum 25(OH)D levels and sun protective behaviors staying in the shade, wearing long sleeves, and using sunscreen) questionnaire responses of 9143 adults were extracted from the US National Health and Nutrition Examination Survey 2011–2016. VD deficiency was compared between rare and frequent users of sun protection with age, sex, education, VD supplementation, milk consumption and BMI covariates using logistic regression. Results: Non-Hispanic Whites and Mexican Americans who frequently stay in shade were more likely to be VD deficient than those who rarely do so (OR 2.16; P<.001; OR 1.74; P=.039). Non-Hispanic Whites who frequently use sunscreen were less likely to be VD deficient than those who rarely do so (OR 0.60; P=.002). Multiracial people who frequently use sunscreen were more likely to be VD deficient than those who rarely do so (OR 4.41; P=.012). Discussion: VD deficiency among groups with lower skin phototypes types (SPT) has some association with a higher frequency of seeking shade. Groups with higher SPT have no association between VD deficiency and sun protection frequency suggesting sun protection may not contribute to an already reduced amount of VD production in the skin due to pigmentation.

LB923

COVID-19 vaccine immunity in hidradenitis suppurativa patients receiving TNF-alpha inhibitors

A. Nosrati, M. E. Torpey, G. D. Ball, N. Shokrian, K. L. Campton, S. R. Cohen
Albert Einstein College of Medicine, Bronx, New York, United States

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease treated with a multi-tiered approach that includes TNF-alpha inhibitors (infliximab, adalimumab). While COVID-19 mRNA vaccines are the standard of care, concerns about vaccine immunity in the setting of immunosuppressive therapies remain. Nonetheless, there is a consensus recommendation for mRNA vaccination in these patients. We studied COVID-19 mRNA IgG spike antibody responses in HS patients on TNF-alpha inhibitors. Previous studies have shown that in healthy vaccinated individuals the median immunoglobulin levels peaked at 7-weeks after the first vaccine dose at approximately 4,000 au/mL. An IRB-approved retrospective chart review of patients receiving care at the Einstein/Montefiore HS Center (HSC) (n=93) was limited to those fully vaccinated with an mRNA-based COVID-19 vaccine and screened for vaccine immunity with a SARS-COV-2 IgG spike antibody test [AdviseDx SARS-CoV-2 IgG II assay, reported as arbitrary units (au/mL), Abbott Laboratories]. We compared HS patients receiving TNF-alpha inhibitors to those not on immunosuppressive drugs. Fifty-six patients (60%) were treated with a TNF-alpha inhibitor [adalimumab(n=10); infliximab(n=46)]. There were no significant differences between users and non-users regarding the mean number of days since vaccination (147.2±76.7 and 149.4±56.8, respectively) and vaccine brands [users: Pfizer(n=34); Moderna(n=22) versus non-users: Pfizer(n=28); Moderna(n=9)]. There were no significant differences in mean IgG spike antibody levels among patients receiving TNF-alpha inhibitors and those not on immunosuppressive drugs (5196.9±10811.4 au/mL and 6844.9±10872.3 au/mL)(p=0.884). In our cohort, immune responses to mRNA COVID-19 vaccines among HS patients are not significantly affected by therapy with TNF-alpha inhibitors. Further investigations to determine long-term differences in antibody levels, maintenance of antibody levels over time, and variances in antibody levels across different doses of infliximab and adalimumab are warranted.

LB924

Ceftazidime-avibactam: A novel treatment for advanced hidradenitis suppurativa

M. Torpey, A. Nosrati, K. L. Campton, S. Cohen
Dermatology, Albert Einstein College of Medicine, Bronx, New York, United States

Introduction: Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease manifest as painful nodules, abscesses, and draining sinus tracts ("tunnels"). While treatment consists of antibacterial, anti-hormonal, and anti-inflammatory modalities, advanced and recalcitrant HS poses a formidable challenge. We have found penem-class antibiotics are uniquely efficacious; however, flares often signal the emergence of penem-resistance requiring an alternative approach. We report dramatic regression of advanced HS with ceftazidime-avibactam. Methods: We conducted an IRB-approved retrospective chart review at the Montefiore Medical Center/ Albert Einstein HS Center. Twelve patients with advanced HS completed a course of intravenous ceftazidime-avibactam (AbbVie), 2.5 grams IV every 8 hours for 21-42 days. Patient demographics (age, gender), concurrent therapies, disease severity (HS-Physician Global Assessment [HS-PGA]), and Numerical Rating Scale (NRDS) pain scores were documented before, during, and at the conclusion of treatment. Results: The mean age of our cohort was 34.3±14.3 years (n=12); 9 were female. Concurrent therapies included topical medicaments (100%), anti-hormonal therapy (83%), and an anti-TNF biologic (100%). Ceftazidime-avibactam was associated with highly significant reductions of HS-PGA and NRS pain scores. Before treatment: HS-PGA 3.8±1.2; NRS pain score 3.0±2.1. After treatment: HS-PGA 2.8±1.4 ($p<0.0001$); NRS pain score 0.54±1.3 ($p<.03$). Conclusion: To our knowledge, this is the first report of intravenous ceftazidime-avibactam for advanced HS. We found significant reductions in both disease severity and pain. Ceftazidime-avibactam is a novel addition to the HS treatment arsenal but further experience is needed to optimize the length of therapy and understand its mechanism of action.

LB925

Bullous pemphigoid associated with immune checkpoint inhibitor therapy: A systematic review of the literature

M. S. Asdourian[1], N. Shah1, T. V. Jacoby[1], K. Reynolds[2], S. Chen[1]
[1]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Medicine, Massachusetts General Hospital, Boston, Massachusetts, United States

Bullous pemphigoid (BP) is a rare and debilitating autoimmune blistering disorder reported to occur secondary to immune checkpoint inhibitors (ICIs). Management is challenging, often necessitating ICI discontinuation and prolonged immunosuppressive treatment. There is limited original research comprehensively characterizing ICI-induced BP (ICI-BP), with most existing studies being small case-based studies. We thus sought to summarize the risk factors, presentation, diagnostic findings, treatments, and outcomes of ICI-BP. A systematic review of several databases was performed according to PRISMA guidelines. Studies reporting data on individual patients meeting pre-established inclusion criteria were selected and a predefined set of data was abstracted. 531 articles were initially screened, and 70 studies reporting data on 127 individual patients were ultimately included and quantitatively combined whenever possible. Patients ranged in age from 35-90. ICI-BP often occurred during the course of anti-PD-1 (84.7%), PD-L1 (3.9%), or CTLA-4 (2.4%) therapy but was also found to develop after treatment cessation. 48.2% of patients experienced prodromal symptoms (e.g. pruritus). Immunotherapy was discontinued after ICI-BP development in 55.1% of patients. Common treatments were systemic (84.2%) and topical (53.5%) corticosteroids. Biologic and targeted agents, used predominantly in steroid-refractory cases, led to marked symptomatic improvement in most patients (13/16, 81.3%). Although rituximab and IVIG are included in the National Comprehensive Cancer Network treatment algorithm for ICI-induced bullous dermatoses, we do not have clear safety data and anecdotally avoid them. As steroid-sparing agents mitigate the need for ongoing immunosuppression and other negative sequelae, further research is needed to allow for the earlier utilization of targeted and immunomodulatory therapies in affected patients.

LB926

COVID-19 vaccine hesitancy in a cohort of hidradenitis suppurativa patients

M. Torpey, A. Nosrati, K. L. Campton, S. Cohen
Dermatology, Albert Einstein College of Medicine, Bronx, New York, United States

Introduction: Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease manifest as painful nodules, abscesses, and draining sinus tracts ("tunnels"). Numerous studies of SARS-CoV-2 and currently available mRNA vaccines have been published in recent months, including investigations into the phenomenon of vaccine hesitancy. However, the rationality driving COVID-19 vaccine hesitancy has not yet been thoroughly explored or described in patients with HS. Methods: An IRB-retrospective chart review and telephone survey of patients receiving care at Einstein/Montefiore HS Center (MMC-HSC) were conducted between September-December 2021. Patient demographics, vaccine status, and reasoning for vaccine abstinence (hesitancy) were documented. Results: Of 66 patients who agreed to participate, the mean age was 35.2±14.0; 44 (67%) were female. Of the 13 (20%) non-vaccinated patients, 11 (83%) stated that there wasn't enough information known about the long-term consequences of the vaccine; 5 (42%) expressed concerns about side effects; 3 (25%) identified apprehension about exacerbating HS; 2 (17%) expressed concern about negative interactions with HS medications, and 2 (17%) listed "other reasons" for vaccine hesitancy. Conclusion: Data from this pilot study strongly suggest that fear of long-term consequences and/or side effects associated with COVID-19 vaccination are major reasons for the phenomenon of vaccine hesitancy in our HS cohort. We propose that greater emphasis on patient education regarding COVID-19 vaccination may neutralize negative attitudes and provide stronger motivation for protection against SARS-CoV-2.

LB927

Evaluating patterns of co-occurrence between cutaneous and non-cutaneous immune-related adverse events among cancer patients after immune checkpoint blockade

M. S. Asdourian[1], N. Shah[1], T. V. Jacoby[1], Y. Semenov[1], L. L. Thompson[1], K. Reynolds[2], S. Chen[1]
[1]*Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States,* [2]*Medicine, Massachusetts General Hospital, Boston, Massachusetts, United States*

Immune checkpoint inhibitors(ICIs) have been associated with the development of multiple cutaneous and/or extracutaneous immune-related adverse events(cirAEs/irAEs). Given the prevalence and morphologic diversity of cirAEs, multiple distinct toxicities are particularly common in the skin, but these patterns remain poorly characterized. We sought to examine associations among multiple cirAE status, cirAE features, and irAE risk in patients receiving ICIs. In this retrospective cohort study, we screened patients initiating ICI therapy at MGH (1/1/16-6/29/21) who developed cirAEs and abstracted clinical history. Patients were grouped into those developing multiple(mcirAE) or single (scirAE) cirAEs. irAE diagnoses were categorized into 10 organ-based classes: cardiac, neurologic, renal, endocrine, GI, hepatobiliary, pulmonary, rheumatologic, HEENT, and other. Multivariate logistic regression was used to assess associations between cirAE features and risk of irAE development. Among 628 cirAE patients, 49.7% (n=312) also developed a non-cutaneous irAE and 89 developed multiple cirAEs. mcirAE patients commonly had initial and subsequent cirAEs with different morphologies, and on multivariate analysis had a higher odds of developing multiple non-cutaneous irAEs (OR: 1.88, 95% CI: 1.07-3.31, p=0.028). Among all patients, those with psoriasiform eruptions were significantly more likely to develop any irAE (OR: 2.93; 95% CI: 1.26-6.85, p=0.013). Almost half of the cirAE patients in our cohort had co-occurring irAEs, and mcirAE patients were at increased risk for multimorbid toxicity, with most developing >1 non-cutaneous irAEs. Although prior studies have suggested associations between specific irAEs after psoriasiform cirAE and mucositis, this study shows an overall elevated risk of irAE after ICI-psoriasis. Further research will be valuable in informing the optimal management of sequential and co-occurring immunotoxicities.

LB928

Distinguishing morphologic characteristics of cutaneous immune-related adverse events among patients receiving immune checkpoint inhibitors combined with other anti-cancer therapies

M. S. Asdourian[1], T. V. Jacoby[1], N. Shah[1], Y. Semenov[1], N. LeBoeuf[2], K. Reynolds[1], A. K. Dewan[3], S. Chen[1]
[1]*Massachusetts General Hospital, Boston, Massachusetts, United States,* [2]*Brigham and Women's Hospital, Boston, Massachusetts, United States,* [3]*Vanderbilt University Medical Center, Nashville, Tennessee, United States*

Novel combination regimens with immune-checkpoint inhibitors (ICIs) and targeted agents, chemotherapy, and non-ICI immunotherapies have led to unprecedented clinical outcomes in cancer patients, though there is little research describing the morphological patterns of cutaneous toxicities resulting from such regimens. We sought to characterize the clinical morphology of cutaneous immune-related adverse events (cirAEs) caused by ICIs alongside other anti-cancer therapy compared to ICI monotherapy. In this multi-institutional retrospective cohort study, we identified patients receiving ICI therapy at MGH (n=628; 1/1/16-6/29/21) and VUMC (n=6; 7/1/17-1/1/22) who developed cirAEs. Patients were categorized by regimen into those receiving ICI monotherapy (ICI-only) or ICI alongside other targeted agents, chemotherapy, or non-ICI immunotherapy (ICI-plus). Multivariate logistic regression was used to assess differences in cirAE characteristics based on ICI-only vs. ICI-plus status. Among 634 cirAE patients, 96 were ICI-plus and 538 were ICI-only. ICI-plus patients had a lower odds of developing isolated pruritus compared to ICI-only cases (OR: 0.19, 95% CI: 0.07-0.54, p=0.002). ICI-plus cirAEs were more likely to present as maculopapular (OR: 2.19, 95% CI: 1.26-3.80; p=0.005) and morbilliform (OR: 5.23, 95% CI: 1.34-20.37, p=0.017) eruptions compared to ICI-only. cirAEs were commonly described as having a single morphology regardless of ICI-plus status, suggesting that the concurrent use of ICIs and other anti-cancer therapies leads to synergistic cutaneous toxicities presenting with singular primary morphologies. Further research into how morphologic subtypes of cirAEs manifest in the setting of combination therapy will allow for the earlier recognition and improved management of cutaneous toxicities.

LB929

Opioid use disorder in a cohort of hidradenitis suppurativa patients

A. Nosrati, M. E. Torpey, K. L. Campton, S. R. Cohen
Albert Einstein College of Medicine, Bronx, New York, United States

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease. While HS treatment consists of a multi-tiered approach, many patients also seek therapy for debilitating pain. An increased risk of long-term opioid use disorder (OUD) has been intensively explored in patients with HS; however, there is a paucity of information associated with 'screening' for OUD. The Prescription Opioid Misuse Index (POMI), a 6-point questionnaire, is a specific and sensitive instrument for identifying patients with OUD. All patients seen in the Einstein/Montefiore HS Center (HSC) between September 1 and December 31, 2021 were invited to participate in this study. A total of 66 patients volunteered to complete telephone surveys. The presence of opioid use within this cohort was evaluated with the POMI. Thirty of 66 participants (45%) reported taking prescription opioids for HS-related pain. Nineteen (63%) of the opioid users were female; 16 (53%) self-identified as Black/African American, 10 (33.3%) as Spanish/Hispanic/Latino, 5 (17%) as White, and 1(3%) as Asian. The POMI questionnaire identified 5 (17%) patients taking higher doses of opioids than prescribed; 4 (13%) reduced the time between doses; 6 (20%) reported early refills; 4 (13%) described feeling "high" or "getting a buzz" after using the medication; 2 (7%) reported using the medication to relieve or cope with problems other than pain; 2 (7%) went to multiple physicians or an emergency room, seeking more medication to supplement the original prescription. Overall, the POMI identified 7 (23%) patients with OUD. Hidradenitis suppurativa is associated with debilitating pain that has led to an increased risk of long-term opioid use and an increased prevalence of substance use disorder. More than one of every five participants (23%) in this pilot study screened positive for OUD. Further screening for OUD in HS patients should be examined more closely.

LB930

Mental health professional utilization within a cohort of hidradenitis suppurativa patients

A. Nosrati, M. E. Torpey, K. L. Campton, S. R. Cohen
Albert Einstein College of Medicine, Bronx, New York, United States

Hidradenitis suppurativa (HS) is a chronic, debilitating inflammatory skin condition. HS patients are known to have increased rates of anxiety, depression, and suicidal ideation. There is a paucity of information about how HS exacerbations affect depression and mood. We decided to study the utilization of mental health professionals by a cohort of patients with HS. An IRB-approved retrospective chart review and telephone survey were conducted on patients treated at the Albert Einstein/Montefiore HS Center (HSC). All patients seen between September 1 and December 31, 2021 were invited to participate in this study. A total of 64 patients volunteered to complete telephone surveys. The survey consisted of demographics, a Patient Health Questionnaire-2 (PHQ-2) screening test, and patient perception regarding the utilization of mental health support. The mean age of participants (n=64) was 35.3±14.1, and 43 (67%) were female. The average HS-PGA for this cohort was 3.4±1.3. Twenty patients (31%) screened positive for depression using the PHQ-2. By contrast, when recalling times during which they experienced HS flares, 37 (58%) patients screened positive for depression. When asked if they experienced an improvement in mood with successful control of HS disease activity, 45 (70%) answered yes, 10 (16%) answered no, and 9 (14%) were unsure. Twenty-four (38%) had pursued support from a mental health professional (MHP); 12 (50%) felt that a MHP helped them cope with HS, 18 (75%) recommended seeking out a MHP to other HS patients. When asked if they would be willing to speak to a mental health professional in the future, 43 (67%) answered yes, 15 (23%) answered no, and 6 (9%) were unsure. This pilot study identifies a propensity for states of clinical depression during periods of worsened HS disease activity. It is remarkable that two-thirds (67%) of this cohort would seek the care of a MHP. Our data underscore the need for incorporating mental healthcare into the standard management of HS patients. Further studies are essential to define how to make these resources available.

LB931

Examining real-world treatment patterns of locally advanced basal cell carcinomas

N. Gupta[1, 2], V. Forrester[3], W. Su[4], E. J. Anstadt[4], J. Schoenfeld[5], A. Krausz[6], J. Lukens[4], S. Koyfman[7], F. Murad[1], E. S. Ruiz[1]

[1]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Case Western Reserve University School of Medicine, Cleveland, Ohio, United States, [3]Dermatology, Cleveland Clinic, Cleveland, Ohio, United States, [4]Radiation Oncology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [5]Radiation Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States, [6]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [7]Radiation Oncology, Cleveland Clinic, Cleveland, Ohio, United States

Locally advanced basal cell carcinomas (LaBCCs) require multi-disciplinary treatment including surgery, radiation, and systemic therapy. There is limited real-world data on treatment patterns for these advanced tumors. A multicenter retrospective chart review was performed of all laBCCs. Tumors were included if they underwent advanced surgery (amputation, exenteration, bone resection, auriculectomy, excision on the face and scalp > 4 cm2), radiotherapy, or systemic therapy. Data on achievement of no evidence of disease (NED) and poor outcomes (including local recurrence, nodal metastasis, distant metastasis, and disease-specific death) were collected. Cox proportional Hazard modelling was utilized to evaluate the impact of number of treatments required to clear the tumor on poor outcomes. A total of 494 laBCCs were identified, of which 414 (84%) achieved NED within the first three lines of treatment. 89 (21%) of tumors developed a poor outcome. 363 (73%) of tumors received surgery, 84 (17%) of tumors received radiation, and 47 (10%) of tumors received systemic therapy as first line treatment. 323 (65%) of tumors that required first line treatment, 78 (48%) of tumors that required second line treatment, and 13 (33%) of tumors that required third line treatment achieved NED. Tumors that required a second line treatment were more likely to develop poor outcomes compared to tumors that required only a first line treatment (HR:2.03, 95% CI: (1.3-3.3)). LaBCCs that required a second line treatment were twice as more likely to develop a poor outcome compared to laBCCs that only required a first line treatment. Further studies are needed to validate these results.

LB932

Dermatomyositis following treatment with cyclin-dependent kinases inhibitors: A single-center retrospective cohort study

A. Jfri[1, 2, 3], T. Harp[4], R. Vleugels[1, 2], N. LeBoeuf[1, 2, 3], L. Guggina[1, 2, 3]

[1]Dermatology, Harvard Medical School, Boston, Massachusetts, United States, [2]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [3]Cutaneous oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States, [4]Medicine, Rocky Vista University College of Osteopathic Medicine, Parker, Colorado, United States

The cyclin-dependent kinases 4/6 inhibitors (CDK4/6i) palbociclib, ribociclib, and abemaciclib have been reported to show an association with new-onset subacute lupus erythematosus (SCLE) but CDK4/6i's role in new-onset dermatomyositis (DM) is unexplored. We evaluated the clinical characteristics of patients presenting with new-onset DM following CDK4/6i. In this retrospective cohort study we queried data registries of Mass General Brigham and Dana-Farber Cancer Institute from October 1, 2014 - November 30, 2021, excluding patients with rash pre-initiation or post-termination of CDK4/6i. Of 428 patients with rash post-CDK4/6i, 5 had dermatomyositis (1.16%). All were female, mean age 65.2 years. Of these, 3 had stage IV invasive ductal carcinoma of the breast on abemaciclib (n=2) or palbociclib w/letrozole (n=1); 2 had (metastatic) lung adenocarcinoma and were on palbociclib w/binimetinib (n=2). Rash appeared 2 weeks-36 months post-CDK4/6i initiation, with median presentation of 9 months. Elevated creatine kinase (n=2) and aldolase (n=3) were found. Only 1 patient had a positive myositis-specific antibody (MDA5). CDK4/6i was terminated in 80% (n=4) due to DM development, with subsequent rapid improvement of symptoms. CDK4/6i was continued in 1 patient with mild symptoms who developed mild flares managed with topical steroids. Most patients had disease progression upon re-staging after CDK4/6i discontinuation (n=4; 80%) while one had stable disease. We present the first reported cases of CKD4/6i-associated dermatomyositis. We favored drug-induced DM over cancer-associated DM given symptoms improvement post-CDK4/6i termination and mean DM onset 3+ years after the cancer diagnosis. The findings of this study suggest the importance of re-staging and a possible shared role of the drug and malignancy in disease presentation.

LB933

Systemic corticosteroid exposure during cutaneous immune related adverse events worsens overall survival

N. Shah[1, 2], T. V. Jacoby[1, 3], M. S. Asdourian[1, 4], N. LeBoeuf[1], Y. R. Semenov[1], K. Reynolds[1], S. Chen[1, 4]

[1]Dermatology, Mass General Brigham Inc, Boston, Massachusetts, United States, [2]Virginia Commonwealth University School of Medicine, Richmond, Virginia, United States, [3]University of Hawai'i at Manoa John A Burns School of Medicine, Honolulu, Hawaii, United States, [4]Harvard Medical School, Boston, Massachusetts, United States

Although immune checkpoint inhibitor(ICI) therapy represents a breakthrough in advanced cancer treatment, further research into immune related adverse events (irAEs) and their management is warranted. Cutaneous irAEs (cirAEs) represent an early irAE for which systemic corticosteroids(SCS) are recommended, depending on their severity and morphology. We sought to investigate the impact of SCS exposure and dose on survival outcomes when administered during cirAE episodes. In this retrospective, single-institution study, we screened patients receiving ICI therapy between 1/1/16-6/29/21 and confirmed cirAE status using previously defined criteria. Patients identified as having cirAE were chart reviewed for additional demographic and clinical information including SCS exposure. SCS exposure was categorized by indication (cirAE, irAE, or other medical condition) and dosage ("low," ≤7.5 mg prednisone equivalents (eqs)/day for ≥2 months; "moderate," >7.5 mg prednisone eqs/day for ≥2 months; "high," ≥1mg/kg prednisone eqs/day for ≥1 week). Cox proportional hazard models were used to assess the relationship between SCS and survival outcomes, such as progression free survival (PFS) and overall survival (OS) while controlling for potential confounders. Of 628 patients who developed cirAEs, one-third (n=209, 33.3%) of patients were exposed to SCS during their cirAE episode for any cause. Out of those receiving SCS, 66.0% (n=138) received a "low" dose, 12.0% (n=25) received a "moderate" dose, and 22.0% (n=46), received a "high" dose. SCS use was associated with decreased OS (HR 1.41, p<0.05). When comparing high-dose versus low- and moderate-doses, no difference was found in PFS (HR: 0.79, p-value=.22) or OS (HR: 0.96, p-value=0.20). Results suggest SCS exposure during cirAE may worsen OS. Oncologists and dermatologists should avoid SCS exposure if possible.

LB934

Metastatic squamous cell carcinoma is associated with a lower disease-specific survival in immunosuppressed patients: A matched case-control study

M. E. Wackel, C. Georgensen, A. Wysong, M. J. Whitley

Dermatology, University of Nebraska Medical Center, Omaha, Nebraska, United States

Cutaneous squamous cell carcinoma (cSCC) is the second most common non-melanoma skin cancer with over 1 million cases and an estimated 3,932 to 8,791 deaths each year in the United States. cSCC patients have a 1-3% chance of developing metastasis which increases to 7-13% in immunosuppressed patients. The new 8th edition American Joint Committee on Cancer (AJCC8) classification system is currently used to stage cSCC, however immunosuppression is not included as a staging criterion. A review of patients with a diagnosis of cSCC seen at a tertiary referral center was conducted from 2010-2020. Pathology database review yielded five cases of metastatic cSCC with a known primary tumor and immunosuppression. These patients were 2:1 case-matched using age, gender, and diameter with 3 other cohorts: immunosuppressed patients with non-metastatic cSCC, immunocompetent patients with metastatic cSCC with known primary, and immunocompetent patients with non-metastatic SCC. The majority of metastatic cSCC in immunosuppressed and immunocompetent patients had a T stage of T3. Immunosuppressed patients with metastatic cSCC have worse prognosis for survival compared to their immunocompetent counterparts or those with non-metastatic disease with only 50% disease-specific survival (DSS) at one year. The disease-specific survival for immunocompetent patients with metastatic cSCC was 90% at one year (p<0.0001). A 40% decline in probability of survival from metastatic immunocompetent patients when compared to immunosuppressed patients suggests that immunosuppression is an important risk factor in determining DSS and should be taken into account when caring for these individuals.

LB935

Psoriasis association with stem cell transplantation for cancer treatment: A retrospective cohort and literature review

Y. Kost, A. Muskat, B. McLellan
Dermatology, Albert Einstein College of Medicine, Bronx, New York, United States

Background: Hematopoietic stem-cell transplantation (SCT) is a form of immunotherapy for leukemia, multiple myeloma, and relapsed lymphoma treatment. Given substantial advances in safety since its introduction, SCT is increasingly studied as treatment for refractory autoimmune diseases like psoriasis. Psoriasis is a multifactorial autoimmune disease mediated by T-helper type 1 cell response to an unidentified antigen. Anecdotal reports describe the impact of SCT for cancer treatment in triggering new-onset psoriasis or psoriasis resolution. However, the association of psoriasis and SCT has not yet been comprehensively assessed. Methods: We conducted a literature review using terms related to "stem cell transplant" and "psoriasis" via PubMed and EMBASE. We also conducted a retrospective chart review including patients with a history of psoriasis before or after SCT at our institution. Results: 30 studies with 55 cases were extracted from the literature and 4 patients were identified from chart review. In our chart review, we identified one never-described case of a patient with psoriasis skin lesions and psoriatic arthritis flaring 5 months after autologous SCT for multiple myeloma. Among all cases, remission of psoriasis following SCT was most common, accounting for 74.58% (44/59) of cases. Typical clinical presentation was that of an older (mean age 48 years) male (31/44, 70.45%) with multiple myeloma (15/44, 34.09%) following either allogeneic or autologous SCT at similar rates (23/44, 52.27% vs 21/44, 47.72% respectively). Less frequently, we identified new-onset psoriasis triggered by SCT in 23.72% (14/59) cases. Patients were younger (mean age 25 years) males or females (5/11, 45.45% vs 6/11, 54.54%) and most commonly presented with AML (4/14, 28.57%). Most cases (13/14, 92.85%) followed allogeneic rather than autologous SCT. Conclusion: We demonstrate that psoriasis remission, onset, and flaring can occur following SCT. Further research is needed to understand the mechanisms underlying these phenomena before employing SCT for psoriasis treatment.

LB936

Cutaneous T-cell lymphoma (CTCL) following B-cell lymphoma: A case series

A. Vander Does[2], A. Nichols[2,1]
[1]*Sylvester Comprehensive Cancer Center, Miami, Florida, United States,* [2]*Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States*

Cutaneous T-cell lymphoma (CTCL) is associated with an increased risk of developing secondary malignancies, particularly lymphomas. However, the diagnosis of lymphoma prior to CTCL is very rare. Herein we present the cases of two patients diagnosed with lymphoma prior to a diagnosis of CTCL. The first patient was a female in her late 50s with a history of diffuse large B-cell lymphoma (DLBL) of her breast status post chemo plus radiation who developed poikilodermatous scaly patches on her arms and legs one year after her DLBL diagnosis. Her condition was initially managed as an eczema refractory to treatment until a biopsy performed at an outside clinic several years later revealed likely CTCL followed by confirmative biopsies demonstrating poikiloderma atrophicans vasculare. She was treated with acitretin 10 mg five times weekly and narrowband UVB twice weekly for 12 treatments, but self-discontinued both. She recently initiated monthly extracorporeal photopheresis. The second patient was a female in her 70s with history of melanoma who presented to outpatient dermatology for evaluation of erythematous macules on her groin pending radiation treatment for DLBL of her breast. The rash was refractory to topical therapies and a biopsy later demonstrated mycosis fungoides. The patient initially preferred conservative therapy with natural sunlight and topical hydrocortisone, but ultimately elected for monthly photopheresis as her disease progressed. The response of both patients to photopheresis is yet to be determined. These cases illustrate the importance of full workup for erythematous, scaly rashes especially in the setting of other malignancy and in rashes refractory to topical treatments for other conditions. Both cases had at least one biopsy unrevealing of the true underlying CTCL diagnosis, showing that multiple biopsies may be necessary to capture this diagnosis.

LB937

Presentation and management of immune checkpoint inhibitors-induced psoriasis: A single-center Experience

A. Jfri[1,2,3], B. Leung[3], N. LeBoeuf[1,2,3]
[1]*Dermatology, Harvard Medical School, Boston, Massachusetts, United States,* [2]*Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States,* [3]*Center for cutaneous oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States*

Cutaneous immune-related adverse events (irAEs) are the most common irAEs caused by immune-checkpoint inhibitors (ICI). We assessed the clinical presentation and treatment outcomes of ICI-mediated psoriasiform eruptions in our center. In this retrospective study we queried data registries of Dana-Farber Cancer Institute/Mass General Brigham through February 2020. Patients with de novo ICI-induced psoriasis or flared up psoriasis post-ICI were included. Known psoriasis who did not flare up were excluded. Out of 1354 ICI-treated patients, 270 patients were included: 59.3% male (160) median age 67. Most frequently used ICI was pembrolizumab (39.4%) followed by nivolumab (38.6%), atezolizumab (10.6%), ipilumumab (9.8%) and ipilumumab w/nivolumab (3.7%). Most common ICI-treated cancer was lung (26%) followed by melanoma (22%). De novo psoriasis was 64.5% and flare of preexisting psoriasis was 35.5%. Median time from ICI initiation to the development of de novo vs. flare of psoriasis was 120 and 54 days, respectively. Most common location was upper extremities (66.7%) followed by lower extremities and anterior trunk, with 50.7% for both. Pruritis was reported in the majority (86.2%). Psoriatic arthritis was reported in 9.3%, mostly concomitant with the rash onset in 85.7% and after eruption in 14.3%. Other irAE within 6-week period before or after the rash was observed in 18.1%. Only 9.7% of cases required ICI interruption/discontinuation due to the rash. Most-used topical treatment was topical corticosteroids in 97.3%, Systemic treatment was acitretin 55.6% followed by oral steroid 27.5%, apremilast 11.1%, biologics 5.3% and other systemic agents 21.%. Response to topical vs systemic treatment was complete resolution in 35.3% vs 81.8%. In this largest single study cohort, ICI-induced psoriasis either de novo or flare-up of pre-existing was manageable in most cases and rarely lead to ICI interruption or discontinuation at our institution.

LB938

Use of a scalp clarifying shampoo in the treatment of lichen planopilaris and female pattern hair loss

S. Ali[1], M. Collins[1], I. Pupo Wiss[1], J. Pathoulas[1], K. Flanagan[1], M. Senna[1, 2]
[1]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Harvard Medical School Department of Dermatology, Boston, Massachusetts, United States

Patients routinely inquire whether shampoos are helpful in the treatment of hair loss disorders. Despite evidence that suggests scalp health is important for hair growth,1 many haircare product formulations contain known scalp irritants and/or allergens.2,3 In this randomized control trial, we evaluated the use of a non-medicated, hypo-allergenic scalp-clarifying shampoo on patient reported outcomes and clinical severity of lichen planopilaris (LPP) and female pattern hair loss (FPHL). Thirty-five women (80% Caucasian), mean age 52.2 years, were randomized 1:1 to either clarifying shampoo or the shampoo they already used. Patients were assessed at baseline and at 6 months. At both visits, subjects completed surveys including the Sinclair Shed Scale and a single investigator performed the Lichen Planopilaris Activity Index (LPPAI) on patients with LPP.4 The experimental group reported improved hair volume, and more comfort showing their hair without camouflage (p=.041). In subjects with LPP, the experimental group had a higher percentage of patients who reported “My hair looks great” compared to controls (p=.040). The experimental group also reported a significant decrease in hairs shed per week compared to the control group, which showed an increase in hair shedding at 6 months (-122 vs +102, p=.034). Subjects with LPP also had significant reduction in their LPPAI score (2.01 vs 0.96, p=.010). Our results suggest that use of a scalp clarifying shampoo may be helpful for hair loss treatment. This may be due to avoidance of potentially irritating ingredients, or better penetration of topical medications due to reduced scalp residue. Larger studies should be done to evaluate this further.

LB939

Novel recommendations to minimize cSCC risk in SOTR

B. D. Kwinta[1], S. Khan[2], R. Carvajal[2], B. Izar[2], D. Queen[2], T. Garcia-Saleem[2], L. Geskin[2]
[1]Columbia University Vagelos College of Physicians and Surgeons, New York, New York, United States, [2]Columbia University Irving Medical Center, New York, New York, United States

Solid organ transplant recipients (SOTR) are at significantly increased risk for the development of cutaneous squamous cell carcinomas (cSCCs). Based on previously published data, our Columbia University Cutaneous Oncology Group has developed novel clinical management guidelines aimed at reducing the development of cSCCs in SOTR. Currently published data contain the following broad recommendations that may reduce development of cSCCs in SOTR: 1) Use of mTOR inhibitors, particularly sirolimus, in conjunction with mycophenolate, compared to alternative immunosuppressants; 2) Chemoprevention with acitretin and niacinamide (limited data is available for the use of niacinamide in immunocompromised patients); 3) Use of capecitabine (small case series have shown reduction of cSCCs in SOTR). Based on the above data, our group proposes the following algorithm: 1) Considering the excellent safety profile of niacinamide, all SOTR with risk factors for skin cancers should start niacinamide; 2) For SOTR with 1 low-risk cSCC/year, AJCC/BWH stage T1, continue niacinamide and consider adding acitretin. 3) For 2-10 low-risk cSCCs/year, add acitretin and consider immunosuppression modification. 4) For >10 low-risk cSCCs/year, consider adding capecitabine. 5) For SOTR with 1 high-risk cSCC, AJCC T3 or BWH stage T2B, continue niacinamide, add acitretin, and consider immunosuppression modification. 6) For ≥2 high-risk cSCCs, reduce immunosuppression and consider adding capecitabine. Niacinamide is efficacious in immunocompetent patients and has an excellent safety profile. Therefore, our group proposes early administration of niacinamide in SOTR, recognizing the need for additional investigation and dedicated trials focused on niacinamide in this population. Our institutional guidelines allow for an algorithmic and standardized approach to the current preventative paradigm for cSCCs in SOTR, and enable the building of a unified platform for future studies.

LB940

A comprehensive evaluation of thoracoscopic sympathectomy for severe primary palmar hyperhidrosis

L. Bosacker[1], I. Diaz Gutierrez[2], M. Hordinsky[3], R. Andrade[2]
[1]University of Minnesota Medical School Twin Cities, Minneapolis, Minnesota, United States, [2]Thoracic Surgery, University of Minnesota Health, Minneapolis, Minnesota, United States, [3]Department of Dermatology, University of Minnesota Health, Minneapolis, Minnesota, United States

We assessed the efficacy of thoracoscopic sympathectomy (TS) in patients with severe primary palmar hyperhidrosis (PPH) with objective and subjective measures. We conducted two prospective institutional review board-approved studies. We evaluated healthy volunteers (controls) with measurement of palmar transepidermal water loss (TEWL; g/m2/h) and standardized questionnaires (Hyperhidrosis Disease Severity Scale [HDSS], Dermatology Life Quality Index [DLQI]). We evaluated PPH patients the same way at baseline, and 1 month and 1 year after TS and compared them to controls. We report medians and IQR (two-sample Wilcoxon for continuous variables; Chi-square for categorical variables). We evaluated 50 controls and 127 PPH patients. Control palmar TEWL was 106 g/m2/h (IQR 70), HDSS was 1 (IQR 0), and DLQI was 0 (IQR 0). PPH palmar TEWL was 146 g/m2/h (IQR 83), HDSS was 4 (IQR 1), and DLQI was 13 (IQR 8.5). Palmar TEWL and questionnaire scores were higher in PPH patients than in controls (TEWL p<0.05; HDSS and DLQI p<0.0001). 21 PPH patients underwent TS and the 1 month postop evaluation; 10 patients had a 1 year postop evaluation. Palmar TEWL 1 month after TS was 40 g/m2/h (IQR 13), and 1 year after TS was 28 g/m2/h (IQR 9.5); both were lower post TS than in controls (p<0.0001). Palmar TEWL 1 month and 1 year after BTS was lower than PPH palmar TEWL preop (p<0.004). At 1 month and 1 year post TS, DLQI scores were not different from controls, and they were lower than preop scores (1 month post TS PPH = 0 [IQR 2], 1 year post TS = 2.5 [IQR 5] p < 0.003). Post TS, HDSS scores at 1 month were 1 (IQR 0) and at 1 year were 2 (IQR 1), both values were higher than in controls (p<0.003). One-month and 1-year post TS HDSS scores were lower than preop scores (p<0.007). We demonstrate that after TS, PPH patients significantly improve and compare favorably to healthy controls.

LB941

Importance of six-month dosing with QTORIN rapamycin to achieve maximal effect in patients with pachyonychia congenita

J. Teng[1], J. Martini[2], T. Funk[3], J. Connor[4], E. Cook[2], D. Hansen[5], A. Paller[6]
[1]Dermatology, Stanford University School of Medicine, Stanford, California, United States, [2]Palvella Therapeutics, Wayne, Pennsylvania, United States, [3]Oregon Health & Science University, Portland, Oregon, United States, [4]Confluence Statistics, Wayne, Pennsylvania, United States, [5]University of Utah Hospital, Salt Lake City, Utah, United States, [6]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

PC is a rare, debilitating disease with lifelong limited mobility caused by mutations in KRT6, 16, or 17. There are no approved therapies. mTOR regulates expression of KRT6 and its binding partners KRT16 and 17. We completed Ph. 2/3 and 3b trials with QTORIN rapamycin for PC. We identified a cohort of subjects, gradual responders, who accrued a higher response between 12 and 24 weeks of therapy. The Ph. 2/3 trial was a randomized withdrawal trial, where all subjects received 12-weeks of QTORIN rapamycin. The primary endpoint was the Patient Global Assessment of Activities Difficulty (PGA-AD). To enrich the withdrawal study, subjects who met a 2-point improvement seamlessly rolled into the Ph. 3 randomized withdrawal period. All subjects (Ph.3 qualifiers and non-qualifiers) were invited into the Ph. 3b OL trial after washout. 21 subjects in the Ph. 3b study completed 24 weeks with QTORIN rapamycin reported a mean improvement in PGA-AD of 2.74 points (p<0.0001). Improvements were observed in Worst Pain, CGI-S, and PROMIS-PF (p<0.0001). At 24 weeks, “qualifiers” had a 3.02-point improvement (n=13) while “non-qualifiers” (n=8) had a 1.41-point improvement in PGA-AD at 12 weeks and reached a 2.28-point improvement at 24 weeks, indicating these subjects are gradual responders since they continued to improve between 12 and 24 weeks. We identified a cohort with clinically meaningful responses to QTORIN rapamycin: gradual responders. After 12-weeks of treatment, gradual responders, achieved a PGA-AD improvement of <2 points but continued to improve over weeks 12-24, indicating these gradual responders have a full clinical benefit that takes >12 weeks to achieve. This result supports the 24-week treatment period with QTORIN rapamycin for the ongoing Ph. 3 study in PC.

LB942

Prospective randomized controlled trial on the effects of almonds on the gut microbiome in association with the gut-skin axis and skin related effects

A. Pan[1, 2, 3], I. Rybak[2], A. Shakhbazova[2, 3, 4], M. Chakkalakal[3], C. J. Chambers[3, 1, 5], R. K. Sivamani[1, 2, 3]
[1]California Northstate University College of Medicine, Elk Grove, California, United States, [2]Dermatology, University of California Davis, Sacramento, California, United States, [3]Integrative Skin Science and Research, Sacramento, California, United States, [4]University of California Riverside, Riverside, California, United States, [5]Pacific Skin Institute, Sacramento, California, United States

Almonds are a rich source of fatty acids, phytochemical polyphenols, and antioxidants such as vitamin E. We previously showed that 24 weeks of almond consumption significantly decreased facial wrinkle severity and pigmentation. Sebum excretion increased in the control group while there was no increase in the almond group. Here, we analyze how the gut microbiome was altered after almond consumption and how this may contribute to the gut-skin axis. A prospective, randomized, placebo-controlled study included postmenopausal women (n=56) who consumed 20% of their daily energy consumption in either almonds (n=28) or calorie-matched snacks (n=28) for 24 weeks. Stool samples were analyzed with whole-genome sequencing to detect microbial abundance as well as functional gene expression. In the almond intervention group, there was a significant increase in Bacteroidetes phylum and in Butyricimonas virosa abundance ($p<0.05$), which are known short-chain fatty acid producers. The functional analysis demonstrated an increase in microbial queuosine biosynthesis, glycolysis VI, and mannitol cycle, and decrease in formaldehyde assimilation II (RuMP cycle) and superpathway of L-serine and glycine biosynthesis I in the almond intervention group ($p<0.05$). On the other hand, control snack supplementation was augmented for microbial glycogen degradation I ($p<0.05$), suggesting that there was an increased load of glucose to the gut in the control group, in agreement with the increased glucose content in the control group. The daily consumption of almonds may beneficially alter the gut microbiome in association with previously noted changes in the skin. Changes in the gut microbiome may also reflect the glycemic load of the food interventions.

LB943

Tofacitinib treatment of inflammatory skin conditions in patients with Down Syndrome

C. A. Dunnick[1], A. Rachubinski[2], B. Enriquez-Estrada[2], K. Worek[2], M. Galbraith[2], K. Smith[2], A. Hill[2], E. Gurnee[1], E. Wallace[1], D. Norris[1], J. Espinosa[2]
[1]Dermatology, University of Colorado, Aurora, Colorado, United States, [2]Linda Crnic Institute for Down Syndrome, University of Colorado, Aurora, Colorado, United States

Individuals with Down syndrome (DS, trisomy 21) display consistent activation of the interferon (IFN) response, hyperactive JAK/STAT signaling, and chronic dysregulation of the immune system, which could be explained by the fact that four IFN receptors are encoded on chromosome 21. IFN hyperactivity may explain the high prevalence of immune skin conditions in this population, including alopecia areata (AA), hidradenitis suppurativa (HS), psoriasis, atopic dermatitis, and vitiligo. We investigated the use of the JAK inhibitor Tofacitinib in an open label clinical trial enrolling individuals with DS ages 12-50 with moderate-to-severe AA, HS, psoriasis, atopic dermatitis, and/or vitiligo. All participants received 5mg of Tofacitinib (Xeljanz, Pfizer) BID. Transcriptional IFN scores, plasma cytokine scores, and skin pathology were assessed at baseline and 16 weeks. Results from the first 10 participants indicate that Tofacitinib is well tolerated in people with DS. Both IFN scores and cytokine scores were significantly decreased at 16-weeks ($p<0.05$). Overall skin pathology improved in 7 of 10 participants (IGA, $p<0.05$). For AA, 5 of 6 participants responded to Tofacitinib treatment (SALT, $p<0.05$), as did the two participants with atopic dermatitis, and the single participant with psoriatic arthritis. Two of five participants with HS showed improvement. Tofacitinib appears to be a safe treatment for patients with DS. For individuals with DS and AA, treatment is efficacious in stimulating hair re-growth, and on-label use for psoriatic arthritis can be appropriate in DS. Additional research is required in individuals with DS and HS. Tofacitinib represents a viable treatment option for people with DS and immune skin conditions when clinically indicated.

LB944

Crowdsourcing for the rapid and accurate visual assessment of treatment efficacy in dermatology clinical trials: A case study in the treatment of eczema.

Z. Wu, R. Kong, G. Hillebrand
Amway Corp, Ada, Michigan, United States

Visual grading of standardized before/after images is a mainstay clinical outcome in dermatology/cosmetic science. However, the method is typically time and labor intensive. Here we use crowdsourcing to measure visible treatment efficacy quickly and accurately in an eczema clinical trial. The double-blind, randomized, vehicle-controlled study enrolled 17 men and 25 women ranging in age from 18 to 64 and having at least one active eczema lesion. Subjects were randomized to either active (rosemary extract containing carnosic acid, a Staphylococcus aureus quorum sensing inhibitor, n=26) or vehicle (n=16). Treatments were twice-a-day for 28 days with standardized images collected at baseline, 7, 14 and 28 days. Dermatologists (n=10) and naïve panelists (n=50) reviewed 246 image pairs (e.g. baseline vs Day 28, fully randomized) with a forced choice method (left or right is better) using an online crowdsourcing service (Amazon Web Services). In-house expert graders served as gold standard comparison. Other clinical outcomes were ADSI clinical grading, self-assessment of itch and the Dermatology Life Quality Instrument (DLQI). Crowdsourced dermatologists' data showed lesion improvement in the active group exceeded that of the vehicle group at all time points with Day 14 being significantly different ($p = 0.02$). For the crowdsourced naïve panel data, a Bradley-Terry ranking model yielded perceived improvement magnitude. Active group showed significant improvement vs. vehicle at day 7 ($p = 0.047$) and at day 28 ($p = 0.003$). Crowdsourced results were corroborated by the in-house expert grades and other outcomes. Compared to vehicle, subjects in the active group reported significantly less itching on Day 14 ($p=0.02$) and were significantly less embarrassed/self-conscious about their skin at Day 28 ($p=0.042$). Active group showed significant improvement at Day 28 vs. baseline in 5 out of 6 ASDI scoring parameters. The speed and ease of conducting crowdsourcing along with low cost make it an attractive alternative for gathering visual perception data of clinical photography.

LB945

CBP-201, a novel and differentiated IL-4Rα targeting antibody being evaluated in Th2 inflammatory diseases

X. Yang, L. Zhang, Y. Ding, Q. Wang, W. Pan, Z. Wei, P. A. Smith
Connect Biopharma, Suzhou, China

CBP-201 is a novel IgG4 targeting IL-4Rα. In a Phase 2b trial (WW001, NCT04444752), all three doses of CBP-201 met the primary endpoint in the treatment of moderate-to-severe atopic dermatitis (AD) with significant reductions in Eczema Area Severity Index scores observed at Week 16. Here, we report the first description of the immunological profile of CBP-201 from our in-house preclinical experiments, including all comparisons to dupilumab. CBP-201 exhibited higher binding affinity (20.7 pM) to human IL-4Rα than dupilumab (45.8 pM), based on surface plasmon resonance. CBP-201 did not bind to monkey or mouse IL-4Rα. CBP-201 and dupilumab utilized distinct binding epitopes, as shown by amino acid mutation in human IL-4Rα and co-crystallization studies. CBP-201 potently inhibited signaling of IL-4 and IL-13 in a cell-based STAT6 reporter assay (IC50 = 7.0 ± 2.5 and 6.6 ± 1.5 ng/mL, respectively) and significantly reduced IL-4 and IL-13 dependent TF-1 cell proliferation (IC50 = 8.0 ± 1.6 and 9.7 ± 0.8, respectively). TARC/CCL17 is a known biomarker of AD inflammatory activity. CBP-201 inhibited IL-4 and IL-13 induced TARC production in activated human peripheral blood mononuclear cells (IC50 = 59.1 ± 1.2 and 13.6 ± 1.1, respectively). CBP-201 and dupilumab were similarly effective at reducing IL-4 and IL-13 mediated activation of primary human B-cells. The *in vivo* efficacy of CBP-201 was profiled using an allergic inflammation model in transgenic mice expressing human IL-4Rα, and IL-4. CBP-201 reduced eosinophilic tissue infiltration ($p<0.0001$) and antigen-specific IgE ($p<0.0001$). This is the first description of the immunological profile of CBP-201, demonstrating reductions in clinically validated biomarkers of Th2-driven inflammation. Additionally, we report distinct binding epitopes, higher binding affinity and increased potency demonstrating improved target engagement properties with CBP-201 compared to dupilumab. CBP-201 is under evaluation in AD (NCT04444752 and NCT05017480) and persistent asthma (NCT04773678).

LB946

Clinically meaningful responses achieved with tralokinumab in adults with moderate-to-severe atopic dermatitis who did not meet IGA 0/1 at initial 16-week treatment

E. Simpson[1], A. Blauvelt[2], J. I. Silverberg[3], M. Cork[4], N. Katoh[5], T. Mark[6], S. Schneider[7], A. Wollenberg[8]

[1]Oregon Health & Science University, Portland, Oregon, United States, [2]Oregon Medical Research Center, Portland, Oregon, United States, 3The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, United States, [4]The University of Sheffield, Sheffield, United Kingdom, [5]Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan, [6]LEO Pharma A/S, Ballerup, Denmark, [7]LEO Pharma Inc, Madison, New Jersey, United States, [8]Ludwig Maximilian University of Munich, Munich, Germany

In 2 monotherapy phase 3 studies (ECZTRA 1 and 2) of patients with moderate-to-severe atopic dermatitis (AD) treated with tralokinumab, one of the primary endpoints was IGA 0/1 (clear/almost clear) at Week 16. However, in a heterogeneous disease like AD, other important and clinically meaningful parameters include improvement in signs, symptoms, and/or quality of life. We assessed the impact of tralokinumab on AD signs, symptoms, and quality of life in patients with IGA>1 at Week 16. Partial responders, defined as patients with IGA>1 at Week 16, were included in a post-hoc analysis of ECZTRA 1 and 2. Non-responder imputation (NRI) was used for patients who utilized rescue medication or had missing data, and as observed to address missing data (AO). Clinically meaningful changes were defined as EASI-50, ≥3-point improvement in Worst Daily Pruritus NRS (itch), and ≥4-point improvement in DLQI. At Week 16, 960 patients on tralokinumab and 358 patients on placebo had IGA>1 by NRI (AO: tralokinumab n=898, placebo n=327). 49% (NRI), 82% (AO) of the patients with IGA>1 in the tralokinumab arm achieved clinically meaningful changes in EASI, itch, and/or DLQI. 33% met EASI-50, 23% achieved ≥3-point improvement in Itch NRS, and 41% achieved ≥4-point improvement in DLQI by NRI (AO: 55%, 40%, 71%). At Week 16, tralokinumab patients with IGA>1 achieved significantly (P<0.001) greater responses across all endpoints versus placebo. At 16 weeks of treatment with tralokinumab, partial responders achieved clinically meaningful improvement in AD signs, symptoms, and quality of life.

LB947

An open-label study of topical ruxolitinib in necrobiosis lipoidica

P. Bhullar[4], B. Boudreaux[4], K. Severson[4], N. Zhang[1], R. Butterfield[1], C. Brumfiel[4], M. Patel[4], X. Li[2], A. Hughes[4], S. Zunich[4], E. Branch[3], S. Nelson[4], A. Sekulic[4], M. R. Pittelkow[4], A. R. Mangold[4]

[1]Research and Biostatistics, Mayo Clinic Arizona, Scottsdale, Arizona, United States, [2]Division of Biomedical Statistics and Informatics, Mayo Clinic Minnesota, Rochester, Minnesota, United States, [3]Health Sciences Research, Mayo Clinic Arizona, Scottsdale, Arizona, United States, [4]Dermatology, Mayo Clinic Arizona, Scottsdale, Arizona, United States

Necrobiosis lipoidica (NL) is a chronic granulomatous disease of the skin. A growing body of literature suggest that janus kinase (JAK) inhibitors for may be effective in the treatment of granulomatous skin diseases, including NL. Prior translational research identified activation of JAK-STAT pathways by interferon gamma (IFNγ) and other cytokines is integral to macrophage activation in these diseases. We aimed to evaluate the safety and efficacy of topical ruxolitinib, a JAK-1/2 inhibitor, for the treatment of NL. An open-label, single-arm study of 12 patients was conducted at Mayo Clinic Arizona (NCT04492618). The primary endpoint was the change in NL score (0-12) of the index treatment lesions (weeks 0 and 12). Additional endpoints included: Physician Global Assessment (PGA), body surface area (BSA), lesion count, Skindex-16, Pruritus NRS. Mean NL lesion score decreased from 4.4 (SD 1.5) at baseline to 1.7 (SD 1.0) at week 12 (P=0.003). Nine (81.8%) of patients were responsive by PGA at week 12. Mean BSA involvement decreased by 0.4% (SD 0.8, P=0.034). Mean Skindex-16 score improved from 33.8 (SD 19.4) at baseline to 11.8 (SD 13.2) at week 12 (P=0.03). There was no significant change in lesion count or pruritus NRS from baseline to week 12. This study shows that topical ruxolitinib may be an effective treatment for NL. Larger randomized controlled trials are needed to better characterize effectiveness and long-term safety of topical ruxolitinib for the treatment of NL.

LB948

Oral cannabidiol treatment of seborrheic dermatitis in patients with Parkinson's disease

C. Zagona-Prizio, T. E. Sivesind, M. D. Szeto, E. Wallace, S. H. Sillau, Y. Liu, M. A. Leehey, C. A. Dunnick, R. Dellavalle

University of Colorado Denver School of Medicine, Aurora, Colorado, United States

Seborrheic dermatitis (SD) is a common skin condition highly associated with the progressive neurogenerative disorder Parkinson's disease (PD). Conventional SD treatment typically includes topical agents; however, recent evidence suggests oral cannabidiol (CBD) may simultaneously ameliorate neurologic symptoms of PD while reducing the increased sebum production involved in SD pathogenesis. As part of a larger PD patient study, CBD treatment for SD was investigated with a parallel, double-blind randomized controlled trial collecting facial images before and after a 3-week treatment with 2.5mg/kg/day oral CBD solution (n=29) or placebo (n=29). Images were randomized and assessed independently by two board-certified dermatologists using the Seborrheic Dermatitis Area and Severity Index (SEDASI) quantitative instrument. After calculating averages of SEDASI score ratings, assessors determined whether SD improved, worsened, or did not change. Multiple regression models evaluated the effect of CBD on these categorical SD outcomes with gender, age, and log-scaled PD disease duration as interacting covariates. Overall levels of SD disease severity were low in the cohort at onset, with average SEDASI scores falling in the mild category for both CBD (5.33) and placebo (3.55) groups. Pre-treatment, 48.3% of CBD-treated patients and 58.6% of placebo patients had SD, compared to 37.9% CBD-treated and 69.0% placebo post-treatment. Overall, while potential predictors of treatment effect did not reach statistical significance (p=0.05 threshold), CBD treatment was found to be slightly protective with p=0.0528. Notably, although this study had adequate power for the primary objective of assessing the efficacy of CBD for PD motor symptoms, it was underpowered to detect the impact of CBD on SD, encouraging future investigation. These promising preliminary results indicate a potential role for CBD in the treatment of SD in patients with PD.

LB949

A systematic review and meta-analysis on RCTs evaluating combination laser treatment in skin rejuvenation

A. Pour Mohammad[2], A. Goodarzi[1], M. Gholizadeh Mesgarha[2]

[1]Dermatology Department, School of Medicine, Iran University of Medical *Sciences, Tehran, Tehran, Iran (the Islamic Republic of), [2]Iran University of Medical Sciences, Tehran, Iran (the Islamic Republic of)*

Background:Lasers have earned renown in skin rejuvenation, however, their efficacy in combination use is yet controversial. This systematic review aims to investigate the effectiveness and safety profile of combination laser therapies for skin rejuvenation in recent literature. Study Design/ Material and Method: A comprehensive search was conducted in major databases considering PRISMA guidelines; with related keywords encompassing wrinkle, skin aging, laser, rejuvenation and related MESH terms, from 2010 to 2020. Then, RCTs in the human population were selected, which used a combination of two laser modalities for wrinkle improvement and photo aging treatment, reporting the efficacy and safety. The exclusion criteria were comparative studies of two laser types, non-laser modalities, absence of at least one combination arm, or studies of exclusive scar resurfacing. The pooled estimate of Odds Ratio(OR) with confidence interval(95%CI) were computed using random-effects models. Results: Five studies compared a laser combination therapy with laser monotherapy, recruiting 206 cases in total; comprising 105 cases as treatment group (combination therapy) and 101 cases as control group (monotherapy). Considering site-specific sub-group analysis, odds of clinical improvement in combination therapy in the facial region were almost equal to that of laser monotherapy (OR: 0.99, 95%CI 0.45–2.18). Nevertheless, the pooled OR was increased to 3.19 in laser combination therapy of hand region (95% CI 1.14–8.93). Odds of occurrence of adverse effects, comprising erythema and swelling were not significantly different in two groups (OR: 1.00, 95% CI 0.10–10.15) and (OR: 1.00, 95%CI 0.19–5.37), respectively. Conclusion:Regarding facial rejuvenation, no significant difference was observed between laser combination therapy and laser monotherapy in terms of clinical improvement rate or adverse events. However, in hand rejuvenation, combination therapy seems to enhance the clinical outcomes.

LB950

A randomised trial of a wearable uv dosimeter for skin cancer prevention

G. I. Varghese[1], P. D. Kaplan[2], C. Do[3], M. Barrer[1], K. Ezzedine[4], J. Zippin[1], E. Dumont[2, 5]

[1]Dermatology, Weill Cornell Medicine, New York, New York, United States, [2]Shade, Nutley, New Jersey, United States, [3]Pathology, NYU Langone Health, New York, New York, United States, [4]Dermatology, Hopital Henri Mondor, Creteil, Île-de-France, France, [5]Hackensack Meridian Health Center for Discovery and Innovation, Nutley, New Jersey, United States

Background: Non-melanoma skin cancer (NMSC) is the most prevalent cancer in the United States. Despite guidelines on UV radiation avoidance, it remains difficult for people to assess their individual exposure, as UV is invisible and the onset of UV-induced symptoms is delayed. Objective: To measure the clinical impact of a wearable UV dosimeter that provides real-time information to users. Methods: In a prospective randomised trial, ninety-seven elderly patients with a history of actinic keratoses (AK) were enrolled and followed over six months. Fifty patients were given a wearable UV dosimeter that provided real-time and cumulative daily UV exposure, and forty-seven patients received UV protection counseling by a dermatologist. Results: After 6 months of intervention, when comparing the device group to the control group, we observed a 20% lower ratio of incidence rates of AKs (95% CI = [-41%, 55%], p-value = 0.44) and a 95% lower ratio of incidence rates of NMSCs (95% CI = [33%, 99.6%], p-value = 0.024). Limitations: The study has a short duration and a small sample size. Conclusion: This pilot clinical trial suggests that providing real-time UV exposure data using a wearable UV dosimeter may assist in the reduction of NMSC in an elderly population.

LB951

Assessing penetration, delivery, and mode of action of dissolving microneedle patches for reducing under-eye wrinkles using reflectance confocal microscopy

S. Razi[1], S. Ouellette[1, 2], B. Rao[1, 2]

[1]Rao Dermatology, New York, New York, United States, [2]Dermatology, Rutgers University New Brunswick, New Brunswick, New Jersey, United States

The purpose of this study was to visualize penetrance of self dissolving microneedles into different layers of the epidermis, visualize subsurface anatomical changes and calculate distance between microneedles and visualize deposition of drugs in the dermis. We recruited 8 subjects of different age groups to better understand how the microneedling patches containing hyaluronic acid and botulinum polypeptide-1 work under the eye to reduce wrinkles. Clinical pictures were taken and reflectance confocal microscopy (RCM) was performed before application and after removal of the patch to view subsurface anatomical changes. Each under eye patch was left for at least 6 hours overnight before performing a confocal scan. In 1 subject, we performed confocal microscopy after 1 hour and 2 hours of patch application to better understand the mode of action of microneedle patches. We were able to visualize the area of penetration of microneedles into the epidermis. Distance between microneedles was calculated. We were clearly able to visualize deposition of drugs into the dermis in 3 subjects, as well as in the subject that was confocaled sequentially after 1 and 2 hours. Dissolving microneedle patches allow for targeted drug delivery and are available over the counter for various aesthetic reasons, including wrinkles. RCM can be a useful tool to study microneedle penetration and drug delivery. Furthermore, it can help visualize and determine which areas of the skin would be ideal for transdermal drug delivery.

LB952

A phase 1 trial of DSG3-CAART cells in mucosal-dominant pemphigus vulgaris (mPV) patients: Preliminary data

D. J. Chang[1], S. Basu[1], R. Micheletti[2], E. Maverakis[4], M. Marinkovich[3], D. L. Porter[2], M. Abedi[4], W. Weng[3], K. Hoffman[1], J. Volkov[1], D. Nunez[1], M. C. Milone[2], G. K. Binder[1], A. S. Payne[2]

[1]Cabaletta Bio Inc, Philadelphia, Pennsylvania, United States, [2]University of Pennsylvania, Philadelphia, Pennsylvania, United States, [3]Stanford University, Stanford, California, United States, [4]University of California Davis, Davis, California, United States

mPV is mediated by anti-desmoglein 3 (DSG3) autoantibodies (Abs) and treated with chronic, broad immunosuppressive therapy. Based on the long-lasting remission of B cell cancers with chimeric antigen receptor T (CART) cells, we genetically modified autologous T cells to express chimeric autoantibody receptors comprising the DSG3 autoantigen (DSG3-CAART) to target only anti-DSG3 B cells. In this ongoing open-label study of adults with active, anti-DSG3 Ab-positive mPV, subjects are assigned to receive 2e7,1e8, 5e8, 2.5e9 and 5-7.5e9 DSG3-CAART after discontinuing immunosuppressives or tapering steroids. The primary endpoint is related adverse events (AEs) within 3 months of infusion. Screening characteristics of the 9 subjects in the first 3 cohorts who have completed 3 months (median (range)): 67% female; age 53y (32-70); disease duration 4.0y (0.3-15.4); PDAI 12 (1-20); anti-DSG3 Ab 104 U/mL (51-169). Prior medications: prednisone (9), rituximab (6) and mycophenolate (6). No dose-limiting toxicities or serious AEs were observed over 3 months. Disease was clear or almost clear (PDAI 0-1) in 0, 1, 2, 5 and 3 of the 9 subjects at screening, baseline and 1, 2 and 3 months after treatment, respectively. Anti-DSG3 Ab levels through month 3 were increased, stable (+/-20%) or decreased in 3, 5 and 1 subjects, respectively. There was a dose-dependent increase in DSG3-CAART persistence (rs=0.85) within 29 days, but at much lower levels than for cancer CART therapy. DSG3-CAART from all subjects exhibited specific in vitro lysis of anti-DSG3-expressing cells. The favorable DSG3-CAART safety profile along with the preliminary clinical and biological data from the first 3 cohorts provide rationale to evaluate higher doses and manufacturing enhancements in future cohorts.

LB953

Cure rate of curettage alone for basal cell carcinoma

G. O'Neill[1], A. M. Dean[1], R. Bednarek[1], L. Scherz[2], E. W. Hossler[1]

[1]Dermatology, Geisinger Health, Danville, Pennsylvania, United States, [2]Geisinger Commonwealth School of Medicine, Scranton, Pennsylvania, United States

The purpose of this study was to demonstrate the effectiveness of curettage alone for treatment of basal cell carcinoma (BCC). We conducted a prospective single center study examining the cure rate of curettage alone for 305 BCCs from 218 unique patients with a follow up of at least 3 years. After excluding deceased patients and those without clinical follow-up, 99.6% of BCCs showed no recurrence after a year (95% CI: 97.8%, 100.0%), and 99.0% of BCCs showed no recurrence after three years (95% CI: 96.5%, 99.9%). To the best of our knowledge, this is the first prospective study examining the efficacy of curettage alone as treatment for BCC and provides clinical evidence to support the use of this treatment modality.

LB954

Assessment of a topical bakuchiol containing cream for facial photoaging

A. Shakhbazova[1, 2], A. Pan[3, 2], R. K. Sivamani[2, 4]
[1]University of California Riverside, Riverside, California, United States, [2]University of California Davis Department of Dermatology, Sacramento, California, United States, [3]California Northstate University College of Medicine, Elk Grove, California, United States, [4]California State University Sacramento Department of Biological Sciences, Sacramento, California, United States

Currently, retinoids are the first-line noninvasive treatment for decreasing the appearance of fine lines and wrinkles. Although retinol does decrease signs of photodamage and aging, it has a side effect profile of erythema, peeling, pruritus, dryness and burning and/or stinging. Bakuchiol is a natural retinol alternative with anti-aging properties and less side effects. It is a meroterpene phenol found in the seeds and leaves of the plant Psoralea coryfolia and has anti-inflammatory, antiproliferative, and antiacne properties. We assessed the efficacy and side-effect profile of bakuchiol in decreasing the appearance of fine lines and wrinkles, erythema and pigmentation. Thirty four participants were asked to apply a sunscreen with SPF 30 containing bakuchiol twice daily. A facial photograph and analytical system captured photos of the subjects' faces at 0,4,8 and 12 weeks and a board certified dermatologist graded the facial erythema. After 12 weeks of use, bakuchiol containing sunscreen significantly reduced the surface area of subsurface spots ($p=0.04$), especially spots less than 2 mm2 ($p<0.001$) and between 2-4 mm2 ($p=0.002$). The spot count also significantly decreased ($p<0.001$). Grading done by a board-certified dermatologist revealed decreased erythema in the subjects ($p<0.001$). Our study demonstrates that bakuchiol containing sunscreen may decrease facial pigmentation and facial erythema.

LB955

Adherence in dermatology during the COVID-19 pandemic: A review

S. G. Bridgeman[1], P. O. Perche[1], S. R. Feldman[1,2,3]

[1]Dermatology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States, [2]Pathology, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States, [3]Social Sciences & Health Policy, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States

The purpose of this review is to investigate the extent to which the COVID-19 pandemic has affected adherence in dermatology. A literature search was performed using PubMed to identify articles relevant to the topic of adherence in dermatology during the COVID-19 pandemic. Search terms included: adherence, dermatology, COVID-19, Sars-CoV-2, and pandemic. In this review, adherence ranged from 31.5% to 100% across 11 studies. Non-adherence was primarily linked to concern about risk of COVID-19 infection with long-term use of immunomodulatory and immunosuppressive medications. In some cases, non-adherence was associated with feelings of depression, anxiety, or perceived stress. High adherence was attributed to regular and convenient communication between patients and dermatology providers though the effective use of telemedicine and electronic messaging. In conclusion, the COVID-19 pandemic has introduced new barriers to adherence in dermatology. Improved implementation of virtually-integrated and multidisciplinary treatment plans may help maintain and improve adherence in dermatology in the setting of COVID-19.

LB956

Dermatology on reddit: Analysis of content and quality on major dermatologic conditions

H. Kang[1], A. Wan[3], B. Desai[4], M. Beveridge[1], B. R. Rohr[1], D. Barlev[1], C. Wong[1], J. F. Scott[2]

[1]UH Cleveland Medical Center, Cleveland, Ohio, United States, [2]Johns Hopkins Medicine, Baltimore, Maryland, United States, [3]The University of Tennessee Health Science Center, Memphis, Tennessee, United States, [4]Case Western Reserve University School of Medicine, Cleveland, Ohio, United States

The majority of dermatologic content on Reddit seeks medical advice from the public community. Reddit is one of the most rapidly growing social media sites with 52 million daily users. Despite its prominence, little is known regarding the quality of dermatology content on Reddit. Our aim is to evaluate the accuracy, completeness, and likelihood of causing harm for posts seeking medical advice on the most popular skin-related subreddit, "r/skincareaddiction." Posts were queried for major dermatologic conditions, including atopic dermatitis, acne vulgaris, urticaria, psoriasis, sebaceous filaments and post-inflammatory hyperpigmentation. The top five posts for each condition within a year were evaluated with a Visual Analogue Scale (VAS) by board-certified dermatologists on accuracy, completeness, and likelihood of causing harm. Statistical analysis was performed to assess associations between conditions, mean post quality, and user activity. The responses' mean accuracy, completeness, and likelihood of causing harm were significantly lower than the null hypothesis of 5/10 on the VAS (0.458 ± 0.150, 0.436 ± 0.124, 0.281 ± 0.135, respectively; $p<0.005$). Accuracy was lowest for responses in the sebaceous filament category ($p<0.005$) and likelihood of causing harm was highest for the atopic dermatitis category ($p<0.05$). This study highlights how accuracy and safety varies based on dermatologic condition. Overall, Reddit dermatology content is unlikely to cause harm but is slightly below average accuracy and completeness. By determining the quality of dermatologic information on Reddit, this study highlights Reddit as a public source of information and misinformation for patients. We propose Reddit as a potential platform for dermatologists to improve the quality of widely disseminated dermatologic knowledge and better understand ongoing dialogues that affect patient safety.

LB957

Post-traumatic stress disorder in patients with alopecia areata

L. E. Drake[1,2], J. Li[1], S. Reyes-Hadsall[3,2], A. Mostaghimi[2]

[1]Tufts University School of Medicine, Boston, Massachusetts, United States, [2]Brigham and Women's Hospital Department of Dermatology, Boston, Massachusetts, United States, [3]University of Miami School of Medicine, Miami, Florida, United States

Post-Traumatic Stress Disorder (PTSD) occurs secondary to exposure to a traumatic event. Alopecia areata (AA) is an autoimmune condition causing unpredictable hair loss. Disorders such as anxiety and depression are comorbid with AA,1 and lack of highly efficacious treatment options can be distressing.2 This cross-sectional survey study examines the prevalence of PTSD among patients with AA. The PTSD Checklist for DSM-5 was distributed through the National Alopecia Areata Foundation.3 1449 completed surveys (rate 79.6%); most respondents were female (83.8%) and white (76.6%), with 91.4% experiencing active hair loss. 37.3% had history of AA, 12.8% AT, and 50.0% AU. A total of 33.9% screened positively for PTSD. Feelings of intrusion (Cluster B) and avoidance (Cluster C) were predominant symptoms. AT had the highest average PCL-5 score ($p=0.003$) and the highest percentage of PTSD, followed by AU, and AA. Our results reflect that one in three patients in this cohort meet the screening criteria for PTSD. These results build on the literature of psychological comorbidities of AA suggesting that ramifications extend beyond active disease. Dermatologists should consider implications of treatment nonadherence to avoid triggering PTSD. Additionally, dermatologists can better support patients by validating their responses to triggers, utilizing systems like telehealth to be available for patients during crisis, and referring patients for psychiatric evaluation. Generalizability is limited by the utilization of the NAAF database, with most respondents identifying as white females. 1.Toussi A, et al. Psychosocial and psychiatric comorbidities and health-related quality of life in alopecia areata: A systematic review. Journal of the American Academy of Dermatology. 2021;85(1):162-175. 2.Hunt N, et al. The psychological impact of alopecia. Bmj. 2005;331(7522):951-3. 3.Weathers FW, et al. The PTSD Checklist for DSM-5 (PCL-5). The National Center for PTSD at www.ptsd.va.gov

LB958

A retrospective study of skin biopsy diagnostic accuracy among dermatologists throughout the day: Do physicians experience diagnostic or procedure fatigue?

F. Dadrass[1], C. Joyce[3], E. Lake[2]

[1]Loyola University Chicago Stritch School of Medicine, Maywood, Illinois, United States, [2]Department of Medicine, Division of Dermatology, Loyola University Medical Center, Maywood, Illinois, United States, [3]Department of Biostatistics, Loyola University Medical Center, Maywood, Illinois, United States

Background: A skin biopsy is a diagnostic procedure which is the standard of care to confirm or rule out certain dermatologic conditions. Given the high volume of patients seen by dermatologists, it is important to consider if fatigue leads to changes in accuracy throughout the day or week. Methods: We performed a retrospective chart review of patients who underwent a skin biopsy at Loyola University Medical Center between October 2019-December 2020. Skin biopsies were identified by CPT codes, and covariates included: same day or return visit biopsy, skin cancer history, Fitzpatrick skin type, and biopsy type. The dermatologist differential diagnosis was compared to dermatopathology (referent standard) to assess accuracy. Mixed effects logistic regression was performed to test the association between biopsy timing and accuracy. Results: Between October 2019-December 2020, 2090 biopsies were performed. The mean patient age at visit was 59±19, 51% were female, 85% were White, and 84% had skin type I-III. Overall accuracy was 60.9%, and for each hour later in the day, the odds of an accurate diagnosis decreased by 2% (OR: 0.98, 95% CI: 0.94-1.02; $p=0.29$). This change in accuracy was driven by significantly decreasing specificity (OR: 0.95, 95% CI: 0.90-0.99; $p=0.04$) as sensitivity was similar across time (OR: 1.00, 95% CI: 0.90-1.11; $p=0.99$). Compared to the rest of the week, Fridays featured lower sensitivity (76.7% vs 86.5%; $p=0.08$) and higher specificity (66.4% vs 51.0%, $p=0.001$). Conclusions: A negative trend in accuracy of skin biopsies was observed throughout the day. It would be helpful for dermatologists to take this into account and consider restructuring clinic days to account for procedure fatigue.

LB959

The influence of social media on non-surgical cosmetic dermatologic procedures: A qualitative study using survey responses

E. Albazi[1], F. Khalil[1], J. Pestenariu[2], A. Rau[2], K. Ashack[3]
[1]Michigan State University College of Human Medicine, East Lansing, Michigan, United States, [2]University of Michigan, Ann Arbor, Michigan, United States, [3]Dermatology Associates of West Michigan, Grand Rapids, Michigan, United States

The rise of social media in recent years has changed the landscape of social interaction and profoundly impacted culture– this study aims to identify its role in influencing society's perceptions of non-surgical dermatologic procedures. We accomplished this by conducting a cross-sectional Qualtrics survey distributed via Instagram, Facebook, GroupMe, Twitter, and email. Questions addressed frequency and type of social media use, exposure to cosmetic procedures through these platforms, and their desires to seek out cosmetic procedures. The participants were categorized by age, quantity of time spent on social media, and general perceptions of cosmetic procedures. Of 357 participants, 93.28% (n=333) of participants used social media daily. Of those 333 daily users, 85.29% (n=284) see cosmetic procedures weekly to daily on their feeds. We also found that 69.67% (n=232) of daily users are either currently considering non-surgical cosmetic procedures (including botulinum toxin, dermal filler, polydioxanone threads, etc.), or have considered it in the past year. 64.15% (n=211) of daily users also stated that they find it beneficial when they see cosmetic procedures shared and discussed on social media. However, only 17.58% (n=58) of daily users stated that if they were to have a procedure done they would share it on social media. Despite apprehension with sharing their own photos online, 72.67% (n=242) of daily users stated that people in their social groups are more open to talking about their cosmetic desires than ever before. This suggests that social media is generating increased awareness of cosmetic procedures leading to a shift in how they are perceived. By gaining a deeper understanding of how social media contributes to public perceptions of cosmetic dermatologic procedures, we may be better able to use these platforms as a resource to educate and provide free and accessible information.

LB960

Clinical and economic trade-offs of non-pregnancy laboratory monitoring in persons on isotretinoin acne therapy

E. D. Borre, S. C. Chen, M. Nicholas
Duke University, Durham, North Carolina, United States

Objective: To project the 1-year clinical and economic trade-offs of current practice versus cessation of laboratory monitoring for patients with acne on isotretinoin. Design: Markov model of isotretinoin administration and laboratory monitoring comparing: 1) current practice (CP), and 2) cessation of non-pregnancy laboratory monitoring (NoLabMon). In the simulation, 20-year-old persons initiating isotretinoin were maintained on therapy for six months, unless taken off early due to laboratory abnormalities. Simulated patients experienced weekly probabilities of cell-line abnormalities (0.12%) and early cessation of isotretinoin therapy after detection (2.2%, CP only). Patients with cell-line abnormalities experienced weekly probabilities of serious adverse event (0.1%) and mortality (5%). Utilities for fully-treated patients were 0.93 (0.89 for early cessation). We included costs of isotretinoin ($110/week), laboratory monitoring ($5/week), and specialist visits ($220/visit) from a healthcare payer perspective. Distributions were assigned to conduct probabilistic uncertainty analysis (PUA). Results: Over 1 year, and for 200,000 people on isotretinoin in the US, NoLabMon resulted in 40 additional quality-adjusted life-years (QALYs) compared to CP. CP resulted in 1.6 isotretinoin-related deaths and the NoLabMon strategy resulted in 1.9 deaths. Total 1-year costs were $819M for CP and $795M for NoLabMon. NoLabMon was more effective and less costly than CP. In sensitivity analysis, the cost-effectiveness of NoLabMon was most sensitive to variations in the cost of lab monitoring and the probabilities of cell-line abnormality development. No variation of a single parameter across its plausible range changed our cost-effectiveness findings. In PUA, NoLabMon was the dominating strategy in >99% of 10,000 simulations. Conclusions: Laboratory monitoring for patients on isotretinoin as currently practiced is unlikely to be an efficient use of resources. Cessation of laboratory monitoring could realize savings of $24M/year and improve patient outcomes, with negligible effects on adverse events.

LB961

Clinical contexts of long-term antibiotic prescriptions for acne: A qualitative study

R. Festok, A. Ahuja, J. Chen, J. Barron, H. Yeung
Department of Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States

Dermatologists prescribe more oral antibiotics per clinician than all other specialties. Despite clinical guidelines that recommend limitation of long-term oral antibiotic treatments for acne, there is little evidence to guide implementation of antibiotic stewardship programs in clinical practice. This study sought to examine provider knowledge, preferences, facilitators, and barriers that underpin oral antibiotic prescriptions for acne with a duration longer than 3 months. Semi-structured interviews were conducted with 30 key stakeholders: 22 academic and community dermatologists, including experts from the American Acne & Rosacea Society and acne clinical guideline authors, 4 infectious diseases physicians with expertise in antibiotic stewardship, 2 dermatology advanced practice practitioners, and 2 dermatology residents. Interviews were transcribed, coded by 2 independent coders, and analyzed deductively using the Theoretical Domains Framework to generate salient themes and sub-themes. Knowledge of the guideline recommendations on limiting long-term antibiotics prescription was high. Most believe that antibiotic stewardship is a professional responsibility. Perceptions of limited effective and safe alternative options, insurance and regulatory barriers, lack of time and clinical inertia, discomfort and stigma surrounding contraceptive discussion, patient insistence on continuing successful oral antibiotic regimens, limited capacity for follow up appointments, medication refill protocols, and lack of a formal feedback program were some notable factors influencing long-term oral antibiotic prescriptions for acne. Salient patient-, clinician-, and systems-level factors were identified to inform the design and implementation of future antibiotic stewardship interventions.

LB962

Barrier integrity test for *in vitro* permeation testing of topical and transdermal products

G. Krishnan[1], N. Nalamothu[2], B. Holland[3], R. Palacharla[4]
[1]Curtin University, Perth, Western Australia, Australia, [2]Cary Academy, Cary, North Carolina, United States, [3]Virginia Tech University R B Pamplin College of Business, Blacksburg, Virginia, United States, [4]Florida Agricultural and Mechanical University, Tallahassee, Florida, United States

In vitro skin permeation testing (IVPT) is often used to ensure sufficient efficacy of dermaceuticals. IVPT studies utilize viable tissue membrane to represent *in vivo* conditions. The intact barrier of such tissue membrane provides biological relevance, in vitro. Transepidermal water loss (TEWL) measures changes in water vapor density on the skin surface. The purpose of this study was to evaluate TEWL as an appropriate barrier integrity test, in vitro, and determine an acceptable range using a Delfin® Vapometer. Eight pieces of unoccluded dermatomed human abdominal skin (500±250µm thick) from 2 donors were mounted on Franz diffusion cells, with PBS in the receptor chamber. Four pieces were subjected to 20x tape stripping, effectively removing the stratum corneum (SC) to mimic a compromised barrier. Four tissue pieces were left untreated (n=4). After 2 hours of hydration, TEWL measurements were periodically taken over 72 hours by placing the Vapometer on the SC. The uncompromised skin presented TEWL values of 8.1-14.3 g/m2/h, whereas the compromised skin presented significantly higher values of 18.8-47.0 g/m2/h. At T0, the intact-barrier tissue presented TEWL of 10.95 g/m2/h (1.93 SD), while the tape-stripped skin presented TEWL of 31.35 g/m2/h (10.93 SD). After 72 hours of exposure to a controlled environment, the TEWL measurements presented similar values of 7.5 g/m2/h (4.1 SD) and 24.8 g/m2/h (12.1 SD), respectively. The current study reaffirmed the acceptance criterion (5-25 g/m2/h) set for skin samples representing *in vivo* tissue with intact barriers suitable for in vitro permeation testing. While a larger data set with different tissue types is necessary to narrow this range, these TEWL values can reasonably estimate the integrity of barriers for in vitro permeation studies.

LB963

Single-cell transcriptomics of viable epidermis reveals inflammation along the skin furrows of barrier-disrupted skin.

M. Yokota[1], M. Katagiri[2], S. Nomura[2], S. Aihara[1], Y. Tokudome[3], T. Yoshino[1], T. Sakurai[1]
[1]FANCL Research Institute, FANCL Corporation, Yokohama, Kanagawa, Japan, [2]Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Bunkyo-Ku, Tokyo, Japan, [3]Laboratory of Cosmetic Sciences, Regional Innovation Center, Saga University, Honjo-Machi, Saga, Japan

The prevalence of dry skin, one of the most common skin conditions worldwide, is estimated at 29% to 85%. As the interface between the environment and the living body, a functional stratum corneum (SC) is essential for barrier homeostasis. Thus, the disruption of SC lipids results in skin dryness, which impacts the quality of life. However, subtle responses of healthy skin to disruptions of SC lipids is not well understood. Here, we investigated alterations of the epidermal transcriptome associated with SC lipid disruption at a single-cell resolution. First, we established a dry skin model using *ex vivo* explants from the same donors and compared a water-treated control with an Acetone:MeOH=1:1 treated dry skin model. In the dry skin model, the viability and histological features of the epidermis remained unchanged, but the outside-in barrier and water content evaluated by Raman imaging was decreased. Also, an X-ray diffraction study of the SC showed a shift to higher q values in the dry skin model, suggesting smaller repeat distances and/or a reduced presence of the long periodicity phase of the lamellar phases. We then conducted single-cell RNA-seq analysis to characterize the behavior of viable epidermis immediately beneath the disrupted SC. Most of the differentially expressed genes belonged to the KRT17 positive cluster. Pathway analysis indicated that signaling associated with Type I hemidesmosome assembly, Senescence-Associated Secretory Phenotype and Interleukin-4 and -13 signaling were upregulated in the dry skin model. Finally, immunohistochemical analysis revealed that the KRT17 positive cluster was localized along the skin furrows. Taken together, our data suggest that keratinocytes existing along skin texture lines might be an inflammatory niche in healthy skin.

LB964

TET2 participates in the dysregulation of skin barrier in psoriasis

H. Zhang, X. Zhang, S. Geng
Dermatology, Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, Shaanxi, China

Psoriasis is a chronic inflammatory skin disease. Dysregulation of the epidermal barrier in psoriasis has been demonstrated and epigenetic modifications have been found to be associated with psoriasis. TET2 has dioxygenase activity and oxidizes 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), and is a key regulator of DNA demethylation. There is a lack of studies on the effect of TET2 on the epidermal barrier in psoriasis. We observed reduced expression of TET2 and 5hmc in the lesions of psoriasis patients. In imiquimod psoriatic dermatitis mice, we obtained similar results by immunohistochemistry. We constructed epidermal TET2 conditional knockout mice (TET2CKO). In the imiquimod psoriasis dermatitis model, TET2CKO mice presented more severe erythema, scaling and skin thickness. The TEWL assay was performed on imiquimod-treated mice and was elevated in the TET2CKO group. This indicates that epidermal TET2 deficiency exacerbates psoriatic dermatitis in mice. We next examined the expression of K14, K10, filaggrin, involucrin, which represent proliferation and differentiation, TET2CKO mice showed K14, involucrin increased and K10, filaggrin decreased. TET2 absence may accelerate the proliferation and inhibit differentiation of keratinocytes. We subsequently observed that intercellular linker molecules Claudin-1 and E-cadherin were both downregulated in TET2CKO mice. In conclusion, deletion of epidermal TET2 is involved in dysregulation of the skin barrier. By RNAseq, we found that FLG mRNA is reduced after TET2 knockdown. Deficiency of filaggrin encoded by the FLG gene, leads to impaired epidermal barrier. Filaggrin expression reduced but mutations in the FLG gene have been reported to be rarely observed in psoriasis, therefore we suppose that FLG gene expression might be regulated by TET2, which is an alternative mechanism for barrier dysregulation in non-mutant FLG patients.

LB965

Sonic hedgehog signaling promotes aberrant hair follicle stem cell differentiation and subcutaneous ossification formation in a mouse model of albright hereditary osteodystrophy

P. J. McMullan[1, 2], P. F. Maye[2], Q. Yang[1, 2], E. Germain-Lee[1, 2, 3]

[1]*Pediatrics, UConn Health, Farmington, Connecticut, United States,* [2]*Center for Regenerative Medicine and Skeletal Development, UConn Health, Farmington, Connecticut, United States,* [3]*Albright Center, Division of Pediatric Endocrinology, Connecticut Children's Medical Center, Hartford, Connecticut, United States*

Albright hereditary osteodystrophy (AHO) is a disorder caused by GNAS heterozygous inactivation, and results in the spontaneous development of subcutaneous ossifications (SCOs) in the dermis and subcutaneous tissue. We have generated an AHO mouse model through global Gnas heterozygous inactivation (Gnas+/-) that phenocopies the human disorder and develops SCOs that consistently surround hair follicles (HF). Therefore, this study examined the cellular and molecular etiologies contributing to SCO formation within the HF microenvironment of Gnas+/- mice. We crossed Gnas+/- mice with an Osterix-mCherry model, which allowed for visualization of osteoprecursors *in vivo* by mCherry expression. Dorsal skin histology revealed Osterix expression within HF dermal sheath cells in both WT;Osx-mCherry and Gnas+/-;Osx-mCherry. However, Gnas+/- mice exhibited Osterix+ cell expansion in the dermis surrounding HFs and along the SCO bone surface, which suggests that these populations contribute to SCO formation. Gene expression studies on WT and Gnas+/- skin biopsies by RT-PCR array were performed, and Gnas+/- SCO biopsies displayed elevated ($p<0.0001$) sonic hedgehog-related transcripts Gli1 (10-fold), Gli2 (5-fold) and Ptch1 (10-fold) when compared to WT and Gnas+/- unaffected biopsies. Gnas+/- mice also displayed an expansion of Gli1+ cells in the dermis and SCO bone surface by immunofluorescence. Finally, treatment of dermal fibroblast cultures with purmorphamine, a hedgehog agonist, resulted in a significant upregulation of Gli1 ($p<0.001$) within Gnas+/- mice when compared to WT. These data demonstrate the involvement of HF dermal sheath cells in SCO development and suggest a role for sonic hedgehog inhibitors as a potential therapeutic modality for SCO prevention and treatment.

LB966

Molecular profiling of immune checkpoint inhibitor induced bullous pemphigoid

M. Janeczek[1], A. Shahin[2], X. Li[3], A. Hughes[1], R. Butterfield[4], P. Bhullar[2, 1], B. Boudreaux[1], M. R. Pittelkow[1], A. R. Mangold[1]

[1]*Dermatology, Mayo Clinic Arizona, Scottsdale, Arizona, United States,* [2]*Mayo Clinic School of Medicine - Scottsdale Campus, Scottsdale, Arizona, United States,* [3]*Heath Sciences Research, Mayo Clinic Minnesota, Rochester, Minnesota, United States,* [4]*Qualitative Health Sciences, Mayo Clinic Arizona, Scottsdale, Arizona, United States*

Bullous pemphigoid (BP) is a blistering cutaneous disorder that has been associated with immune checkpoint inhibitor (ICI) therapy. This cutaneous adverse event may be dose limiting and cutaneous treatment options may reduce the efficacy of ICI therapy. The goal of this study was to identify key molecular pathways in ICI-BP to elucidate targeted treatments. RNA sequencing was performed on skin biopsies of lesional ICI-BP samples (n=7) and normal skin (controls, n=5). Differential gene analysis was performed using Reactome to identify differentially expressed genes (DEGs). Gene function enrichment analysis (GFEA) and network hub gene analysis was performed to identify key differentiating molecular pathways. We identified 1037 upregulated and 898 downregulated genes in ICI-BP samples compared with controls. The most significantly upregulated gene pathways in ICI-BP were those involved in innate immunity, neutrophil degranulation, cytokine-mediated signaling, IL-4 and IL-13 signaling, interferon-alpha and beta signaling, and degradation of extracellular matrix. PD-1 signaling was enriched. The most significant downregulated genes in ICI-BP were related to keratinization, transport of nucleosides, vitamins, and fatty acids, ion hemostasis, lipid particle organization, and muscle contraction. Based on the top 10 closely related upregulated immune and inflammatory response pathways identified by GFEA, interaction network analysis elucidated the following hub genes: CD4, PTPRC, ITGAM, TLR4, IL10, CD86, TYROBP, TLR2, ACTB, and ITGAX. ICI-BP cases exhibit broad immune activation as well as evidence of PD-1 inhibition within the skin. The identified hub genes and functionally enriched pathways represent potential drug targets for future novel therapies.

LB967

Monogenic mutations implicate STAT1 in hidradenitis suppurativa pathogenesis.

M. Youssef[1], E. Baugh[1], A. Colvin[1], K. Babbush[2], T. Adriano[2], G. Benesh[2], M. E. Torpey[2], A. Nosrati[2], K. R. van Straalen[3], L. C. Tsoi[3], A. T. DeWan[4], S. M. Leal[1], R. Eisenberg[2], J. E. Gudjonsson[3], J. Milner[1], S. R. Cohen[2], L. Petukhova[1]

[1]*Columbia University, New York, New York, United States,* [2]*Montefiore Medical Center, Bronx, New York, United States,* [3]*University of Michigan, Ann Arbor, Michigan, United States,* [4]*Yale University, New Haven, Connecticut, United States*

Hidradenitis suppurativa (HS) is a prevalent and debilitating skin disease with many unmet needs. Genes that underlie rare monogenic etiologies demonstrate greater success as drug targets. HS gene discovery has been limited. Transcriptomic studies show that upregulated STAT1 has a prominent role in HS pathogenesis, supporting previous evidence implicating activators of STAT1 (TNFα, microbial signals, IFNγ). Patients with heterozygous STAT1 gain-of-function (GOF) mutations can develop clinical features that overlap with HS (abscesses, colitis, autoimmunity, SCC). Thus, we hypothesize that some HS patients may carry rare monogenic GOF mutations in STAT1. To test this, we analyzed sequence data for STAT1 in 219 HS cases and 10,863 healthy controls; experimentally validated mutations; and investigated phenotype coherency. We identified 5 cases (2.3%) with 4 different STAT1 mutations, while only 0.13% of controls were carriers ($p=3x10-5$). Functional analysis of PBMCs from HS carriers of STAT1 mutations (I83M, T450M, V712I) showed enhanced short-term cytokine-mediated STAT1 phosphorylation compared to non-carrier healthy controls (IL6 p=.019; IL-27 p=.01). *Ex vivo* total STAT1 expression was higher in T-cells from HS carriers (p=.006); as was IL-27 activated PBMC expression of STAT1 target genes SOCS1 and CXCL9. These molecular phenotypes, along with some clinical phenotypes of the mutation carriers (histories of viral and fungal infections, dental abscesses, thyroid dysfunction, asthma, and spondylitis), are consistent with those observed in well characterized STAT1GOF patients. Taken together our data indicate that rare STAT1GOF variants may underlie risk for HS, provide a rationale for ongoing investigation into JAK-STAT inhibition as a potential HS treatment, and invite a precision medicine approach to HS management.

LB968

COL7A1 frame restoration by CRISPR/Cas9-facilitated, NHEJ-mediated removal of mutant exons as a reliable broad-range therapeutic strategy for RDEB

A. Mencia[1], J. Bonafont[2], R. Murillas[1], S. Modamio[3], A. Nystrom[4], G. Zambruno[5], B. Duarte[1], M. Del Río[6], S. Llames[7], F. Larcher[1, 7, 6]

[1]*Centro de Investigaciones Energeticas Medioambientales y Tecnologicas, Madrid, Spain,* [2]*Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom,* [3]*Hospital Universitario La Paz, Madrid, Spain,* [4]*Uniklinik-Freiburg, Freiburg, Germany,* [5]*Ospedale Pediatrico Bambino Gesu, Roma, Lazio, Italy,* [6]*Bioengineering, Universidad Carlos III de Madrid - Campus de Leganes, Leganes, Comunidad de Madrid, Spain,* [7]*Centro de Investigacion Biomedica en Red de Enfermedades Raras, Valencia, Spain*

Bi-allelic frameshift mutations leading to PTC in COL7A1 are frequent causal mutations of recessive dystrophic epidermolysis bullosa (RDEB). Several studies including ours showed that COL7A1 exons encoding the collagenous domain of type VII collagen (C7) could be therapeutically skippable without apparent functional consequences for protein function. However, the long-term *in vivo* phenotypic consequences of targeted exon deletion and concomitant COL7A1 reframing have been assessed only for the small exon 80 (36 bp) but are not yet clear for other larger exons. Thus, here we chose exons 73 and 105 to determine whether their targeted removal by means of a CRISPR/Cas9-facilitated, NHEJ-mediated approach could be made broader-range. Exon 73, the largest COL7A1 exon (201bp), contains the highest number of reported mutations. Exon 105 (81bp) is also larger than the average COL7A1 exons. Electroporation of the ribonucleoprotein complexes containing the optimal sgRNA guide pairs for each exon (73 and 105) resulted in efficient removal (80% for E73 and 75% for E105) with concomitant C7 expression restoration in RDEB patient cells with frameshift mutations in the targeted exons. In vitro tests showed normal stability of the C7 variants and *in vivo* analyses of the regenerated skin after grafting to immunodeficient mice of the ΔE73 or ΔE105 RDEB cells demonstrated proper deposition of human C7 at the BMZ, presence of anchoring fibrils and normal dermo-epidermal adherence. Overall this study shows the broad applicability of the exon removal approach for the treatment of RDEB.

LB969

Cicatricial junctional epidermolysis bullosa (CJEB): A forgotten phenotype

M. Hunjan[1], D. Balacco[2], D. Wen[1], A. Bardhan[1,2], N. Harper[1], A. Heagerty[1]
[1]University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom, [2]University of Birmingham Institute of Clinical Sciences, Birmingham, United Kingdom

CJEB, a subtype of EB first described in 1985, is characterized by blistering with scarring that can result in a degree of pseudosyndactyly and scarring alopecia. Phenotypically it may resemble recessive dystrophic EB (RDEB) and be misdiagnosed as such; however on electron microscopy a split is seen at the level of the lamina lucida. We report 2 cases of patients with CJEB. Patient 1 is a 34yr old female with a homozygous in-frame deletion of exons 16 and 17 in COL17A1, and Patient 2 a 21yr old female with a homozygous nonsense mutation of LAMB3(c.1702C>T, p.Q568X). We used in silico approaches to investigate genotype-phenotype correlation. InterPro was used to perform functional analyses and domain predictions. Regarding Patient 1 we determined that the mutation resulted in a truncated type XVII collagen(COL17) protein lacking the transmembrane domain . As a result, the extracellular domain of COL17 is unable to extend from the lamina lucida into the lamina densa and establish protein-protein interactions essential for skin integrity. When compared with other JEB patients with COL17A mutations, although interaction with other proteins is diminished, the transmembrane domain is preserved. For Patient 2, we hypothesize that the LAMB3 p.Q568X mutation disrupts the formation of disulphide bridges that are pivotal for the assembly of the laminin 332 trimer, which interacts with other proteins including collagen VII(COL7). Based on these preliminary results, we propose that loss of the COL17 transmembrane domain and the disruption of disulphide bridge formation in laminin 332 dramatically attenuate their indirect interactions with COL7, resulting in phenotypic manifestations typically associated with COL7 deficiency as seen in RDEB. These examples serve to highlight a forgotten subtype of JEB and the power of combining bioinformatic, molecular and deep phenotyping approaches for improved rare disease genotype-phenotype correlation to inform development of effective therapeutic strategies.

LB970

Effect of Oleogel-S10 (birch triterpenes) on dressing change frequency and wound infection in epidermolysis bullosa: Analysis from the EASE study

L. Wine Lee[1], J. S. Kern[2], D. Murrell[3], S. Löwe[4], L. Maher[4], T. Cunnigham[4]
[1]Medical University of South Carolina, Charleston, South Carolina, United States, [2]The Royal Melbourne Hospital, Parkville, Victoria, Australia, [3]University of New South Wales, Sydney, New South Wales, Australia, [4]Amryt Research Limited, Dublin, Ireland

EASE (NCT03068780) was a randomized, Phase 3, double-blind, controlled study evaluating Oleogel-S10 efficacy and safety in patients with dystrophic or junctional EB. The primary endpoint was met with a higher percentage of target wound closure by Day 45 with Oleogel-S10 vs control gel (p=0.013). We report analyses of dressing change frequency and wound infection in patients treated with Oleogel-S10 vs control gel (n=205). A reduction in the frequency of weekly dressing changes was observed with Oleogel-S10 (–0.5; equating to one dressing change every two weeks) compared with no change for control gel (0.1) by Day 90. Analysis of the subset of patients who had daily dressing changes at baseline showed that at Day 90, a higher percentage of Oleogel-S10 patients no longer required daily dressing changes vs control gel (14.7% vs 6.1%). The incidence and severity of wound infections was evaluated over time. At Day 90 target wound infection incidence was low (Oleogel-S10 0.9%; control gel 4.4%); without any cases classified as moderate or severe in the Oleogel-S10 arm vs 3.5% moderate or severe events with control gel. Infection rates in other treated wounds followed a similar trend with lower incidence in the Oleogel-S10 arm (11.0%) vs control gel (15.8%). Severity in the Oleogel-S10 group was mostly mild (7.3%), with low moderate or severe rates (1.8% and 0.9%, respectively) vs control gel which were mostly mild and moderate (5.3% each) over severe (2.6%). In summary, patients treated with Oleogel-S10 had reduced requirements for daily dressing changes and had fewer, less severe wound infections. Reducing the frequency of painful dressing changes and decreasing wound infection is an important factor in alleviating the burden of disease for patients and caregivers.

LB971

HiCuT: An efficient and low input method to identify protein-centric chromatin interactions.

S. Sati[1], P. Jones[1], H. S. Kim[1], L. A. Zhou[2], E. Rapp-Reyes[1], T. H. Leung[1]
[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Genetics, University of Pennsylvania, Philadelphia, Pennsylvania, United States

Protein-DNA interactions regulate gene expression, and some interactions occur over large distances, such that they are nearby in 3-D space but are separated by many nucleotides in the linear genome. These long-range chromatin loops are essential for gene regulation but remain difficult to interrogate. Methods to capture these chromatin interactions mediated by a specific protein factor include Hi-C sequencing coupled with ChIP-seq, ChIA-PET, PLAC-seq, and HiChIP. These methods all require high amounts of starting material (0.5M – 100M cells) and sequencing at high depth (a minimum of 150M reads per sample), which limits their general use. Here, we describe Hi-C Coupled chromatin cleavage and Tagmentation (HiCuT), an enzyme-based tagmentation strategy that provides efficient and high-resolution protein-centric chromatin mapping from as few as 100,000 cells and 12M sequencing reads per sample. Activated transposase generates fragment libraries with extremely low background signal that are easily interpreted with minimal computational processing. This permits cost-effective protein-centric 3D genome profiling in systems previously unmeasurable, including primary cells and human tissue samples. We validated HiCuT method for two different proteins, CTCF and RNA polymerase 2 in GM12878 cells. Next, we performed HiCuT on primary skin cells with an anti H3K27ac antibody and identified previously validated long-range chromatin interactions. Importantly, the high resolution generated by HiCuT permitted annotation of previously identified single nucleotide polymorphisms (SNPs) in skin disease to potential target genes. Thus, HiCuT will permit protein-centric 3-D genome binding assessment in rare cell populations that were not feasible previously, including primary cells, human tissue samples, and personalized epigenomics.

LB972

Defensins and neutrophil-specific defensin receptors prevent skin dysbiosis and infection

X. Dong[1], L. S. Miller[2], N. Archer[2], L. Garza[2], X. Dong[1]
[1]Neuroscience, Johns Hopkins University, Baltimore, Maryland, United States, [2]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Antimicrobial peptides (AMPs) are secreted by host cells to kill invading pathogens and are evolutionarily conserved critical components of host-microbe interfaces. Despite their importance, the mechanisms by which AMPs interact with microbes and the host immune system remain unexplored due to the lack of genetic tools. The largest family of AMPs in the mouse genome, defensins, has over 50 functionally redundant members. To overcome this problem, we generated a Defensin cluster knockout (Def cKO) mouse in which all Def genes are removed from the skin. This stringent loss-of-function analysis revealed that neutrophils are key target cells of defensin signaling and led to the discovery of orphan G protein-coupled receptors (GPCRs) Mrgpra2a/b as neutrophil-specific defensin receptors. Def and Mrgpra2 mutant animals both exhibited skin microbiome dysbiosis and severe defects in anti-S. aureus immunity. This study demonstrates the importance of epithelial-neutrophil signaling via the defensin-Mrgpra2 axis in maintaining healthy skin ecology and promoting antibacterial host defense.

LB973

Cutaneous surgical wounds have distinct microbiomes from intact skin

S. Gupta[4], A. J. Poret[1], D. Hashemi[4], A. Esenou[4], S. H. Yu[4], J. D'Gama[4], V. A. Neel[4], T. D. Lieberman[1, 2, 3]
[1]Institute for Medical Engineering and Science; Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States, [2]Broad Institute, Cambridge, Massachusetts, United States, [3]Ragon Institute of MIT, MGH, and Harvard, Cambridge, Massachusetts, United States, [4]Department of Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Surgical site infections are common following surgical procedures, despite aseptic technique and prophylactic antibiotic use. Although gut commensals are believed to limit certain infections in the intestine, an analogous role for skin commensals has not been described. To identify bacteria with the potential to protect against infection, we characterized the wound microbiome in 52 patients who underwent skin cancer surgery and healed without signs or symptoms of infection. Compared to intact, non-operated skin from the same patients, we observed several species of bacteria with significant differences in relative abundance one week after surgery. The most common bacteria found on intact skin, Cutibacterium acnes, was depleted in wounds by 5-fold. Surprisingly, Staphylococcus aureus, a frequent cause of postoperative skin infections, was enriched by 6.4-fold in clinically non-infected wounds, suggesting active suppression of this pathogen. Finally, members of the Corynebacterium genus were the dominant organism is postoperative wounds, making up 37% of the average wound microbiome. Future studies focused on the biological and clinical significance of the wound microbiome may shed light on normal wound healing and potential therapeutic opportunities to mitigate infection risk.

LB974

Blastomycosis-like pyoderma: A diagnosis of correlation

R. O. Prakash, T. Chakrala, S. McGaugh, S. Saikaly, K. Motaparthi
University of Florida, Gainesville, Florida, United States

A 63-year-old fisherman presented for a chronic and progressive eruption, beginning on his right hand and extending up his right arm over the past 8 years. He reported that he spent time in forests and handled raw fish without gloves frequently; he also recalled an inoculation injury while in a forest. The patient denied previous exposure to halogens. On examination, he had multiple edematous verrucous plaques with crust and elevated borders on the dorsal and ventral aspects of his right hand and right upper extremity. The clinical differential diagnosis included sporotrichosis, chromomycosis, and atypical mycobacterial infection (particularly that due to M. marinum). Punch biopsy of a right forearm lesion revealed pseudoepitheliomatous hyperplasia with prominent intraepithelial abscesses surrounded by suppurative granulomas. These changes were limited to the upper half of the dermis; there was fibrosis in the lower half of the dermis. A tissue culture identified Staphylococcus aureus. While nonspecific in isolation, each of the clinical, histopathologic, and microbiologic features when correlated confirmed the diagnosis of blastomycosis-like pyoderma (BLP). He was prescribed a three-month course of acitretin and cefadroxil. Follow-up was limited due to difficulty contacting patient after treatment. BLP is characterized by clinical, pathologic, and microbiologic features which are individually nonspecific but when correlated permit diagnosis of this rare and recalcitrant bacterial infection. Bacteria such as Staphylococcus aureus, Streptococcus spp., Pseudomonas aeruginosa have been implicated. A diagnosis of BLP is made based on clinical and histopathologic findings, and exclusion of other diseases that can cause similar vegetative plaques such as deep fungal and mycobacterial infections and halogenoderma. Treatment modalities include oral and topical antibiotics, acitretin, corticosteroids, curettage, cryotherapy, potassium iodide, laser therapy, and surgical excision.

LB975

The role of gut flora metabolite butyrate in inhibiting mast cell activation via deacetylase in chronic spontaneous urticaria

X. Zhang, D. Che, K. Guo, S. Geng
Dermatology, Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, Shaanxi, China

Chronic spontaneous urticaria (CSU) is an immune-related skin disease characterized by abnormal activation of mast cells (MCs). With the advanced research on the gut flora in immune-related diseases, it is suggested that dysbiosis of the gut flora may contribute to the pathogenesis of CSU, but the mechanisms involved are unknown. Butyrate, a metabolite of the gut flora, is involved in a variety of cellular physiological processes as a histone deacetylase inhibitor (HDACi) and exerts beneficial effects on the intestinal tract and other organs. It is not clear whether gut flora can be involved in the pathogenesis of CSU by affecting mast cell activity through its metabolite butyrate. In the present study, we detected the gut flora of CSU patients and healthy individuals by 16s rRNA massive sequencing. The differential analysis of microbiota composition showed that the relative abundance of the Firmicutes was significant decreased in CSU patients. A variety of bacteria in Firmicutes, such as Clostridium and Streptococcus, are major producers of butyrate. And the decrease in these bacteria results in lower levels of butyrate in the body. In vitro experiments, we used DNP-Bull serum albumin (BSA) to induce anaphylactic reactions and degranulation of mast cells (MCs). We found that butyrate pretreatment of MCs significantly inhibited Fc epsilon RI (FcεRI) -mediated MCs activation, including degranulation and release of cytokines. Our results provide evidence that dysbiosis of gut flora leads to reduced levels of butyrate in CSU patients, and butyrate inhibits IgE-mediated MCs activation. We hypothesize that butyrate acting as an HDACi to affect the expression of MCs signaling pathway proteins, which will be confirmed in further experiments. This study will help to reveal the mechanism of the involvement of gut flora in the pathogenesis of CSU and provide a basis for the search of new treatment strategies for CSU in the future.

LB976

Skin-gut inflammatory crosstalk: First experimental murine model of pyoderma gangrenosum with spontaneous colonic inflammation

S. Jatana, A. Ponti, N. Rebert, E. Johnson, E. Maytin, A. Fernandez, J. Achkar, C. McDonald
Cleveland Clinic, Cleveland, Ohio, United States

Pyoderma gangrenosum (PG) is a debilitating skin condition characterized by deep, pus-filled, non-healing ulcers packed with neutrophils and is a common extraintestinal manifestation (EIM) of inflammatory bowel disease (IBD). Strikingly, ~40% of people with an initial presentation of PG go on to be also diagnosed with IBD, suggesting common factors drive the pathogenesis of these diseases. The molecular and cellular mechanisms of PG and IBD co-development are currently unknown. Impeding the development of effective treatments for these individuals is the absence of an animal model that exhibits features of both diseases. We have developed the first preclinical mouse model of concurrent PG and IBD. We hypothesize that neutrophil dysfunction mediates skin-intestine inflammatory crosstalk that drives disease pathogenesis in PG-IBD mice. Our preliminary data show that topical application of a pyrimidine synthesis inhibitor on wounded skin of mice generates skin ulcers enriched in neutrophil extracellular traps (NETs), mimicking the characteristic PG skin phenotype. The proinflammatory milieu of the skin in PG-IBD mice is enriched in IL-1β, CXCL1, TNF-α, IL-17A/F, and IL-6, similar to the profile observed in human PG skin biopsies. These mice also develop spontaneous gut inflammation resembling colitis indicated by inflammatory changes quantified by elevated fecal lipocalin-2 and histologic damage characterized by epithelial ulceration, crypt hyperplasia, submucosal swelling, and immune infiltrates in the distal colon. These mice have elevated circulating immature low-density granulocytes primed with IL-1β that undergo enhanced NET formation. Citrullinated Histone 3, a marker of dysregulated NET formation, is elevated in PG-IBD mouse serum and distal colon tissue suggesting that neutrophils are major components of the pathology in this model. These findings demonstrate that immature neutrophils potentially contribute to inflammatory cross-talk between the skin and gut in this novel mouse model.

LB977

NK cells in the pathogenesis of hidradenitis suppurativa (HS)

C. Raman[1], M. P. Kashyap[1], B. Mishra[2], J. Deshane[3], S. M. Mukhtar[2], M. Athar[1]
[1]Dermatology, University of Alabama at Birmingham, Birmingham, Alabama, United States, [2]Biology, University of Alabama at Birmingham, Birmingham, Alabama, United States, [3]Medicine, University of Alabama at Birmingham, Birmingham, Alabama, United States

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease with deep-seated lesions in the apocrine gland areas of the body. Recruitment and activation of innate and adaptive immune cells around hair follicles drive initiation and progression of HS; however, the nature of the cell populations and underlying mechanisms remain unclear. scRNAseq revealed that NK cells and CD4 T cells were major lymphocyte populations in HS. Confocal microscopy showed that HS, but not normal skin, contained elevated numbers of CD56dim and CD3+CD56bright NK cells. These CD56dim classical highly cytolytic NK cells were primarily within the epidermis and sinus tracts of HS and they expressed high levels of perforin and granzyme A. In contrast, CD3+CD56bright NK cells (NK-T cells) were primarily restricted to sinus tracts. The NK-T cells associated with α-SMA expressing sinus tracts were enriched in fibroblasts, a feature of fibrosis. Notably, CD56dim NK cells also expressed high levels of CD2, a receptor that signals augmented NK cell cytolytic activity and increased production of IFN-γ. These NK cells associated with keratinocytes expressing CD58 (LFA-3), the counter-receptor for CD2. The keratinocytes contained elevated levels IL-15 and IL-18; cytokines induced after engagement of CD58 with CD2. HS skin also expressed elevated levels of miR150 and miR155, micro RNAs necessary for the NK cell maturation and cytotoxicity, respectively. To test that CD2 blockade will be beneficial, skin from HS patients were cultured in presence of anti-CD2 mAb or control IgG. CD2 blockade led to significant decrease in HS-associated inflammatory cytokines (IL-6, IL-15, IL-18, IFN-γ) and chemokines (IL-8, MIP1α, RANTES, IP10). In sum, this study show that distinct NK cell populations are the major contributors to cytolytic activity as well as fibrosis in HS. CD2 blockade is likely to be a viable novel therapy for HS.

LB978

High relative abundance of bacillales is associated with epidermolysis bullosa (EB) at different stages of wound healing

D. Balacco[1], A. Bardhan[1,2], M. M. Grant[1], S. Kuehne[1], J. Hirschfeld[1], A. Heagerty[2], I. Chapple[1]
[1]School of Dentistry, University of Birmingham College of Medical and Dental Sciences, Birmingham, Birmingham, United Kingdom, [2]Epidermolysis Bullosa Unit, Department of Dermatology, University Hospitals Birmingham NHS Foundation Trust, Birmingham, Birmingham, United Kingdom

EB is a prototypic and heterogeneous group of inherited bullous disorders characterized by detachment of the epithelium following minimal mechanical trauma. The phenotypic spectrum is broad, with persistent blistering, inflammation, delayed re-epithelialization, dysfunctional wound healing and often infection, leading to disability and, in the most severe cases, death. EB is classified into four subtypes: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler EB (KEB). Skin provides a protective barrier from thermal, mechanical, and physical trauma. Its microbiome, pH, structural and thermoregulatory properties, and resident immune cell population contribute to making the skin an active organ of the immune system. In health the human skin microbiome has adapted to produce molecules that inhibit colonization of pathogenic microorganisms. We hypothesize that in EB patients the skin barrier is broken down, and the balance between commensal and pathogenic microorganisms is hence altered. We used whole-genome sequencing to characterize the microbiome from blisters and adjacent healthy skin in EB patients. Metagenomic sequence data were processed through the MG-RAST pipeline. We explored shifts from a health- to disease-associated skin microbiome in three groups of EB (EBS, JEB, and DEB). We observed a higher relative abundance of Bacillales associated with healthy skin in people affected by EBS. In addition, blister skin sampled 48 hours after blister formation exhibited a higher relative abundance of Staphylococci in patients affected by DEB. These shifts may contribute to delayed wound healing, and a deeper understanding may present opportunities for improved intervention.

LB979

Mast cell activation via mas-related g protein-coupled receptor X2 is regulated by ryanodine-sensitive calcium stores

X. Zhao, W. Zeng, S. Geng, Z. Wang
Department of Dermatology, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

Mast cells (MCs) are tissue-resident immune cells, which play a pivotal role in skin hypersensitivity reactions. MCs activation via Mas-Related G Protein-Coupled Receptor X2 (MRGPRX2) is independent of the classical IgE-mediated pathway, which gained much interest recently. Calcium mobilization is crucial to mast cell activation and functional regulation. Ryanodine receptor (RYR) expresses on the sarcoplasmic reticulum and regulates intracellular calcium liberation. Although MRGPRX2 activation induces boosted intracellular calcium levels, the role of RYR in MRGPRX2 mediated MC activation has not been fully addressed. We confirmed RYR expression in human skin-derived MCs and recognized the most abundant expression of the RYR3 isoform. Human MC line (LAD2 cells) was utilized in the functional study triggered by MRGPRX2 agonists (c48/80 and Substance P). MCs pretreated with RYR inhibitor (dantrolene) suppressed both intracellular calcium release and extracellular calcium entry stimulated by MRGPRX2 ligands. Degranulation assessed by β-hexosaminidase release was likewise inhibited by dantrolene. IL-13, IL-8 and CCL-2 induction by MRGPRX2 activation were suppressed at mRNA level when MCs were pre-treated with RYR inhibitor. The results demonstrate the role of the RYR in MRGPRX2 triggered calcium signaling, and provide a potential approach for MRGPRX2-mediated disorders.

LB980

Skin microbiome restoration after transplantation for DOCK8 deficiency

Y. Che[1], J. Han[1], A. F. Freeman[3], H. C. Su[3], C. J. Holmes[1], C. E. Gonzalez[2], N. N. Shah[2], J. A. Segre[4], H. H. Kong[1, 2]

[1]Dermatology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, United States, [2]Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, United States, [3]Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, United States, [4]National Human Genome Research Institute, Bethesda, Maryland, United States

Patients with the rare primary immunodeficiency due to dedicator of cytokinesis 8 (DOCK8) mutations commonly suffer from atopic disorders, recalcitrant viral infections, potential malignancy, and early mortality. Even in the absence of viral skin lesions, DOCK8-deficient skin has been previously shown to have an abundant virome. Hematopoietic stem cell transplantation (HSCT) is potentially curative for this disease and provides a unique opportunity to investigate the influence of host immunity on skin microbial communities. Using shotgun metagenomic sequencing, we analyzed longitudinal skin samples from 18 DOCK8-deficient patients. HSCT induced pronounced shifts in skin microbial composition with strong individuality and skin site-specificity. DNA viruses (e.g. papillomavirus and polyomavirus) predominated before HSCT, but significantly decreased after withdrawal of immunosuppression (e.g., inner elbow, 83.3 +/- 22.7% to 5.6 +/- 12.0%; P < 0.01), with corresponding increases in bacteria and fungi. After HSCT, patients on immunosuppression retained significantly higher relative abundances of skin DNA viruses, in contrast to patients who were weaned from immunosuppression (e.g. inner elbow, 64.1 +/- 18% vs. 5.8 +/- 12.0%; P < 0.01). Although the predominant viruses pre-transplant substantially decreased after weaning of immunosuppression, less abundant viruses pre-transplant, including papillomavirus and polyomavirus species, persisted after HSCT, suggesting differential control of viruses by the donor immune system. This study underscores how host immunity is critical in shaping the skin microbiome.

LB981

Pandemic associated chilblain-like lesions result from an inducible type 1 interferon response to SARS-CoV-2

L. Arkin[1], A. Costa da Silva[2], J. Mays[2]

[1]University of Wisconsin System, Madison, Wisconsin, United States, [2]National Institutes of Health, Bethesda, Maryland, United States

Chilblain-like lesions (CLL), known in the lay press as “COVID toes,” increased significantly during the COVID-19 pandemic. The phenotypic similarity of chilblains in the monogenic type 1 interferonopathies, coupled with the consistent clinical phenotype across multiple countries and temporospatial association with COVID-19 spread, suggest a SARS-CoV-2 triggered immune phenomenon. Yet direct evidence of this relationship has been limited due to low rates of SARS-CoV-2 positivity utilizing conventional testing. We prospectively enrolled a cohort of 79 patients with CLL across 4 waves of the SARS-CoV-2 pandemic in Wisconsin collecting serial blood samples and lesional skin biopsies. Immunophenotyping including the type 1 interferon (IFN-1) signature was investigated utilizing multiplex immunohistochemistry in affected tissue. Proteomics and RNA sequencing were performed on the peripheral blood at serial time points. RNAscope for S gene and depositional immunohistochemistry for evidence of SARS-CoV-2 were performed on tissue. Antibody responses and T-cell specific responses to SARS-CoV-2 were performed and an animal model (golden hamster) provided mechanistic evidence of dissemination of viral RNA to acral sites with local IFN-1 activation. Our results support an inducible local and peripheral IFN-1 signature, which abrogates within weeks, with evidence of viral SARS-CoV-2 RNA as the trigger.

LB982

IL-33 enhancement of histaminergic itch is dependent on IL-13

A. M. Trier[1], A. Ver Heul[2], A. Fredman[1], J. Meixong[3], F. Wang[1], X. Dong[3], B. Kim[4]

[1]Center for the Study of Itch, Washington University in St Louis School of Medicine, St Louis, Missouri, United States, [2]Division of Allergy and Immunology, Washington University in St Louis, St Louis, Missouri, United States, [3]Solmon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [4]Kimberly and Eric J. Waldman Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, New York, United States

Histamine is a critical mediator if itch in urticaria, however, many patients with chronic spontaneous urticaria (CSU) itch in spite of treatment with antihistamines. Further, it is widely known that in the CSU, patients over time can lose clinical itch responses to antihistamines as well. We hypothesized that the limitation of these therapies is partially due to other immune mediators amplifying the histaminergic itch pathway. We focused specifically on IL-33, an alarmin cytokine that we and others have recently shown is critical for the development of chronic itch in different mouse models through its direct signaling in sensory neurons. Surprisingly, while IL-33 notably enhanced histaminergic itch, this effect was not dependent on IL-33 receptor (IL-33R) expression in sensory neurons. Instead, IL-33R expression in the immune cell compartment, and specifically the presence of mast cells, was required for IL-33-dependent augmentation of histaminergic itch. It has been previously shown that IL-33 can augment mast cell production of IL-13, a canonical type 2 cytokine that can directly activate sensory neurons and induce itch. Thus, we sought to investigate if IL-13 may be necessary for the enhancement of histaminergic itch by IL-33. We found that global deletion of IL-13 ameliorated the ability of IL-33 to potentiate histaminergic itch. These data highlight the dynamic mechanisms by which IL-33 promotes itch, providing insight on the therapeutic potential of IL-33 blocking therapies currently in development. Strikingly, these findings also challenge current binary paradigms of histaminergic versus nonhistaminergic itch.

LB983

Genomic variation in staphylococcus aureus stress response and virulence in diabetic wounds

A. Campbell[1], A. McCready-Vangi[1], S. Gardner[2], E. Grice[1]

[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]College of Nursing, University of Iowa, Iowa City, Iowa, United States

Diabetic foot ulcers (DFUs) are a common, dangerous, and costly complication of diabetes. Over half of DFUs develop infections, often requiring lower extremity amputations. While not all DFUs become infected, they are all colonized by a diverse microbiome which includes opportunistic pathogens and commensal organisms alike. Staphylococcus aureus is a prevalent and abundant bacterial pathogen in the DFU microbiome, but its presence alone does not necessarily lead to infection and other complications; instead, its impact differs between strains within the species, and even closely related strains of S. aureus can have drastically different effects on DFU healing. With the goal of identifying genomically encoded S. aureus virulence adaptations underlying strain-specific effects on DFU healing, we conducted parallel genomic and phenotypic analyses on 222 S. aureus isolates cultured from 62 DFUs representing both healing and nonhealing clinical outcomes under standardized treatment conditions. We found that in vitro production of the antioxidant pigment staphyloxanthin was higher in S. aureus isolates cultured from nonhealing DFUs, and that strains producing higher levels of the pigment were more detrimental to wound healing in a diabetic mouse model. Through detailed genomic comparisons of closely related high and low staphyloxanthin-producing isolates, we identified a single nucleotide variant in a stress-responsive regulator of the Staphylococcal transcription factor sigma B which drastically decreased staphyloxanthin production and attenuated the strain’s impairment of wound healing *in vivo*. These results motivate our ongoing investigations into the interactions between S. aureus stress adaptations and host wound healing processes, as well as genome-wide markers for strain-dependent differences in staphyloxanthin production and other S. aureus virulence phenotypes which could yield novel therapeutic targets for diabetic wound care.

LB984

Intralesional sodium thiosulfate as a reversal agent for calcium hydroxylapatite soft tissue filler: An in vitro and *ex vivo* comparison

M. A. Dirr[1], R. Christensen[1], N. Anvery[1], E. A. Merkel[1], B. Worley[2], V. Harikumar[1], K. Lu[1], S. Evans[1], E. Poon[1], M. Alam[1]
[1]*Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States,* [2]*Florida Dermatology and Skin Cancer Centers, Lake Wales, Florida, United States*

CaHA microspheres are the active component of an injectable filler material commonly used to correct fat and dermal atrophy. While use of CaHA in soft tissue augmentation is generally safe and well-tolerated, dissolution may be required for unwanted nodule formation or, rarely, for vascular occlusion. Though there is no approved reversal agent, preliminary reports have suggested STS may be considered. In this translational study, we sought to understand the effect of sodium thiosulfate (STS) on calcium hydroxylapatite (CaHA) microsphere size and dispersion. First, we assessed the effect of incubating STS with CaHA at standardized dilutions at room and physiologic temperatures to determine the effect on microsphere size. Regardless of dilution, in-vitro samples of CaHA were significantly decreased in size when incubated with STS compared to saline control samples at both temperatures, except for the 1:4 (CaHA:STS) dilution under physiologic conditions ($p<0.05$). Hence, STS reduces the effective particle size of CaHA microspheres. Second, in an ex-vivo viable human tissue model under physiologic conditions, the degree of CaHA dispersion was comparable when flooded with both normal saline (2.88 ± 0.78 mm average maximum distance traveled) and STS solutions (2.87 ± 0.82 mm average maximum distance traveled) (1:4, CaHA:dilutent). Thus, STS increases the distance between CaHA microsphere particles. As such, saline flushing of the injection site may be a back-up strategy for managing nodule formation when STS is unavailable. These findings clarify the role of STS in the management of filler complications.

LB985

A three-dimensional analysis of the collagen network in rats treated with hyaluronic acid-based soft tissue filler

A. B. Kutikov[1], G. Ferron[2], A. Pierce[1], A. Knesis[2], S. Linehan[2], T. Ragan[2], L. Nakab[1]
[1]*Allergan Aesthetics, an AbbVie Company, Irvine, California, United States,* [2]*TissueVision Inc, Newton, Massachusetts, United States*

This study evaluates the tissue integration and collagen network effects of the HA-based soft tissue filler Juvéderm Voluma XC in a rat model using a novel three-dimensional (3-D) imaging platform and image analysis algorithms. Rats were injected subcutaneously with 125 µL of Juvéderm Voluma XC. After 4- or 12-weeks post-injection, the second harmonic generation (SHG) signal of collagen and tissue autofluorescence within the entire filler bolus was assessed using the TissueVision TissueCyte Serial Two-Photon Plus (STP2) imaging platform. The extent of tissue integration and collagen quality metrics were evaluated through image analysis of 3D collagen integration into the filler bolus, collagen fiber remodeling (anisotropy), and collagen fiber bundle frequency. SHG imaging demonstrated a robust fibrillar collagen network within the filler bolus at 4- and 12- weeks after injection. An image analysis algorithm that measured the density and uniformity of tissue throughout the bolus (3D integration metric) indicated a 2.1-fold increase in collagenous tissue integration from 4 to 12 weeks. A 1.3-fold increase in collagen fiber bundle remodeling (anisotropy) and 1.4-fold increase in bundle frequency were observed throughout the bolus from 4 to 12 weeks. This study demonstrates that the HA-based soft tissue filler Juvéderm Voluma XC supports the development of a 3D fibrillar collagen network that becomes more robust over the course of 12 weeks.

LB986

A novel three-dimensional imaging and analysis of imiquimod-induced mouse skin

L. Khrimian[1], G. Ferron[2], A. Knesis[2], S. Haxhinasto[1], A. N. Economides[1], S. Linehan[2], T. Ragan[2]
[1]*Regeneron Pharmaceuticals Inc, Tarrytown, New York, United States,* [2]*TissueVision Inc, Newton, Massachusetts, United States*

Here we report a novel three-dimensional (3D) assessment technique for TLR7/8 agonist-induced mouse skin samples using the TissueCyte Serial Two-Photon Plus (STP2) imaging platform. Wild-type (WT) mouse skin was treated with either TLR7/8 agonist imiquimod (IMQ) cream, a commonly used method for modeling psoriasis in mice, or vehicle. Explants were sectioned serially and imaged to visualize tissue autofluorescence and collagen fibril signatures using second harmonic generation (SHG) imaging. The hypodermis, dermis, and epidermis skin layers across each section were segmented and the layer volume (millimeter3) was computed. Results indicate an increase in volume of all assessed tissue layers of the IMQ-treated versus vehicle-treated skin, by 1.6-fold in the hypodermis, 1.4-fold in the dermis, and 3.0-fold in the epidermis. This study demonstrates that IMQ cream produces inflammation throughout the hypodermis, dermis, and epidermis of mouse skin. The ability to process TLR7/8 agonized mouse skin samples using the STP2 imaging platform demonstrates a new method to produce a 3D model and analyze total gross morphology with collagen fibril signatures. The techniques developed in this study hold promise for assessing inflammation type and disease severity of psoriasis, with future application to thoroughly evaluate pharmacodynamics and pharmacokinetics of drug treatment.

LB987

Tapinarof inhibits the formation, cytokine production, and persistence of resident memory T cells *in vitro*

N. Mooney[1], J. Teague[1], A. Gehad[1], K. McHale[2], D. S. Rubenstein[2], R. A. Clark[1]
[1]*Brigham and Women's Hospital, Boston, Massachusetts, United States,* [2]*Dermavant Sciences, Morrisville, North Carolina, United States*

Tapinarof, a novel, first-in-class small-molecule topical therapy with combined aryl hydrocarbon receptor (AhR) agonist and antioxidant activities, is effective in the treatment of both psoriasis (PsO) (NCT03956355; NCT03983980; NCT04053387) and atopic dermatitis (AD) (NCT02564055). In previous experiments in culture, tapinarof increased keratinocyte expression of skin barrier proteins and reduced T cell production of IL-17A and IL-17F. Psoriasis patients in phase 3 trials who cleared after topical tapinarof remained clear for a median of four months after therapy was discontinued, as defined by a PGA of 0 (clear) or 1 (almost clear) while off therapy after achieving complete disease clearance (PSOARING studies 1, 2, and 3). Tapinarof is therefore unique in its combined AhR agonist and antioxidant activity, ability to affect both immune and stromal cells in skin, topical efficacy in both PsO and AD, and induction of durable remissions. The efficacy of tapinarof in both AD and PsO and its ability to induce lasting remissions suggests that it targets mechanisms common to both diseases and may affect resident memory T cell (TRM) activation or persistence. IL-17 drives PsO lesion formation and evidence suggests that reactivation of IL-17 producing pathogenic TRM induces recurrence of lesions in the same locations after therapy is discontinued. We tested tapinarof in an in vitro human TRM differentiation assay and found that it significantly reduced CD4+ CD69+CD103+ TRM generation ($p=0.03$) and reduced CD4 TRM production of IL-17A and TNFα. Furthermore, 3-week tapinarof treatment of TRM from healthy human skin reduced the number of surviving CD4+ CD69+CD103+ TRM by 47% and reduced the number of IL-13 producing CD4+ TRM by 44%. In summary, our results demonstrate that tapinarof reduces TRM generation, inhibits TRM Th17 and Th2 cytokine production and inhibits the survival of CD4+ TRM. These effects may be responsible for its ability to treat both AD and PsO and induce durable remissions in PsO.

LB988

FlowSkin®: A microfluidic human skin model to study pharmacokinetic & biotherapeutic diffusion

E. Raude[1], E. Pages[1], M. Meseguer[1], D. Twum[2], P. Descargues[2], L. Malaquin[3], N. Gaudenzio1, [4]

[1]Genoskin SAS, Toulouse, France, [2]Genoskin Inc., Salem, Massachusetts, United States, [3]Laboratoire d'Analyse et d'Architecture des Systemes du CNRS, Toulouse, Occitanie, France, [4]INSERM UMR1291, Toulouse, France

Predicting response to biotherapeutics remains a major challenge in the field of bioengineering, especially since the diffusion process of high molecular weight molecules in human tissue remains largely unknown. This study presents an innovative microfluidic system to study the diffusion of therapeutic formulations in *ex vivo* human skin explants. The FlowSkin® model is composed of a human skin biopsy, which is preserved in a proprietary matrix to keep skin cells and appendages functional, and into which a custom-made porous catheter is implanted. The model's configuration allows the flow in the catheter to exchange with the tissue via an automated fluidic system without detectable alteration of tissue viability for 10 days, while preserving physiological levels of structural cell proliferation. A combination of X-ray tomography and molecular analyses was used in order to examine the model's relevance to study the diffusion of molecules with different molecular weights. The obtained results position the FlowSkin® model as a relevant tool for efficacy, toxicity and pharmacokinetic studies of systemically and subcutaneously administered drugs directly in human skin.

LB989

Differentiation of therapeutic antibodies targeting IL-23

J. G. Krueger[1], K. Eyerich[2, 3], C. Greving[4], K. Sachen[4], D. Hammaker[4], P. Bao[4], E. Lacy[4], M. Elloso[5], Y. Orlovsky[5], I. McInnes[6], A. Fourie[4]

[1]Rockefeller University, New York, New York, United States, [2]Technical University, Munich, Germany, [3]Karolinska Institute, Stockholm, Sweden, [4]Janssen R&D, San Diego, California, United States, [5]Janssen R&D, Spring House, Pennsylvania, United States, [6]University of Glasgow, Glasgow, United Kingdom

Clinically relevant differences between therapeutic antibodies against the same target may relate to their unique molecular attributes. Differences in therapeutic profile across the domains of psoriatic disease between guselkumab (GUS) and risankizumab (RIS) have been observed. To explore potential mechanisms underpinning this, we studied GUS, a fully human IgG1 specific for IL-23 with a native Fc region, and RIS, a humanized anti-IL-23 IgG1 with a mutated Fc region. We compared binding and functional characteristics of the antigen-binding and Fc regions of these antibodies. GUS and RIS displayed comparable picomolar affinities for binding IL-23 by KinExA and surface plasmon resonance assays, and equivalent potency (IC50 0.2 nM) for inhibition of IL-23-induced STAT3 phosphorylation in human peripheral blood mononuclear cells. However, in cells transfected with individual Fcγ receptors (FcγRs), GUS showed strongest binding to CD64 (FcγR1), while RIS showed negligible binding to any FcγRs, by virtue of its mutated Fc region. Furthermore, in IFNγ-primed human monocytes, labeled GUS showed dose-dependent binding to CD64 by flow cytometry, while RIS did not. GUS binding to CD64 on monocytes did not trigger activation as shown by lack of cytokine or chemokine production. Importantly, CD64-bound GUS was able to bind IL-23, as detected by anti-p40 staining. In conclusion, compared with RIS, GUS uniquely binds both CD64+ myeloid cells and IL-23. CD64+ mononuclear phagocytes are enriched in psoriatic skin and serve as the dominant IL-23 source. Taken together, GUS presence may be enriched within the inflamed tissue microenvironment by binding to CD64, neutralizing IL-23 at its cellular source, potentially leading to durable response and observed therapeutic differences within the class. Further studies are warranted to generate additional evidence supporting this hypothesis.

LB990

Phase 2 randomized, double-blind, vehicle-controlled trial of NFX-179 gel in neurofibromatosis type 1 patients with cutaneous neurofibromas.

K. Y. Sarin[1], C. O'Mara[2], P. Horwath[2], B. Beger[2], G. Kochendoerfer[2], S. Plotkin[3], C. Powala[2], M. Bradshaw[2], G. Webster[2]

[1]Dermatology, Stanford University School of Medicine, Stanford, California, United States, [2]NFlection Therapeutics, Boston, Massachusetts, United States, [3]Neurology, Harvard Medical School, Boston, Massachusetts, United States

Cutaneous neurofibromas (cNFs) are a major cause of morbidity in individuals with Neurofibromatosis Type 1 (NF1), as they can cause irritation, pain, disfigurement, and social anxiety. cNFs arise due to loss of function of neurofibromin, a negative regulator of RAS activity. Thus, the Ras/MEK pathway represents a compelling therapeutic target. We recently reported the development of a topical MEK inhibitor, NFX-179 Gel, for the treatment of cNFs. NFX-179 is designed to be metabolically labile so it can suppress the RAS pathway in cNFs while minimizing systemic exposure. We recently completed a double-blind vehicle-controlled Phase 2a trial of NFX-179 in 48 individuals with NF1. In the Phase 2a study, NFX-179 gel (0.05%, 0.15%, and 0.5%) or vehicle was applied topically once daily to 5 target cNF tumors for 28 days and tumors were removed for p-ERK biomarker analysis at the end of the study. NFX-179 gel led to a dose-related reduction in p-ERK in cNFs at Day 28 with a 47% reduction in the 0.5% NFX-179 Gel group compared with vehicle ($p < 0.0002$). In addition, a dose-related reduction in tumor volume at Day 28 was observed, with a 17% mean reduction in the 0.5% NFX-179 Gel group compared to 8% in the vehicle group ($p = 0.073$). No significant local or systemic toxicities were observed, including acneiform rash which has been reported with systemic inhibitors. This first-in-human study of NFX179 in cNF tumors demonstrated a good safety and tolerability profile and potential therapeutic benefit by biomarker and clinical evidence. We will be sharing the results of our Phase2a study and presenting the design of the current randomized, double-blind, vehicle-controlled Phase 2b study of NFX-179 Gel in NF1 patients with cNFs, which is currently enrolling.

LB991

Clinical serum biomarker profiling in TRuE-AD1 and TRuE-AD2 studies after 8 weeks of treatment with ruxolitinib cream

H. Liu, X. Gong, S. H. Smith

Incyte Corp, Wilmington, Delaware, United States

Ruxolitinib cream has been approved by the United States Food and Drug Administration for the treatment of mild to moderate atopic dermatitis (AD). Ruxolitinib is an inhibitor of Janus family of protein tyrosine kinases (JAK) 1 and 2. Two randomized, vehicle-controlled phase 3 studies with identical design were conducted in adolescent and adult patients with up to 20% body surface area (BSA) affected by AD (TRuE-AD1 and TRuE-AD2). Serum samples for biomarker analysis were collected from all patients at baseline and week 8. The week 8 visit corresponded to the primary endpoint for efficacy and the end of the double-blind period. Select samples (representing 816 samples from 408 patients) were analyzed using the Olink® Target 48 Cytokine panel. This panel includes proteins, such as interleukin (IL) 4, IL13, C-X-C motif chemokine ligand 10 (CXCL10), and C-C motif chemokine ligand 13 (CCL13), which have been reported as increased in association with AD. The goal of the analysis was to understand whether the pharmacodynamic effects of ruxolitinib cream were primarily local or systemic. Changes in analyte expression patterns were evaluated in relation to blood plasma drug levels and clinical parameters. Across the full analyte list, there were analytes showing significantly changed expression levels following treatment. Nevertheless, none of the changes in serum biomarkers showed a strong correlation ($R \geq 0.5$) with plasma drug concentrations at steady state. Further, the correlation remained weak when considering only the samples with the highest blood plasma levels of ruxolitinib, which is a more stringent comparison. In contrast, more than 15 analytes correlated well with clinical parameters, including Eczema Area and Severity Index (EASI) scores. Together, these data suggest that changes in systemic protein levels in AD patients treated topically with ruxolitinib cream reflect local drug effects and improved clinical condition and are not correlated to plasma levels of ruxolitinib.

LB992

NTX-2009, a novel charged sodium channel blocker has significant anti-inflammatory activity in an atopic dermatitis model and produces sustained inhibition of chloroquine-induced itch.

J. Ellis[1], S. Chaumeron[2], K. LaVigne[1], M. Lipp[1], A. Malekiani[1], B. M. Cole[1]
[1]Nocion Therapeutics, Waltham, Massachusetts, United States, [2]Oncodesign, Villebon-sur-Yvette, France

Charged sodium channel blockers (CSCBs) such as NTX-2009 do not cross membranes, and selectively inhibit sensory nerves by gaining access to nociceptors through large pore channels (eg TRPV1, TRPA1) that are open due to inflammation. Evidence suggests that sensory nerves transmit itch and pain signals to the CNS and play an important role in driving the immune response in psoriasis and atopic dermatitis. We examined whether NTX-2009 blockade of sodium channels in skin sensory nerves would have anti-inflammatory activity as well as anti-pruritic effects in models of atopic dermatitis (AD). A topical gel formulation of NTX-2009 was tested for its ability to inhibit chloroquine (CQ)-induced scratching. Injection of CQ produced an eight-fold increase in the scratching duration compared to saline injected mice. NTX-2009 gel (0.1 - 1.0%) applied 3 hours before CQ injection dose dependently decreased scratching duration, with 0.3% producing a 73% inhibition and 1% a complete inhibition. The duration of action of 1% NTX-2009 was characterized with >70% reduction in the scratching response maintained out to 15 hrs. NTX-2009 (0.3% gel QD) was evaluated in the calcipotriol model of AD. Calcipotriol (CP) was administered to mice once daily for 10 days and ruxolitinib (10 mM, QD) was used as a positive control. Experimental endpoints were skin clinical score (erythema, edema and desquamation), gene expression and histology. NTX-2009 significantly reduced the clinical score producing a greater effect than ruxolitinib. CP increased IL-4 gene expression while decreasing Filaggrin-2 expression. Both effects were significantly reversed by NTX-2009 and ruxolitinib. Dermal thickness and mast cell infiltration produced by CP were both inhibited by NTX-2009 and ruxolitinib. This data demonstrates the anti-inflammatory and anti-pruritic potential of a new class of CSCBs, such as NTX-2009.

LB993

Kinetics of IRAK4 degradation and impact on functional response in circulating immune cells and skin cell subsets

E. Lurier[1], J. Sullivan[2], S. Skouras[1], V. Massa[1], M. Fitzgerald[2], A. Wang[1], X. Zheng[2], D. Walther[2], C. Browne[2], J. Dey[2], A. McDonald[2], J. Gollob[2], N. Mainolfi[2], A. Slavin[2], V. Campbell[2]
[1]Immunology, Kymera Therapeutics, Watertown, Massachusetts, United States, [2]Kymera Therapeutics, Watertown, Massachusetts, United States

Kymera has developed selective IRAK4 degraders for the treatment of autoimmune and inflammatory diseases where IL-1R/TLR activation plays a central role in driving the inflammatory response. In autoimmune diseases of the skin, like hidradenitis suppurativa, keratinocytes are active inflammatory mediators that are triggered by IL1R signaling. IL1R signals through the myddosome which is dependent on the kinase and scaffold functions of Interleukin-1 receptor associated kinase 4 (IRAK4) to induce downstream production of proinflammatory cytokines like IL6 and IL8. Selective IRAK4 degraders can achieve max. degradation of >90% in several immune cell types in vitro after 24 hours of treatment that is associated with max. inhibition of pro-inflammatory cytokine production following IL1R stimulation. To investigate the kinetics of IRAK4 degradation and impact on functional response in disease-relevant skin cell types, keratinocytes were treated with IRAK4 degraders for up to 4 days in vitro. 24 hours of degrader treatment led to 60% degradation of IRAK4 while a longer exposure time of 72 hours led to maximal degradation, with continued and sustained Dmax observed up through the 4th day under basal and stimulated conditions. Maximal inhibition of IL6 and IL8 induction occurred following 72-hour degrader treatment. While the kinetics of IRAK4 degradation between leukocytes and skin cell subtypes may differ, similar maximal degradation can be observed leading to comparable cytokine inhibition. These findings translate to the clinic in healthy volunteers, where similar IRAK4 knockdown is achieved in circulating mononuclear cells and skin biopsies following 14 days of multi-dosing. Taken together, these data demonstrate the broad impact across multiple cell types and further support the development of IRAK4 degraders in patients with HS and other IL1R/TLR driven autoimmune diseases.

LB994

A novel MIF inhibitor for treatment of melanoma

P. Pham[1], Z. Garrison[1], T. Clister1, M. Chang[1], R. Meza-Romero[1, 2], J. King[3], A. Vandenbark[1, 2], R. P. Kulkarni[1, 2]
[1]Oregon Health & Science University, Portland, Oregon, United States, [2]Portland VA Medical Center, Portland, Oregon, United States, [3]Virogenomics, Portland, Oregon, United States

Macrophage migration inhibiting factor (MIF) is expressed by both immune and tumor cells and MIF overexpression is increasingly recognized as implicated in worse outcomes in melanoma patients and thus is a novel therapeutic target for melanoma. This is particularly important as although checkpoint inhibitor blockade has positively impacted advanced melanoma outcomes, there are few treatment options for non-responders (primary resistance) or those who have resistance after initial response (secondary resistance). MIF functions by binding its receptor CD74 (present on melanoma) and activating multiple pro-growth pathways. Thus, targeting the CD74/MIF pathway may be a promising therapeutic strategy in melanoma. We have developed the novel third generation DRQ class of biologic compounds that are potent inhibitors of MIF binding to CD74 and have shown promise in preclinical models of melanoma. DRQ is well tolerated and has minimal toxicity in mouse models. In our melanoma tumor models, MIF was highly expressed and its production downregulated by DRQ. DRQ also significantly controlled tumor growth in two localized intradermal melanoma tumor models (B16F10 and YUMMER1.7) when compared with vehicle control ($p<0.05$). DRQ exerted its effects by increasing immune infiltration into the tumor microenvironment, in particular increasing CD8+ T lymphocyte infiltration into the tumors. RNA seq data demonstrated that DRQ decreased ERK and STAT3 expression in B16F10 melanoma cells. Furthermore, rechallenge experiments demonstrated the generation of persistent and specific immune anti-tumor memory responses that were promoted by DRQ administration. These results provide support for modulation of the CD74/MIF axis as a novel therapeutic strategy for melanoma and suggest that DRQ is a unique class of melanoma-active biologic agents that could fill a critical unmet therapeutic need.

LB995

Salicylic acid attenuates UVA-induced photoaging by inhibiting extracellular matrix degradation

H. Zhou, Z. Wang, W. Zeng
Department of Dermatology, Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, Shaanxi, China

Ultraviolet A (UVA) radiation is one of the main causes of skin photoaging. UVA is the principal component of solar UV radiation and penetrates deeper into the skin than UVB and UVC. Salicylic acid (SA) is widely used in skin rejuvenation as a superficial chemical exfoliator and has shown a potential anti-photoaging property. However, the role and mechanism of SA in UVA-induced photoaging have not been fully addressed. In the present study, we established a UVA-induced photoaging mouse model by 10J/cm2/day UVA exposure for 10 days, SA was applied 30 min before UVA radiation. Topical SA pretreatment ameliorated skin coarseness induced by UVA in the photoaged mouse model. Hematoxylin and eosin (HE) and Masson's Trichrome staining were performed for histopathological evaluation and showed that SA remarkably inhibited extracellular matrix degradation induced by UVA. Human skin fibroblasts were isolated and exposed to UVA (8J/cm2) with SA pre-incubation for the investigation of cellular events. Reactive oxygen species (ROS) expression evaluated by flow cytometry indicated that SA pretreatment prevented ROS production induced by UVA. NF-κB mRNA expression was suppressed by SA pretreatment, TNF-α, IL-6, and MMP-1 were as well downregulated. Moreover, SA pretreatment resulted in a higher cell number than UVA treated group by prohibiting apoptosis induced by UVA. Our finding indicated that SA prevents UVA-induced photoaging by inhibiting MMP-1 induced extracellular matrix degradation and cell apoptosis induced by UVA of human skin fibroblast.

LB996

Lipid droplets are a metabolic vulnerability in melanoma

D. Lumaquin[1, 2], E. Montal[1], A. Baggiolini[3], Y. Ma[1, 2], C. LaPlante[1, 2], T. Huang[1], S. Suresh[1], L. Studer[3], R. White[1]
[1]Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, New York, United States, [2]Tri-Institutional MD-PhD Program, Weill Cornell Medicine, New York, New York, United States, [3]Center for Stem Cell Biology and Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, New York, United States

Melanoma cells exhibit numerous transcriptional cell states including developmental programs such as neural crest-like and pigmented melanocytic cells. These cell states endow tumorigenic properties like sustained growth, immune evasion, therapy resistance, and metastasis. Here, we use a zebrafish melanoma model to identify a transcriptional program linking the pigmented melanocytic cell state to a dependence on lipid droplets, the specialized organelle responsible for lipid storage. Single-cell RNA-sequencing of these tumors show a concordance between genes regulating pigmentation and those involved in lipid and oxidative metabolism. This transcriptional state and metabolic phenotype is conserved in human melanoma specimens. This state demonstrates increased fatty acid uptake, an increased number of lipid droplets, and dependence upon oxidative metabolism. Genetic and pharmacologic suppression of lipid droplet production is sufficient to disrupt oxidative metabolism and slow melanoma growth *in vivo*. Because the pigmented cell state is linked to poor outcomes in patients, these data indicate a metabolic vulnerability in melanoma that depends on the lipid droplet organelle.

LB997

Detecting the world's smallest solid malignant tumor: The role of reflectance confocal microscopy in the diagnosis and management of a micro-melanoma

A. Witkowski[3, 1], J. Ludzik[3], J. Chung[3], K. White[3], J. Leitenberger[3], C. Lee[3], E. Berry[3], R. Samatham[3], S. Esener[3], G. Pellacani[2], S. Leachman[3]
[1]Uniwersytet Jagiellonski w Krakowie, Krakow, Małopolska, Poland, [2]Universita degli Studi di Roma La Sapienza, Rome, Lazio, Italy, 3Oregon Health & Science University, Portland, Oregon, United States

Early detection of melanoma is a vital determinant in achieving a favorable prognosis and patient outcomes which led to innovations in skin imaging modalities that improved the diagnostic accuracy and early detection of melanoma. Studies reported the detection of skin cancer in its early stages using dermoscopy and reflectance confocal microscopy, however there are limited reports detecting micro-melanomas less than 2-mm in diameter. Previously, the smallest reported melanoma, measuring 0.9-mm was detected using dermoscopy. We present a case of micro-melanoma measuring 0.65-mm, the smallest malignant solid tumor reported to date, that was detected with a combination of dermoscopy and reflectance confocal microscopy. A 61-year-old woman presented for a focused skin exam which revealed a small hyperpigmented dot on her right cheek. Dermoscopy showed a dark brown, perifollicular macule with hyperpigmentation present at the periphery of 60% of a single hair follicle and reflectance confocal microscopy demonstrated folliculotropic, dendritic melanocytes within the follicle. Initial histologic assessment diagnosed the lesion as a solar lentigo, however deeper sections were stained with Melan-A and SOX10 and a final diagnosis of melanoma-in-situ was made. Additionally, the lesion underwent 23-gene expression profiling which yielded a malignant result. Based on measurements made with reflectance confocal microscopy, we believe this micro-melanoma represents the smallest skin cancer ever recorded. Our findings attest to the diagnostic capabilities of reflectance confocal microscopy and highlight its potential utility in developing our current understanding of early tumor formation, progression, and thereby improve treatment and patient outcomes

LB998

Lymph node delivery of immunstimulatory agent monophosphoryl lipid A via bioadhesive nanoparticles in the treatment of cutaneous melanoma

J. Chang, K. Shin, J. Lewis, H. Suh, M. Bosenberg, W. Saltzman, M. Girardi
Yale School of Medicine, New Haven, Connecticut, United States

Monophosphoryl lipid A (MPLA) is a low-toxicity derivative of LPS with immunomodulating properties. Nanoparticle (NP) encapsulation of immunomodulatory agents may allow for effective delivery to tumor sites as well as tumor draining lymph nodes (TDLN) and stimulation of anti-tumor immune responses while minimizing systemic side effects. Our bioadhesive NP (BNP) platform has been used for delivery of chemotherapy drugs with enhanced efficacy in pre-clinical models of solid tumors including SCC. To evaluate the cellular association and retention of BNP in TDLN, we used flow cytometry to assess immune cell uptake after in-vivo intratumoral (i.t.) delivery of Cy5-BNP to established PDVC57 SCC tumors. Cy5-BNP were present in 58.4 ± 9.7% of TDLN dendritic cells (DC) and 62.1 ± 7.7% of CD68+ macrophages (Mac) 24 hr post injection, with sustained uptake one week after administration (DC: 13.9 ± 1.2%, Mac 23.1 ± 0.8%), while <1% of DC or Mac in the contralateral LN were Cy5+ at either timepoint. We then examined whether MPLA activity is affected by NP encapsulation by measuring NF-kB inducibility in THP-1 monocytes. We observed equivalent dose-dependent NF-kB induction with free MPLA and BNP-MPLA. We also assessed induction of bone marrow-derived DC (BMDC) maturation and found that BNP-MPLA were superior after 24 hr (57.0% CD11c+MHCII+) and 48 hr (65.7%) of incubation relative to equivalent doses of free MPLA (24 hr: 51.5%, 48 hr: 59%, p= 0.0040). We further assessed inhibition of melanoma tumor growth *in vivo* using the immunogenic YUMMER1.7 model. Tumor bearing mice received two once weekly i.t. injections of BNP-MPLA, free MPLA, or vehicle. BNP-MPLA treated tumors showed significantly reduced tumor volume, with tumor resolution in 12.5% of mice, and improved survival (vehicle v MPLA p =0.8147, vehicle v BNP-MPLA p= 0.0076, MPLA v BNP-MPLA p= 0.0060). These results suggest that BNP-MPLA may be effectively delivered to TDLN and help promote anti-tumor immune response against local melanoma.

LB999

Basal levels of MCL1 and its binding partners contribute to a higher sensitivity to MCL1 inhibitors in uveal vs cutaneous melanomas

N. Mukherjee, C. Dart, K. Lambert, D. Norris, Y. Shellman
Dermatology, University of Colorado Denver School of Medicine, Aurora, Colorado, United States

The two subtypes of melanomas, uveal (UM) and cutaneous (CM), originate from melanocyte transformation, but differ in their genetic etiology and signaling pathways. MCL1 and BCL2, the anti-apoptotic members of the BCL2 family, induce anti-cancer treatment resistance. Here, we evaluate their basal levels and role in inducing response to MCL1 inhibitor (MCL1i) in CM and UM. We used in vitro assays (viability, immunoblot and shRNA), and bioinformatics analyses of the TCGA database. UM cell lines have higher BCL2 and lower MCL1 protein expressions compared to CM. Mechanistic studies using shRNAs suggest knockdown (KD) of MCL1 in UM led to ~30% reduction in cell viability and sensitized them to MCL1i ($p<0.001$); however, KD of MCL1 in CM did not significantly alter viability or sensitivity to MCL1i. KD of BCL2 did not have significant effect in either CM or UM. Thus, MCL1 may play a larger role in UM, and to further understand, we looked at the binding partners of MCL1: BOK, BAK, BIM, NOXA and PUMA. UM had higher BOK, BAK and PUMA and lower BIM and NOXA compared to CM. KD of PUMA and BIM led to partial protection against MCL1i induced cell death in multiple UM cell lines ($p<0.05$), while the effects of KD of NOXA were variable. Further studies with KD of BAK and BOK are in progress. Results suggest low MCL1 expression and high pro-apoptotic BCL2 family members that bind to MCL1 contributes to a high sensitivity of UM to MCL1 inhibitors. To determine the clinical relevance, we analyzed the gene expression of TCGA database of 34 cancer types. UM had much higher expression of BCL2, BOK and PUMA and lower MCL1, NOXA and BIM compared to CM ($p<0.001$). In addition, UM had the highest level of BCL2 and PUMA. Thus, our studies with cell lines are consistent with clinical samples. Overall, our data indicates that the basal level of MCL1 and its binding partners contributes to differences in UM and CM's response to MCL1i, and UM is a good candidate for treatment with MCL1 inhibitors.

LB1000

Potential role of cold atmospheric plasma in improving drug resistance of BRAFi/MEKi and immune checkpoint blockade agents in melanoma cells.

C. Yan, L. Zhao, S. Geng, K. Guo
Dermatology, Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, Shaanxi, China

Cold atmospheric plasma (CAP) has been found effective in tumor treatment and promises to be adjuvant agents for various tumors. In melanoma, it has been reported that CAP can alter metabolism, senescence, migration, apoptosis and autophagy, and improve efficacy of chemotherapeutic agents. Current first-line therapeutic agents of advanced melanoma are immune checkpoint blockade and BRAF inhibitor (BRAFi)/MEK inhibitor (MEKi). These agents have definite effects with fatal limitation, drug resistance. The effect of CAP on first-line agents treatment remains unveiled. In this study, next-generation mRNA sequencing technology was utilized to detect drug resistance gene-related changes in CAP-treated A875 human melanoma cell line. Main pathways and molecules were confirmed by Real-time Quantitative Polymerase Chain Reaction (qRT-PCR). 33 differentially expressed genes were found in melanoma drug resistance-related pathways, among which 16 were up-regulated and 17 were down-regulated. MAPK pathway, PI3K/Akt pathway and P53 pathway were dominant molecules involved in drug resistance. Among them, TP53, MAPK2, MAPK3K1 and MAPK3K2 were up-regulated, while other genes such as NRAS, BRAF, MAPK1, PTEN, MITF, AKT3 and PIK3CB were down-regulated. It is the first time to explore effect of CAP on current first-line agents of melanoma. We found that CAP treatment of A875 melanoma cell line carrying BRAFV600E mutations has a potential significant impact on improving drug resistance to BRAFi/MEKi and immune checkpoint blockade.

LB1001

Effectiveness and differentially expressed genes analysis of melanoma cells treated with cold atmospheric plasma

L. Zhao, C. Yan, X. Zhang, T. Jia, S. Geng, K. Guo
Xi'an Jiaotong University Second Affiliated Hospital, Xi'an, Shaanxi, China

Cold atmospheric plasma (CAP) has attracted increasing attention due to its anti-bacterial and anti-tumor effects. Melanoma is an aggressive malignancy that is increasingly common and with a poor prognosis. By evaluating cell viability, detecting cells apoptosis, this study explored the differences of the biological effect between melanoma cells and human keratinocyte cell lines (HaCaT) treated with CAP, and combining the reactive species injection efficiency and tumor cell physiological characteristics system analyzed the biological safety of CAP. The results showed that under the same injection condition, CAP has a more significant biological effect on melanoma in inhibiting proliferation and apoptosis. Based on the effect study, RNA-sequencing was performed to further elucidate the differential gene expression of CAP treated melanoma cells. The results showed that besides MAPK and p53 apoptosis pathways, autophagy and necroptosis also had important roles in CAP-induced melanoma cells death. Our results provided genomic resources for the study of the apoptosis mechanism of CAP treated melanoma cells, and in particular, provided new insights into the molecular mechanisms in autophagy and necroptosis.

LB1002

Melanocyte depletion in vitiligo and canities is associated with M2 macrophage deficiency and responds to modulation by M2-secreted soluble mediator maresin 1 *in vitro* and *in vivo*

M. Su, G. Leung, J. P. Dutz, Y. Zhou
Dermatology and Skin Science, The University of British Columbia Faculty of Medicine, Vancouver, British Columbia, Canada

Melanocyte depletion occurs in vitiligo and aging associated hair greying (canities). Currently available therapies are unsatisfactory due to limited efficacy or therapy-related adverse effects. The purpose of our study is to uncover previously unknown pathogenic pathways for vitiligo and canities and develop therapies that can block these pathways. Transcriptome sequencing-based immune cellular profiling was performed on skin biopsies from healthy volunteers (N=9) and patients with vitiligo (N=36) and chronic dermatitis (N=15), revealing a 3-fold reduction of M2 macrophages in vitiligo skin lesions (p=0.037). There was no M2 macrophage reduction in the lesions of dermatitis. In addition, serum levels of maresin 1 (a soluble functional mediator of M2 macrophages) in aged C57BL/J6 mice with canities were significantly lower than that of the age-and sex matched mice without canities (p=0.026). Further, addition of maresin 1 to the culture medium increased survival of melanocytes by more than 50% (p<0.05). Intraperitoneal injection of maresin 1 into C57BL/J6 mice inhibited vitiligo development by 43% (p<0.05) after TRP-2 immunization, and abolished development of spontaneous aging-associated hair greying (canities) (p=0.0125). Taken together, our results suggest that M2 macrophage deficiency is a shared pathogenic mechanism for melanocyte depletion in vitiligo and canities, and that M2 agonists, such as maresin 1 and other specialized pro-resolving molecules, may be effective for the prevention and treatment of skin conditions caused by melanocyte depletion, including vitiligo and canities.

LB1003

The 31-GEP stratifies risk of recurrence and metastasis in 894 medicare-eligible patients with cutaneous melanoma

B. Martin[1], S. K. Morgan-Linnell[1], S. Kurley[1], M. Goldberg[1, 2], J. Siegel[1], A. Jarell[3]
[1]Castle Biosciences Inc, Friendswood, Texas, United States, [2]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [3]Northeast Dermatology Associates PA, Portsmouth, New Hampshire, United States

Background: The incidence of cutaneous melanoma (CM) is rising, particularly among older patients who generally have worse outcomes than younger patients. The 31-gene expression profile (GEP) test stratifies patient risk of recurrence and metastasis into low (Class 1A), intermediate (Class 1B/2A), and high risk (Class 2B). The study goal was to demonstrate the 31-GEP provides critical risk information for Medicare-eligible patients, allowing providers to identify patients who may benefit from increased surveillance or therapeutic treatment. Methods: Retrospective analysis of patients over 65 years old enrolled in previous studies who received 31-GEP testing (n=894). Recurrence-free survival (RFS) and distant metastasis-free survival (DMFS) were assessed using Kaplan-Meier, log-rank test, and Cox regression multivariable analysis. Results: Patients with a Class 1A 31-GEP result had higher 5-year RFS (93.9% vs. 52.0%, p<0.001) and DMFS (96.3% vs. 64.8%, p<0.001) than patients with a Class 2B result. A Class 2B result was a significant predictor for RFS (HR=4.66, p<0.001) and DMFS (5.34, p<0.001) in multivariable analysis including AJCC 8th edition stage. Stage II (RFS: HR=2.37, p<0.001; DMFS: HR=1.83, p=0.033) and stage III disease (RFS: HR=6.22, p<0.001; DMFS: HR=6.20, p<0.001) were also significant. Older patients with a negative SLNB and a Class 1A 31-GEP result had higher 5-year RFS (88.2% vs. 53.0%, p<0.001) and DMFS (93.0% VS. 69.4%, p<0.001) than those with a Class 2B result. Class 1A patients who did not receive a SLNB had high RFS (98.2%) and DMFS (99.1%). Conclusions: The 31-GEP test stratified risk of recurrence and survival in Medicare-eligible patients and added independent prognostic information to current staging methods. In patients with a potentially large number of comorbidities, identifying those who can forego more extensive therapy is vital.

LB1004

miR-4521 is over-expressed in human lentigos and downregulates components of the autophagic pathway in keratinocytes.

H. Maeno[1], Y. Suzuki-Horiuchi[1], A. Funakoshi[2], T. Shimizu[2], Y. Satou[2], T. Ishii[2], J. T. Seykora[1]

[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Rohto Research Village Kyoto, Rohto Pharmaceutical Co Ltd, Kizugawa, Kyoto, Japan

Lentigo is a common cutaneous pigmented lesion associated with age and sun exposure where keratinocytes demonstrate increased melanin pigmentation. Prior studies have been investigated the molecular pathogenesis of lentigos; however, the mechanisms leading to lentigo formation remain poorly understood. As a first step in exploring lentigo pathogenesis, we performed laser-capture microdissection of human lentigo tissue sections capturing lesional epidermis from the five patients and from three matched control skin samples. Total RNA was isolated from these dissected tissues and then pooled into 'lentigo' and 'epidermal' samples. These RNA samples were analyzed on an Affymetrix GeneChip® Human Gene 2.0 ST array for gene expression profiling. A total of 1777 genes were differentially expressed at least 1.8-fold with a FDR < 0.1. One differentially expressed transcript was miR-4521 which was found to be 38.31 fold higher in human lentigos. Additional studies showed that mir-4521 transcript levels were 17-fold higher in lentigos (N=5) compared to control subjects (N=3) by qRT-PCR ($p<0.05$) which was further confirmed by RNA in situ hybridization showing increased transcript in lentigo keratinocytes. In silico analysis indicated that mir-4521 targets GABARAPL2 and other genes associated with autophagy. Downregulation of both GABARAPL2 mRNA and protein was confirmed using mir-4521 microRNA precursor transfection experiments in HaCaT cells. Immunohistochemistry for GABARAPL2 showed positive staining of keratinocytes in the upper layers of the epidermis in human lentigos, in contrast to positive staining in basal layer keratinocytes in unremarkable epidermis. These data indicate that mir-4521 expression is increased in lentigos and that it may regulate expression of genes in the autophagic pathway in keratinocytes.

LB1005

Skin marking for reflectance confocal microscopy

C. M. Verenzuela[1], K. L. Hanlon[1, 2], L. Correa-Selm[1, 2]

[1]Dermatology and Cutaneous Surgery, USF Health Morsani College of Medicine, Tampa, Florida, United States, [2]Cutaneous Surgery and Oncology, Moffitt Cancer Center, Tampa, Florida, United States

Reflectance confocal microscopy (RCM) generates serial depth images with either 8mm mosaic or 0.6mm field views with the Vivascope 1500 or 3000, respectively. Using 830nm light produces greyscale cellular level images of features that refract light at that narrow portion of the spectrum. Margin mapping for lentigo maligna and lentigo maligna melanoma with RCM can be challenging, especially without clear lesion boundaries or visible markings within confocal images. In some cases, orientation of anatomical direction is important, while in other cases, marking the boundaries of a particular finding is critical. Surgical marking pen ink is invisible to the confocal microscope, making alternative marking approaches that can be visualized within confocal images desirable. A simple and efficient way to mark the skin that does not smear or spread around the field when index matching oil is applied or when wiped with alcohol is using nail polish. This can be a color that either reflects or absorbs the near-infrared light. Silver nail polish refracted the light, appearing bright; white nail polish absorbed the light, appearing dark. The nail polish is visible at every depth from the stratum corneum down to the dermis. Having multiple "colors" may be advantageous for delineating separate areas or those that overlap. Often the orientation to a margin or a scar from a prior excision is critical data, in other cases the distance in millimeters from a location is in question or the outline of an otherwise subtle finding may be important. The nail polish strategy has proven useful for both the Vivascope 1500 for mosaic images as well as the handheld device for real-time assessments. The approach also increased confocal workflow efficiency, since the physician can determine the areas for assessment and then a technician or other staff member can orient the images and localize the findings. This expands on prior work using silver sharpie or adhesive tape windows.

LB1006

Primary radiotherapy for the treatment of keloids: Pilot study preliminary results

Y. Kost[1], A. Muskat[1], K. Mieczkowska[1], A. Deutsch[1], C. Zouzias[1], B. McLellan[1], K. Mehta[2], J. Klein[2]
[1]Dermatology, Albert Einstein College of Medicine, Bronx, New York, United States, [2]Radiation Oncology, Albert Einstein College of Medicine, Bronx, New York, United States

Introduction:Keloids are a benign excessive proliferation of connective tissue, with associated pruritus, pain, and appearance that can be distressing to patients. Post-excision radiation therapy (RT) can decrease keloid recurrence with excellent cosmetic results, few long-term toxicities, and no RT-attributable cancers. When surgical excision is not feasible, RT monotherapy may be a successful alternative. However, lack of standardization of an RT-only protocol has precluded wide implementation. Methods:We initiated a prospective trial evaluating feasibility and tolerability of RT alone to treat unresectable keloids. Keloids were treated with 15 Gy delivered in three fractions, a widely used standard for postoperative keloid treatment selected for known excellent safety profile. We assessed RT-related toxicity using Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, changes in keloid dimensions, and patient-reported quality of life (QOL) outcomes using the SKINDEX-16 survey (SD-16, 0-96 points) at baseline (before RT), 0 (during RT), 2, 6, and 10 weeks and 6, 9, and 12 months after RT completion. Results:We report preliminary results in 4 patients. The maximum CTCAE toxicity score was grade 1 (faint erythema or dry desquamation) and scores for all patients were maintained at grade 0 after 6-weeks follow up. Time to lowest mean keloid height (-42.86% vs baseline) was 10 weeks. Three patients have completed 12-month follow up, at which point mean keloid height remained lower (-28.57% vs baseline) while length (+0.75%) and width (+1.53%) were stable. SD-16 scores demonstrated improved QOL at 12 months overall (mean score Δ: -65.67) and in SD-16 domain scores (mean score Δ vs baseline in symptoms: -17, emotion: -23, functioning: -11.33). Conclusion:Preliminary results support the safety, feasibility, and efficacy of a three-fraction, 15 Gy RT-only protocol for unresectable keloid treatment. Further study is ongoing to validate these findings.

LB1007

Hair loss and traction alopecia in the Sikh population

H. Dhinsa[2], J. Powers[1]
[1]Dermatology, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States, [2]The University of Iowa Roy J and Lucille A Carver College of Medicine, Iowa City, Iowa, United States

Traction alopecia is common in individuals of the Sikh faith who wear tight bun hairstyles covered by turbans. An IRB-approved 50-question survey self-assessing hair loss and various hair care practices was offered voluntarily through email distribution to national Sikh organizations. Out of the 156 total respondents, 82% indicated they wore a turban daily. Of the participants who specified they wore a turban, 50% kept it on for at least 6-12 hours a day while 19.5% wore it 12-18 hours and 10% for 18-24 hours. The majority of subjects (75.5%) felt that they tied their turban “just right” while 23.7% preferred to tie it tightly. Most of the participants (79.5%) indicated they were experiencing hair loss somewhere on the scalp, with 72.4% specifying that hair loss occurred primarily at the frontal and temporoparietal regions of the scalp. Those experiencing frontal-temporal hair loss also noted experiencing itching (34.3%), thin, broken hairs on the scalp region (22.9%), blisters or bumps (13.3%), and shiny or scarred skin (10.5%). In response to a question querying if participants felt wearing a turban contributed to their hair loss, 43% agreed with the statement while 29% disagreed. While 73% of subjects noted being unhappy with their hair loss and 63.4% indicated feeling anxiety or worry over it, only 18.5% had seen a physician for care. Of those declining to see a physician for hair loss, 51% gave the reason that they were unsure if the condition was treatable. Those attempting to treat their hair loss at home tried hair oils (35.4%), tying their hair more loosely (23%), or tying the turban more loosely (12%). This pilot data confirms there are knowledge and treatment gaps in the Sikh community regarding alopecia, suggesting that many have active symptoms and associated mental health concerns. Further studies should explore the correlation between Sikh hair care practices and traction alopecia.

LB1008

Prophylactic treatment of auricular keloid recurrence post excision using topical imiquimod

L. Chan[1], H. Greenberg[2]
[1]University of Nevada Las Vegas Kirk Kerkorian School of Medicine, Las Vegas, Nevada, United States, [2]Las Vegas Dermatology, Las Vegas, Nevada, United States

Introduction: Keloid scars are difficult to treat and result from aberrant wound healing and have recurrence rates of 50% to 80% without treatment. Clinicians who are comfortable with surgical removal often offer patients post excision prophylactic options such as glucocorticoid creams, pressure dressings, and radiation. However, these therapies can be timely for patients and may involve poor compliance. The exact mechanism of keloid growth is unknown, but TGF-β has been theorized to play a role in fibroblast activation. Keloids are benign fibroproliferative disorders but demonstrate cancer like properties including: regrowth, lack of spontaneous regression, and uncontrolled growth. Various studies have examined Imiquimod (Aldara) in prophylactic treatment of keloids post excision with success, thus shave excision plus topical Imiquimod can be a novel keloid scar solution for patients preventing keloid regrowth post excision. Method: A retrospective case series of 6 adults with auricular keloids on initial presentation, 83% were female, mean age 30. Shave excision was performed on all keloids; twice daily cleaning of surgical site with H2O2 and nightly Imiquimod application was prescribed with clinical follow up at 2, 6 and 12 weeks. One patient had four staged excisions of the right auricular keloid. In this cohort there was 0% keloid growth recurrence. Result: All 6 patients demonstrated normal wound healing post-keloid excision with zero recurrences. Discussion: We solely used topical Imiquimod 5% to evaluate its full clinical use in prophylaxis of keloids and determined it is suitable as a single therapy for post excisional keloid prophylaxis. Although Imiquimod 5% is a recommended option, there haven’t been many case series in recent years demonstrating results or defining the treatment period. Our study found nightly Imiquimod 5% topical application for six weeks as an efficacious post keloid excision treatment. <div id=”accel-snackbar” style=”left: 50%; transform: translate(-50%, 0px); top: 50px;”>

LB1009

Sun protection behaviors among Hispanic subgroups

R. Chen[2, 1], A. Ahmad[3, 2], N. Trepanowski[4, 2], D. Y. Kim[5], R. Hartman[2, 6]
[1]Emory University School of Medicine, Atlanta, Georgia, United States, [2]Dermatology, Brigham and Women’s Hospital, Boston, Massachusetts, United States, [3]The University of Texas Health Science Center at Houston John P and Katherine G McGovern Medical School, Houston, Texas, United States, [4]Boston University School of Medicine, Boston, Massachusetts, United States, [5]Harvard Medical School, Boston, Massachusetts, United States, [6]Dermatology, VA Integrated Service Network 1 (VISN-1), Jamaica Plain, Massachusetts, United States

Introduction: The Hispanic population is rapidly growing in the United States, but the grouping of these individuals into one category can obscure heterogeneity. This in turn may limit our understanding of sun protective behaviors among Hispanics, especially given the significant variations in sun protection practices among subpopulations. We aim to explore these nuances. Methods: For the years 2005, 2010, and 2015, sun protection behaviors, skin cancer risk factors and demographic characteristics were obtained from the National Health Interview Survey (NHIS). Univariate and multivariate logistic regressions were utilized to compare sun protection and risk behaviors among Hispanic subgroups. P-values were adjusted with Benjamini-Hochberg Procedure. Results: With Mexican, the largest grouping, as the reference category, Cubans had lower adjusted odds of practicing sun avoidance ($p = 0.008$), while Puerto Ricans (PR), Cubans, and C/S Americans had lower adjusted odds of wearing sun protective clothing ($p = 0.050$, $p < 0.001$, $p < 0.001$, respectively). Mexican Americans, Cubans, C/S Americans, and other Hispanic had higher unadjusted odds of using sunscreen ($p < 0.001$ for all). Mexicans and other Hispanics had higher unadjusted odds of having multiple sunburns ($p = <.001$ for both), while Cubans and Dominicans had lower adjusted odds (<0.001 for both) of having multiple sunburns. Discussion: There are important differences between sun protective and risky sun behaviors among Hispanic subpopulations. Recognizing the differences will allow for further targeted recommendations toward specific sub-populations to mitigate skin cancer risks and mortality.

LB1010

Cutaneous manifestations post stem cell transplant

S. Rangarajan[1], N. Lalefar[2]
[1]*Western University of Health Sciences, Pomona, California, United States,* [2]*Pediatrics Hematology-Oncology, University of California San Francisco, Fresno, California, United States*

Allogenic stem cell transplant (SCT) and bone marrow transplant (BMT) are curative of conditions including Leukemias, Sickle cell disease, and Thalassemia. Cutaneous manifestations in a post-transplant patient may be an early sign of acute graft versus host disease (aGvHD), a serious and potentially life-threatening reaction to the transplant. Other causes can include medications, and reactivation of latent viral infections, owing to their immune-compromised state1. We present a case of a 28-year-old female with a history of sickle cell disease, status post allogenic SCT day 62, and HHV-6 infection on day 24, who presented with pruritic rash that started on the back of her right shoulder two weeks prior, and then spread to other parts of her body. Initially, the rash presented as scattered papules along dermatomes with a few on her scalp. She was prescribed acyclovir, however discontinued following infectious disease specialist ruling out varicella infection. She was later referred to a dermatologist who noted several dozen scattered erythematous to violaceous plaques with collarette scale ranging from few mm to > 1 cm symmetrically distributed along Langer's lines involving mainly upper chest, back, arms, neck, and a few on lateral cheeks, scalp, and medial thighs. Biopsy of the lesion revealed vesicular spongiotic dermatitis with eosinophils consistent with Pityriasis Rosea. aGvHD is a serious reaction to BMT, and typically involves skin manifestations. There have been studies demonstrating atypical clinical presentations of aGvHD1, 2 including those that appear to be viral exanthems. Early skin biopsies become diagnostic and are therefore necessary to rule out aGVHD and to tailor treatment plans accordingly. 1. Aractingi, S. and O. Chosidow, Cutaneous graft-versus-host disease. Arch Dermatol, 1998. 134(5): p. 602-12. 2. Cornejo, C.M., et al., Atypical manifestations of graft-versus-host disease. J Am Acad Dermatol, 2015. 72(4): p. 690-5.

LB1011

Oncogenic ras mutation induces spatiotemporally specific tissue deformation through converting fluctuated into sustained ERK activation

T. Xin[1], S. Regot[2], V. Greco[1]
[1]*Department of Genetics, Yale School of Medicine, New Haven, Connecticut, United States,* [2]*Department of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, Maryland, United States*

Tissue regeneration and maintenance rely on coordinated stem cell behaviors. This orchestration can be impaired by oncogenic mutations leading to tissue architecture disruption and ultimately cancer formation. However, it is still largely unclear how oncogenes perturb stem cells' functions to break tissue architecture. Here, we utilized intravital imaging and novel signaling reporter to investigate the mechanisms of oncogenic Kras-induced tissue disruption in the hair follicle. Through longitudinally tracking the same hair follicles in live mice, we found KrasG12D, a mutation that can lead to squamous cell carcinoma, induces epithelial tissue deformation in a spatiotemporally specific manner. This is linked with a spatial dysregulation of stem cell proliferation and migration in hair follicle regeneration. By using a reporter mouse that allows to capture real-time ERK signal dynamics at the single cell level in live animal, we discovered that KrasG12D converts the transient ERK signal fluctuation in the stem cells into sustained activation. In contrast, hair follicles carrying oncogenic mutation HrasG12V, which does not disrupt the tissue, still exhibit fluctuated ERK activation. Furthermore, by combining drug treatment with longitudinal imaging, we demonstrated that inhibiting ERK signal reverts the KrasG12D-induced tissue deformation, suggesting the alteration of the ERK signal dynamics led to tissue disruption in Kras mutant. Intriguingly, low number of KrasG12D cells were insufficient to induce sustained ERK activation and deform the tissue, suggesting a collective effect from a large group of mutant cells is required for tissue disruption. Altogether, our work supports a niche-dependent mechanism for oncogene-induced tissue disruption. Oncogenic mutations induce tissue abnormalities when spatiotemporally specific conditions are met, which allows mutant stem cells disturb local cell coordination through altering dynamic signal communications.

LB1012

Dermal sheath mechanosignaling activation of TGF-β controls progenitor death during hair follicle regression

N. Heitman, P. Martino, D. Srivastava, R. Sunkara, M. Rendl
Icahn School of Medicine at Mount Sinai, New York, New York, United States

Epithelial-mesenchymal crosstalk is vital for balancing expansion and regression of the progenitor cell pool during tissue turnover. In the hair growth cycle, little is known about the mechanisms underlying the regulated cell death during follicle regression that precedes each regeneration and hair re-growth. Here, we discover that the dermal sheath smooth muscle, which lines all follicles, controls progenitor death via transforming growth factor (TGF)-β mechanosignaling. The sheath secretes TGF-β, which is first stored as an inactive, latent complex in the basement membrane extracellular matrix. The sheath then contracts to free TGF-β for essential signaling to progenitors for cell death regulation and regression. Finally, we identify Integrin-alpha-V on the sheath surface as the molecular link for force-mediated TGF-β release. Our study establishes the dermal sheath as a mechanosignaling niche that controls progenitor pool reduction through mechanical force, signal activation and cell-cell communication.

LB1013

Nuclear transcriptomics reveals the determinants of eccrine sweat gland fate and differentiation

H. L. Dingwall, R. R. Tomizawa, B. Kokalari, Q. Qiu, P. Hu, H. Wu, Y. G. Kamberov
Genetics, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States

Eccrine sweat glands are the predominant ectodermal appendage of human skin and are essential in human thermoregulation. Despite their importance, the developmental program and the cellular interactions that drive eccrine gland formation are poorly understood. In mouse volar skin, to which eccrine glands are restricted in this species, the expression of the transcription factor Engrailed-1 (En1) in basal ectoderm is required for the specification of eccrine placodes, while in humans, the upregulation of EN1 in a hallmark of eccrine-forming skin. Accordingly, we used En1 as a molecular node to interrogate the eccrine gland developmental program. To this end, we adapted single-nucleus RNA sequencing technology to profile the transcriptomes of developing volar skin of wildtype and inducible, basal ectoderm-specific, En1 knock-out mice (cEn1KO). We found that cEn1KO skin is characterized by the loss of subsets of basal keratinocyte and dermal nuclei. Computational analyses and *in vivo* validation of the top markers of cEn1KO-depleted keratinocyte nuclei revealed expression signatures of eccrine gland developmental progression from placode specification through nascent gland differentiation. Moreover, through analyses of the top differentially-expressed markers of the cEn1KO-depleted dermal population, we identified, for the first time, a population of mesenchymal cells that are specifically associated with developing eccrine anlage. Targeted ablation of this dermal population using the inducible and cell type-specific expression of Diphtheria toxin led to a depletion of eccrine placodes in mouse volar skin. Our findings reveal novel cellular and molecular paradigms driving eccrine gland development, and highlight the potential of nuclear transcriptomics for interrogating the functional heterogeneity of the skin.

LB1014

Hedgehog signaling reprograms hair follicle mesenchyme toward a hyper-activated state

Y. Liu[1, 5, 2], C. F. Guerrero-Juarez[1, 4, 3], F. Xiao[2], R. Liu[6], Z. Yu[6], Q. Nie[1, 4, 3], J. Li[5], M. Plikus[1, 2, 3]
[1]*Department of Developmental & Cell Biology, University of California Irvine, Irvine, California, United States,* [2]*Sue and Bill Gross Stem Cell Research Center, University of California Irvine Sue and Bill Gross Stem Cell Research Center, Irvine, California, United States,* [3]*Center for Complex Biological Systems, University of California Irvine, Irvine, California, United States,* [4]*NSF-Simons Center for Multiscale Cell Fate Research, University of California Irvine, Irvine, California, United States,* [5]*Department of Dermatology, Central South University, Changsha, Hunan, China,* [6]*Department of Nutrition and Health, China Agricultural University, Beijing, China*

Dermal papilla (DP) is the key mesenchymal niche cell type, that regulates cyclic regeneration of hair follicles by orchestrating paracrine signaling crosstalk with epithelial progenitors. We discovered that leptin receptor (Lepr) is a robust marker gene of adult DP fibroblasts in mice and that constitutive Cre and inducible CreER genetic tools, whose activity follows the expression of the Lepr isoform B, can efficiently target DPs for labeling and gene modulation during both growth (anagen) and rest (telogen) phases of the hair growth cycle. Using Lepr-based DP targeting, we show that Hedgehog signaling activation in adult DPs prominently accelerates cyclic growth of hair follicles and induces formation of new hair follicles from the sides of pre-existing follicles in unwounded skin. By single-cell RNA-sequencing we fully define cellular heterogeneity of DP fibroblasts, that increases upon Hedgehog activation, and establish new gene regulatory network of hyper-activated DP state. We also identify new Hedgehog-regulated signaling factors and reveal the role of selected factors in physiological growth of hair follicles. Considering current scarcity of genetic tool that allow specific, robust, and time-dependent targeting of adult DP fibroblasts, newly identified Lepr-based drivers, that will become broadly publicly available, will enable rapid testing of candidate signaling in hair follicle niche biology.

LB1015

Exosome therapy in hair regeneration: A literature review of the evidence, challenges, and future opportunities

Y. Kost[1], A. Muskat[1], N. Mhaimeed[2], R. S. Nazarian[1], K. Kobets[1]
[1]Dermatology, Albert Einstein College of Medicine, Bronx, New York, United States, [2]Dermatology, Eastern Virginia Medical School, Norfolk, Virginia, United States

Alopecia is a common chief complaint and is challenging to treat. Exosomes, extracellular vesicles formed by sequential invagination of membranes between intracellular components, are involved in cell communication, homeostasis, and differentiation, and play a key role in hair morphogenesis and regeneration with potential for alopecia treatment use. To summarize the current body of knowledge surrounding exosomes and identify areas for research, a literature review was conducted using keywords including "exosome," "alopecia," and "hair loss" on PubMed, EMBASE, and Google Scholar databases to assess exosome use, postulated underlying mechanisms, and clinical applications. Reference lists of identified articles were reviewed. 47 studies were included. Clinical trial databases were searched, however no relevant alopecia trials were identified. Preclinical studies demonstrate benefits of exosomes in regenerative medicine. Evidence for their use in alopecia is most abundant for exosomes from dermal papilla cells (6 studies) and adipose-derived stem cells (4 studies). Other exosome sources include: the hair outer root sheath, bone marrow, dermal fibroblasts, myeloid-derived suppressor cells, macrophages, and scalp commensal bacteria. Mechanistically, upregulation of the Wnt/β-catenin pathway was most frequently described, and TNF-α and vascular endothelial growth factor signaling cascades upregulation was noted in adipose-derived stem cell exosomes. Exosomes from immune cells showed decreased T cell hyperreactivity via increased FoxP3 and arginase 1. We also identified several clinical pilot studies, case series, and patented studies in mouse models showing exosome benefits in alopecia treatment. Importantly, we noted gaps in knowledge required for effective clinical translation including safety, pharmacokinetic profile, delivery method, and exosome source. While exosome treatment for alopecia is on the horizon, further high-quality mechanistic studies and clinical trials are required.

LB1016

Gradual differentiation uncoupled from cell cycle exit generates heterogeneity in the epidermal stem cell layer.

K. Cockburn[1], K. Annusver[2], D. Gonzalez[1], S. Ganesan[1], D. May[1], K. Kawaguchi[3], M. Kasper[2], V. Greco[1, 4]
[1]Genetics, Yale University, New Haven, Connecticut, United States, [2]Cell & Molecular Biology, Karolinska Institutet, Stockholm, Stockholm, Sweden, [3]Cluster for Pioneering Research, Rikagaku Kenkyujo, Kobe, Japan, [4]Dermatology, Yale University, New Haven, Connecticut, United States

In the mammalian skin epidermis, it has long been unclear how molecularly heterogenous stem/progenitor cell populations fit into the complete trajectory of epidermal differentiation. Here, we combine *in vivo* imaging with single-cell RNA sequencing to define the early stages of epidermal stem cell differentiation at the molecular and behavioral level. We show that differentiation, from commitment to exit from the stem cell layer, is a multi-day process wherein cells transit through a gradual continuum of transcriptional changes. By tracking epidermal regions over many days, we find that differentiation-committed cells remain capable of dividing to produce daughter cells fated to further differentiate, demonstrating that differentiation is uncoupled from cell cycle exit. These cell divisions occur as a response to local density changes, and are not required as part of an obligate transit amplifying program but instead help to buffer the differentiating cell pool upon disruption of the epidermal barrier. Thus, instead of distinct contributions from multiple progenitor types, a single continuous differentiation process shaped by feedback from the local environment fuels lifelong turnover of the epidermis.

LB1017

A novel link between human hair follicle neuroimmunology and mitochondrial biology: Substance P increases intrafollicular oxidative stress and mitochondrial biogenesis

E. J. Horesh[1], J. Chéret[1], J. O'Sullivan[1], R. Paus[1, 2, 3]
[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States, [2]Monasterium Laboratory Skin & Hair Research Solutions GmbH, Munster, Nordrhein-Westfalen, Germany, [3]CUTANEON, Hamburg, Germany

Substance P (SP) is the prototypic neuropeptide associated with emotional stress and neurogenic perifollicular inflammation and is released by sensory nerve fibers. It also promotes human hair follicle (HF) immune privilege collapse and catagen development *ex vivo*, and has thus been implicated in stress-related hair loss, ranging from telogen effluvium to alopecia areata. Since SP stimulates the mitochondrial release of reactive oxygen species, we have asked how SP impacts on human HF mitochondrial biology. When organ-cultured anagen scalp HFs were treated with SP (10-10 and 10-12M), this showed that the mitochondrial-encoded subunit 1 of cytochrome c oxidase (MTCO1), a key enzymatic marker of oxidative phosphorylation, and mitochondrial activity and biogenesis, was significantly upregulated. This was coupled with significantly increased expression of the mitochondrial mass and biogenesis marker, VDAC1/porin, a channel found in the mitochondrial membrane. SP also significantly increased mTORC1 activity (measured by p-S6 immunofluorescence), a known promoter of ROS production. Since we had previously shown that stress-induced HF greying is associated with VDAC1/porin upregulation, these SP effects suggest a hitherto unknown strong mitochondrial response of human scalp HFs to this stress- and sensory nerve fiber-associated neuropeptide. This invites therapeutic interventions with SP antagonists that target this novel link between mitochondrial biology and neuroimmunology of the HF under conditions of perceived stress in the future management of stress-associated human hair greying and hair loss.

LB1018

ALRN-6924, a dual inhibitor of MDMX and MDM2, protects human scalp hair follicles and their epithelial stem cells from paclitaxel-induced toxicity

J. Gherardini[1], A. D. Annis[2], J. Chéret[1], M. Aivado[2], R. Paus[1, 3]
[1]Dr. Phillip Frost Dept. of Dermatology & Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, United States, [2]Aileron Therapeutics, Inc., Boston, Massachusetts, United States, [3]CUTANEON, Hamburg, Germany

Taxanes like paclitaxel (PTX) cause severe and often permanent chemotherapy-induced alopecia (CIA). We have previously shown that PTX damages human scalp hair follicles (HFs) by inducing massive mitotic defects and apoptosis in hair matrix (HM) keratinocytes as well as bulge stem cell DNA damage, and that pharmacological induction of transient G1 arrest can be HF- and stem cell-protective. We tested whether ALRN-6924 can protect human HFs from PTX-induced toxicity *ex vivo*. ALRN-6924 is a clinical-stage MDM2/MDMX dual inhibitor that activates p53 to upregulate p21, transiently arresting and selectively protecting healthy normal cells from chemotherapy in patients with p53-mutant cancers without protecting cancer cells. In vitro studies confirmed that ALRN-6924 protects human fibroblasts (HS68) from multiple chemotherapies, but not p53-mutant breast cancer cells (BT-474). When organ-cultured anagen scalp HFs were pre-treated with ALRN-6924 or vehicle, followed by PTX or vehicle, ALRN-6924 significantly increased the number of p21+ HM and bulge stem cells compared to vehicle or PTX alone, confirming cell cycle arrest *ex vivo*. Importantly, the number of melanin clumps, a sensitive HF cytotoxicity and dystrophy marker, as well as apoptosis, pathological mitosis, and DNA damage (gH2AX) of K15+ stem cells in PTX-treated human HFs were reduced by ALRN-6924 pretreatment. Despite its cell cycle inhibition, ALRN-6924 did not promote catagen development and even reduced the level of PTX-induced pathological epithelial-mesenchymal transition in HF stem cells *ex vivo* (K15/vimentin double-immunostain by quantitative immuno-histomorphometry). These results show that transient cell cycle arrest protects HFs from PTX-induced toxicity and irreversible stem cell damage, supporting clinical investigation of ALRN-6924 to prevent both acute and permanent CIA.

LB1019

The early region of trichodysplasia spinulosa polyomavirus drives proliferation, altered differentiation, and ectopic expression of hair follicle differentiation markers in interfollicular tail epidermis

L. Syu[1], D. Wilbert[1], E. van der Meijden[2], M. Feltkamp[2], A. A. Dlugosz[1]
[1]*Dermatology, University of Michigan, Ann Arbor, Michigan, United States,* [2]*Leids Universitair Medisch Centrum Afdeling Infectieziekten, Leiden, Zuid-Holland, Netherlands*

Trichodysplasia spinulosa (TS) is a rare hair follicle disorder that develops in immunosuppressed patients and is associated with productive infection by the TSPyV polyomavirus. Affected follicles contain an attenuated hair matrix, expanded compartment of aberrantly proliferating inner root sheath (IRS)-like cells containing viral particles, and a disorganized mass of hair shaft-like cells. The expression of viral early region (ER) genes encoding T antigens (TAgs) that drive cellular proliferation and viral genome replication is tightly restricted to hair follicle matrix cells and IRS-like cells. To probe the potential impact of TAg expression on other aspects of epithelial biology, we generated bitransgenic iK5;TSER mice harboring Cre-inducible TSPyV ER genes that could be activated in K5-expressing cells and their progeny using tamoxifen. As we previously described, expression of TSPyV TAgs in skin of adult mice led to development of numerous dysmorphic hair follicles. Additionally, interfollicular tail epidermis was hyperplastic and contained small collections of TAg+ cells, some of which were multinucleated, with a clear cytoplasm. Many of these cells were proliferating (Ki67+) and strikingly deficient in epidermal keratins (K5, K14, K1, K10). Remarkably, a subset of these aberrant interfollicular cells ectopically expressed the IRS marker trichohyalin and hair-specific keratin. Our findings suggest that in addition to the primary function of ER genes in driving cell cycle progression needed for viral genome replication, TAgs may also contribute to human pathology by modulating the differentiation status of target cells.

LB1020

Scurvy: A forgotten illness?

S. Mahlberg[1], S. Rangarajan[2], T. Sharma[1]
[1]Dermatology, University Hospitals, Cleveland, Ohio, United States, [2]Western University of Health Sciences, Pomona, California, United States

Scurvy is classically thought to be a historical disease rarely found in developed countries, thanks to increased awareness of the role of ascorbic acid in this disease. A non-verbal 19-year-old female with a history of developmental delay, G-tube (and by mouth intake), gumline bleeding, and poor dietary intake, presented with bilateral lower extremity non-palpable petechial, perifollicular eruptions. She denied any significant pain, itch, or irritation from the rash. Perifollicular petechiae and scattered corkscrew hairs were found on dermoscopy examination. A suspicion of Scurvy from her history and physical examination was confirmed by the serum levels of vitamin C <0.1 mg/dL. Although, the diagnosis is clinical, a histopathologic examination showed hyperkeratosis with irregular acanthosis of the epidermis and follicular spongiosis with erythrocyte extravasation adjacent to a hair follicle consistent with Scurvy. Scurvy is a well-documented disease caused due to a deficiency in Vitamin C, a known cofactor of enzymes involved in the synthesis of collagen, carnitine and catecholamines, and in gene expression and maintenance. Thorough dermascopic examination will reveal symptoms such as perifollicular petechiae and corkscrew hairs1 - some of the diagnostic clues to lack of functioning collagen, that occur with Scurvy. Other symptoms of collagen dysfunction include gingival bleeding, ecchymosis, epistaxis, and bone hemorrhage. In developed countries, patients with avoidant-restrictive food intake or those with renal imbalances are at risk of acquiring Scurvy. Therefore, it is important to keep Scurvy in the differentials in patients presenting with petechiae and perform a comprehensive physical exam, to avoid misdiagnosis. 1. Hirschmann, J.V. and G.J. Raugi, Adult scurvy. J Am Acad Dermatol, 1999. 41(6): p. 895-906; quiz 907-10. https://doi.org/ 10.1016/s0190-9622(99)70244-6.

LB1021

Single-cell dynamics of fibroblast states during skin wound healing in mice

A. A. Almet[1, 2, 3], Q. Nie[1, 2, 3], M. Plikus[4]
[1]NSF-Simons Center for Multiscale Cell Fate Research, University of California Irvine, Irvine, California, United States, [2]Center for Complex Biological Systems, University of California Irvine, Irvine, California, United States, [3]Department of Mathematics, University of California Irvine, Irvine, California, United States, [4]Department of Developmental & Cell Biology, University of California Irvine, Irvine, California, United States

Wound healing is a highly dynamic process over spatial and temporal scales. Key to wound healing outcomes are fibroblasts, whose precise functions in tissue repair are only partially understood. We constructed an atlas of mouse skin wound healing using several published single-cell RNA-seq (scRNA-seq) datasets. Using scRNA-seq data integration across wound healing time, we identified key cell types and states that are both shared across as well as unique to specific post-wounding timepoints. We reveal that fibroblasts dramatically alter after wounding as compared to unwounded skin fibroblasts, and also remain highly dynamic over time of healing. These transcriptional dynamics are not paralleled in other major wound-resident cell types. Furthermore, by "supervised clustering" and training-interpretable machine learning models we infer that these state dynamics are largely driven by selected gene categories, that are essential for key functions of fibroblasts. Finally, RNA velocity and pseudotime analysis suggest that fibroblast dynamics during small wound healing is an abridged version of large wound healing dynamics. Taken together, our findings reveal a highly dynamic transcriptomic landscape of fibroblasts during wound healing.

LB1022

Complete regeneration of secretory glands in salamander skin via blastema-independent mechanism

R. Hou, A. Tsao, N. Kwang, C. Kuan, M. Plikus
Department of Developmental and Cell Biology, University of California Irvine, Irvine, California, United States

While human and other mammals are limited in their abilities to regenerate, amphibians, including salamander species such as the axolotl (Ambystoma mexicanum), are highly regenerative. Contrary to the prevailing notion that healing via embryonic-like regeneration necessarily requires a specialized "blastema" tissue, we show that axolotls regenerate new skin with anatomically complex secretory glands without a blastema and that, in fact, formation of blastema represses gland-bearing skin regeneration. We studied regenerative healing of excisional skin wounds in axolotls with live confocal imaging by which same wounds are consecutively re-imaged over the span of several weeks. This enabled us to record the entire process of gland neogenesis at single-cell resolution and show that specialized fibroblasts closely associate with epithelial gland primordia already at the onset of regeneration. We then profiled regenerating skin wounds with single-cell RNA-sequencing (scRNA-seq), which is enabling us to identify key epithelial and mesenchymal cell populations engaged in gland neogenesis. In mammals, such as in laboratory mice, new hair follicles can regenerate following large excisional wounding. However, this phenomenon, termed wound-induced hair neogenesis (WIHN) is typically restricted to the center of the wound, such that wound edges repair with a hairless scar. Unlike WIHN in mice, gland neogenesis in axolotls is more complete, occurring across the entire wound area. Our study on axolotls will establish blastema-independent mechanism for skin regeneration and will likely illuminate understandings of broader skin regeneration mechanisms.

LB1023

Deletion of bardet biedl syndrome 1 (BBS1) gene in T cells impairs wound healing in mice

M. Stump[1, 2], D. Guo[3], K. Rahmouni[4]
[1]Dermatology, The University of Iowa, Iowa City, Iowa, United States, [2]Physician Scientist Training Program, The University of Iowa, Iowa City, Iowa, United States, [3]Neuroscience and Pharmacology, The University of Iowa, Iowa City, Iowa, United States, [4]Neuroscience and Pharmacology, The University of Iowa, Iowa City, Iowa, United States

Primary cilia play an important role in cell directional migration and tissue repair. Ciliary function depends on several proteins including the BBSome, a complex of eight conserved Bardet-Biedl syndrome (BBS) proteins, such as BBS1, which transport cargos to and from cilia. The immune synapse (IS), the nano-scale gap between T cells and antigen presenting cells, in non-ciliated T cells is functionally homologous to the primary cilium. During the formation of the IS, the BBSome appears to be critical for centrosome translocation toward the IS interface within T cells. Data from our lab demonstrate that BBS1 hypomorphic mice (containing a systemic homozygous knock-in missense mutation) characteristically exhibit impaired cilia function, altered fibroblast function, and reduced wound healing/tissue repair. The skin is residence for most lymphocytes in the body. To investigate the role of BBS1 in T cells in wound healing, we generated a novel transgenic mouse model in which the BBS1 gene was conditionally deleted in T cells. For *in vivo* wound healing, 2 mm full-thickness punch biopsy was performed on the shaved backs of 9 to 11 weeks old male and female T-BBS1-/- mice and their wild-type littermate controls and assessed for rate of wound closure by secondary intention between day 0 and day 7. The wounds formed fibrin clots at similar times in both T-BBS1-/- and littermate controls. However, male T-BBS1-/- mice demonstrated significantly delayed wound healing at day 7 compared to controls (4.061±1.79%, p=0.0342, n=10-12/group). The female mice show a trend toward reduced would healing at 7 days as well. These results demonstrate that BBS1 plays an important role in T cell mediated skin repair and that this effect may be sex specific.

LB1024

Ashwagandha (Withania somnifera) seed plant exosome-like nanovesicles promote in vitro cellular regeneration of human dermal hair follicle, endothelial and fibroblast cells

G. Dellacqua, R. Scotland
Research and Innovation, Nutrafol, New York, New York, United States

To overcome inherent drawbacks associated with human-derived platelet-rich plasma (PRP) and exosome therapeutics in the treatment of hair thinning and other dermatological conditions, plant exosome-like nanovesicles were derived from Ashwagandha (Withania somnifera) seeds (Ash-PELNs) to test their regenerative capacity. Prior Ash-PELN studies indicate topical human safety, tolerability and anagen active hair growth prolongation. The regenerative capacity of Ash-PELNs was evaluated in vitro by cellular proliferation, angiogenesis, and growth factor stimulation measures. Ash-PLEN 1E+07-10 nanovesicles/ml concentrations were assessed. Proliferation was measured by bromodeoxyuridine incorporation in stressed (300 nM hydrocortisone) human hair follicle dermal papilla cells (hHFDPCs). Angiogenesis was evaluated via migration assay (Oris™, Platypus® Technologies) in human endothelial cell (HUVEC) and dermal fibroblast (hDF), HUVEC tubulogenesis by fluorescence microscopic tubule quantitation, and hDF VEGF-A protein expression ELISA (Quantikine®, R&D Systems). hHFDPC secreted growth factor protein expression was quantitated by immunoassay (MAGPIX®, Luminex®). One-way analysis of variance with Tukey's post-hoc statistical analysis was performed (n=3; p<0.05 considered significant). Ash-PELNs increased hHFDPC proliferation (1E+10; 11.9%±6.8%, p<0.05), HUVEC and hDF migration (1E+10; 82.8%±6.8% and 67.0%±15.2%, respectively, p<0.05), HUVEC tubulogenesis (1E+09; 54.9%±10.7, p<0.001) and hDF VEGF-A expression (1E+09; 39.9%±14.7%, p<0.05). Ash-PELNs also dose-dependently increased hHFDPC secreted LIF, PLGF-1, FGF-2 and VEGF-A growth factor expression (1E+10; 115.1%±26.0%, p<0.01; 139.1%±21.9%, p<0.01; 90.2%± 24.4%, p<0.05; and 102.8%±9.0%, p<0.01, respectively). Ash-PELNs show regenerative capacity in several cell lines suggesting a potential use as a plant-derived topical alternative or adjunct to human-derived PRP or exosome treatments for hair, scalp, and skin conditions.

LB1025

Histopathologic scarring outcomes of cutaneous surgery in the setting of immunosuppression

T. Greif[1], V. Liu[1, 2], A. Steahr[1], J. Powers[1]
[1]Dermatology, The University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States, [2]The University of Iowa Hospitals and Clinics Department of Pathology, Iowa City, Iowa, United States

Background: Cutaneous wound healing and scarring are poorly understood in the setting of immunosuppressive therapies for anti-rejection post solid organ transplantation. Purpose/Hypothesis: To investigate whether the concurrent use of immunosuppressive agents during cutaneous surgery correlates with less favorable scarring outcomes. Methods: A prospective cohort study at the University of Iowa studied patients undergoing elliptical excision for treatment of non-melanoma skin cancers. Clinical scarring assessments (photographs, scar width, Konica Minolta CM-700d Spectrophotometer, POSAS=patients and observer scar assessment scale, and bacterial culture swabs) as well as tissue samples from the excision/scar site collected at day 0 and day 14. Tissue samples were analyzed by an independent, blinded dermatopathologist and graded in categories of: acute inflammation, chronic inflammation, fibroblast density, collagen fiber characteristics and density of blood vessels. Categorical scores were summed to produce a total wound score with higher scores indicating features more consistent with increased scar maturity. Results: Of 23 patients recruited (all white/Caucasian, mean age 64), 17 completed requirements for analysis. 4 subjects were receiving anti-rejection immunosuppressive therapy for solid organ transplant at the time of excision. Of those on immunosuppressants, levels of acute/neutrophilic inflammation were significantly increased (MD -1.27, P=0.044, 95%CI -2.50 to -0.04) and total wound scores were significantly lower (MD 2.75, P= 0.038, 95% CI 0.18 to 5.32) indicating less mature scar formation by day 14. Conclusions: Patients on immunosuppressive anti-rejection therapies during cutaneous surgery show scars with decreased histologic markers for scar maturity indicating delayed wound healing.

LB1026

Role of skin cellular senescence in chronic wound healing

P. Dashti, T. Pirtskhalava, B. Tekin, C. Inman, L. Sales Gomez, A. Lagnado, L. Prata, D. Jurk, J. Passos, T. Tchkonia, J. Kirkland, S. Wyles
Mayo Clinic Minnesota, Rochester, Minnesota, United States

Wound healing is an essential physiological process to restore normal skin structure and function after injury. Cellular senescence, an irreversible cell cycle state in response to damaging stimuli, has emerged as a paradigm shift to disrupt the traditional concept of wound healing beyond the described inflammatory, proliferative, and remodeling phases. We hypothesized that skin senescent cells, particularly fibroblasts, play an inhibitory role in the wound healing process and contribute to delayed healing. We generated an acute wound versus chronic wound mouse model to compare cellular senescence expression and their senescence-associated secretory phenotype (SASP) including growth factors, cytokines, and extracellular matrix remodeling factors. To validate our model, we first examined wound closure, degree of inflammation, epidermal hyperplasia, and degree of dermal fibrosis among others. Then, we assessed SASP profile in normal skin, acute wound, and chronic wound. Our data demonstrate Mmp3 (matrix metalloproteinase 3), Mmp9, Mcp (membrane cofactor protein), TGF-β (transforming growth factor β) which are key SASP factors involving in delayed would healing, significantly increased in chronic wounds. RNA in situ hybridization indicated increased p16ink4a (Cdkn2a) in chronic wounds, which is an intrinsic mediator in transition to cellular senescence. Furthermore, senescence-associated beta galactosidase staining showed enhanced senescent cells in chronic wounds. Taken together, cellular senescence may play a inhibitory role in chronic wound healing, and senescent cells clearance through systematic or topical senolytic treatment, may present a novel therapeutic option in the treatment of chronic wound disorders, including diabetic ulcers.

LB1027

An adipose-derived stem cell-engineered patch represents a promising treatment for chronic wounds.

N. C. Brembilla[1, 2], A. Modarressi[3], D. André-Lévigne[3], H. Vuagnat[4], S. Durual[5], L. Marger[5], W. Boehncke[1, 2], K. Krause[6, 2], O. Preynat-Seauve[6]
[1]Dermatology, Hopitaux Universitaires Geneve, Geneve, Switzerland, [2]Pathology and Immunology, Universite de Geneve Faculte de Medecine, Geneve, GE, Switzerland, [3]Division of Plastic, Reconstructive and Aesthetic Surgery, Hopitaux Universitaires Geneve, Geneve, Switzerland, [4]Program for Wounds and Wound Healing, Hopitaux Universitaires Geneve, Geneve, Switzerland, [5]Laboratory of Biomaterials, Universite de Geneve, Geneva, Switzerland, [6]Laboratory of Therapy and Stem Cells, Hopitaux Universitaires Geneve, Geneve, Switzerland

Mesenchymal stem cell (MSC)-based therapies are emerging as promising approach to treat chronic wounds, although uncontrolled/sub-optimal delivery represents a major threat to their wide-spread clinical adoption. Here we report a comprehensive pre-clinical evaluation program on the mechanism of action, efficacy and safety of an easy-to-use patch that concentrates MSC extracted from the human fat (ASCs) in a clinical-grade sponge made of porcine crosslinked-gelatin. ASC were derived from the abdominal/skin fat of four ischemic patients. Microarray data revealed that ASC grown within the patch upregulate the transcription of genes important for wound healing, including VEGF, CXCL12 and FGF7. The patch acted both as a concentrator and a reservoir of ASC-derived factors and sponge-derived fragments having healing capacity. The ASC-patch promoted dermal fibroblast survival, epidermal epithelialization and neovascularization, as shown in a chick chorioallantoic membrane model. No tumor formation was observed in immunodeficient Nude mice subcutaneously transplanted with the ASC-patch. Finally, ASC-patches prepared from syngeneic rats promoted faster healing and re-vascularization of full-thickness skin defects in a pe-clinical rat animal model of ischemic wounds. Together, we provide here compelling pre-clinical evidence that patches concentrating ASCs within crosslinked gelatin may represent a convenient and effective tool for the management of chronic wounds.

LB1028

Neuropeptides influence stem cell fate during wound healing.

A. Khalifa[1, 2, 3], B. Abegaze[1, 2], T. Weisenberger[1, 2], S. Sanad[3], T. AbuElnasr[3], R. Ghadially[1, 2]
[1]Dermatology, University of California San Francisco, San Francisco, California, United States, [2]dermatology, San Francisco VA Health Care System, San Francisco, California, United States, [3]zoology, Zagazig University, Zagazig, Sharkia, Egypt

Little is known about the kinetics of hyperproliferation in wound healing, the role of stem cells (SC) and transit amplifying cells (committed progenitors - CP), and relevant SC signaling pathways. To study SC and CP proliferation during wound healing, 6mm wounds were made on the flanks of mice and cell divisions assessed 4d post-wounding using fluorescence microscopy; *in vivo* using tubulin expression and in vitro using Numb and keratin 1 expression. In wounded vs. intact skin *in vivo* there were increased basal, but not suprabasal, divisions, with an increase in both perpendicular (asymmetric self-renewal- ASR) and parallel (symmetric) divisions in in the basal layer (4.9 vs. 1.3 /10 cm P=0.001 and 6.8 vs. 4.6 /10 cm P=0.01, respectively, n=4). In vitro, where ASR and symmetric self-renewal (SSR) SC divisions can be distinguished from symmetric differentiation (SD) of CPs, an increase in both ASR and SSR divisions per 100 cells was seen, (3.3 vs. 2.1, P=0.01 and 0.7 vs. 0.3, P=0.03 - n=4) with no significant change detected in symmetric differentiation (8.5 vs. 8.3, ns), suggesting that the proliferative response early on is confined to SCs. Substance P is important for wound healing and induced epidermal proliferation, improved wound healing, and increased epidermal thickness, as previously shown. Substance P treatment of wounds increased total, ASR, and SD divisions per 100 cells, but not SSR (total 12.9 vs. 9.0, ASR 3.1 vs. 1.9, SSR 0.9 vs. 0.7, and SD 9.1 vs. 6.7, all P<05, except SSR P=.2). In summary, while psoriasis is associated with increased ASR and squamous cell carcinoma with increased SSR, wound healing is associated with increases in both ASR and SSR. Substance P induced improvement of wound healing is associated with increased asymmetric SC self-renewal divisions. Understanding the effects of neuropeptides on SC behavior is important in the search for better wound healing therapies.

LB1029

Correlation of merkel virus-specific CD8 T cells with response to immunotherapy in merkel cell carcinoma

T. Pulliam[1], S. Jani[1], L. Jing[1], J. Zhang[2], R. Kulikauskas[1], C. Church[1], C. Garnett-Benson[3], K. Paulson[4], D. Pardoll[2], D. Koelle[1], S. Topalian[2], P. Nghiem[1]

[1]Medicine, University of Washington, Seattle, Washington, United States, [2]Bloomberg~Kimmel Institute for Cancer Immunotherapy, Johns Hopkins University, Baltimore, Maryland, United States, [3]Bristol-Myers Squibb Co, New York, New York, United States, [4]Swedish Cancer Institute, Seattle, Washington, United States

PD-1 pathway blockade has revolutionized oncology, though most patients do not derive durable benefit and accurate prediction of response is not currently possible. Cancer-specific CD8 T cells are thought to mediate responses, however identifying these cells is difficult because tumor antigens are typically patient-specific. Merkel cell carcinoma (MCC), driven by Merkel cell polyomavirus (MCPyV) oncoproteins in ~80% of cases, is an attractive cancer for the study of tumor-specific T cells due to shared, almost invariant viral antigens and a low tumor mutational burden. Using samples from a recent trial of neoadjuvant nivolumab in MCC (NCT02488759), we studied anti-PD-1 resistance mechanisms by interrogating MCPyV-specific CD8 T cells over the course of therapy. We used a newly expanded panel of 16 MCPyV-specific HLA-I tetramers with 26-plex flow cytometry to study T cells in the blood of 35 patients (21 virus-positive with HLA-matched tetramers) before, 2 and 4 weeks after anti-PD-1. Strikingly, patients with detectable circulating MCPyV-specific CD8 T cells before treatment (n=11) had longer recurrence-free survival compared to patients without these T cells in blood (n = 3; p = 0.00021). Using scRNAseq to compare MCPyV-specific CD8 T cells in blood vs tumor from 7 separate non-trial patients, we found that 0-50% vs >90% of cells, respectively, were within "dysfunctional clusters" expressing genes encoding Tox, PD-1 and Lag3. In summary, cancer-specific CD8 T cells in blood were less dysfunctional than their intratumoral counterparts and their frequency strongly correlated with response to anti-PD-1 in MCC. These results suggest adoptive cell therapy or vaccines that augment cancer-specific T cell numbers or function may benefit patients with anti-PD-(L)1-refractory MCC.

LB1030

Automated image analysis methods to detect mast cells in skin

K. Tsuji, N. Barrezueta, E. Price, B. Klein

Merck & Co Inc, Kenilworth, New Jersey, United States

Mast cells are a key cell in the innate immune system. These cells are tissue resident and are considered to act as sentinels for the detection of a multitude of different ligands. Activation of mast cells via stimulation of IgE receptors underlies allergic reactions to food, pollen, pet dander, and many other allergens. Stimulation of mast cells results in their activation leading to degranulation – a process in which the cell releases extracellular mediators like histamine, proteases, and cytokines. These mediators can then go on to activate other cell types nearby or can recruit more immune cells to the tissue. Some chronic pruritic diseases are thought to involve mast cells in the skin and many studies have demonstrated an increase in total mast cell number in skin from atopic dermatitis (AD) or allergic contact dermatitis (CAD). Previous methods relied on staining of skin biopsy sections with a chromogenic stain (like toulidine blue), then manual counting of the mast cells in small sections of the biopsy. More recently, the use of fluorescently-tagged avidin has been used to detect mast cells in skin biopsies, which represents a more specific detection method. A challenge still remains in the differentiation between quiescent (resting) and active (or degranulated) mast cells. Here we describe a method for the automated detection and quantification of quiescent and resting mast cells based on cell morphology, using commercially available image software analysis tools. Our method was applied to human skin biopsies that were treated to induce degranulation and to skin biopsies collected from AD patients. In contrast to earlier results, we do not observe an increase in total mast cell number nor in the number of active mast cells in the skin of AD patients. This method allows for the unbiased detection of mast cells in human skin with out the need to develop de novo proprietary algorithms.

LB1031

Using social media to elucidate the patient experience with common acne treatments

B. Chandani[1], J. Jueng[1], R. Dellavalle[2], I. Brooks[2], O. Burton[2], S. Shaikh[2], V. Bhupalam[1], J. Solomon[1, 2]

[1]University of Central Florida College of Medicine, Orlando, Florida, United States, [2]University of Illinois Urbana-Champaign, Urbana, Illinois, United States

Acne affects 50 million Americans annually and can have marked psychological effects on patients, who may also have poor experiences with different treatments. Our goal was to determine emotional sentiment associated with patient global impressions of change (PGIC) for common treatments. Through Brandwatch, a social media analysis tool, our team at The University of Illinois mined 90,121 social media posts pertaining to key words in relation to acne and expressions of satisfaction or disappointment, for twelve common treatments: adapalene, azelaic acid, benzoyl peroxide, clindamycin, dapsone, doxycycline, erythromycin, isotretinoin, minocycline, salicylic acid, spironolactone, and tretinoin. Using SPSS software, an independent samples t-test was conducted comparing positive and negative underlying emotional sentiments in conjunction with a positive PGIC. The percentage of posts with a positive PGIC out of the total number of posts ranged from 63% (salicylic acid) to 84% (spironolactone), indicating a degree of patient satisfaction with the results of these treatments. Each treatment group had a greater percentage of positive PGIC posts than negative or neutral. The independent samples t-test comparing means of negative and positive emotional sentiment in conjunction with a positive PGIC was statistically significant across all treatments ($p < 0.008$). Most patients perceived a positive clinical change with these twelve medications but had negative emotional sentiments. This tells the story of a patient's struggle with common regimens. The large source of data that exists on social media can provide physicians with a means to discover ways to best support patients through treatment.

LB1032

Influence of the covid-19 pandemic on emergency room usage for dermatologic ailments

S. Gotewal, A. Good, M. Wilkerson

The University of Texas Medical Branch at Galveston School of Medicine, Galveston, Texas, United States

We performed a cross-sectional retrospective analysis to explore the effects of COVID-19 on United States ER visits for various acute dermatologic diagnoses. ER visits from 2010-2020 were identified on the TriNetX database and divided into three groups of diagnoses using ICD-10 codes: Herpes Zoster, Cellulitis, and SJS. Data was stratified by 1-year intervals and age (pediatric <18 years-old or adult ≥18 years-old). Data was analyzed using chi-square with $p<.05$ considered significant. Of the 24,620,393 ER visits identified between 2010-2020, 543,121 Herpes Zoster, 2,825,192 Cellulitis, and 6,888 SJS cases were identified. ER visits for Herpes Zoster, Cellulitis, and SJS decreased by 7.3%, 10.8%, and 17.8% during 2020 compared to prior years, respectively. Pediatric patients had a greater decrease in Herpes Zoster and Cellulitis-related ER visits than adults (49.1% and 23.4%, respectively). All the above differences were statistically significant ($p<.05$). There was no significant difference in the proportion of pediatric ER visits for SJS in 2020 compared to prior years ($p=.12$). In conclusion, ER visits for Herpes Zoster, Cellulitis, and SJS declined significantly during the COVID-19 pandemic. Avoidance of the ER due to fears of COVID-19 infection could delay treatment of acute dermatological conditions, potentially increasing the risk of complications, morbidity, and mortality for those populations who rely on the ER because they lack access to telemedicine and other healthcare resources.

LB1033

Thrombin contributes to atopic dermatitis pathogenesis and staphylococcus aureus skin colonization in children

A. Filuta[4], P. K. Amezcua[4], W. Chang[4], J. Biagini[4], J. Kroner[4], H. He[3], B. Grashel[4], C. Almasri[4], L. Martin[3, 2], J. S. Palumbo[1, 2], G. K. Khurana Hershey[4, 2], M. G. Sherenian[4, 2]

[1]*Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States,* [2]*Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, United States,* [3]*Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States,* [4]*Asthma Research, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States*

Rationale: Adults with atopic dermatitis (AD) have an increased risk for thromboembolic events. Clotting factors have been shown to impact Staphylococcus aureus (S. aureus) colonization. However, the interplay between pediatric AD, thrombin, and S. aureus remain under investigated. Methods: To assess thrombin in pediatric AD we performed a plasma thrombin generation assay on samples from the Mechanisms of Progression of Atopic Dermatitis to Asthma in Children (MPAACH) cohort. We used SCoring Atopic Dermatitis (SCORAD) score, transepidermal water loss (TEWL), and S. aureus colonization as AD measures. To determine whether thrombin contributes to AD pathogenesis we used dabigatran, a direct thrombin inhibitor, in our established murine model of AD. All mice underwent objective disease severity assessments and TEWL evaluations. Analyses were performed using GraphPad Prism, version 9.0.0. Results: In children, we found that increased total thrombin generation was associated with moderate-to-severe AD (P-value=0.02) and increased TEWL at lesional (P-value=0.001) and never-lesional (P-value=0.02) sites; increased peak thrombin was associated with moderate-to-severe AD (P-value=0.05) and increased lesional TEWL (P-value=0.01). Peak thrombin was also associated with skin colonization by S. aureus (P-value=0.01). In our mouse mode of AD, thrombin inhibition attenuated disease development by decreasing disease severity (P-value<0.001) and TEWL (P-value<0.001). Conclusions: Our combined human and murine findings highly demonstrate a key role for thrombin in AD pathogenesis. Further, these data support a role for thrombin in the colonization of skin by S. aureus in children with AD.

LB1034

Chromatin remodeling by warfare arsenicals in porcine skin

R. Srivastava, J. Khan, S. Muzaffar, P. Guroji, M. Athar

Dermatology, The University of Alabama at Birmingham School of Medicine, Birmingham, Alabama, United States

Accidental or deliberate exposure to highly toxic chemical warfare agents and many industrial chemicals pose significant threat to environment and human health. Skin exposure to arsenical vesicants, developed as chemical weapons in World War I/II, causes painful inflammation and skin wounds. The lack of human relevant animal models has been a key impediment for defining the underlying mechanism/s of these devastating chemicals. The Gottingen minipig skin closely resembles to human skin and we demonstrate that cutaneous exposure to molar equivalent doses of lewisite or structurally related arsenicals [diphenylchlorarsine (DPCA), diphenylcyanoarsine (DPCYA), diethylchlorarsine (DECA)] leads to skin inflammation and wound that progresses in dose- and time-dependent manner. Cutaneous arsenical exposure leads to overexpression of BRD4, an epigenetic reader. This results in lysine hyperacetylation of H3 and H4 histone target proteins, H3K9ac, H4k5ac and H4k8ac. IHC analysis confirmed that BRD4, H3 and H4 histone hyperacetylation were not only confined to epidermal cells but were also seen in dermally infiltrated immune cells. Transcriptomic analysis of lewisite-challenged skin demonstrated significant alterations in expression of BRD4-regulated inflammatory and tissue damage related genes. Although, lewisite and other structurally related arsenicals manifested similar molecular changes, they differed in magnitude. The response was lewisite>DPCA =DPCYA>DECA. Employing human skin keratinocytes in cultures, we demonstrated that BRD4 inhibitors CPI0610, ZL0420, JQ1 and iBET significantly reduced arsenical-induced changes in the expression of BRD4 and acetylation H3 and H4. Expression of pro-inflammatory cytokines was also decreased. Our data suggest that the porcine skin model can recapitulate the molecular pathogenesis of vesicants that is observed in humans. BRD4 is a critical epigenetic regulator of arsenical-mediated cutaneous inflammation and can serve as a viable druggable target for developing antidotes against these and similar chemicals.

LB1035

Three-dimensional structural and transcriptomic analyses reveal disruption and dysfunctional repair of the dermal extracellular matrix in early striae gravidarum

F. Wang, K. Calderone, L. C. Tsoi, J. Voorhees, G. J. Fisher

University of Michigan, Ann Arbor, Michigan, United States

The pathogenesis of striae gravidarum (SG) remains unclear. We obtained samples of early, abdominal SG emerging within 4-45 days and adjacent, involved skin (control) in pregnant women (n=8). We performed second harmonic generation (SHG) imaging coupled with multiphoton microscopy, and transcriptomic profiling (RNA-Seq). In SG, SHG imaging revealed that dermal type I collagen bundles appeared disorganized and separated. In the "spaces" between bundles, there were thin, loosely packed, wavy collagen fibrils, which were 47% less dense and 56% thinner (cross-sectional width 12.6±1.3 μm [mean±SEM]) than intact collagen bundles (28.3±2.0 μm). In these areas, immunofluorescence staining showed disrupted elastic fibers, which appeared as short, disorganized, thread-like "fibrils" that occupied 10% more volume but were 54% thinner (2.0±0.2 μm) than intact elastic fibers (4.3±0.3 μm). Three-dimensional video rendering of images revealed many type I procollagen-producing fibroblasts within areas of disrupted extracellular matrix (ECM). Compared with control, transcriptomic profiling of SG revealed 860 differentially expressed genes (at least 2-fold change, p<0.05, 443 upregulated, 417 downregulated). Among ECM-related (matrisome) genes, collagens (e.g., types 1/3/5), glycoproteins (e.g., CTGF, fibrillins 1/2), proteoglycans (e.g., lumican, biglycan), ECM regulators (e.g., LOX), and secreted proteins (e.g., VEGFC) were upregulated. Genes associated with the WNT, FGF and TGF-β pathways were downregulated. Pathway analysis revealed enrichment of genes involved in ECM organization, collagen biosynthesis/assembly, ECM degradation, elastic fiber formation, and angiogenesis. These observations indicate that disorganization of the ECM, including type I collagen and elastic fibers, occurs early in SG development. Novel insights into altered molecular pathways provided by global transcriptome analysis suggest that inability to restore collagen bundle formation may lead to the development and persistence of "mature" atrophic SG.

LB1036

Artificial intelligence for the automated assessment of psoriasis severity

T. Okamoto[1], M. Kawai[2], S. Shimada[1], T. Kawamura[1]

[1]*Dermatology, Yamanashi Daigaku Igakubu Daigakuin Sogo Kenkyubu Igakuiki, Chuo, Yamanashi, Japan,* [2]*Pathology, Yamanashi Daigaku Igakubu Daigakuin Sogo Kenkyubu Igakuiki, Chuo, Yamanashi, Japan*

PASI score is globally used to assess disease activity of psoriasis. However, it is relatively complicated and time-consuming, and the score will vary due to the inconsistent subjectivity between dermatologists. Therefore, an AI system capable of assessing psoriasis severity will be useful. We showed the progress of our AI research at SID meeting last year. Recently, we have established a novel AI platform for the evaluation of psoriasis severity by using deep convolutional neural networks. 705 psoriasis images of the trunk's front and back were used in our research. Considering the relatively small number of images, we used data augmentation techniques to expand the data. A psoriasis expert's scores were used as teacher data. Various convolutional neural network models and hyperparameters were adjusted using a five-fold cross validation. From these adjustments, we discovered that fine-tuning Imagenet2012-pretrained InceptionV3 whose last linear layer was replaced by two-layer perceptron (30 hidden units and five output units) exhibited the best performance. To validate our deep learning system, 10 images were selected as test sets and were excluded from the training sets. The AI assessment was almost consistent with the clinical severity. We examined whether AI assistance would affect human scoring. 13 dermatologists and nine medical students were invited as evaluators. Mean absolute differences from AI scores and standard deviation among evaluators reduced with AI assistance. Moreover, the evaluator's scores got close to the teacher's score with AI's assistance. We have developed an AI system capable of assessing psoriasis severity simply by uploading a single clinical image. An easy-to-use scoring system would help dermatologists and patients with psoriasis.

LB1037

Aberrant regulation of protein translation controlled by eukaryotic initiation factor 4F (eIF4F) in the pathogenesis of hidradenitis suppurativa (HS) and associated cutaneous squamous cell carcinoma (cSCC)

L. Jin, M. P. Kashyap, C. Raman, C. A. Elmets, M. Athar
Dermatology, University of Alabama at Birmingham, Birmingham, Alabama, United States

HS is a chronic inflammatory skin disorder and often associated with neoplastic growth of cSSC. Between 1%-3.2% of HS patients develop cSCC, however, the molecular pathogenesis of this disease remains undefined. Here, we demonstrate the pathogenic role for the 5'cap-binding protein complex eIF4F in HS and lesion-associated cSSC. This complex is composed of the scaffold protein eIF4G, the cap-binding protein eIF4E and a DEAD-box helicase eIF4A1. Employing confocal microscopy, we observed elevated expression of eIF4E, eIF4G, and eIF4A1 in HS skin that co-localized in hyperproliferative keratinocytes and associated with enhanced eIF4E translation targets, Cyclin D1 and c-Myc. Proliferating cell nuclear antigen (PCNA) positive cells were also positive for c-Myc expression in the HS epithelium. From HS global transcription profiles from two public databases, we identified 734 eIF4F-related genes that included those involved in tumorigenesis and skin inflammation. Among these, eIF4F complex-associated RAS/MEK/ERK and PI3K onco-signaling pathways were significantly elevated in HS lesional skin. BrdU-labeling and cell cycle assays revealed that keratinocytes from HS lesional skin elicit rapid G1/S progression. The overexpression of eIF4E/eIF4A1/eIF4G proteins was contiguous throughout HS lesion and associated cSCC. Oncogenic Cyclin D1 and c-Myc were co-localized in the nucleus within some hyperproliferative outer epidermal cells of the tumor invading dermis, thereby confirming their role in cSCC pathogenesis. These onco-signatures were also present in some epithelial cells of HS tunnels. We also observed activation of ERK signaling, which under clinical circumstances have been shown to be involved in the pathogenesis of cSCC in humans and experimental animals. In summary, our data indicates that the 5'-cap-dependent translation pathway is critical in the underlying pathogenesis of hyperproliferative HS lesions as well as associated cSCC.

LB1038

Development of experimental models for hidradenitis suppurativa (HS).

M. P. Kashyap[1], R. Sinha[1], K. Goliwas[2], L. Jin[1], J. Deshane[2], C. A. Elmets[1], C. Raman[1], M. Athar[1]
[1]*Department of Dermatology, The University of Alabama at Birmingham School of Medicine, Birmingham, Alabama, United States,* [2]*Pulmonary, Allergy and Critical Care Medicine, The University of Alabama at Birmingham School of Medicine, Birmingham, Alabama, United States*

HS is inflammatory skin disease with painful, deep-seated, inflamed lesions in the apocrine gland-bearing skin areas. The lack of an animal model, which could faithfully recapitulate most disease features, is a major impediment in defining HS pathobiology and developing effective treatment. In this study, we developed experimental HS models to elucidate the underlying mechanisms of HS pathobiology, and screen potent drug molecules. Keratinocyte cultures from HS skin provide one such model. Profiling of gene signatures showed that these keratinocytes retain the expression of many inflammatory and cell signaling signature genes altered in HS skin and these signatures remained intact up to three passages. These HS keratinocytes cultured in multiwell plates can be used in high throughput assays to test the efficacy of various anti-inflammatory molecules. Furthermore, we established three *ex vivo* HS skin tissue culture models. These are air-liquid (A-L) interface, liquid-submersion (L-S) and three-dimensional perfusion bioreactor (Bio) culture approaches for *ex vivo* HS skin tissues. We established that these platforms were effective for culturing skin samples up to 14 days. Inflammatory gene profiling at day-3 and day-14 of cultures showed that all three-culture platforms provide dynamic modulation of genes. Within each of the cultures, there were clusters of genes that were unchanged from that on day 0; however, between cultures there were differences in the set of genes unchanged from day 0. Based on profiling of cytokines/chemokines in supernatants from vehicle- or drug-treated (JQ1 and Lenalidomide) HS skin cultures, we determined that the culture platforms are informative for screening HS-specific anti-inflammatory drugs. We are currently developing a humanized (NGS) mouse model grafted with human HS skin as a novel *in vivo* strategy to study HS and develop effective treatments.

LB1039

New insights into neuronal itch mechanisms by targeting IL-13Rα1 with eblasakimab

Y. Miron[1], P. Miller1, C. Firth[2], F. Cevikbas[2]
[1]*AnaBios Corp, San Diego, California, United States, 2Aslan Pharmaceuticals, Menlo Park, California, United States*

Chronic itch is a cardinal feature of multiple type-2 driven skin disorders exemplified by atopic dermatitis (AD). The signaling of itch in AD has been recently postulated to be amplified by the inflammatory cytokines present within the skin. In inflammatory skin diseases, cytokines exacerbate the immune responses, disrupt the skin barrier, and drive the disease pathology. The direct neuronal activation by type-2 canonical cytokines was first described with Interleukin-31 (IL-31). Recently, it has been shown that IL-13 acts as a neuronal enhancer for a multitude of different itch pathways in human neurons. Our objective is to understand the relevance of targeting the IL-13 receptor alpha 1 (IL-13R α1), on human sensory neurons and how this might result in cellular and intracellular changes altering neuronal activity. To study these phenomena, we used eblasakimab, a monoclonal human IgG4 antibody, which binds to the human IL-13Rα1 with nanomolar affinity. By binding to the receptor, eblasakimab prevents signaling of IL-4 and IL-13 through the type-2 receptor, which is expressed on a multitude of different immune and non-immune cells except for T-cells. Using an ex-vivo human neuronal model system, human dorsal root ganglia (hDRG) neurons were treated with IL-4 or IL-13 alone, or in combination, and were subsequently subjected to pruritogens. Neuronal responses were captured by live cell calcium imaging. Our data with human sensory neurons pre-stimulated with IL-4, IL-13 and their combination showed a neuronal enhancer effect for IL-4, and IL-13 with no obvious synergy or combined additive enhancer effects on pruritic pathways. Eblasakimab significantly reduced neuronal responses to IL-4, IL-13, and their combination by more than 40% to control conditions ($p = 0.0001$). Moreover, our finding that eblasakimab treatment reduced neuronal responses below vehicle group suggests that IL-13Rα1 has an additional role in neuro-immune modulation beyond the cytokine-related neuronal itch sensitization in inflammatory diseases.

LB1040

Treatment of sweet syndrome by molecular identification of PIK3R1 mutation

S. Bhattacharya[1], E. Sheng[1], C. Murphy[1], J. Wei[1], A. Kersh[1], C. Nelson[2], J. Bryer[1], H. Ashchyan1, K. Steele[1], A. Forrestel[1], J. T. Seykora[1], R. Micheletti[1], W. James[1,3], M. Rosenbach[1], T. H. Leung[1,3]
[1]*Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States,* [2]*Dermatology, Yale University, New Haven, Connecticut, United States,* [3]*Medicine, VA Medical Center Corporal Michael J Crescenz, Philadelphia, Pennsylvania, United States*

Acute febrile neutrophilic dermatosis (Sweet syndrome) is a potentially fatal multiorgan inflammatory disease characterized by fever, leukocytosis, and rash with a neutrophilic infiltrate. Disease pathophysiology remains elusive. Corticosteroids and steroid sparing agents remain mainstays of treatment, but refractory cases pose a clinical challenge. Whole genome sequencing and transcriptomic profiling of a refractory Sweet syndrome patient identified elevated IL-1 signaling in lesional skin caused by a PIK3R1 gain-of-function mutation in neutrophils. Targeted treatment with an IL-1R1 antagonist resulted in a dramatic therapeutic response and enabled tapering of corticosteroids. Molecular study of this patient improved our understanding of pathophysiology and guided therapy.

LB1041

Machine learning-based single cell analysis of prurigo nodularis identifies a unique population of CD14+pERK+ macrophages correlating with itch intensity

J. Patel, J. Deng, V. Parthasarathy, M. Szeto, M. Marani, C. Trinh, K. Lee, O. Olapido, M. Kwatra, S. Kang, M. P. Alphonse, S. G. Kwatra
Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Prurigo nodularis (PN) is a chronic inflammatory skin disease characterized by intense itch and predominantly affects African Americans (AA). Little is known about its pathogenesis and there are currently no FDA approved therapies for PN. To further characterize single cell interactions in the skin we performed imaging mass cytometry (IMC) from prospectively collected formalin-fixed, paraffin-embedded skin biopsies of 12 PN patients and 4 healthy controls (HC). Sections were stained with a panel of 19 isotopic antibodies against immune and epithelial markers. Machine learning was used to segment cells via the bodenmiller pipeline with analysis done in histoCAT. Unsupervised clustering revealed PN patients have unique populations of CD14+ macrophages, keratinocytes (KC), CD11C+CD14+ myeloid dendritic cells (mDC), CD3+ T cells, sMA+ endothelial smooth muscle cells (ESM), CD63+ cells, CD33+ cells, and CD15+ myeloid cells. PN also had an increase in CD15+ myeloid cells (50±87 vs 21±19, p=0.02), CD14+ macrophages expressing pERK1/2 (47±60 vs 9±5, p=0.002), and CD14+KCs (331±565 vs 21±33, p=0.008). Both percentage and absolute number of circulating blood eosinophils correlated positively with CD3+ cells (r=0.955, p=0.003; r=.8929, p=0.01) and negatively with vimentin (r=-0.8469, p=0.02; r=-0.8214, p=0.03) and pERK (r=-0.8108, p=0.04; r=-0.7857, p=0.05). Neighborhood-based single cell analysis revealed CD14+ KCs interacting with CD14+ macrophages and ESM interacting with CD14+ macrophages, CD63+ cells, and CD11C+CD14+ mDCs (p<0.05). Itch intensity positively correlated with frequencies of CD14+ pERK macrophages (r=0.861, p=0.006). The discovery of CD14+ pERK macrophages correlating with itch and interacting with activated KCs may serve as a target for future therapies in PN.

LB1042

Neuroimmune mediators of pruritus in scalp psoriatic itch: An immunofluorescent analysis in a Hispanic population

L. A. Nattkemper[1], Z. Lipman[1], G. Ingrasci[1], C. Maldonado[2], J. Garces2, A. Hawash[1], E. Loayza[2], G. Yosipovitch[1]
[1]Dermatology, University of Miami School of Medicine, Miami, Florida, United States, [2]Departamento de Dermatología, Hospital Luis Vernaza, Guayaquil, Ecuador

Background: Scalp psoriatic itch is a prevalent yet understudied condition with numerous associated treatment challenges. Objective: The current study aims to enhance our understanding of scalp psoriatic itch pathophysiology in order to guide current management and identify novel treatment targets. Methods: Immunohistochemical analysis of known neuroimmune mediators of pruritus was conducted using scalp biopsies from 27 patients with scalp psoriasis. Patients were grouped per their itch NRS rating (taken at time of biopsy) into mild (NRS 1-3), moderate (NRS 4-6), and severe (NRS 7-10). Results: Intraepidermal nerve fiber (IENF) density, Substance P (SP), PAR2, TRPV3, TRPM8, and IL-23 expression were all significantly elevated in scalp psoriasis patients compared and significantly correlated with itch intensity. TRPV1, TNFα, and IL17 expression was increased in scalp psoriasis but did not correlate with itch intensity. Conclusion: Our results are congruent with prior data that scalp psoriasis pathophysiology is largely non-histaminergic and rather, driven by a Type 1 and IL-17 dominant immune response. Additionally, scalp psoriatic itch may have a greater neurological component than previously thought.

LB1043

COVID-19-associated pruritus is non-histaminergic mediated.

A. Labib[1], L. A. Nattkemper[1], A. Vander Does[1], T. Ju[1], S. Cacciapuoti[2], M. Vastarella[2], G. Fabbrocini[2], G. Yosipovitch[1]
[1]Dermatology, University of Miami School of Medicine, Miami, Florida, United States, [2]Dermatology, Universita degli Studi di Napoli Federico II, Napoli, Campania, Italy

Background: Infection of coronavirus-19 (COVID-19) has been shown to cause numerous types of skin findings in approximately 20% of affected individuals. Skin manifestations include exanthema, vascular changes, urticaria, and acral eruptions. Namely, the acral lesions consisting of a chilblain, pernio-like phenotype has a high prevalence. Pruritus and mild pain are the most reported symptoms associated with this skin lesion; however, the mechanism of itch has yet to be explored. In this study, we aimed to identify itch-related biomarkers in samples of lesions from COVID-19 patients and correlation with itch intensity to shed light on the underlying pathogenesis and guide treatment. Materials and Methods: Skin biopsy samples were collected from 19 patients with COVID-19-associated skin lesions and 5 healthy individuals. At the time of biopsy, itch intensity was quantified using itch NRS. Samples were treated with fluorescent immunohistochemistry to evaluate for various pruritic biomarkers. Results: Two itch-associated biomarkers, PAR2 and tryptase, were found to be upregulated following analysis. Both PAR2 and tryptase were positively correlated with itch intensity in patients with COVID-19-associated skin lesions. Histamine was not found to be elevated or correlated with itch intensity. Additionally, TRPA1 and Substance P were also found to be over expressed in COVID-19-associated skin lesions. Conclusion: The findings of this study suggest that itch in COVID-19-associated skin manifestations is medicated by non-histaminergic mechanisms. Given these results, it would be inadvisable to use antihistamines to treat this condition. Treatments that would reduce the activity of PAR2, such as topical or oral minocycline, would be a more appropriate choice of treatment.

LB1044

Inhibitors of CDK4/6 and HIF2a induce immunogenic cell death in merkel cell carcinoma cells

J. H. Lee[1], J. Lee[2], T. Pulliam[1], K. Paulson[3], V. Voillet[3], A. Berndt[1], C. Church[1], K. Lachance[1], S. Y. Park[1], E. Cromwell[3], R. Gottardo[3], A. Chapuis[3], P. Nghiem[1]
[1]University of Washington, Seattle, Washington, United States, [2]University of Washington, Seattle, Washington, United States, [3]Fred Hutchinson Cancer Research Center, Seattle, Washington, United States

Cyclin-dependent kinase 4 and 6 (CDK4/6) are critical components of the cell cycle and have been implicated in carcinogenesis and, more recently, immunosurveillance. This effect may be mediated by upregulation of PD-L1 expression as well as cell death via reactive oxygen species (ROS). Indeed, CDK4/6 inhibition alters mitochondrial function to release ROS. Merkel cell carcinoma (MCC) is an aggressive form of skin cancer that can be controlled with PD-1-based immune therapy in about half of cases. Treating MCC cells with a CDK4/6 inhibitor, palbociclib, increased transcription from the PD-L1 gene and elevated PD-L1 cell surface expression. The increase in PD-L1 gene expression was due to induction of HIF2a, a transcriptional activator of PD-L1. A HIF2a-specific inhibitor (TC-S7009), was used to test the importance of blocking the activity of HIF2a, which prevented palbociclib-induced PD-L1 transcription and, interestingly, potently enhanced MCC cell death. We further studied the mechanism of cell death and found that targeting both CDK4/6 and HIF2a caused suppression of a cysteine uptake regulatory protein, SLC7A11, critical for cellular redox balance. This was mediated by upregulation of an antisense long noncoding RNA known to block expression of the SLC7A11 redox regulatory protein. Reduction in SLC7A11 level has been associated with ferroptosis, an immunogenic, iron-dependent cell death. Indeed, when co-treatment was tested in several MCC cell lines, the level of SLC7A11 was dramatically reduced, and cell death that was immunogenic in nature was markedly augmented. Thus, our studies indicate that targeting CDK4/6 plus HIF2a can promote immunogenic cell death in MCC cells. Taken together, these findings suggest that hypoxia induced by cell cycle inhibition could be exploited to augmented effective cancer immunotherapy.

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