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IL-17A induces heterogeneous macrophages, and it does not alter the effects of lipopolysaccharides on macrophage activation in the skin of mice
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Macrophages are central to inflammatory response and become polarized toward the M1 or M2 states upon activation by immunostimulants. In this study, we investigated the effects of lipopolysaccharides (LPS) and interleukin (IL)-17A on the activation of macrophages in vivo in mouse skin. We examined whether macrophages are activated in the skin of immunogold (IMQ)-treated mice, a model for IL-17A-induced peri-orificial skin inflammation, and flaky-tail (ft) mice. IL-17A induced non-uniform macrophage activation in the skin of both ft mice and normal mice. IL-17A independently increased the expression levels of iNOS, CXCR1, CD206, phospho-STAT1 and phospho-STAT3 proteins in the skin of ft mice, and the effects of LPS were not altered by IL-17A. The expression levels of these proteins were increased in the skin of ft mice, compared with normal mice, but remained unchanged in the skin of normal mice treated with LPS and IL-17A. However, percentages of IFN-$\gamma$-secreting CD8$^+$ T cells were decreased in B cell-specific PTEN-deficient mice. In regard to Granzyme B secretion by tumor-infiltrating NK cells, there was no difference between both mice. The numbers of tumor-infiltrating Bregs were significantly increased in B cell-specific PTEN-deficient mice. More than 20% of the tumor-infiltrating B cells consisted of Breg in both B cell-specific PTEN-deficient mice and control mice. In addition, most of tumor-infiltrating Bregs consisted of B1 B cells, but not B2 MZ B cells. Adoptive transfer of CD5$^+$ B cells into IL-17A-treated mice increased melanoma growth while non-B1 B cells had no effect. These results suggest that tumor-infiltrating CD5$^+$ Bregs negatively regulate tumor immunity by reducing Th1 cytokine production of tumor-infiltrating CD8$^+$ T cells.

T cell specific microRNA-155 regulates the immune landscape of B16f10 melanoma
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MicroRNA-155 (miR-155) has recently been shown to regulate anti-tumor immune responses. However, its specific functions within distinct immune cell types during this process have not been delineated utilizing conditional knockout mouse models. In recently published work, we discovered a role for miR-155 expression within the T cell compartment during the immune response to syngeneic B16f10 mouse melanoma tumors. We found that miR-155 expression within T cells is required to limit syngeneic tumor growth and promote interferon gamma (IFN$\gamma$) production within the tumor microenvironment. Consequently, miR-155 expression by T cells was necessary for proper tumor associated macrophage (TAM) expression of interferon gamma inducible genes. We also found immune checkpoint blocking (ICB) reduces tumor-infiltrating lymphocytes (TIL) and cytotoxic T lymphocyte associated proteins 4 (PD-1/PD-L1) and cytotoxic T lymphocyte associated protein 4 (CTLA-4) restored antitumor immunity in miR-155 T cell conditional knockout mice by rescuing T cell IFN$\gamma$ expression, TAM activation, and T cell expression of multiple activation and effector genes expressed by tumor infiltrating CD8$^+$ T cells and CD4$^+$ T cells. ICB partially restored expression of several derepressed miR-155 targets in CD8$^+$ tumor infiltrating T cells, suggesting that miR-155 and ICB regulate overlapping pathways to promote anti-tumor immunity. More recently, we performed 10X single cell sequencing of tumor infiltrating CDA5$^+$ immune cells from the tumors of miR-155 T cell conditional knockout mice and further characterized the role of miR-155 expression within T cells in the process of shaping the immune landscape of the tumor microenvironment. This work will hopefully culminate in the ability to modulate miR-155 within the T cells of cancer patients to promote antitumor immune responses and improve clinical outcomes.
Propose that a decreased number of Tr1 cells in the blood of psoriasis patients could allow for the passive transfer of polyclonal or mouse models. One is a passive-transfer BP model that reproduces subepidermal separation shown to be effective for patients with steroid-resistant BP in clinical practice. However, the Chiba, Japan and 4 Hokkaido University, Sapporo, Hokkaido, Japan

Activated T(CD3+ CD4+ CD8- CD69+) cells. First, the proportion of activated T cells was analyzed 12 psoriasis patients’ blood & skin tissues, and 8 control blood samples to compare expression in psoriasis lesions are extremely low, we hypothesized that impaired immune regulators of peripheral immune tolerance. Recently it was identified that surface expression of FOXP3+ regulatory T cells (Tregs) tend to be inversely correlated with PASI, but the correlation was not significant (p=0.18%) compared to normal subjects (mean 0.51%) (OR 0.18). In contrast, the proportion of Tr1 cells was lower in psoriasis patients (mean 0.015). These results suggest that Tregs play a pathogenic role in SSc, while Breg plays a protection role. At the moment, we are developing an experimental therapeutic strategy for SSc via alteration of effector and regulatory T cell balance.

Proportion of CD4+ CD49b+ LAG-3+ T regulatory cells in the blood of psoriasis patients inversely correlates with psoriasis area and severity index

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First, the proportion of activated T cells was higher in psoriasis patients than in controls (mean 0.42% compared to 0.05%) (p<0.015). In contrast, the proportion of Tr1 cells was lower in psoriasis patients (mean 0.18%) compared to normal subjects (mean 0.51%) (OR 0.016). The proportion of conventional Tregs tended to be higher in psoriasis patients (mean 0.01%) compared to normal subjects (mean 0.09%), but the difference was not significant (p=0.132). Second, the proportion of activated T cells was positively correlated with PASI (r=0.65, p=0.002). The proportion of T cells was inversely correlated with PASI (r=-0.61, p=0.002). The proportion of Tregs tended to be inversely correlated with PASI, but the correlation was not significant (r=-0.35, p=0.127). Lastly, 4-color immunofluorescence identified CD4+ CD49b+ LAG-3+ DAP1+ cells in psoriasis non-lesional skin, but not in lesional skin. We propose that a decreased number of T cells in the blood of psoriasis patients could allow for excess expansion of psoriasis disease-related T cells in either lymph nodes or cutaneous compartments and thus lead to psoriasis disease progression.

Intravenous immunoglobulin reduces pathogenic antibodies, serum IL-6 levels and disease severity in experimental bullous pemphigoid

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Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by autoantibodies to type XVII collagen (COL17). Currently, systemic corticosteroids are used as first-line treatments for BP, additionally, intravenous administration of high-dose IgG (IVIG) has been shown to be effective for patients with steroid-resistant BP in clinical practice. However, the effects of IVIG on BP has not been fully investigated. To examine the effects and mechanisms of actions of IVIG, we first performed IVIG experiments using two experimental BP mouse models. One is a passive-transfer BP model that reproduces subepidermal separation in neonatal COL17-humanized (COL17-/-hu1) mice by the passive transfer of polyclonal or monoclonal murine IgG to human COL17-/-hu1 or IgG from BP patients. The other is an active BP model that is generated by the adoptive-transfer of immunized splenic cells reacting with human COL17 into adult immunodeficient COL17-humanized mice. This model continuously reproduces disease in adult mice and the disease scores in all models. Injected IVIG distributed throughout the dermis and the intercellular space of the lower epidermis. Notably, IVIG inhibited the increase of circulating IL-6 in both models, and suppressed the production of IL-6 by keratinocytes in vitro. These results suggest that IVIG on BP is associated with the reduction of pathogenic IgG and the modulation of IL-6 production.

An exploration of the histological features of dermatomyositis

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Systemic sclerosis (SSc) is an autoimmune disease characterized by inflammation in the skin and lung. Over 90% of patients are positive for autoantibodies. In addition, BAF, a strong stimulus for T cells is up-regulated in patients with SSc and correlated with the severity and activity of SSc. Thus, B cell had been considered to have a pathogenic role in SSc. However, there are two opposing B cell subsets; regulatory B cell (Breg) and effecter B cell (Beff). IL-10-producing Breg negatively regulates immune response, while IL-6-producing Beff positively regulates it. Therefore, a protocol that selectively depletes Breg while sparing Beff is a potent therapy for SSc. The aim of this study was to investigate the roles of Breg and Bef of Beff in SSc, and to develop the new treatment strategy against B cells. We generated mixed bone marrow chimeric mice with Beff-specific deletion in SSc. Chimeric model-induced SSc and dermato-sclerodermia model was induced in chimeric mice. Furthermore, we examined whether BAF regulates cytokine producing B cells and sclerodermia model. IL-6 producing Beff was increased and infiltrated into the inflamed skin in bleomycin-induced sclerodermia model. The skin and lung fibrosis of bleomycin-induced sclerodermia model was attenuated in B cell specific IL-6-deficient mice, while B cell specific IL-10-deficient mice showed more severe skin and lung fibrosis. BAF increased IL-6 production of Breg while BAF attenuated IL-10 producing Beff. Furthermore, BAF antagonist attenuated skin and lung fibrosis in bleomycin-induced sclerodermia, through reduction of Beff but not Breg. The current study indicates that Beff plays a pathogenic role in SSc, while Breg plays a protective role. BAF inhibition is a potential therapeutic strategy for SSc via alteration of effector and regulatory T cell balance.

Role of epithelial cell adhesion molecule (EpCAM) in iniquinum-induced psoriasis like dermatitis

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Psoriasis is a chronic skin disorder that is characterized by excessive skin inflammation with scaly erythema and epidermal thickness. Epithelial cell adhesion molecule (EpCAM) is a cell surface protein that is expressed by Langerhans cells (LCs), a population of epidermal dendritic cells, and normal epithelial cells. LCs control initial immune response to allergens or pathogens and therefore we hypothesized that EpCAM expression by LC might be important in regulating inflammatory immune responses. Mutations in EpCAM have been reported in patients with congenital cutaneous T-cell lymphoma, CD, who suffer from severe diabetes and high mortality rate. Therefore, we propose EpCAM as an important target of pharmacological intervention for the intestinal epithelial homeostasis. In the knockout mice lacking EpCAM in LC using human promoter, contact hypersensitivity responses were enhanced. In those mice, LC migration from the epidermis was impaired. The role of EpCAM in psoriasis, however, is still unclear. Here, we evaluated the function of EpCAM in iniquinum (IMQ)-induced psoriasis-like dermatitis using the conditional knockout mice that lacked EpCAM expression in a tamoxifen-inducible manner. Treatment with tamoxifen intraperitoneally for the first 2 days in ROSA-CreERT2 EpCAM/- mice lead to loss of EpCAM expression in skin. IMQ was applied to the ear skin after tamoxifen treatment for consecutive 6 days. Skin inflammation and epidermal thickness were attenuated in EpCAM KO mice 6 days after IMQ treatment compared with control mice. The results suggest that EpCAM may have a crucial role for the development of IMQ-induced psoriasis. We are in the process of determining which cytokines or chemokines regulate skin inflammation influenced by EpCAM and elucidating the role of EpCAM in psoriasis.
Cholesterol 25-hydroxylase expressing CD4+ T cell regulates skin inflammation

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Excessive inflammation leaves unnecessary damage in the body and must be strictly regulated by multiple mechanisms. But the details have not been fully elucidated yet. The purpose of this study is to identify a new regulatory function of CD4+ T cells. During Th subset differentiation, subset-determining cytokines always restrain differentiation to undesired subsets but the details have not been fully elucidated yet. The purpose of this study is to identify a new regulatory function of CD4+ T cells. During Th subset differentiation, subset-determining cytokines always restrain differentiation to undesired subsets. Therefore, when naive T cell is stimulated with IL-27 and TGF-β, the stimulated T cell should obtain functions that have never been identified in those four subsets. Transcriptome analysis showed that one of the genes uniquely expressed in IL-27 and TGF-β-stimulated cells was cholesterol 25-hydroxylase (CH25H), an enzyme converting cholesterol to 25-hydroxycholesterol (25HC). QPCR and mass spectrometry revealed that IL-27-stimulated CD4+ T cells expressed CH25h in Stat1-dependent manner and secreted 25HC. Exogenous 25HC induced cell death in proliferating T cells in vitro, but not in unstimulated ones. Transcriptome analysis of 25HC-treated T cells revealed that cholesterol synthesis was suppressed. Since exogenous cholesterol restored the cell death, T cells died of insufficient supply of cholesterol that is highly demanded in proliferating cells. To assess CH25h roles in T cells in vivo, interface dermatitis model that Drs-5-specific (hT) T cells directly attack epidermis was employed. Strikingly, CH25h+ H1 T cells produced IFN-γ and IL-17a, and exacerbated the disease more notably than wild-type H1 T cells did. Collectively this study revealed a new regulatory function of CH25h+ CD4+ T cells that selectively targets activated T cells to prevent excessive inflammation.

Deficiency of the G protein-coupled receptor HCA2 alters the phenotype and function of dendritic cells

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Short chain fatty acids (SCFA), bacterial products of fermentation of fiber, exert anti-inflammatory effects in the colon and the skin via induction of regulatory T cells (Treg). The most relevant receptors for SCFA is the G protein-coupled receptor 109a/HCA2. To understand the mechanism by which Treg are induced via HCA2, we generated SCFA non-responsive mice deficient in the HCA2 gene (HCA2-/-). We employed CD4+CD25- dendritic cells as an in vivo assay. HCA2-/- BMDC were less potent in taking up FITC-OVA compared to WT BMDC. The corresponding MHCII on the surface was lower in HCA2-/- BMDC indicating a reduced presentation of antigen. Moreover, we were not able to detect help to T cells in HCA2-/- BMDC indicating a reduced induction of Treg. Therefore, we conclude that HCA2 plays a role in the regulation of dendritic cells.

FcRβ1 deficiency accelerates immunoglobulin class switch and pemphigus vulgaris

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Class switch recombination (CSR) is crucial for humoral immunity but its regulation has not been sufficiently clarified. Pemphigus vulgaris (PV) is an autoimmune bullous disease caused by anti-desmoglein 3 antibody (Ab) that potently blocks desmoglein-3 (Dsg-3) crosslinking. It is not clear why anti-Dsg-3 Ab in PV patients induce CSR to IgG2b and not to IgG1 which is a general response to anti-Dsg-3 Ab. We hypothesized that the FcRβ1 deficiency could be a reason for the CSR to IgG2b in PV patients. Accordingly, we isolated IgM+ B cells from the spleen of FcRβ1-/- mice and stimulated them with NP-OVA. The frequency of switched IgG2b+ B cells and the proportion of switched IgG2b in the total switched B cells were not significantly different between WT and FcRβ1-/- B cells. However, when the mice were sensitized with TNCB, FcRβ1-/- mice had more switched IgG2b+ B cells and a 2.3 fold increase of IgG2b in the serum. In addition, the expression of IgG2b in FcRβ1-/- mice was not induced by anti-IgE AAbs to cause FcεRI expression in these mice. This indicates that FcRβ1 deficiency accelerates CSR to IgG2b in PV patients.

The argyriosporin receptor agonist 4-n-propionylphenol switches non-regulatory T cells into a regulatory phenotype

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4-n-propionylphenol (NP), an argyriosporin receptor (AHR)-agonist, was found to inhibit the pro-inflammatory Th17 cell phenotype and to promote the production of Treg. Although dendritic cells were identified as one of the major targets for NP in its immunosuppressive features, it is unknown whether NP can directly affect T cells as well. To address this issue, non-regulatory T cells (CD4+CD25-5) were isolated from trinitrochlorobenzene (TNCB)-sensitized mice and stimulated with NP or left untreated. After 48 hours cells were counted intravenously into naive recipients which were sensitized after injection; Treg (CD4+CD25-5) served as a control. Ear challenge with TNCB was significantly suppressed in CD4+CD25-5 treated with NP compared to the Treg group. Extraction of the fraction of untreated CD4+CD25-5 did not affect CHS in the recipients. Flow cytometry analysis revealed a significant increase of the Treg markers Foxp3 and Garp in response to NP, suggesting that NP shifts non-regulatory T cells into a regulatory phenotype. However, NP-Treg did not suppress the elicitation phase of CHS since the ear challenge response was not suppressed upon intravenous injection of NP. Treg-T into already sensitized mice. When injected subcutaneously into the ears of sensitized mice before challenge the ear swelling response was significantly reduced, indicating that NP-Treg upon intravenous injection may migrate into the lymph nodes but not into the skin. This may be due to the expression of specific tissue homing receptors. To prove whether NP induces Treg also in the human system CD4+CD25-5 cells were isolated from peripheral blood mononuclear cells, incubated with NP and transferred into an in vitro suppression assay. NP-Treg suppressed the proliferative capacity of the responder cells, whereas untreated CD4+CD25-5 did not. Together this implies that activation of the AHR switches non-regulatory T cells into a regulatory phenotype which is functionally suppressive.
**019**

KLK6-PAR1 signaling drives psoriasisiform manifestations in skin and bone

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Kallikrein-related peptidase 6 (KLK6) is a serine protease hypothesized to promote inflammation via cleavage of PAR1. We identified activated receptors PAR1 and PAR2. KLK6 and PAR2 were co-expressed in skin, however their biological roles remain unclear. RNAseq of lesional skin from psoriasis (Ps) patients identified KLK6 as the most increased KLK family member (n=6; 13-fold; P<0.01). Ps patients treated with etanercept show rapid KLK6 decreases that correspond to disease improvement (n=9; P<0.05). As KLK6 protein localized largely to keratinocytes (KCs), we engineered a tetrarepressible KC-KLK6 overexpressing mouse (KLK6+) to study KLK6 function. KLK6+ mice spontaneously develop a skin phenotype that ~7wks of age characterized by a ~5-fold increase in acanthosis (~19-28; P<0.001), increased IgE and phospho-Stat3+, a dense inflammatory infiltrate containing CD4+ T cells, CD11c+ DCs, F4/80+ macrophages and micro-abscesses containing Gr1+ neutrophils. RNAseq of KLK6+ mouse skin revealed correspondence with human Ps (r=0.38 and 7-16; P<0.01). By ~10wks of age, KLK6+ mice develop a psoriatic arthritis (PsA)-like phenotype, including forelimb dactylitis, and decreased bone mineral density, periarticular osteopenia with fusion and erosive changes in the sti joint and pulc symphysis. Gene repression for 4wks by doxycycline in KLK6+ mice with established disease reversed the PsA-like phenotypes. To study KLK6-PAR1/2 signaling, KLK6+ mice with or without PAR2-KO mice. KLK6+ and PAR2-KO mice showed similar skin inflammation and a delay in PsA-like outcomes. Our results identify a critical role for KLK6 in promoting Ps-like inflammation via PAR1 signaling and suggest targeting PAR1 may provide a cytokine-independent approach for treating psoriasis.

**020**

The combination of intratumoral delivery of inactivated modified vaccinia virus Ankara with systemic delivery of immune checkpoint blockade enhances antitumor immunity in many cancer patients. Poxviruses, such as modified vaccinia virus Ankara (MVA), have the potential as cancer immunotherapeutic agents. We recently showed that intratumoral (IT) delivery of inactivated modified vaccinia virus Ankara (iMVA) induces antitumor systemic immunity via the STING-mediated cytosolic DNA-sensing pathway and Baf31-dependent CD11b+CD8+ dendritic cells (DCs). The combination of iMVA and systemic delivery of ICB is highly effective in treating large established tumors and distant metastasis (Dai et al., Science Immunology, 2017). In this study, we investigated the immunological mechanisms underlying the superiority of combination therapy over monotherapy with either IT Heat-iMVA or with systemic ICB alone. Using a bilateral tumor implantation model, in which only the larger tumors were injected with iMVA and the smaller tumors were treated with systemic ICB induced higher numbers of activated CD8+ and CD4+ T cells and higher levels of type I IFN and proinflammatory cytokines and chemokines in both injected and non-injected tumors, and more antitumor-specific memory CD8+ T cells in the spleens of mice compared with IT-iMVA alone. In addition, concomitant IT-iMVA and IT-ICB at one ten of the dose used for systemic delivery elicited superior antitumor effects compared with IT-iMVA alone. In mice with established melanoma, we observed that systemic treatment alone failed to elicit significant antitumor responses, which were enhanced by combination therapy.

**021**

PD-1 on radio-resistant cells negatively regulates effecter CD8+ T-cell activation during the elicitation phase of contact hypersensitivity

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The programmed cell death protein 1 (PD-1)/PD-1 ligand 1 (PD-L1) pathway is a negative regulator of CD8+ T cells, and is suggested to be involved in the regulation of various inflammatory diseases, including contact dermatitis. However, the precise regulatory mechanisms of PD-1/PD-L1 pathway in contact dermatitis remain unclear. To address this issue, we studied PD-1-deficient (PD-1/-) mice to contact hypersensitivity (CHS). PD-1/- mice exhibited significantly enhanced and prolonged ear swelling responses and increased IFN-γ production by CD8+ T cells in the skin compared to wild-type (WT) mice. PD-1/- mice also exhibited significant accumulation of lymph node (LN) cells from irradiated mice, but showed exacerbated CHS responses, while WT mice transferred with LN cells from PD-1/- mice showed normal CHS responses. In addition, administration of PD-1 blocking antibody during the elicitation phase, but not during the sensitization phase, caused enhanced CHS responses in WT mice compared to administration of isotype antibody, indicating that the PD-1/PD-L1 pathway works mainly during the elicitation phase. Bone marrow (BM) chimera experiments revealed that PD-1+ mice transplanted with BM cells from WT mice exhibited significantly increased CHS responses compared to WT mice transplanted with BM cells from PD-1/- mice, suggesting that radio-resistant cells are responsible for the PD-1/PD-L1 independent regulation. Consistently, among the radio-resistant cells in the skin, mast cells, blood endothelial cells, and lymphatic endothelial cells highly expressed PD-1 during the elicitation phase. Taken together, our data indicate that PD-1 on radio-resistant cells regulates CD8+ T-cell activation during the elicitation phase of CHS.

**022**

Distinct transcriptome signature of skin-resident memory T cells and migratory memory T cells in atopic dermatitis

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Memory T cells in human skin has been composed of two major subsets, called migratory memory T cells (TMM) and skin-resident memory T cells (TRM). The best characterized are TRM cells that bear the CD69 and CD103. To characterize and identify a gene expression signature of skin TMM and TRM cells in atopic dermatitis, we evaluated and verified the cytokine signatures and various genes associated with tissue egress and residency specific to TRM cells compared to TMM cells isolated from a skin T-cell migration assay in human normal and AD skin. We found that skin TMM cells were transcriptionally distinct from TRM cells using principal component analysis and correlation matrix analysis. AD CD69+ TSM cells also showed a significant level of genes related with tissue-residency compared to TMM cells. Gene set enrichment analysis further showed that skin TMM cells were significantly enriched for various immune-related signature genes compared to TMM cells from AD skin. Interestingly, AD TMM produced multiple cytokines, such as IL-4, IL-17, IL-22, and IFN-γ. These results indicate that highly-mutifunctional AD TMM could be a main mechanism resistant to conventional treatments, suggesting a new therapeutic target for the treatment of AD.

**023**

Inducible endothelial cell (EC)-specific RAMP1 knockout (KO) mice have immunity biased away from IL-17A and toward IFNγ and IL-22 expression

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We have reported that calcitonin gene-related peptide induces ECs to bias in vitro antigen presentation towards Th17 T cells. To evaluate the expression of Ag presentation to T cells away from an IFNγ effect on IL-17A expression, we exposed epidermal BALB/c EC or, separately, T cells from Th1 responses. To test the in vivo relevance of this finding, we engineered an inducible EC-specific RAMP1 KO mouse model by mating mice with floxed RAMP1 (exon 2) mice carrying vascular endothelial (VE)-cadherin-CreER2. Mice carrying floxed RAMP1 but not the (VE)-cadherin-CreER2 allele were used as controls. Mice were injected intraperitoneally with 0.05 ml of 20 mg/ml tamoxifen (Tx) on 3 separate days starting at 2 weeks of age, and injected 2 additional times at 3 weeks of age. We monitored excision of exon 2 in mice by PCR analysis of genomic DNA. Mice were used in turn with Tx injection in the regulatory fragment of chicken ovalbumin, cOVA323339) to IL-6 or medium alone for 3 h. Then, all cells were washed 4 x. IL-6-exposed or medium-exposed LCs were cultured with DO11.10 T cells not exposed to IL-6 along with Ag. Supernatants were harvested 72 h later and analyzed by ELISA for cytokine content. Exposure of LCs to IL-6 led to significantly enhanced production of IL-6 and IL-17A with significantly reduced IFNγ production. When analogous experiments evaluated exposure of LCs to IL-6, a much smaller, but still significant, effect on production of IL-6 and IFNγ was observed. Exposure of doxycycline to TMM cells isolated from a skin T-cell migration assay in human normal and AD skin showed CD8+ T-cell activation during the elicitation phase of CHS. Taken together, our data indicate that PD-1 on radio-resistant cells regulates CD8+ T-cell activation during the elicitation phase of CHS.

**024**

Exposure of murine Langerhans cells (LCs) to IL-6 biases antigen (Ag) presentation toward an IL-17A response

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Exposure of endothelial cells (ECs) to the neuropeptide calcitonin gene-related peptide endows ECs with the ability, acting as bystanders, to bias the outcome of LC Ag presentation to T cells away from Th1 responses and toward Th17 responses, and IL-6 by ECs appears to mediate most of this effect. To determine if IL-6 action on LCs alone, or T cells alone, is sufficient for this phenomenon, we exposed epidermal BALB/c LCs or, separately, T cells from DO11.10 mice (BALB/c background; DO11.10 mouse T cells that respond to a fragment of chicken ovalbumin, cOVA323339) to IL-6 or medium alone for 3 h. Then, all cells were washed 4 x. IL-6-exposed or medium-exposed LCs were cultured with DO11.10 T cells not exposed to IL-6 along with Ag. Supernatants were harvested 72 h later and analyzed by ELISA for cytokine content. Exposure of LCs to IL-6 led to significantly enhanced production of IL-6 and IL-17A with significantly reduced IFNγ production. When analogous experiments evaluated exposure of ECs to IL-6, a much smaller, but still significant, effect on production of IL-6 and IFNγ was observed. Exposure of doxycycline to TMM cells isolated from a skin T-cell migration assay in human normal and AD skin showed CD8+ T-cell activation during the elicitation phase of CHS. Taken together, our data indicate that PD-1 on radio-resistant cells regulates CD8+ T-cell activation during the elicitation phase of CHS.
Walnut antigen may trigger pathogenic autoantibody development in pemphigus vulgaris

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In human skin, epidermal Langerhans cells, as well as dermal dendritic cells, express CD1 proteins, which are MHC class I like antigen presenting molecules specifically equipped to present lipid antigens. In addition to the abundance of lipid antigens presented in the epidermis, the physiological functions of lipid-specific CD1 T cells remain incompletely understood. Previously, we have described the presence of CD1a-autoreactive T cells in normal skin and have identified several skin lipid antigens that can function as antigens for these T cells. More recently, published studies in both CD1a transgenic mice as well as in patients with inflammatory skin disease have suggested a role for CD1a and lipid antigen presentation in the pathogenesis of allergic contact dermatitis, atopic dermatitis and psoriasis. Thus far, we and others have primarily relied on functional readouts to identify lipid-reactive T cells. These functional readouts preclude ex-vivo phenotypic and genetic expression analysis, since functional readouts require T cell activation that can alter both surface phenotype and gene expression profiles of responding T cells. To circumvent this, we have developed fluorescence-activated cell sorting (FACS)-based sheep red blood cell (SRBC) assay, which can be used to detect CD1-restricted T cells, and allow for their quantification, isolation and analysis. CD1a T cells loaded with small hydrophobic lipids, including fatty acids, were shown to stain a subset of CD1a-autoreactive T cells among a polyclonal population of T cells isolated from normal skin. CD1a T cells loaded with sulfated fatty acids, which can be used as a marker to detect CD1-restricted T cells, and allowing for their isolation, quantification and analysis. CD1a T cells loaded with small hydrophobic lipids, including fatty acids, were shown to cause a subset of CD1a-autoreactive T cells among a polyclonal population of T cells isolated from normal skin. CD1a T cells loaded with sulfated fatty acids, which can be used as a marker to detect CD1-restricted T cells, and allowing for their isolation, quantification and analysis.
IgG from atopic dermatitis patients induces IL-17 and IL-10 production in infant intra-thymic TCD4 and TCD8 cells

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IgG modulates αβ T cell cytokine production during the maturation process in human thymus.

We aimed to investigate whether IgG from atopic dermatitis (AD) patients can modulate in vitro cytokine production of infants. Thymic tissue was obtained from newborn children from non-atopic mothers and thymocytes were cultured for 6 days with purified IgG from AD patients, with IgM or mock conditions as controls. Cells were gated as double positive T cells (CD4+CD8+), TCD4 cells or TCD8 cells, and levels of IL-17, IFN-γ, TNF-α, IL-10 and TGF-β were evaluated by flow cytometry. IgG of AD individuals induced enhanced IL-17 (p < 0.05) and IL-10 (p < 0.05) production by intra-thymic TCD4 and TCD8 cells of infants and of TGβ-f cells. Moreover, IgG from AD patients reduced IFN-γ production (p < 0.05) in TCD4 cells. IgG of AD patients can stimulate cytokine production in infant thymocytes and resembles the peripheral profile observed in adults. These findings suggest a novel mechanism that can contribute to AD pathogenesis.

Increased expression of IL-31, IL-31RA and OSMRβ in bullous pemphigoid

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Bullous pemphigoid (BP) is characterized by intense pruritus. Serum levels and tissue expression of Interleukin-31 (IL-31) have been found to be increased in pruritic skin disorders such as atopic eczema (AE) and prurigo nodularis (PN). We aimed to investigate the expression of IL-31 and its receptors (IL-31 receptor alpha (IL-31RA) and oncostatin M receptor beta (OSMRβ)) in the skin of classical and atypical forms of BP patients. Immunohistochemical staining was performed in skin biopsy samples from these age groups. We performed immunohistochemistry and RNA transcriptome analyses of LCs from young (n=5) and old LCs (n=5) using Next Generation Sequencing (Hiseq4000). STAR was used to align raw sequencing reads to the mouse genome. Read counts were calculated using HTSeq-count. Differential expression analysis was performed using the Deseq2 package. We identified 42 genes that were up-regulated by more than 2-fold and selected 5 genes that were significantly upregulated in LCs from old mice (PRX2, C121H7, C209D9, CASPI2, CADPS2) (padj < 1×10^-7) and three genes (CXXL2, MEF2C, RGS7BP) that were significantly down-regulated in older mice (padj < 1×10^-5). We validated these changes using qPCR. These genes are involved in metabolic signaling, cell proliferation, cell migration, cell adhesion, antigen processing, protein degradation, and apoptosis. We hypothesize that some of these changes in gene expression may contribute to age-associated decline in immune function including the robustness of LC antigen presentation for IL-4, IL-17A, and IL-22 responses.

031

Staphylococcus aureus enterotoxins modulate IL-22-secreting cells in adults with atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease with skin colonization by Staphylococcus aureus. Interleukin (IL)-22 triggers antimicrobial peptide (AMP) elaboration and enhances immune responses. In AD, IL-22 is related to epidermal hyperplasia, keratinocyte apoptosis, and inhibition of AMP production. We aimed to evaluate the impact of staphylococcal enterotoxins (SEA and SEB) on the Th22/Tb22 induction in the peripheral blood of AD patients and on CD4+ T cells expressing IL-22 in AD skin. Our study showed inhibition of SEA and SEB response by Th22 cells (p < 0.05) in AD patients, and an enhanced response to the bacterial stimuli by Th22 cells (p < 0.05). In AD skin, we detected increased IL-22 transcript expression and T lymphocytes expressing IL-22 (p < 0.01). Together, our results provide two major findings in response to staphylococcal enterotoxins in adults with AD: a dysfunctional CD4+ T cell cytokine response and increased expression of IL-22 in T cells. Our hypothesis reinforces the relevance of CD6 T cells modulated by staphylococcal enterotoxins as a potential source of IL-22 in adults with AD, which is relevant for the maintenance of immunological imbalance.

032

Langerhans cells from aged mice display changes in gene expression and antigen presentation to responsive T cells

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Langerhans cells (LC) are antigen presenting cells of the epidermis that initiate antigen-specific immune responses under some circumstances while, in the steady state, they may also serve to down-regulate immune responses. Important LC functions decline with age; LCs from aged mice have impaired migratory behavior and impaired ability to promote CD4+ and CD8+ T cell (TC) proliferation. We have found that LCs from older mice (>12 months) are less efficient at eliciting IL-4, IL-17A and IL-22 responses from antigen-specific CD4+ T cells compared to LCs from younger mice (323-339) to responsive TCs (from DO11.10 mice) and validated by qPCR after in vitro co-culture of LCs and T cells with 1pmol CT. To explore the mechanism of these age-related functional changes in vivo, we performed RNA transcriptome analyses of LCs from young (n=5) and old LCs (n=5) using Next Generation Sequencing (Hiseq4000). STAR was used to align raw sequencing reads to the mouse genome. Read counts were calculated using HTSeq-count. Differential expression analysis was performed using the Deseq2 package. We identified 42 genes that were upregulated by more than 2-fold and selected 5 genes that were significantly upregulated in LCs from old mice (PRX2, C121H7, C209D9, CASPI2, CADPS2) (padj < 1×10^-7) and three genes (CXXL2, MEF2C, RGS7BP) that were significantly down-regulated in older mice (padj < 1×10^-5). We validated these changes using qPCR. These genes are involved in metabolic signaling, cell proliferation, cell migration, cell adhesion, antigen processing, protein degradation, and apoptosis. We hypothesize that some of these changes in gene expression may contribute to age-associated decline in immune function including the robustness of LC antigen presentation for IL-4, IL-17A, and IL-22 responses.

033

Keratinocyte-derived IL-15 links oxidative stress to melanocytes immunologic destruction in vitiligo

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Oxidative stress is critical for T cell-mediated killing of melanocytes in vitiligo. IL-15, a pleiotropic cytokine, acts on virtually each cell type of the innate and adaptive immune system. However, whether IL-15 plays an immune-related pathologic role in vitiligo and its mechanism is still unknown. We hypothesize that oxidative stress could activate CD8+T cell-mediated autoimmune destruction of melanocytes by enhancing IL-15 expression and its membrane trans-presentation in KCs. KC-derived IL-15 may be a potential novel therapeutic target for vitiligo treatment.
**037**
Neutrophil-derived exosome drives the autoinflammatory responses of generalized pustular psoriasis via activating NOD2 in keratinocytes


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Glycoprotein nonmetastatic melanoma protein B (GPNMB) is a glycosylated transmembrane protein expressed in various types of cells, such as osteoclasts, dermal cells, melanocytes, and keratinocytes. GPNMB was known to be involved in a variety of biological processes including tissue repair, cell proliferation, and cell differentiation. GPNMB also has an important axis driving crosstalk between keratinocytes and myeloid cells to mediate skin inflammation. Neutrophils are the most abundant leukocytes present in human blood and in the lesional skin of GPP patients. Though short-lived, neutrophils can immediately secrete cytokines, chemokines, and vesicles. Our study aimed to function the neutrophils in the immune disorder of GPP. Herein, we demonstrated that the neutrophil to lymphocyte ratio (NLR) was correlated with the severity of GPP, and decreased dramatically after effective treatment, which indicated that the NLR score could be a marker for the severity and progression of GPP, and neutrophil might play a critical role in the pathogenesis of GPP. Besides, keratinocytes co-cultured with GP neutrophils indirectly produced more CXCL1, CXCL2, CXCL8, CCL20, IL16G, and TNF-α than those in the direct co-culturing system. Further, exosomes derived from GPP neutrophils could enter and activate keratinocytes to secrete the above-mentioned mediators. The secretion of IL-36γ in GP neutrophils was significantly increased while IL-36α antagonist was particularly prominent in the M2b lineage. IL-36γ increased the production of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) from M2a macrophages. Taken together, our data support a pro-inflammatory role for IL-36 in myeloid cells, and may be an important axis of neutrophils to keratinocytes and M2a macrophages to mediate skin inflammation.
043 
Blockade of OX40 signal ameliorates the mortality and activity of systemic lupus erythematosus

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The diversity of blood T cells decreases in the 7th and 8th decades, leading to reduced immunocompetence. T cells in the skin of older individuals are diverse and highly functional, and their responses to pathogens are more pro-inflammatory than those of younger individuals. However, the precise mechanisms underlying these differences are not fully understood.

044 
Paradoxical psoriasis induced by anti-TNF is dependent on unaltered type-I interferon-driven innate IL22 overexpression

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Although anti-Tumour necrosis factor (TNF) agents are highly effective in the treatment of psoriasis, 2-5% of treated patients develop psoriasiform-like skin lesions called paradoxical psoriasis. The pathogenesis of this distinct form of psoriasis is largely unknown. We find that skin lesions from patients with paradoxical psoriasis are characterized by a selective overexpression of type I interferons, dermal accumulation of plasmacytoid dendritic cells (pDC), and reduced pathogenic T cell numbers, when compared to classical psoriasis. Using a newly established mouse model, we find that anti-TNF treatment prolongs type I interferon production by pDCs through inhibition of their maturation. The resulting type I interferon overexpression is responsible for the skin phenotype of paradoxical psoriasis, which, unlike classical psoriasis, is independent of T cells. Albeit a strong Th1/Th17 signature profile, IL17 blockade did not lead to amelioration of the phenotype, while IFNβ blockade even led to aggravation. In contrast, blockade of IL22, which is potentially induced by type-I interferon, inhibits the development of paradoxical psoriasis. Taken together, these findings suggest that paradoxical psoriasis is not solely driven by an exaggerated pro-inflammatory process without T cell autoimmunity, driven by pDC-derived type I interferon and mediated by downstream IL22.

045 
T cells in the skin of older individuals are diverse and highly functional

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The diversity of blood T cells decreases in the 7th and 8th decades, leading to reduced immunocompetence. The effects of aging on skin T cells have not been studied, and it is unknown if a blockade of OX40 signal can be a therapeutic option for lupus. To examine the effects of OX40 signal blockade in lupus, we used topical imiquimid (IMQ)-induced lupus model in WT and OX40L KO mice. We also used control IgG and anti-OX40L blocking mAb. Topical IMQ application for 12 weeks resulted in the death of 50% of WT mice and 15% of OX40L KO mice. Glomerulonephritis, glomerular immune complex and Cl deposition, serum creatinine level, and urine protein level in OX40L KO mice were milder than those in WT mice. The number of mature T follicular helper (Tfh) cells and the activation of dendritic cells (DCs) in the spleen were significantly impaired in OX40L KO mice compared with WT mice. These data suggested that OX40 signal promotes pathogenic Tfh cell responses, DC activation, and lupus nephritis. To address if biologics against OX40L can be therapeutic options for lupus, WT mice topically applied IMQ for 4 weeks (urine protein began detecting at this time point) were divided into two groups; control IgG group and anti-OX40L blocking mAb group. Control IgG and anti-OX40L mAb were administered i.p. three times per week. Like OX40L KO mice, the administration of anti-OX40L mAb was impaired pathogenic Tfh cell responses, DC activation, and lupus nephritis, thereby leading to the improvement of mortality. Collectively, OX40 signal exacerbates lupus by promoting lupus nephritis. However, it is still unknown if a blockade of OX40 signal can be a therapeutic option for lupus.

046 
Plexin-B2-Semaphorin 4D dampens regulatory T-cell functions leading to CD8+ T-cell resistance in oral lichen planus

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Bullous pemphigoid (BP) is an autoimmune-mediated disease associated with autoantibodies against type XVII collagen (Col17). Several animal models mimicking important features of human BP have been developed. Complication avoidance is essential for blister formation, but recent findings indicate complement-independent mechanisms to add to blister formation in vivo. Here, we show that indeed both complement-dependent and -independent mechanisms are operative in experimental BP. In different experimental settings, complement deposition at the dermal-epidermal junction did not correlate with disease activity. In C5s, mice, independent of injected anti-Coll7 IgG dose, disease activity was reduced by about 50%. Also, Casr1-/- mice did not develop disease, whereas the extent of skin lesions was increased in Casr2-/- animals. Phenotypic pharmacological inhibition of Csr2 led to reduced disease activity. While Csr2 was crucially involved in neutrophil migration in vivo, its role for Col17-/-Col7 IgG immune complex-mediated release of ROS from neutrophils was minor. Our data show that complement-dependent and -independent mechanisms drive autoimmune-mediated tissue destruction in BP, and Csr2 seems to play opposing roles in this process, while Csr1 exerts its primary role during the early phase disease.
049

Development of active mouse models to recapitulate pemphigus subtypes and to evaluate response to therapy

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Pemphigus, a complex disease is characterized by bullous skin disease caused by loss of adhesion caused by autoantibodies (Titan) against keratinocyte antigens, desmogleins (Dsg and Dsg) and non-Dsg proteins, such as acetylcholine receptors (ACHr) or Desmocollin (Discl). Different PVLG result in diverse clinical phenotypes. In order to recapitulate the features of human pemphigus, to evaluate the role of different antigens, and to test the response to therapy, we generated an active mouse model. We produced mune recombinant proteins (Dsg, Dsg, Dsc, ACHrMA, ACHRMA2 and ACHRMA2a). We then immunized Dsg-/- mice with mouse (Dsg), and adoptively transferred their splenocytes into C57BL6 Rag2-/- (Rag2) immunodeficient mice, that express Dsg1. Resident mice stably produce the pathogenic anti-Dsg IgG and exhibit the features of pemphigus vulgaris, as shown by clinical score and Dsg3 levels (ELISA). We also generated the pemphigus foliaceus active mouse model, by breaking the immunological tolerance to Dsg1-Dsg3 in Dsg1-/- Dsg3-/- mice by transferring the combined splicenocytes from mice immunized with Dsg1 and mice immunized with Dsg3 into Rag2 mice. The Dsg/Dsg mouse model displays the most severe phenotype, reproduces symptoms of mucocutaneous PV in patients, as well as the same level of intraepidermal detachment and interkeratinocyte deposits of IgG. Moreover, only the Dsg/Dsg mice significantly responded to treatment with methylprednisolone in a time-dependent manner (clinical score and ELISA). In conclusion, we created new tools that could improve the knowledge on pemphigus pathomechanisms, 2. serve for the identification of new therapeutic targets; 3. allow a long-term pre-clinical observation with or without current and novel therapies.

050

Patients with hirudinetics suppurrativa have altered populations of circulating B cells

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Hirudinetics suppurrativa (HS) is a chronic inflammatory disease characterized by painful deep-seated recurrent nodules and abscesses in axillary, inguinal, perineal and mammary areas. The disease is polymorphic but commonly involves lung, breast and skin. HS is an immune-mediated disease that triggers several factors as well as abnormal immune functions. B cells are the key mediators of humoral immune responses by producing antigen-specific antibodies and by orchestrating CD4+ T cell activation. It is becoming clear that abnormalities during the B cell development and differentiation give rise to a diverse array of inflammatory diseases including autoimmunity and immunodeficiency. In this study, we investigated the B cell subsets in the peripheral blood mononuclear cells (PBMC) from HS patients compared to healthy controls (HC). PBMCs from HS patients (n=10, 80% female and 20% male with a mean age 37.8 ± 6.2 years) and HC (n=11, blood transfusion donors) were surface stained using B cell-specific markers and B cell populations were detected by flow cytometry. We observed that HS patients had significantly elevated frequencies of IgG CD27 CD138 CD24+ transitional B cells (p<0.001) and IgG CD27 CD138 CD24+ naive B cells (p<0.001) compared to healthy controls. The proportion of IgG CD27+ non-switched memory B cells (p<0.01) and IgG CD27+ switched memory and IgG CD27 CD138 CD24+ naïve B cells did not differ between the two groups. Our finding suggests that B cells may play an important role in the pathogenesis of HS. Increased frequencies of transitional and antibody-secreting B cells are associated with autoimmune disorders. Although HS is not considered as an autoimmune disease, our results support the complexity of the condition and identify B cells as a new potential therapeutic target.

051

Antimicrobial activity of cytotoxic Th17 cells targeting propionibacterium acnes

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Propionibacterium acnes, a normal skin flora, is a complex pathogen that has been shown to kill extracellular bacteria by membrane-pore formation. To uncover the antimicrobial pathways involved, we stimulated P. acnes-specific Th17 clones, performed RNAseq, and used bioinformatics to identify genes present in modules associated with cytolytic Th17 activity. We identified several highly expressed genes in cytolytic Th17 cells that encode for secreted antimicrobial proteins targeting P. acnes. Altogether, our data suggest that P. acnes strains may differentially modulate the CD4+ T cell response, leading to the generation of Th17 cells that may contribute to either homeostasis or disease pathogenesis.

052

Vitiligo is characterized by multi-axis T-cell activation in blood

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Vitiligo is the most common autoimmune depigmenting skin disorder. Scarcity of immune profiling studies limits targeted therapeutic advancements. Skin-homing/CLA+ T-cells have been shown to be centered around perilesional melanocytes, but were not further characterized in vitiligo patients. We sought to evaluate CD4+/CD8+ T-cell differentiation to IFN-γ/IL-13, IL-22, IL-17A and IL-9 producing T-cells within the skin-homing/CLA+ and systemic/CLA- compartments in blood of 14 vitiligo patients compared to 25 controls. Blood from patients and controls was activated by PMA/ionomycin and stained by a 12-color antibody panel for flow cytometry analyses. We measured increased cutaneous/CLA+ and systemic/CLA- CD22+ T-cells in vitiligo patients. Vitiligo patients had significantly elevated frequencies of Th17 CD45RA+ CD62L- T-cells compared to healthy controls (p<0.05) and Th1 CD45RA CD62L- T-cells (p<0.001). Overall, our data suggest that CLA+ T-cells were detected in vitiligo patients vs. controls. Trend for positive correlation between IL-22 frequencies and vitiligo severity (Vitiligo Area Severity Index/AIS) was demonstrated (p=0.12). Overall, our data show diverse circulatory cytokine axes activation with Th2 and Th22/Th17 predominance in vitiligo patients. In light of the extensive involvement of IL-22 and IL-17 cytokines in autoimmune disorders, future functional and targeted therapeutic studies will have to determine the relative pathogenic role of each axis dysregulation.

053

Lipid scavenger molecule CD36 regulates free fatty acid uptake and peripheral maintenance of tissue resident memory T cells

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The mechanisms by which tissue-resident memory T cells (TRM) survive in peripheral tissues for long periods of time remains obscure. Cytokine, Recent studies revealed that CD36+ TRM generated by skin vacuosis (VACV) infection express strikingly high levels of several molecules involved in the uptake and intracellular transport of free fatty acids (FFA) and other lipids. These molecules include fatty acid binding protein 4 (Fabp4) and Fabp5. Using transgenic mouse models, we have showed mice deficient in both fabp4 and fabp5 develop TRM that are inferior in internalizing exogenous FFA, displayed reduced FFA oxidation and ATP generation, and did not survive as long-term as WT TRM. Another molecule strongly expressed in CD36+ TRM and fabp4-/-/fabp5-/- TRM is immune-inflammatory cytokine TNF-α. The role of CD36 in the maintenance and survival of TRM remains unknown. Here we demonstrated that CD36+ TRM internalized labeled palmitate (1C6 FFA) less efficiently than WT TRM. In vivo competitive experiments by transferring equal numbers of WT and CD36-/- OT-1 cells into the same recipient mice followed by VACV inoculation showed there was a survival disadvantage of CD36-/- TRM in skin beginning at day 45, which became more pronounced through day 90. By contrast, no difference could be observed between CD36-/- and WT TRM over 90 days of experiment. In addition, the defects seen in CD36-/- TRM and fabp4/-/- fabp5/-/- TRM are additive in CD36-/- fabp4/-/- fabp5/-/- OT-1 mice. Thus, these data suggest that CD36 and fabp4/-/- fabp5/-/- are unique lipid metabolic programs intrinsic to TRM and how these programs might be modified therapeutically, will facilitate treatment approaches to TRM-mediated diseases as well as TRM-based vaccines.

054

Immunogenicity to botulinum neurotoxin: long-term clinical relevance in aesthetic and therapeutic injections

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Without any doubt, Botulinum Toxin is an amazing molecule with diverse clinical effects since the 1970's. The clinical use of Botulinum Toxin Type A and B (BONT-A and BONT-B) revolutionized the practice of medicine over the past 30 years. The safety and effectiveness of injectable Botulinum Toxin have been well established in both aesthetic and medical indications. However to reach the desired endpoints and to maintain the efficacy, the frequency of injections is required. By increasing the numbers of patients without favorable therapeutic responses, a new concern has re-emerged on the immunoresistance and loss of effectiveness to treatment. Primary and secondary non-responses have been hypothesized to be associated with the presence of neutralizing antibodies directed against the neurotoxins. Several factors for the production of neutralizing antibodies have been proposed, including the manufacturing process of Botulinum Toxin, the antigenic protein load, the total dose toxin, booster injections, prior vaccination and exposure to fake products on the market. The purpose of this study was to systematically assess published studies and perform an analysis to determine how significant it is clinical relevance of immunoresistance to Botulinum Neurotoxins. A literature search was done by searching Embase, PubMed, Cumulative Index to Nursing and Allied Health Literature (CINAHL), and Cochrane Library databases of clinical trials. The results of this systematic review will be discussed to provide clinicians an updated high level of evidence for clinical importance of immunogenicity to Botulinum toxin formulations.
055 Development of contact dermatitis in mice requires T cell signaling via the neurokinin 1 receptor at the site of dendritic cell T cell synapse
T. Sangueza, Q. T. Pham, T. Villa, K. Park, C. Slongo, A. M. Masson and T. Kupper

Skin scarification with modified vaccinia ankara (MVA) virus generates protective pulmonary immunity

Development of contact dermatitis in mice requires T cell signaling via the neurokinin 1 receptor at the site of dendritic cell T cell synapse. The serological diagnosis of pemphigus, a rare autoimmune blistering disease, using the BIOCHIP has been developed. In all prior studies in humans, MVA has been delivered via intramuscular (im) injection. In this study, we showed that MVA as low as 1.8 X 10^7 pfu could be safely inoculated to scarified skin of immunocompromised mice without causing any morbidity. MVA ss produced more lung CD8 T cells and was superior in protecting mice against lethal respiratory challenge. Overall, our data show that skin scarification with MVA generates protective pulmonary immunity, and could be used as a superior and safe vaccine strategy against smallpox and vaccine vector for other infectious and malignant diseases.

056 Identification of immunomodulator Th2 cell epitopes in Chinese bullous pemphigoid patients
J. Zhang, H. Yang and G. Wang

Identification of immunomodulator Th2 cell epitopes in Chinese bullous pemphigoid patients. Bullous pemphigoid (BP) is a subepidermal autoimmune blistering disease caused by auto- antibodies (autoAbs) targeting the juxtamembranous extracellular noncollagenous 16A (NC16A) domain of human collagen XVII (as seen by Immunostain). Because helper T cells are essential for antibody responses to antigens, we adopted an assay to map the immunomodu- lator Th2-cell epitopes in NC16A. We synthesized 22 overlapping peptides spanning the entire sequence of BP180-NC16A and investigated the reactivity of Th2 cells from BP patients to these peptides by ELISPOT assay. We screened out two epitope peptides, P1B and P21, and confirmed these epitopes play a dominant role in stimulating CD4+ T cells proliferation and NK1R signals in vitro and in vivo. We conclude that, NK1R signaling of T cells by autocrine SP and NK1R cooperates with the TCR for efficient the cellular immunity that mediates CD. NIH R01AI068249 is needed.10.1007/s11280-022-00114-9

057 Variable T<sub>2</sub>T<sub>17</sub>-skewing places Chinese atopic dermatitis and psoriasis on an inflammatory spectrum

Variable T<sub>2</sub>T<sub>17</sub>-skewing places Chinese atopic dermatitis and psoriasis on an inflammatory spectrum. ABSTRACTS | Adaptive and Auto-Immunity

058 Reliability of the BIOCHIP in pemphigus and pemphigoid patients the evaluations of blistering disease experts
S. Liu, A. Yang, M. Melbourne, T. Hashimoto, S. Uzun, M. Daneshpazhooh, I. Yamagami, G. Di Zorzi, J. Mascaro Jr and D. Murrell1 1 University of New South Wales, KIlla, New South Wales, Australia, 2 University of New South Wales, St. George Hospital, Sydney, New South Wales, Australia, 3 St George Hospital, Kogarah, New South Wales, Australia, 4 Department of Derma- tology, Karume University School of Medicine, Tokyo, Japan, 5 Department of Dermatology, Akdeniz University, Antalya, Turkey, 6 Tehran University of Medical Sciences, Tehran, Iran, 7 Department of Dermatology, Keio University School of Medicine, Tokyo, Japan, 8 Molecular and Cell Biology Laboratory, Rome, Italy, 9 Hospital Clinic and Barcelona University Medical School, Barcelona, Spain and 10 University of New South Wales, Sydney, New South Wales, Australia

Reliability of the BIOCHIP in pemphigus and pemphigoid patients the evaluations of blistering disease experts. The cytokine production of autoantigen-reactive B cells associates with pathogenesis in systemic sclerosis
A. Yoshizaki, T. Fukasawa, Y. Asano, T. Kitamori and S. Sato

Reliability of the BIOCHIP in pemphigus and pemphigoid patients the evaluations of blistering disease experts. The cytokine production of autoantigen-reactive B cells associates with pathogenesis in systemic sclerosis.

059 The cytokine production of autoantigen-reactive B cells associates with pathogenesis in systemic sclerosis
A. Yoshizaki, T. Fukasawa, Y. Asano, T. Kitamori and S. Sato

The cytokine production of autoantigen-reactive B cells associates with pathogenesis in systemic sclerosis. The role of autoantigen-reactive B cells is still not clear, because the number of these cells is too small to study them directly. In this study, we investigated the role of autoantigen-reactive B cells directly through medical-engineering collaboration study. Methods: Lymphocytes were obtained from SSc patients. Cytokine production of single B cells was analyzed by the micro-ELISA system which can detect extremely small amounts of analytes. In mouse studies, we assessed antigen affinities and cytokine production of topo I-specific B cells using the BIOCHIP. The multiplex biochip was performed on all patient sera. Six blistering disease experts from around the world (Japan, Turkey, Iran, Italy and Spain) evaluated 312 biochip slides consisting of 1872 photos. The resulting kappa indicated fair agreement for oesophagus and pattern, <0.35 (95%CI, 0.13 to 0.74, p<0.005), 0.55 (95%CI, 0.38 to 0.69, p<0.005) respectively. Oesophagus and pattern both indicated moderate agreement, 0.55 (95%CI, 0.33 to 0.76, p<0.005) respectively. A higher inter-rater agreement was obtained for Salivary Skin and location, 0.5 (95%CI, 0.35 to 0.64, p<0.005), 0.56 (95%CI, 0.40 to 0.72, p<0.005) respectively. Both the BIOCHIP and the other methods were essentially unbiased. This is the first comprehensive molecular profiling of AD and psoriasis in Chinese patients, identifying these diseases as part of an inflammatory spectrum featuring variable T<sub>2</sub>T<sub>17</sub> activation.
061
CD301b+ dermal dendritic cells critically mediate oxazolone-induced contact hypersensitivity response by inducing type 2 immunity
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062
Involvement of β-catenin/CBP-dependent signaling in the emergence of hapten-induced atopic dermatitis-like dermatitis
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063
Activating ILC3 cells suffice to induce psoriasis in human skin in vivo
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064
Impaired peripheral tolerance leads to AIBD phenotype with pathogenic antibodies and blister formation
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065
Circadian rhythm affects the severity of contact hypersensitivity response in mice
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066
Glucocorticoids promote intrinsic human Th17 differentiation
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Although Th17 cells are critical to host protection at epithelial barriers, they can play a pathogenic role in inflammatory and autoimmune diseases. Glucocorticoids (GC) are therapeutically used to suppress undesired inflammation, but the common persistence or even increase of Th17+ Th17 cells expressed IL-10 and their development was associated with an inhibition of Th2, and particularly Th1 differentiation. GC induction of Th17 differentiation was not linked to increased IL-1β, IL-6 and TGF-β, yet GC downregulated IL-2. Consistently, the addition of exogenous IL-2 prevented the GC-induced increase in Th17/Th1 ratios. Also, IL-21/IL-23 enhanced Th17/Th1 ratios, altogether suggesting a mechanistic role for IL-2 regulation in GC-induced Th17 polarization. Functionally, supernatants of GC-differentiated T cells were superior to those of non-GC-differentiated T cells in inducing antimicrobial peptides and pro-inflammatory cytokines in keratinocytes, despite elevated IL-17 in GC-activated cells. Finally, GC also favored Th17 over Th1 among memory T cells from blood and skin. Altogether, our data define GC as human Th17 polarizing factors.
Highly functional skin NKGD2 CD8+ effector memory T cells in vitiligo
C. Jacobse1, C. Martins2, A. Taieb3, J. Seneschal3 and K. Boniface1

Human skin is composed of epidermal and dermal compartments. Both the epidermis and dermis play a crucial role in immune surveillance and defense. In vitiligo, an autoimmune disease characterized by the loss of skin pigment, the skin's immune cells are activated and directed against melanocytes. This study examined the role of NKGD2 CD8+ effector memory T cells in the skin of patients with vitiligo.

Nanoparticle induced immunomodulation in a model of allergic contact dermatitis
S. Chen, Y. Ke, H. Fang, J. Dang, J. Zhang, H. Qiao and G. Wang

The field of drug delivery and transdermal drug administration is rapidly advancing, with significant implications for the treatment of skin diseases. This study explored the use of nanoparticles (NPs) to modulate immune responses in a model of allergic contact dermatitis (ACD).

CD72low B cells are involved in corticosteroids resistance in bullous pemphigoid
MK Sarafian1, G. Hille1, TC Os1, X. Jing2, J. Liu3, Y. Liang4, CC Berthier5, WR Swindell6, M. Patrick7, T. Issu8, R. Uppala9, M. Beamer1, A. Srivastava1, S. Bielas1, P. Harms1, S. Getsios2, J. Elder5, J. Voorhees5, J. Ahmadieh6 and J. Voorhees6

Corticosteroids are a mainstay of treatment for bullous pemphigoid (BP), but resistance to these agents is a significant challenge. This study focused on the role of CD72low B cells in corticosteroids resistance in BP.

Photosensitivity and heightened type I IFN responses in cutaneous lupus are driven by elevated interferon kappa
MK Sarafian1, G. Hille1, TC Os1, X. Jing2, J. Liu3, Y. Liang4, CC Berthier5, WR Swindell6, M. Patrick7, T. Issu8, R. Uppala9, M. Beamer1, A. Srivastava1, S. Bielas1, P. Harms1, S. Getsios2, J. Elder5, J. Voorhees5, J. Ahmadieh6 and J. Voorhees6

Cutaneous lupus is a chronic inflammatory skin disease characterized by photosensitivity and inflammation. Interferon-κ (IFN-κ) has been implicated in the pathogenesis of cutaneous lupus. This study investigated the role of IFN-κ in photosensitivity and type I IFN responses in cutaneous lupus.

CD272low CD19+B subset associated with antibody production and corticosteroids resistance in bullous pemphigoid
S. Chen, Y. Ke, H. Fang, J. Dang, J. Zhang, H. Qiao and G. Wang

B cells are crucial in the adaptive immune response, playing a significant role in antibody production and autoimmune diseases. This study examined the role of CD272low CD19+B cells in CD272low disease.

Human central memory T cells generate superior numbers of resident memory T cells in skin
T. Mateo1, A. Ghezad2, J. Teague2, B. Dying-Anderson2, C. Yang2, J. Malley1, R. Watanabe1, T. Kupper3 and R. Clark4

Memory T cells are crucial for immunity and long-term protection. This study aimed to understand the generation and function of central memory T cells in skin.
The role of T cell immunity in the suppression of papillomavirus

C. Tejeda1, E. Wang1, A. Kwon1, A. Figeroa1 and A.M. Christiano2

Skin homogenates

Anti-laminin-332 pemphigoid sera, and 42 control sera comprising 13 anti-p200 pemphigoid, 11 anti-p200 pemphigus, 12 anti-laminin-332 pemphigoid, and 13 control sera. Anti-laminin-332 pemphigoid and control sera were assayed for antibodies to laminin-332. Anti-laminin-332 antibodies were detected in 13 sera. Serum from patients with AA yielded a mean absorbance value of 0.44 ± 0.15, whereas serum from control subjects yielded a mean absorbance value of 0.12 ± 0.06. The difference in absorbance values was statistically significant (p = 0.001).

Conclusion: The keratinocyte footprint assay is a sensitive and specific assay for detecting anti-laminin-332 antibodies. Keratinocytes cultured in vitro need to demonstrate anti-laminin-332 antibodies in order to be used as a diagnostic tool in AA.

Characterization of a novel long non-coding RNA (GC2608) in psoriasis

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Psoriasis is a chronic inflammatory autoimmune skin disorder characterized by altered epidermal differentiation and hyperproliferation. While major research efforts have been on protein coding genes, little is known about the role of non-coding RNAs in psoriasis. Using RNA-sequencing, we identified over 1,000 novel long non-coding RNAs (IncRNAs) expressed in psoriasis, or healthy skin, with 40% of these being dysregulated in psoriatic skin, and we were able to provide functional inference for many of them. We focused our analyses on the IncRNA, GC2608, which was amongst the most highly induced IncRNA in psoriatic skin (Fold Change (FC)=592, p=0.06, FC<4, p=0.05 respectively, n=4). In contrast, no changes were observed for IncRNA with FC<2 or p>0.1. We further investigated the expression of GC2608 in sentinel lymph node. Genomic sequence analysis revealed close proximity of GC2608 with S100A7, which is a prominently expressed gene in psoriasis and involved in antimicrobial defenses, cell proliferation and matrix remodeling. GC2608 was downregulated in psoriatic skin, indicating a key role in the immune response. Furthermore, we explored the correlation between GC2608 and the expression of other known psoriasis-related genes. GC2608 was found to be negatively correlated with the expression of several genes involved in the inflammatory response, suggesting a potential role in the repression of inflammation in psoriasis.
081 Simvastatin reduces autoimmune alopecia through direct action on T lymphocytes
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Miami, Miami, FL, 2 Diabetes Research Institute University of Miami, Miami, FL and 3 Dermatology and Cutaneous Surgery, University of Miami, Miami, FL

Alopecia areata (AA) is a T-cell-mediated autoimmune disorder involving the lymphocytic infiltration and destruction of the hair follicle during the anagen phase of hair growth. This disease presents with varying patterns of hair loss, from diffuse to patchy to total hair loss on the head or body. Current treatments for AA are associated with troubling side effects, prohibitive cost, or low response rates. Previously, we have shown that systemic administration of simvastatin (Simv) reduces the decreased viability of CTLL-2 cells after 48 hours of statin treatment may suggest an inhibitory effect on the signaling of the pro-inflammatory cytokine IL-2 that these cells rely on for growth.

080 Keratinocytes affect biology of Langerhans cells through mRNA transfer
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Dendritic cell (DC) subsets might acquire specific immune functions based on their tissue of residence. Langerhans cells (LCs), a specific DC population located in the epidermis, are in close contact with epidermal keratinocytes (KC), providing us with an easily accessible model to understand the effect of KCs on infiltrating DCs. Using microarray analysis, RT-qPCR, histology and flow cytometry, we showed that many of the KC-specific molecules, such as keratins and adhesion molecules, can be detected in LCs at mRNA and protein levels. To determine whether these KC-specific genes are accessible for transcription in LCs, we performed APAC-seq on flow-purified LCs. We found that keratin loci of these genes were in the closed conformation in LCs. Therefore, these data support the active transport of mRNAs from the KCs to LCs. Furthermore, we also showed that KC-specific expression of Cre, which is required for the conditional knockout of genes, can be induced in LCs through specific gene knockdown in KCs. These results suggest that keratinocytes can transfer mRNAs that are important for LC development and function, which may shed light on the role of IFNAR in other organ-specific autoimmune diseases, such as multiple sclerosis, in which IFNβ is administered therapeutically.

084 The role of interferon and retinooids in lupus-prone Ro60 knockout mouse skin
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The Ro60 protein is a prominent autoantigen in systemic lupus erythematosus (SLE). Anti-Ro antibodies are strongly associated with photodipsisy in lupus. While it is known that Ro60 binds to small non-coding Y RNAs, it was recently reported that Ro60 also binds to Alu RNAs derived from short interspersed retroelements (SINEs). Loss of Ro60 in human cell lines resulted in the accumulation of Alu RNAs associated with an increase in type I interferon (IFN). Since SINE transcripts have been shown to be increased following DNA damage, we examined the relationship between Ro60, SINEs, and the inflammatory response after UV irradiation in Ro60 knockout (KO) mice that spontaneously develop a lupus-like syndrome. C57Bl/6 wild-type (WT) and RO60 KO mice on the same genetic background were irradiated with UVB resulting in the accumulation of Alu RNAs associated with a type I IFNs. At baseline, Ro60 KO mice exhibited increased expression of ISGs, possibly associated with autoimmunity and the lupus-like syndrome. In contrast to Y RNAs that were undetectable in Ro60 KO mice even after UVB irradiation, SINEs were increased in both Ro60 KO and WT mice after UVB. The accumulation of SINEs transcripts after UVB may overwhelm the binding capacity of Ro60, leading to the increased expression of ISGs. This suggests that SINEs, instead of being regulated by Ro60, may be regulated by the UV-induced inflammatory response. A clearer understanding of the relationship between autoimmunity in Ro60 KO mice and changes in the relative amounts of SINEs versus Ro60 protein could provide a new paradigm of how UV triggers lupus.

083 CXCR4-expressing skin-resident NKT cells develop allergic inflammation in atopic dermatitis
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Adaptive allograft rejection plays key roles in atopic dermatitis (AD). However, it is challenging for non-allergic AD or recalcitrant AD patients, who demand new immunological therapies such as targeting innate immunity to control these conditions. NKT cells share cell-surface proteins with conventional T cells and NK cells that serve as unconventional T cells bridging between innate and adaptive immunity. NKT cells are known for a new player to develop AD, which are collaborated with several chemokines that increase in atopic dermatitis. In this study, we identified that CXCR4 and its cognate ligand CXCL12 were significantly up-regulated in human AD skin by proteomic and transcriptomic analyses, which were consistently elevated in our AD mouse model. Adoptive transfer of allergen-specific NKT cells conferred antigen-specific cutaneous inflammation in our model, and predominant skin NKT cells were CXCR4+ and CD69+, indicating a type of resident LCs. Additionally, YFP+ LCs could be readily identified in LC-depleted KRT14-YFP mice among typically treated mice, as were reduced levels of pSTAT1 and NGK2D in skin-draining lymph nodes. In vitro studies indicate an anti-inflammatory effect of simvastatin in both the cytotoxic T cell line CTL2-2 and primary CHH/HE lymph node cells, suggesting that simvastatin effect on AA is exerted through the direct modulation of T cell activity. Furthermore, the decreased viability of CTL2-2 cells after 48 hours of statin treatment may suggest an inhibitory effect on the signaling of the pro-inflammatory cytokine IL-2 that these cells rely on for growth.

079 Correlation of IgG autoantibodies against acetylcholine receptors and desmogleins in patients with pemphigus vulgaris treated with steroid sparing agents or immunosuppressive therapy
SM Bhatia, R Streilein and R Hall
Miami, Miami, FL, 2 Diabetes Research Institute University of Miami, Miami, FL and 3 Dermatology and Cutaneous Surgery, University of Miami, Miami, FL

Autoantibodies against desmoglein-1 (DSG1) and desmoglein-3 (DSG3) have been shown to be important in the pathogenesis of pemphigus vulgaris (PV). In some patients, however, disease activity does not always correlate with IgG anti-DSG levels. Recent studies have indicated that other non-DSG antibodies may contribute to the pathogenesis of pemphigus, including acetylcholine receptor (AChR) autoantibodies. The purpose of this study is to retrospectively analyze PV patient sera to better understand the role of IgG anti-AChR autoantibodies in patients treated with prednisone and steroid sparing agents (SSA; n=15) or prednisone and rituximab (n=14). Patients were evaluated at two time points, T1 and T2, with a median interval between evaluations of 970 days for SSA treated subjects and 780 days for those treated with rituximab. Sera were tested for the presence of DSG1, DSG3, and AChR IgG autoantibodies by ELISA. Disease activity was assessed using the Pemphigus Disease Area Index (PDAI). Disease activity significantly decreased in both SSA (p<0.001) and rituximab (p<0.005) groups from T1 to T2. SSA patients showed a higher AChR antibody concentration compared to Normal Controls (median PV=19.6 ng/mL, median NC=16.9 ng/mL, p<0.0087). No correlation was found between anti-DSG1 or -DSG3 IgG and IgG anti-AChR antibody levels in either treatment group at T1 or T2. A significant difference in paired IgG anti-DSG1 (p<0.0013) and anti-DSG3 (p<0.002) levels between T1 and T2 was found in both SSA and rituximab subjects. No significant difference, however, was seen in paired AChR antibody levels between T1 and T2 in either treatment group. These findings demonstrate that although IgG anti-AChR autoantibodies were present in PV subjects, they did not decrease with either SSA or rituximab therapy and did not correlate with anti-DSG1 or -DSG3 IgG autoantibody levels.

082 Vitiligo severity is suppressed by type I interferon signaling in resident eosinophils
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Type I interferons (IFN) are pleiotropic cytokines that modulate immune responses in various ways. In fact, they can promote or inhibit inflammation depending on the timing, cell type, and context in which they are secreted. Vitiligo patients present with white, dermgmented patches of skin that arise due to autoimmune destruction of 3D eosine+ T cells that migrate into the epidermis and kill melanocytes. We have reported that IFNγ signaling and IFNα-dependent chemokines are required to drive vitiligo pathogenesis in both mice and humans. Since type I interferon and IFNγ signaling share multiple downstream gene targets, we were interested to determine the role of type I interferon on vitiligo pathogenesis. To do this, we induced vitiligo in mice deficient in the type I interferon receptor, IFNAR. To our surprise, we found that IFNAR+/- mice developed severe and sustained vitiligo compared to WT control mice, suggesting that rather than promoting disease like IFNγ, IFNAR suppresses disease severity. We further found that autoreactive CD8+ T cells do not directly respond to type I IFNs, but rather resident eosinophils require IFNAR signaling to suppress vitiligo. In the absence of type I IFN signaling, autoreactive CD8+ T cells have significantly reduced expression of the effector molecule PD-1 and produce increased amounts of the effector cytokines TNFα and IFNγ. We subsequently found using conditional knockout mice that epidermal Langerhans cells and keratinocytes do not require IFNAR to suppress disease, and thus are currently exploring the role of IFNAR signaling on epithelial melanocytes to regulate tolerance during vitiligo. Our research on the direct role of IFNAR in vitiligo will have broad implications for our understanding of mechanisms of peripheral tissue tolerance and may alter our understanding on the role of IFNAR in other organ-specific autoimmune diseases, such as multiple sclerosis, in which IFNβ is administered therapeutically.
085 TRPV1 and TRPA1 regulate dermal inflammation and epidermal hyperplasia in imiquimod (IMQ)-mediated psoriasis-like dermatis 

Y. Zhou1, J. Gu1, X. Chen1, K. V. Sivakumar1, L. E. Harrington2, D. Hart3, S. Ye1, Y. Li4, M. Carters1, E. Carters1 and S. Hwang1 1 Duke Dermatology, 2 Duke Duke University, 3 Dermatology, Durham, NC, and 4 Dermatology & Immunology, Durham, NC Allergic contact dermatitis (ACD) results from a T-cell response to hapten/allergens applied to the skin. Following sensitization, specific T-cells are first generated and activated in the lymph node, and some Ag-specific T-cells remain in the skin. Thus, exposure to the relevant hapten/allergen initiates the effector phase and clinical expression of ACD, characterized by local T-cell activation and recruitment of specific T cells to the sites of allergic challenge. IL-15 is essential for allergic contact hypersensitivity pathogenesis and plays a pivotal role in both T-cell activation, survival and effector functions and, yet, paradoxically, it also provides anti-apoptotic signals in keratinocytes. IL-15 regulation in the skin is not well known. We made the unexpected observation that in contrast to ACD, patients with the skin of 72 hrs patch-test individuals (effector phase of ACD), IL-15 and anti-apoptotic BCL2 localized to dermal T cell DC clusters and to a much lesser extent to the epidermis. Interestingly, increased BCL2 was expressed in T cells within the clusters and was associated with and directly adjacent to IL-27-producing DC14+ cells in positive patch-tested induced ACD. Monocytic cells, including human DC-like THP1, and murine BMDC up-regulated IL15 mRNA upon IL-27 stimulation. IL-27 was also found to directly stimulate IL-15 mRNA in human epidermal keratinocytes in a STAT1-dependent manner as evidenced by rapid p-STAT1 nuclear translocation and abrogation of this response by silencing STAT1 (p<0.05). We next tested the functional in vivo relevance of IL-27 signaling in a mouse model of hapten/allergen-induced dermal T cell clusters. Administration of IL-27 neutralizing antibody (i.d.) to these mice decreased the number of T cell clusters and resulted in downregulation of BCL2 in T cells supporting a regulatory role of IL-27 on T cell survival possibly via IL-15. Overall, these findings implicate the IL-27 pathway as a potential therapeutic agent in regulating cutaneous T cell immunity.

086 Regulatory function of dendritic cells in psoriasis mediated by CD100-Plexin B1 complex 

B Xu1, X. Liao1, Y. Gu2, G. Wang2 and W. Li3 1 Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi’an, Shaanxi, China and 2 Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China Vitiligo is a chronic auto-immune depigmenting skin disorder that results from a loss of melanocytes. Multiple combinatorial factors have been involved in disease development, with a prominent role of the immune system. Interestingly, several studies observed increased anti-melanocyte antibodies in the sera of patients with vitiligo, suggesting the involvement of humoral immunity in the pathogenesis of vitiligo. Helper CD4+ T (TH) cells are specialized providers of B cell help for the production of antibodies and have been shown to play a central role in the pathogenesis of autoimmune diseases, such as systemic lupus erythematosus (SLE). TH1, mainly produced by TH1 cells, was found increased in vitiligo sera and positively correlated with disease activity, suggesting a possible involvement of these cells in disease pathogenesis. However, the prevalence of TH1 cell subsets has never been previously assessed in vitiligo. We demonstrated TH1 cell subsets in TH1 cells (CD4+CXCR3+CD45RA-) expressing ICOS cell surface markers are increased in active vitiligo patients compared to stable patients or healthy control donors. A comprehensive analysis of circulating TH cell subsets based on the expression of CXCR3 and/or CXCR6 revealed that TH1 cell subsets in vitiligo patients were characterized by a loss of CD4+CXCR6+CD45RA- expressing cells. Both increased IL-15 and anti-apoptotic genes were observed in TH1 cells from patients with vitiligo.

087 IL-27 signaling is relevant for IL-15 production and T cell cluster maintenance 

S. Neubauer1, E. Schmidt1, J. Recke1, KB Yancey3, T Hashimoto4, F Antonicelli5, G Di Zenzo6, D Zillikens7, W Stöcker2 and ENST00000501122.2 may have potential value for the assessment of the disease activity of patients with systemic lupus erythematosus (SLE). Anti-laminin 332 (Lam332) mucous membrane pemphigoid (MMP) is an autoimmune blistering disease with predominant mucosal involvement and autoantibodies against Lam332 and is associated with an increased risk of solid tumors. No standardized detection system for anti-Lam332 serum antibodies is widely available. In this study, a cell-based indirect IF assay was used to detect anti-Lam332 antibodies. Anti-Lam332 serum antibodies is widely available. In this study, a cell-based indirect IF assay was used to detect anti-Lam332 antibodies. The transient receptor potential ion channel receptors TRPV1 and TRPA1 are known to mediate pain from heat and capsaicin (VI) and other noxious chemicals (AI), and may also play roles in mediating pruritus. Their roles in dermal inflammation remains unclear, and we hypothesized that they may regulate dermal inflammation in psoriasis in which pruritus is common. Herein, we assessed transdermal water loss (TEWL), epidermal hyperplasia (by H&E staining), and the dermal inflammatory infiltrate (by Giemsa and immunohistochemical staining) in wild type and TRPV1 and TRPA1 knockout (KO) mice following daily application of topical imiquimod (IMQ) cream for 5 days. Strikingly, TEWL scores were significantly decreased in TRPV1/KO and TRPA1/KO mice by 50.07% and 54.36%, respectively, with WT mice, suggesting a reduction in IMQ-mediated barrier defects. Furthermore, epidermal hyperplasia was decreased in both TRPV1/KO and TRPA1/KO mice by 31.33% and 33.33%, respectively. Additionally, the area of epidermal Munro microabscesses was decreased in TRPV1/KO mice by 27.32% and 68.32% compared with WT mice, suggesting that neutrophil recruitment was impacted in the KO mice. Lastly, mast cells as well as CD31+ blood vascular cells, CD45+ leukocytes, and CD1 T cells were all reduced in the lesional skin of TRPV1/KO, TRPA1/KO mice, suggesting that dermal inflammation is reduced in these KO mice. In summary, key markers for psoriatic inflammation, including dermal inflammation and epidermal hyperplasia, are reduced in TRPV1 and TRPA1 KO mice, suggesting a novel role for these sensory receptors in psoriasiform inflammation and new avenues for therapeutic intervention.

088 Intrafollicular CD4+ T follicular helper lymphocytes in active vitiligo 

C. Jacquemin, A. Taieb, K. Boniface and J. Senechal University of Bordeaux, Bordeaux, France Vitiligo is an inflammatory autoimmune disease triggering skin disorder that results from a loss of melanocytes. Multiple combinatorial factors have been involved in disease development, with a prominent role of the immune system. Interestingly, several studies observed increased anti-melanocyte antibodies in the sera of patients with vitiligo, suggesting the involvement of humoral immunity in the pathogenesis of vitiligo. Helper CD4+ T (TH) cells are specialized providers of B cell help for the production of antibodies and have been shown to play a central role in the pathogenesis of autoimmune diseases, such as systemic lupus erythematosus (SLE). TH1, mainly produced by TH1 cells, was found increased in vitiligo sera and positively correlated with disease activity, suggesting a possible involvement of these cells in disease pathogenesis. However, the prevalence of TH1 cell subsets has never been previously assessed in vitiligo. We demonstrated TH1 cell subsets in TH1 cells (CD4+CXCR3+CD45RA-) expressing ICOS cell surface markers are increased in active vitiligo patients compared to stable patients or healthy control donors. A comprehensive analysis of circulating TH cell subsets based on the expression of CXCR3 and/or CXCR6 revealed that TH1 cell subsets in vitiligo patients were characterized by a loss of CD4+CXCR6+CD45RA- expressing cells. Both increased IL-15 and anti-apoptotic genes were observed in TH1 cells from patients with vitiligo.

089 Sensitive and specific assay for the serological diagnosis of anti-laminin 332 mucus membrane pemphigoid 

S. Goletzi1, E. Prodol1, L. Komorowski2, W. Schlumberger1, N. van Beeck1, M. Holtsche1, A. Recke1, KB Yancey3, J. Hashimoto4, G Di Zenzo5, D. Zillikens6, W. Stöcker2 and ENST00000501122.2 may have potential value for the assessment of the disease activity of patients with systemic lupus erythematosus (SLE). Anti-laminin 332 (Lam332) mucous membrane pemphigoid (MMP) is an autoimmune blistering disease with predominant mucosal involvement and autoantibodies against Lam332 and is associated with an increased risk of solid tumors. No standardized detection system for anti-Lam332 serum antibodies is widely available. In this study, a cell-based indirect immunofluorescence (IF) assay using recombinant Lam32 expressed on the surface cell of HEK293 cells was developed. The IF assay was probed with a large number of anti-Lam332 serum antibodies. A two-tiered scoring system was applied instead of the anti-IgG4 detection antibody. Additionally, a correlation between serum anti-Lam332 IF titers and disease activity was observed. This novel IF-based assay will facilitate the serological diagnosis of anti-Lam332 MMP.
ABSTRACTS | Adaptive and Auto-ImmunitY

091 NativeSkin®, an immunocompetent human skin model to study antigen uptake and processing by Langerhans cells
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Background: NativeSkin® is an ex vivo human skin model which can be kept in culture for 7 days while maintaining normal viability and responsiveness of various immune cells. In this study, we demonstrated the ability of this model to react to topically administered allergens, which were significantly associated with CADRs induced by allopurinol, CBZ, SASP, methotrexate(13.56%), Anti-hyperuricemia agents(12.86%) and antipyretic/analgesic agents(11.68%), respectively. HLA genotyping was performed on patients with CADRs. Patients who were tolerant for the drugs and healthy people from the human MHC database(dbMHC) were defined as controls. In silico docking experiments were conducted and the complex structure model of HLA alleles were created by the molecular modeling software. The results showed that the relative contribution of each of these HLA alleles to drug adverse reactions is its target organs. A previous study showed lesional B cells could produce desmoglein specific antibodies. However, the reason for local antibody secreting and in situ differentiation of B cells remain unclear. Methods: Skin cell suspensions from pemphigus lesions were prepared by incubating skin with a digestion buffer. Then cells were isolated by lymphocyte separation solution. Lesional B cells and peripheral B cells were compared by flow cytometry. Results: The frequencies of CD19+CD27+B cells (80.01% vs. 72.52%, P = 0.001) and CD19+CD38+B cells (27.24% vs. 15.67%, P = 0.001) were significantly higher in the pemphigus lesions than in PBMC. There was a much lower fraction of CD19+CD27-IgD+B cells (3.07±1.66% vs. 10.1±0.61%, P = 0.0226) detected in pemphigus lesions than in PBMC. In addition, a significant higher fraction of CD19+ B cells from the pemphigus lesions could specifically bind to Dsg1 (46.35±9.94% vs. 2.22±1.57%, P = 0.0304) and Dsg3 (35.77±2.34% vs. 6.00±2.63%, P = 0.0099) than CD19+B cell in PBMC. Pemphigus lesions contained a higher fraction of CD19+CD38hiCD77+ centroblasts (17.08±4.61% vs. 0.16±0.06%, P = 0.0341) and CD19+CD27hiCD138hi plasmablasts (26.71±4.64% vs. 3.96±1.29%, P = 0.0028) and plasma cells (7.3±1.05% vs. 4.57±0.14%, P = 0.0005) than those from PBMC. Conclusions: These results suggest that a complete recapitulation of B cell differentiation is ongoing within the pemphigus lesion during the in situ antigen driven cell immune response.

094 The phenotype and differentiation of B cells in pemphigus lesions
S Zhou and M Pan Department of Dermatology, Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Background: Pemphigus is an autoimmune bullous disease with skin and mucous membranes as its target organs. A previous study showed lesional B cells could produce desmoglein specific antibodies. However, the reason for local antibody secreting and in situ differentiation of B cells remain unclear. Methods: Skin cell suspensions from pemphigus lesions were prepared by incubating skin with a digestion buffer. Then cells were isolated by lymphocyte separation solution. Lesional B cells and peripheral B cells were compared by flow cytometry. Results: The frequencies of CD19+CD27+B cells (80.01% vs. 72.52%, P = 0.001) and CD19+CD38+B cells (27.24% vs. 15.67%, P = 0.001) were significantly higher in the pemphigus lesions than in PBMC. There was a much lower fraction of CD19+CD27-IgD+B cells (3.07±1.66% vs. 10.1±0.61%, P = 0.0226) detected in pemphigus lesions than in PBMC. In addition, a significant higher fraction of CD19+ B cells from the pemphigus lesions could specifically bind to Dsg1 (46.35±9.94% vs. 2.22±1.57%, P = 0.0304) and Dsg3 (35.77±2.34% vs. 6.00±2.63%, P = 0.0099) than CD19+B cell in PBMC. Pemphigus lesions contained a higher fraction of CD19+CD38hiCD77+ centroblasts (17.08±4.61% vs. 0.16±0.06%, P = 0.0341) and CD19+CD27hiCD138hi plasmablasts (26.71±4.64% vs. 3.96±1.29%, P = 0.0028) and plasma cells (7.3±1.05% vs. 4.57±0.14%, P = 0.0005) than those from PBMC. Conclusions: These results suggest that a complete recapitulation of B cell differentiation is ongoing within the pemphigus lesion during the in situ antigen driven cell immune response.

096 Demographic and serologic features differentiate bullous pemphigoid with and without preceding neurologic disease
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Background: Pemphigus is a prototypical organ specific autoimmune disease dependent on multiple pathogenic environmental factors. The clinical phenotype and prognosis of each patient is clear that pathogenic factors and host genomics involves HLA genetic susceptibility and multiple autoantibody reactivities to both desmoglein (Dsg) and non-Dsg targets. However, the relative contribution of each of these disease-linked elements to disease is not known. In order to gain new insights into the role of pathogenic factors and develop better tools with clinical utility for disease diagnosis and prognosis, we integrated large scale genetic and proteomic data on 1) PV-associated HLA alleles, 2) 35 autoantibody specificities detected by protein array and 3) 4 antibodies detected by ELISA (against Dsg3, Dsg1, TPO and Tg) in 248 PV patients as well as 131 healthy controls. PBMC. Pemphigus lesions contained a higher fraction of CD19+CD38hiCD77+ centroblasts (17.08±4.61% vs. 0.16±0.06%, P = 0.0341) and CD19+CD27hiCD138hi plasmablasts (26.71±4.64% vs. 3.96±1.29%, P = 0.0028) and plasma cells (7.3±1.05% vs. 4.57±0.14%, P = 0.0005) than those from PBMC. Conclusions: These results suggest that a complete recapitulation of B cell differentiation is ongoing within the pemphigus lesion during the in situ antigen driven cell immune response.

096 Systems medicine for pemphigus: Integrating multiple datasets for improved clinical decision making
L Li, K Seiffert-Sinha1, Y Sun1 and AA Sinha1
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Background: Pemphigus vulgaris (PV) is a prototypical organ specific autoimmune disease dependent on multiple pathogenic environmental factors. It is becoming increasingly apparent that pathogenic factors and host genomics involves HLA genetic susceptibility and multiple autoantibody reactivities to both desmoglein (Dsg) and non-Dsg targets. However, the relative contribution of each of these disease-linked elements to disease is not known. In order to gain new insights into the role of pathogenic factors and develop better tools with clinical utility for disease diagnosis and prognosis, we integrated large scale genetic and proteomic data on 1) PV-associated HLA alleles, 2) 35 autoantibody specificities detected by protein array and 3) 4 antibodies detected by ELISA (against Dsg3, Dsg1, TPO and Tg) in 248 PV patients as well as 131 healthy controls. PBMC. Pemphigus lesions contained a higher fraction of CD19+CD38hiCD77+ centroblasts (17.08±4.61% vs. 0.16±0.06%, P = 0.0341) and CD19+CD27hiCD138hi plasmablasts (26.71±4.64% vs. 3.96±1.29%, P = 0.0028) and plasma cells (7.3±1.05% vs. 4.57±0.14%, P = 0.0005) than those from PBMC. Conclusions: These results suggest that a complete recapitulation of B cell differentiation is ongoing within the pemphigus lesion during the in situ antigen driven cell immune response.

092 Immune response patterns in chronic inflammatory skin diseases as a basis of targeted therapy
E Pagès1, E Descargues1 and S Eyerich1
1 Department of Dermatology and Allergy, Technische Universität München, Munich, Germany and 2 ZAUM Center of Allergy and Environment, Technical University and Helmholtz Center Munich, Germany

Background: Pemphigus vulgaris (PV) is a prototypical organ specific autoimmune disease dependent on multiple pathogenic environmental factors. It is becoming increasingly apparent that pathogenic factors and host genomics involves HLA genetic susceptibility and multiple autoantibody reactivities to both desmoglein (Dsg) and non-Dsg targets. However, the relative contribution of each of these disease-linked elements to disease is not known. In order to gain new insights into the role of pathogenic factors and develop better tools with clinical utility for disease diagnosis and prognosis, we integrated large scale genetic and proteomic data on 1) PV-associated HLA alleles, 2) 35 autoantibody specificities detected by protein array and 3) 4 antibodies detected by ELISA (against Dsg3, Dsg1, TPO and Tg) in 248 PV patients as well as 131 healthy controls. PBMC. Pemphigus lesions contained a higher fraction of CD19+CD38hiCD77+ centroblasts (17.08±4.61% vs. 0.16±0.06%, P = 0.0341) and CD19+CD27hiCD138hi plasmablasts (26.71±4.64% vs. 3.96±1.29%, P = 0.0028) and plasma cells (7.3±1.05% vs. 4.57±0.14%, P = 0.0005) than those from PBMC. Conclusions: These results suggest that a complete recapitulation of B cell differentiation is ongoing within the pemphigus lesion during the in situ antigen driven cell immune response.

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**Tissue-resident memory T cells in psoriasis recurrence**

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Psoriasis is a chronic and recurring inflammatory skin disease. Usually it recurs in previously affected areas, as a pathogenic memory has been proposed. However, the nature of such site-specific memory is unknown. Tissue-resident memory T (TRM) cells are non-recirculating memory T cells that persist long-term in epithelial tissues, including the gastrointestinal tract, lung, and skin. Because they can localize in the epidermal compartment for many months, we speculate that TRM cells may contribute to recurring pathologies. Herein, we investigated whether resolved psoriasis lesions contain TRM cells with the ability to potentially drive psoriasis recurrence. Ten psoriatic patients were included. The flow cytometry, cell culture, and immunofluorescence technique were used. We found that CD8+ epidermal T cells were highly activated even in resolved psoriatic lesions. A high proportion of CD8+ epidermal T cells expressed TRM marker of CD69. This population of CD8+ epidermal T cells persisted at resolved psoriatic lesions as high as in neighboring recurrent psoriatic lesion, which suggests its vital roles in the recurrence of psoriasis. We demonstrated that psoriatic keratinocytes secret high amount of IL-15, and we investigated IL-15’s role in regulation of JAK/STAT5 signaling in TRM cells. Our preliminary study showed TRM cells persisted in clinically resolved psoriatic lesions and produced the cytokines with critical roles in psoriasis pathogenesis. We provide a direct evidence for a site-specific T cell-driven disease memory in psoriasis.

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**Identification of T cell receptor chains responsible for alopecia areata pathogenesis via bulk and single cell TCR sequencing**

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Alopecia Areata (AA) is an autoimmune form of hair loss affecting more than 6 million people in the USA. Our laboratory has recently demonstrated that a subset of CD8+ NKG2D+ T cells, are both necessary and sufficient for development of AA in C3H/HeJ grafted mice. However, the antigens that are targeted by these CD8+ T cells are still unknown. Previously, we performed next generation T cell receptor (TCR) b-chain sequencing on CD8+ T cells isolated from lesional skin and skin-draining lymph nodes in the C3H/HeJ mouse model of AA. This revealed T cell clonal expansion in lesional skin around the time of hair loss, as well as TCR (CD3)b motifs shared between independent lesional skin samples, which supports the notion of the same T cell clone. Moreover, clonal expansion also occurred in lesional skin of human AA patients as well, pointing toward an autocrine drive for human AA. Here, we used single cell TCR sequencing of alpha and beta chains in CD8+ T cells isolated from lesional AA mouse skin to identify the TCR chains that pair with the dominant TCR alpha chains expressed by pathogenic CD8+ T cells. This approach allowed us to pinpoint two TCR alpha and beta chains that were markedly enriched in lesional skin. These two b-chain pairs had similar amino acid motifs, and comprised 16% (13/81) and 18.5% (15/81) of the sequenced cells respectively. We were evaluating the pathogenicity of these TCR pairs by introducing them into retrogene C3H/HeJ mice, and asking whether they are sufficient to induce AA. We identification and validation of pathogenic TCR pairs in AA.

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**Identification of T cell receptor chains responsible for alopecia areata pathogenesis via bulk and single cell TCR sequencing**

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Alopecia Areata (AA) is an autoimmune form of hair loss affecting more than 6 million people in the USA. Our laboratory has recently demonstrated that a subset of CD8+ NKG2D+ T cells, are both necessary and sufficient for development of AA in C3H/HeJ grafted mice. However, the antigens that are targeted by these CD8+ T cells are still unknown. Previously, we performed next generation T cell receptor (TCR) b-chain sequencing on CD8+ T cells isolated from lesional skin and skin-draining lymph nodes in the C3H/HeJ mouse model of AA. This revealed T cell clonal expansion in lesional skin around the time of hair loss, as well as TCR (CD3)b motifs shared between independent lesional skin samples, which supports the notion of the same T cell clone. Moreover, clonal expansion also occurred in lesional skin of human AA patients as well, pointing toward an autocrine drive for human AA. Here, we used single cell TCR sequencing of alpha and beta chains in CD8+ T cells isolated from lesional AA mouse skin to identify the TCR chains that pair with the dominant TCR alpha chains expressed by pathogenic CD8+ T cells. This approach allowed us to pinpoint two TCR alpha and beta chains that were markedly enriched in lesional skin. These two alpha/beta chain pairs had similar amino acid motifs, and comprised 16% (13/81) and 18.5% (15/81) of the sequenced cells respectively. We were evaluating the pathogenicity of these TCR pairs by introducing them into retrogene C3H/HeJ mice, and asking whether they are sufficient to induce AA. We identification and validation of pathogenic TCR pairs in AA.

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**Pathogenic role of interleukin (IL)-26 producing Th17 cells in the acute forms of psoriasis**

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Psoriasis is a chronic and recurring inflammatory skin disease. Usually it recurs in previously affected areas, so a pathogenic memory has been proposed. However, the nature of such site-specific memory is unknown. Tissue-resident memory T (TRM) cells are non-recirculating memory T cells that persist long-term in epithelial tissues, including the gastrointestinal tract, lung, and skin. Because they can localize in the epidermal compartment for many months, we speculate that TRM cells may contribute to recurring pathologies of psoriasis. Here, we demonstrate the critical role of IL-7 in alopecia areata (AA), and furthermore, reversed early-onset AA in C3H/HeJ mice by introducing them into retrogenic C3H/HeJ mice, and asking whether they are sufficient to induce AA. The identification and validation of pathogenic TCR pairs in AA.

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**Alopecia areata is reversed by IL-7R blockade via upregulation of the PD-1 signaling pathway and T cell exhaustion**

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Alopecia areata is an autoimmune skin disease driven by CD8+ T cell effector cells (Teffs) that target melanocytes in the epidermis resulting in visible white spots. Previous studies in our mouse model of vitiligo revealed that Teffs require CXCR3 to migrate to the skin in response to CCL19 and CCL10 chemokine signals. We also found that T regulatory cells (Tregs) suppress depigmentation in vitiligo, so we investigated mechanisms of Treg recruitment into skin during vitiligo. Using flow cytometry, we found that both Teffs and Tregs accumulate in the skin with similar kinetics. Using confocal microscopy in mice and histology in patient biopsies, we observed Tregs and Teffs clustering together, with Tregs in direct contact with Teffs, suggesting that Tregs must colocalize with Teffs to efficiently suppress their function. To investigate chemokine signals that promote Treg migration, we induced vitiligo in RAG-2 mice that received wild-type (WT) or CXCR3-/- Tregs. WT and CXCR3-/- Treg recipients had comparable levels of depigmentation and vitiligo, suggesting that CXCR3 is dispensable for the ability of Tregs to suppress vitiligo in the skin. In previous studies we observed that CCL5 and its receptor CCR5 are highly induced in the skin of vitiligo patients and mice, and published GWAS results identified CCR6 as a vitiligo susceptibility gene. We found that Tregs express CCR5 and CCR6 in mouse and human skin during vitiligo, and that eliminating either chemokine receptor on Tregs inhibited their ability to suppress depigmentation during vitiligo. In addition, the recruitment of CCR6+ Tregs were diminished in the skin of mice with vitiligo compared to mice receiving WT Tregs, supporting the hypothesis that CCR5 and CCR6 promote the recruitment of Tregs to the skin to halt the progression of vitiligo.

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**CXCL9 drives morphea pathogenesis in mice**

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Morphea, also known as localized scleroderma, is a skin disorder that leads to single or multiple sclerotic skin lesions resulting in cosmetic and functional impairment. The pathogenesis of morphea is poorly understood, but morphea is believed to be an autoimmune disease. Prominent theories of pathogenesis involve vascular or immune dysfunction that initially presents as inflammatory changes in the skin. Previous studies showed that morphea patients have elevated CXCL9 and CCL10 chemokines in lesional skin. In order to further explore the role of chemokines in morphea pathogenesis, we used the morphea mouse model, which uses subcutaneous bleomycin injection, to induce morphea-like fibrosis in mice. We induced morphea-like fibrosis in RKO1 mice, which report expression of CXCL9 and CCL10 using fluorescent proteins, and found upregulation of the chemokine reporters via flow cytometry. We next tested mouse strains deficient in CXCL9, CCL10 and CXCR3, and found that CXCL9 and CCL10 were necessary for CXCR3+ Teffs to produce CXCL9 expression in mice compared to vehicle controls. These experiments demonstrate that CXCL9 plays an important role in morphea pathogenesis, and upregulating chemokine production can be an effective treatment strategy.
103 Dissecting autoimmune signaling networks in vitiligo using single-cell RNA-seq of cells isolated directly from lesional skin

IP Strasser1, VN Azzolino2, JP Strassner2, JM Richmond1 and JE Harris1

Vitiligo, an autoimmune disease characterized by patchy, depigmented lesions of the skin. The disease is mediated by autoreactive CD8 T cells that destroy melanocytes. To investigate signaling networks in the skin that lead to this patchy melanocyte destruction, we used a modified suction blistering technique to isolate cells directly from lesional skin and subjected them to single-cell RNA-sequencing. We sequenced cells from both lesional and non-lesional skin in six vitiligo subjects with active disease as well as six healthy controls. We identified multiple cell populations, including melanocytes, keratinocytes, Langerhans cells and immune cells of the innate and adaptive arms of the immune system. We also identified putative regulatory elements that control transcription of novel drug targets in previously unknown pathological signaling pathways in vitiligo and other diseases of the skin.

104 IFNγ and fas ligand are required for melanocyte destruction in a mouse model of vitiligo

VN Azzolino1, JP Strassner2, JM Richmond1 and JE Harris1

Autoimmune CD8 T cells are responsible for the selective destruction of melanocytes in vitiligo. Previous studies by our group and others demonstrated that IFNγ-induced chemo- kines are required for T cell recruitment into the skin and progression of vitiligo. To determine the mechanism of melanocyte killing by autoreactive T cells in our mouse model of vitiligo, we decided to test the role of known CD8 effector molecules. We found that disease severity and T cell accumulation in the skin was reduced in mice receiving adoptive transfer of either IFNγ- or Fas ligand (FasL)+ autoreactive T cells; however, perforin was dispensable. We did not observe defects in expansion or migration any of the transferred T cells. Co-injections of IFNγ and FasL inhibited expansion of T cells in the skin, which correlated with the reduced level of disease as mice injected only with WT T cells. Intriguingly, FasL- and IFNγ- T cells accumulated in greater numbers in the skin than co-injected WT T cells, suggesting that IFNγ and FasL are required locally in the skin to cause disease. To determine if IFNγ has a direct cytotoxic effect on melanocytes, we deleted IFNγR from peripheral blood mononuclear cells using a floxed allele and Cre driven by Tyrrosinase. After inducing vitiligo in these mice, we did not detect any differences in disease development in floxed Cre- mice, indicating that IFNγ has a pleiotropic effect in vitiligo, promoting both migration and function of autoreactive T cells. Furthermore, targeting the IFNγ/FasL axis may be a promising strategy for treating vitiligo.

105 Assessing CEACAM1 blockade as a therapeutic strategy using a CRISPR-Cas9-based mouse melanoma model

C. Kaucik, E. Perez-Guijarro, C. Day and G. Merlino NCI, NIH, Bethesda, MD

CEACAM1 is an immune checkpoint blockade (ICB) target. Unfortunately, melanomas treated with kinase inhibitors typically develop resistance; therefore, ICB has become first line therapy due to more durable responses. However, currently available ICBs, such as anti-CTLA-4 and anti-CD137 (4-1BB), are only effective in a subset of patients, leading to a better understanding of other immune checkpoints as potential therapeutic targets. Carcinomembrynogenic antigen-related cell adhesion molecule 1 (CEACAM1), which is expressed on the surface of activated (but not resting) CD8+ T cells and on many types of tumor cells, has been found to contribute to tumor progression and immune evasion. We examine the relevance of CEACAM1 in a genetically engineered mouse melanoma model harboring BRAFV600E mutation, which represents 40-60% of human melanomas. CEACAM1 is induced by: activation of latent, membrane-bound Braf and neuronal exposure to UV irradiation. We have detected high expression of CEACAM1 on cell surface at protein levels in UV-BRAF melanomas. We hypothesize CEACAM1 is involved in immune evasion, and its inhibition alone or in combination with PD-1/PD-L1 and anti-CTLA-4 blockade could be a new strategy to treat melanoma. To test our hypothesis, we developed an inducible CRISPR-Cas9 system to knockout CEACAM1 expression in UV-BRAF melanoma cells. We are injecting these cells into syngeneic immunocompetent mice to evaluate the effects of CEACAM1 knockdown on tumor growth, and will use immunofluorescent mouse mice to determine the implication of the adaptive immune system in our findings. Future preclinical studies will include the combination of anti-PD-L1 and anti-CTLA-4 treatments with CEACAM1 inhibition to elucidate potential synergistic effects of these ICBs. Overall, we have validated CEACAM1 expression in a mouse model representing a large cohort of human melanoma and created CEACAM1 knockout mouse melanoma cells for mechanistic studies. Amur current immunotherapies, blockade of CEACAM1 may offer a promising alternative treatment strategy for advanced melanoma.

106 Early stage mycosis fungoides has a mutanome distinct from Sezary Syndrome

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Mycosis fungoides and Sezary Syndrome are the most common cutaneous T-cell lymphomas. It is usually characterized by patches and plaques in the early stage; the majority of these patients have indolent disease; however, a subset progress to lethal advanced stage disease. Although Sezary Syndrome accounts for <10% of CTCL cases, most whole exome sequencing (WES) studies have focused on SS because the abundant circulating tumor cells are readily isolated in large numbers. In contrast, in early stage MF, it is difficult to isolate a sufficient enriched population of tumor cells. Accordingly, there is limited published data on the genomic landscape of early-stage MF. We used a novel method of expression microdissection to extract skin T-cells from lesional skin of 29 patients with skin-limited mycosis fungoides, as confirmed by high throughput sequencing of the TCRB gene. 24/29 had stage 1 disease. We performed WES on T-cells from these patients, which we classified into: basal, suprabasal and peripheral blood mononuclear cells. The mean target coverage was 70x in skin samples an 100x in blood samples. The median somatic mutation frequency was 0.6/mb DNA (range 0.05-190), with Stage 1 cases clustering at the lower end. We identified notable recurrent somatic single nucleotide variants and copy number alterations in epigenetic modulators (e.g. KMT2C, KMT2D, TET2, ARID1A), potential tumor suppressors (TPS), CDRK2A, Pten and the MAP kinase signaling pathway. While there was some overlap, the mutational landscape is different than that seen in SS. This study identifies potential tumor drivers and potential new targets in early-stage mycosis fungoides.

107 Chromatin remodeler lymphoid-specific helicase (Lsh) regulates cytotoxic methylation at the DNA repeat elements and prevents autoimmunity inflammation in the epidermis

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Mammalian genomes are highly populated with silent virus-like repeat elements evolutionarily derived from integrated retroviruses. Lsh, a member of the SNF2 chromatin-remodeling family, is involved in the control of DNA methylation during embryonic development. Here, we show that in the epidermis, the Lsh protein is expressed in the basal epidermal keratinocytes (KCs). Constitutive and epidermal-specific Lsh ablation in mice leads to severe skin inflammation associated with epidermal hyperplasia and marked alterations in the epidermal structure. Primary KCs isolated from newborn Lsh KO mice prior to the development of inflammatory phenotype showed substantial alterations in the genome-wide DNA methylation pattern and were defective in controls. Bisulfite sequencing revealed that in Lsh-deficient KCs, the majority of hypomethylated DNA sites are found at the repeat sequences containing Long Terminal Repeats (LTR), Long Interspersed Elements (LINEs) and minor satellites. The global transcriptome profiling and GSEA analysis of Lsh-null KCs revealed a dramatic upregulation of the genes involved in anti-viral defense response, type I interferon- and interferon- mediated signalling pathways, as well as increased expression of Keratin 16/17 and downregulation of Keratin 10, Loricrin, involucrin, filaggrin compared to controls. These data reveal that Lsh is a critical regulator controlling DNA methylation and observed decreased production of dermal T cells. In summary, we find that TGFβ-producing macrophages can support the differentiation of resident memory T cells in human dermis.

108 TGFβ producing macrophages support the differentiation of resident memory T cells in human dermis

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Resident memory T cells develop within peripheral tissues and provide rapid protection against known pathogens but the skin cell types that support TEm differentiation are incompletely characterized. TGFβ producing keratinocytes support TEm generation in the epidermis of mice and humans. Epidermal TEm remain within the epidermis after they differentiate and enter the dermis. However, we find that the majority of human TEm in the skin are located in the dermis and include distinct populations of CD103 expressing CD4+ and CD8+ T cells. These T cells must differentiate into Tem in the dermis and their development is likely supported by dermal, not epidermal skin cells. We studied the localization of TEm in healthy human skin. Many dermal T cells in healthy skin are closely opposed to CD163+ macrophages, a cell type known to produce TGFβ. To test the ability of TGFβ producing macrophages to support the generation of Tem in vitro, we co-cultured human peripheral blood mononuclear cells harvested from healthy donors with Tem blasts in the absence of TGFβ producing macrophages. We found that M2-polarized THP-1 cells, primary human keratinocytes, and plastic. We found that M2-polarized THP-1 cells supported the generation of Tem in vitro. Next, we tested the ability of CD163+ dermal macrophages to support the differentiation of human Tem in cultured human epidermis. In this model, NSG mice are grafted with human foreskin and infused with peripheral blood from a second unrelated human donor. We have previously shown that these mice generate a GVHD-like dermatitis, develop both dermal and epidermal TEm and are an excellent model for studying the differentiation of Tem in vitro and in vivo and are likely a critical cell type for supporting the differentiation of dermal TEm in human skin.
A primary role for human central memory cells in tissue immunosurveillance

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Central memory T cells (TCM) recirculate between blood and lymph nodes providing immunosurveillance. TCM persist long-term in the circulation, have high proliferative capacity and can replenish other memory T cell subsets, including effector memory T cells (TEM). The initial description of human TCM characterized these cells as having poor effector functions and little tissue tropism but these studies did not evaluate the expression of the gut homing addressin a4b7, used a different antibody to detect CLA than the one that identifies cutaneous and little tissue tropism but these studies did not evaluate the expression of the gut homing addressins in human TCM vs TEM from the same donors. In humans, the majority of human TCM were tropic for either skin or gut and the overall tissue tropism of TCM, comparable to the TEM, TCM were sorted from human skin, lung, colon and cervix, suggesting a role for TCM in the primary immunosurveillance of peripheral tissues. TCM also had potent effector functions, 80% of CD8+ TCM produced IFNγ and were also cytotoxic in a 6 hr chromium release assay. TEM injected into human skin-grafted mice migrated into skin & induced inflammatory eruptions comparable to TEM-injected mice. In summary, human TCM express peripheral tissue homing receptors at levels similar to their TCM counterparts, are found in healthy human tissues, have impressive effector functions and can act alone to induce skin inflammation in human engrafted mice. Our work demonstrates that human TCM are different and have the capacity to provide primary immunosurveillance of peripheral tissues. Added to their known abilities to persist long-term in the circulation, proliferate and give rise to additional memory T cell subsets, our work supports a critical role for TCM in providing long-term protection against known pathogens.

Role of platelets in the differentiation of monocytes into dendritic cell-like antigen presenting cells

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Platelet-monocyte aggregates have been documented to result in phenotypic changes in circulating monocytes. To date, studies have overwhelmingly focused on these interactions in the contexts of thrombotic events and platelet-borne disorders. However, an understanding of the specific nature of platelet-monocyte interactions as well as the functional changes induced on the mononuclear phagocytes, particularly in antigen processing and presentation, are yet to be elucidated. In this report, we investigate the effects of platelet-monocyte interaction on the phenotypic, genotypic, and functional properties of peripheral blood-derived monocytes. In the process of this investigation, we discovered that peripheral blood monocytes are able differentiate into professional antigen-presenting cells (APCs) upon interaction with platelets, exhibiting Dendritic Cell (DC)-like properties of antigen processing and cross-presentation as well as functional, antigen-specific stimulation of T cells. We confirmed platelet-induced activation of monocyte differentiation both at a genetic level utilizing single cell RNA-seq analysis, as well as a functional level via antigen-specific T cell readouts. Our results indicate that functional DCs can be generated from monocyte precursors in the absence of exogenous cytokines or growth factors, through ex vivo physiologic platelet-monocyte interactions that may recapitulate monocyte to DC differentiation in vivo.

Epidercutaneous allergen exposure induces pathogenic IL-22-producing T helper cells

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Epidercutaneous allergen exposure induces pathogenic IL-22-producing T helper (T H2) cells and IL-22-expressing Th2 cells. Environmental antigens like food allergens are thought to influence the clinical course of AD. We aimed to study the pathogenic phenotype of food allergen-specific T cells activated through the skin or oral exposure and their potency to transfer skin inflammation. Oral ovalbumin (OVA) TCR-transgenic mice were sensitized epicutaneously with OVA or were fed OVA. CD4+ T cells from skin or mesenteric lymph nodes were phenotyped for cytokine expression and transferred into naive BALB/c mice. Recipient mice were challenged with OVA epicutaneously. Skin inflammation was determined histologically. Importantly, AD-like skin inflammation could only be induced by the transfer of epidercutaneously primed OVA T cells. Analysis of the immune phenotype demonstrated an IL-22/IL-17A-dominated immune phenotype of skin-pathogenic T cells. IL-22 seems to be the critical cytokine for the development of AD and is induced in this model by epidercutaneous sensitization with OVA.

Tumor clone frequency is a robust predictor of progression and survival in patients with Stage III mycosis fungoides

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We previously characterized human skin T cells with low expression of the cutaneous lymphocyte antigen (CLA) and high expression of CRTH2. CLA and CRTH2 are members of the G-protein coupled receptor family, which are expressed on the skin-homing T cells (TCM). Our previous work has demonstrated that the phenotype IL-31-producing T cells is poorly characterized. In the present study, we investigated the effects of platelet-monocyte interaction on the phenotypic, genotypic, and functional properties of peripheral blood-derived monocytes. In the process of this investigation, we discovered that peripheral blood monocytes are able to differentiate into professional antigen-presenting cells (APCs) upon interaction with platelets, exhibiting Dendritic Cell (DC)-like properties of antigen processing and cross-presentation as well as functional, antigen-specific stimulation of T cells. We confirmed platelet-induced activation of monocyte differentiation both at a genetic level utilizing single cell RNA-seq analysis, as well as a functional level via antigen-specific T cell readouts. Our results indicate that functional DCs can be generated from monocyte precursors in the absence of exogenous cytokines or growth factors, through ex vivo physiologic platelet-monocyte interactions that may recapitulate monocyte to DC differentiation in vivo.
miRNAs are required for embryonic development of skin immune cells

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Skin immune cells, including LCs, dendritic epidermal T cells (DETCs), and dermis-resident macrophages (MФs), play an essential role in skin homeostasis and immune response. However, the role of miRNAs in skin immune cell development and function remains unclear. Recently, a myeloid lineage-reporter colony stimulating factor receptor (CSFIR)Cre reporter was used to fate map LC precursor and tissue resident MF development. Given that miR-155 is a key miRNA involved in immune response, to study the potential role of miR-155 in embryonic LCs and resident MФs, we created Csf1rCre;miR-155KO in which both miR-155 and GFP expressions are induced by Csf1rtm1. As expected, the majority of LC precursors and skin-resident MФs highly expressed GFP. Surprisingly, epidermal Vγ1T cells that emerged as early as at E16.5 were also GFP-positive, even though the overexpression of miR-155 did not significantly disturb embryonic LCs, MФs and γδ T cells. These observations were confirmed by Cd11ccre;Rosaa26GFP reporter mice, suggesting that CSFIR promoter drives both embryonic myeloid and lymphoid cell lineages. To further study the role of miRNAs in ontogeny of LCs, skin-resident MФs and DETCs, we next generated Cd11ccre;DicerKO mice with deficient miRNAs, which led to impaired development of LCs andermal MФs starting at E18.5. Likewise, DETCs were almost completely eliminated from the KO epidermis and Vγ1T cells number was severely reduced in KO fetal thymus, due to the survival and maturation defects of thymic Vδ T cells. Overall, this is the first study to demonstrate that Csf1rCre reporter mice are not only good for fate mapping LCs and resident MФs, but also serve as a new tool for the study of skin-resident γδ T cell embryonic development. Using this model, we have uncovered that miRNAs are critical epigenetic regulators in the ontology of skin immune cells.

miRNAs are small, noncoding RNAs regulating immune cell development and function. A significant accumulation of CD4+CD44+CD62L+KLRG1+CCR7low T cells expressing phenotypic markers for tissue residence such as CD69+CD103+/- are amongst the highest PD-1 expressing TILs. Subsequent work will further interrogate the cutaneous immune response to spontaneous melanoma skin cancer and the role of PD-1.
T{	extsubscript{H}}{	extsubscript{17}} recall is critically governed by PD-1:PD-L1/2 cross-talk with classical DCs


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T{	extsubscript{H}}{	extsubscript{17}} recall is rapidly upregulated in 72 hours of viral priming in mouse and human we tested the impact of PD-L1 and PD-L2 (CD273) loss on TRM-based defense against melanoma. We find an acute and transient loss of PD-L1 and PD-L2 in ZBTB46-dependent classical DCs during TRM recall significantly contracts tumors growth and immune response overall survival. These results support classical DCs as a critical regulator of T{	extsubscript{H}}{	extsubscript{17}}-based recall and a dynamic role for the PD-1:PD-L1-2 axis.

The role of circadian clocks in a murine model of antibody-induced skin inflammation


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A central pacemaker coordinates a network of endogenous circadian clocks. The skin itself features a self-sustained, intrinsic clock which may affect our first-line defense against intruders over the course of the day. Epidermal differentiation is under circadian control. Blistering diseases caused by autoantibodies to type-VII collagen. Treatment options are not yet considered satisfactory. Against this background, we aimed at investigating the role of circadian clocks in EBA. We hypothesized that the migration of neutrophils into the skin, which controls the severity of autoantibody-induced tissue damage, is governed by peripheral circadian clocks. To characterize the diurnal regulation of peripheral blood mononuclear cells (PBMCs) under basal conditions, FACs analyses of PBMCs were performed in healthy, male, C57BL/6 mice at different time points. We found that T cells, B cells, monocytes and neutrophils show circadian rhythmicity. Particularly neutrophils, the key players in EBA, show significantly higher numbers in the morning than in the evening, whereas the activation of neutrophils is anti-phasic with higher levels in the evening. The antibody transfer-induced mouse model of EBA was then used to investigate the impact of circadian timing on disease development. Autobodies were applied either in the morning or in the evening. Our results demonstrate a significantly higher disease activity when autobody-injected mice were injected in the morning as measured by a specific clinical score. Additionally, we used two-photon microscopy to visualize neutrophil migration into the skin in Eys-GFP mice, possessing myelomonocytic cells labelled with green fluorescent protein (more Lys-eGFP)+ cells in the morning group. Ultimately, we aim at developing novel chronotherapeutic approaches.

Indispensable role of CD1d in natural IgE production

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Several in vitro and in vivo studies have suggested that IgE naturally produced in the steady state plays an essential role in optimal immune responses and the maintenance of mast cell function. Although antigen-specific IgE production is known to require MHC class II-dependent interaction between T and B cells, the mechanism underlying natural IgE production is poorly understood. Here we report that natural production of IgE depends on expression of a non-classical MHC class I-like molecule, CD1d, rather than MHC class II. We found that serum IgE levels were markedly decreased in CD1d-deficient mice compared to wild-type (WT) mice, while serum levels of other immunoglobulin subclasses were not. Flow cytometric analysis revealed that amounts of IgE bound on skin and peritoneal mast cells were significantly lower in CD1d-deficient mice than in WT mice. In addition, anti-IgE-induced systemic anaphylaxis was markedly impaired in CD1d-deficient mice but was fully restored by administration of IgE 24 h before anti-IgE challenge. WT mice transplanted with bone marrow (BM) cells from CD1d-deficient mice showed significantly lower levels of serum IgE compared with WT mice transplanted with WT BM cells, suggesting that CD1d expression on hematopoietic cells, but not on epidermal/stromal cells, is critical for natural IgE production. Thus, we first examined the requirement of CD1d on B cells for natural IgE production. We generated mixed bone marrow chimera mice where CD1d expression was deficient specifically on B cells and found that natural IgE production was impaired in these mice. Furthermore, we also investigated the involvement of invariant NKT (iNKT) cells in natural IgE production because loss of CD1d expression on hematopoietic cells is known to result in the lack of mature iNKT cells. We found that serum IgE levels of iNKT cell-deficient mice were significantly lower than those of WT mice, indicating that iNKT cells also contribute to natural IgE production. Taken together, our results suggest that iNKT cells and CD1d expression on B cells are essential for natural IgE production in vivo.
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cAMP response element-binding protein 1 (CREB) is a β-catenin-regulated transcription factor in squamous cell carcinoma (SCC) cells

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The β-catenin signaling is essential for maintaining the cancer stem cell phenotype, because that lineage is responsible for the cancer cell linearies. The role of CREB, a hypoxia-inducible protein, and FOXOs, a tumor suppressor, and mTOR complex 2 pathway in in vitro SCC has not been studied yet. The purpose of this study is to investigate CREB and FOXO expression in the SCC and their correlation with proliferation and involution. Primary endobothelial cells were obtained during surgery from 12 infantile hemangiomatosus patients, and the CREB expression was overexpressed. The obtained data identified only a local mechanism under the regulation of cancer stemness by β-catenin in SCC.

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NDRG1 regulates proliferation of endobothelial cells of infantile hemangiomata

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The etiopathogenesis of infantile hemangiomata has not been well understood and is accepted that dysregulation of angiogenic mediators mainly contributes to abnormal formation of hemangiomata. The role of NDRG1, a hypoxia-inducible protein, and FOXOs, a tumor suppressor, and mTOR complex 2 pathway in infantile hemangiomata have not been studied yet. The purpose of this study is to investigate NDRG1 and FOXO expression in the infantile hemangiomata and their correlation with proliferation and involution. Primary endobothelial cells were obtained during surgery from 12 infantile hemangiomatosus patients, and the CREB expression was overexpressed. The obtained data identified only a local mechanism under the regulation of cancer stemness by β-catenin in SCC.

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Analysis of mice skin distribution using MALDI-MSI after subcutaneous injections of a potent novel peptide hair growth promoter, FOL-005

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FOL-005 is an integrin antagonist, which shows a potential to promote hair growth at one of the doses tested. As part of the preclinical program for further development modified osteopontin-derived peptide, FOL-005, has been shown to highly and reproducibly stimulate hair growth in mice. In a recently completed clinical phase IIa study, subcutaneous injections of FOL-005 were shown to be a safe treatment that resulted in 8% increase in hair growth at one of the doses tested. As part of a preclinical program for further development of a new formulation and an oral vaginal treatment, BCC (n = 5), the distribution of FOL-005 into mice was followed for skin up to 24 hours using the MALDI-MSI technology. MALDI-MSI, an advanced label free technique based on the combination of mass spectrometry imaging and histology, provides qualitative and quantitative data on the drug distribution and hence can add information on efficiency or potential toxicity. FOL-005 was found to be distributed exclusively in the treated skin after injection and the concentration was shown to decrease with time. No distribution was detected outside the skin indicating that FOL-005 is locally degraded. The obtained data identify only a local distribution of FOL-005 peptide and further supports the clinical development as a new, dermatological treatment principle for alopecia patients.

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Genetic transformation of keratinocanthoma-type cutaneous squamous cell carcinoma following intrallesional chemotherapy

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Acquired genetic mutations may promote tumorigenesis and can increase the invasive and metastatic potential of tumors. The management of eruptive keratancanthoma-type cutaneous squamous cell carcinoma (KA-type SCC) is difficult, and there is continued debate on the true risk of metastatic potential of these tumors. We report a case of characteristic eruptive KA-type SCCs on the lower leg of a renal transplant patient that recurred, transformed into well-differentiated SCC, developed distant metastases and resulted in fatalit. Multiple lesions had been treated with intrallesional chemotherapy agents, 5-fluorouracil (5-FU) and methotrexate. Relative gene expression was determined using quantitative real-time RT-PCR. Genes of interest included those of basement membrane and extracellular matrix, cell adhesion and immune response. We therefore focused on the expression of a major component of basement membrane, nidogen1 which is derived from stromal fibroblasts. Objectives: To investigate the relationship between components constituting basement membrane and surrounding connective tissue in BCC, we have analyzed the expression of nidogen1 as well as another basement membrane protein, type IV collagen (Col4) using MALDI-MSI. Methods: By immunohistological staining, we assessed the expressions of nidogen1 and Col4 in BCC (n = 5) and compared with those in actinic keratosis (AK) (n = 5) and squamous cell carcinoma (SCC) (n = 5). We also compared the level of nidogen1 expression of tumors to that of normal skin. Results: The levels of the expression of the basement membrane components, nidogen1 and Col4 were both decreased in SCC but not in AK compared to those in normal skin. To the contrary, the expressions of both nidogen1 and Col4 in BCC were much higher than that in normal skin not only at the basement membrane but also in the surrounding strimal tissue. Conclusions: Our findings imply that the surrovaceous environment of KA is closely involved with the development of KA. We therefore focused on the expression of nidogen1 which is derived from stromal fibroblasts. Objectives: To investigate the relationship between components constituting basement membrane and surrounding connective tissue in BCC, we have analyzed the expression of nidogen1 as well as another basement membrane protein, type IV collagen (Col4) using MALDI-MSI. Methods: By immunohistological staining, we assessed the expressions of nidogen1 and Col4 in BCC (n = 5) and compared with those in actinic keratosis (AK) (n = 5) and squamous cell carcinoma (SCC) (n = 5). We also compared the level of nidogen1 expression of tumors to that of normal skin. Results: The levels of the expression of the basement membrane components, nidogen1 and Col4 were both decreased in SCC but not in AK compared to those in normal skin. To the contrary, the expressions of both nidogen1 and Col4 in BCC were much higher than that in normal skin not only at the basement membrane but also in the surrounding strimal tissue. Conclusions: Our findings imply that the surrovaceous environment of KA is closely involved with the development of KA.
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IL-33 - T regulatory cell axis triggers development of a cancer-promoting immune environment in chronic inflammation

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Chronic inflammation is a well-characterized driver of cancer in the skin and other epithelial organs; however, the mechanisms underlying the development of cancer-promoting chronic inflammation is unknown. We previously showed chronic allergic contact dermatitis (ACD) is a type 2 inflammatory disease and potent inducer of squamous cell carcinoma in mice and humans. Here, we describe a role for IL-33 in promoting the development of squamous cell carcinoma in mice and humans. IL-33 expression markedly increased during the transition from acute to chronic ACD, initiating tumor-promoting, type 2 inflammation in chronic ACD. Mice lacking Il-33 or Il-33 receptor (ST2) were protected from ACD-induced skin cancer compared to wild-type controls and Il-33 was required for the progression of inflammatory bowel disease-induced colorectal cancer. Notably, Il-33 direct effect on T regulatory cells was required for the development of a cancer-promoting immune environment in the skin and colon. Our findings elucidate a novel mechanism underlying the formation of a tumor-initiating immune environment in chronic inflammatory diseases and yield novel targets for cancer treatment and prevention in chronic inflammatory contexts.

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Distinctive miRNA expression profiles in tissue and serum of primary extramammary Pagets disease

C. Lee1, B. Qu2, R. Qi3, L. Liu4, M. Ha5, L. Liu6, X. Gao6, Y. Wang1 and H. Chen1 1 Dermatology, No. 1 Hospital of China Medical University, Shenyang, Liaoning, China; 2 Anesthesiology, No. 1 Hospital of China Medical University, Shenyang, Liaoning, China; 3 Pharmacy, Uni- versity of Science and Technology of China, Hefei, China; 4 Physiology, Providence College, Providence, RI; 5 Microbiologists, Nanjing Medical University, Nanjing, Jiangsu, China; and 6 MicroRNA (miRNA) are small noncoding RNAs involved in cancer development. Extramammary Pagets disease (EMP) is a rare cutaneous malignancy and the role of miRNAs in EMP is remained unknown. EMP is always delayed diagnosis due to its specic manifesta- tion. Here, we used TaqMan miRNA arrays to characterize miRNA expression profile in EMP and further validated the candidates by single RT-PCR. In tissue study, using laser capture micro-dissection technique, we collected EMP tumor cells (n=12), normal epithelial cells (n=12) and normal apocrine glands cells (n=7) from fresh frozen EMP tissues. miRNA arrays from two pairs of EMP cells and corresponding normal epithelial cells showed that mir-175, mir-10b, mir-31, mir-31* were differentially expressed. The single RT-PCR further conrmed that mir-375, mir-37 and mir-31* were upregulated in in EMP cell lines. miRNA expression difference between two kinds of EMP and healthy volunteers. Further validation was carried among EMPD(n=16), healthy volunteer(n=17) and the eczemae(n=12) and linea cutis groups. MiR-155 was found stable increased in EMPD.

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Functional role of merkel cell polyomavirus T-antigen regulated microRNAs in merkel cell carcinoma

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Merkel cell carcinoma (MCC) is an aggressive type of skin cancer. About 80% of MCCs harbour integrated Merkel cell polyomavirus (MCPV) genome with a mutation in the large T (LT) gene. MCPV LT-antigens are required for neoplastic transformation and maintenance of cell growth, however the molecular mechanism by which the virus induces tumorigenesis re- mains unclear. In this study we aimed to identify and characterize functional role of MCPV T-antigens in the induction of extracellular acidification was observed 5 minutes after stimulation of 300,000 naive Jurkat T cells upon exposure to anti-CD3/CD28 immunogenic beads in a 2:1 bead to naive T-cell ratio. This information could potentially be used to obtain a comprehensive clinical nature of the assay permits further correlative studies using patient-derived activated T-cells.

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Targeting specific YAP/TAZ functions in melanoma cells using cell-penetrating peptides

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The Hippo pathway is a conserved signaling pathway that controls cell proliferation and cell survival in animal tissues. YAP/TAZ, the downstream substrates of the Hippo pathway are highly related transcriptional co-activators that shuttle between the cytoplasm and the nu- clear where they induce the expression of genes controlling cell cycle progression, apoptosis and cell differentiation by interacting with transcription factors such as TAZ family mem- bers. YAP and TAZ play an important role for cancer initiation, progression and metastasis, and represent potential targets for therapeutic intervention. In this work, we set out to investigate the relevance of cell-penetrating mimetic peptides of defined functional domains of YAP/TAZ as potential drug candidates to target the metastatic activity of melanoma cells. We have encompassing the YAP/TAZ WW domain of TAZ and TAT cell-penetrating domain of the HIV Tat protein transduction domain were synthesized and we determined how they affect melanoma cell behavior in vitro. Our experiments demonstrate that TAT-DW domain peptides strongly inhibit melanoma cell migration, without affecting cell proliferation, both assays performed using the Incucyte technology. TAT-DW also inhibits TAZ-induced TEAD-dependent transcription and target gene expression. TAT-DW cell-penetrating peptide may thus be a promising therapeutic strategy against YAP and TAZ-driven human cancers. Work is in progress to identify the mechanisms whereby peptides mimicking the YAP/TAZ DW domain affect melanoma cell migration.

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A metabolic assay to assess human T-cell activation in squamous cell carcinoma

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Background: T cells exhibit distinct metabolic profiles based on their activation state. Naive T cells are metabolically quiescent and use oxidative phosphorylation (OXPHOS) as their primary pathway of ATP production. Upon T cell antigen receptor (TCR) mediated recogni- tion of antigens and costimulatory signals, T cells become activated and preferentially utilize glycolysis and glutaminolysis metabolism. Methods: An Agilent Seahorse Xp Analyser was used to assess T cell activation in vitro. T cells are exposed to anti-CD3/CD28 immunogenic beads. Then, extracellular acidification is measured in real time as a readout of glycolytic activity. Basic assay parameters, including the immunogenic bead to cell ratio and timing, were established using Jurkat T lymphocytes. CD4 and CD8 T cells were isolated from hu- mogenized squamous cell carcinoma (SCC) tumor samples obtained from patients (n=1) via flow cytometry using a PE-conjugated anti-human CD3/CD28 antibody. Results: Rapid induction of extracellular acidification was observed 5 minutes after stimulation of 300,000 naive Jurkat T cells upon exposure to anti-CD3/CD28 immunogenic beads in a 2:1 bead to cell ratio. These effects persisted for the duration of the 2-hour study. CD4 and CD8 T cells obtained from SCC tumor tissues exhibited similar activation kinetics upon stimulation with anti-CD3/CD28 immunogenic beads. Assessment of the activation potential of T cells ob- tained from patient samples can provide insight into a patients clinical status given that exhausted T cells are correlated with a poor prognosis. In addition, the non-detructive nature of this approach allows for the evaluation of the same sample over time. Conclusion: This is the first report of a kinetic assay to assess activation of SCC patient-derived T cells. This information could potentially be used to obtain a comprehensive clinical profile of the patients tumor microenvironment to tailor patient-specific treatment regimen.

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Mixed phenotype acute leukemia in xeroderma pigmentosum

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Xeroderma pigmentosum (XP) is a rare genodermatosis with a 10,000-fold increased risk for skin and ocular cancers, is caused by an inability to repair UV induced DNA damage. Improved disease management has resulted in patients living longer and developing conditions associated with premature aging (early menopause and internal malignancies). A 19 yr Moroccan girl, XPS-403E, with homozygous mutations in XPC, was seen at NIH under a natural history protocol. Her first skin cancer was at 8 years. She had > 40 skin cancers and 1 eye neoplasm. She had no menses for 1 year and her anti-mullerian hormone level was low consistent with early menopause. Prior to her visit, she had night sweats, shortness of breath climbing stairs, and lumps in her neck and groin. She had generalized lymphadenopathy, tachycardia and pancytopenia: WBC 1.75 K/mL, Hgb 6.8 g/dL, platelets 84 K/mL with 18.2% circulating blasts. Flow cytometry identified 3 distinct immature acute leukemia populations: B cell/myeloid blasts, T cell/myeloid blasts and T cell blasts confirming the diagnosis of a very rare mixed phenotype acute leukemia. No cytogenetic abnormalities were found. Of 117 XP patients seen at the NIH from 1999 to 2017, three died of hematologic malignancies: 2 had myelodysplastic syndrome in their 20s (median age in the general population - 72 yr) consistent with a premature aging XP syndrome. Two had T-cell ALL and myelo- dysplasia. This mutation appeared about 1,250 years ago in the Mediterranean area. XP represents a newly defined genetic predisposing condition for hematologic malignancies. XP patients should be monitored for hematologic malignancies by active surveillance like other leukemia-predisposing syndromes.
Notch signaling modulates BCC persistence in response to anti-hedgehog therapy

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Inhibitors of the Hedgehog (Hh) signaling pathway are effective at repressing basal cell carcinoma (BCC) progression. Vitamin D cells often persist after treatment, indicating the original Hh signaling pathway is not effectively shut down. Using a mouse model of BCC, we show that these tumors are comprised of 2 molecularly and functionally distinct subcompartments. Whereas interior suprabasal cells display elevated Notch signaling and increased apoptosis in response to the Hh inhibitor vismodegib, peripheral basal cells possess increased Hh and persist throughout treatment. We further demonstrate that inhibiting Notch protects tumors against anti-Hh therapy, while activating Notch regresses already established lesions. Finally, we show that some human BCCs contain suprabasal tumor cells that activate Notch, whereas others do not. Altogether, our findings suggest that an interplay of Notch and Hh signaling may determine whether tumor cells persist or apoptosis in response to therapeutic Hh pathway blockade. As Notch mutations are commonly observed in human BCCs, our findings suggest that eliminating Notch-low basal tumor cells is paramount for preventing recurrence.

CD244-CD48 interaction is involved in progression of mycosis fungoides

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CD244 (also called 2B4) is a member of the signaling lymphocyte activation molecule family of membrane receptors and expressed by NK cells, subsets of T cells. CD48, the ligand for CD244, is broadly expressed on hematopoietic cells. CD244 plays an important role in modulating NK cell and CD8+ T cell immunity when engaged by CD48. Although NK cells and CD8+ T cells are anti-tumor immunity, studies on CD244 expression in human malignancies are limited. In this study, we investigated the expression and function of CD244 and CD48 in mycosis fungoides (MF). Both CD244 and CD48 mRNA expression levels in lesional skin of advanced MF (stage IIIb-IVa) were significantly elevated compared to those of normal skin. CD48 mRNA levels were significantly positively correlated with CD244 mRNA levels and CCL17 mRNA levels. Flow cytometric analysis revealed that CD244 was expressed on HH cells, an aggressive cutaneous T-cell lymphoma cell line, and that CD48 was expressed on several MF or aggressive cutaneous T-cell lymphoma cell lines including MyLa cells, M1 cells, and HH cells. CD48 expression in part of MF malignant cells were also confirmed by immunohistochemistry. To elucidate the role of CD244/CD48 interactions in tumor cells, we cultured HH cells with a neutralizing antibody for CD244, resulting in decreased proliferation of HH cells. These results suggest that CD244/CD48 interactions can be involved in MF progression.

Vitamin D hydroxysteroids as therapeutic agents in skin cancer

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Vitamin D hydroxysteroids are known to have anti-malignant properties, suppress certain proliferation, and inhibit histone degradation. Vitamin D hydroxysteroids with or without an OH at C18 inhibited proliferation in both cell lines in a similar manner. However, colony formation and spindled form tests demonstrated that C18a-hydroxylation is a requirement for strong inhibitory effects vs the corresponding precursors that showed weaker or no effects. For example, seco-steroids with potent anti-tumorigenic activity included 25(OH)2D3, 1,20(OH)2D3, 1,20,23(OH)3D3, 1,20,24(OH)3D3, 1,20,25(OH)3D3 and 1,20,26(OH)3D3, whereas, 25(OH)2D3, 1,20(OH)3D3 and 20,23(OH)2D3 showed detectable, but more modest inhibitory effects. Western blot analyses revealed the expression of VDR and ROR in and γ in A311 and VDR and ROR in A3101 cancer cells lines. Histochemical analysis performed on human biopsies of SCC in situ, invasive SCC and BCC (n=10 for each category) showed nuclear expression of all of these target genes. Our study identified several vitamin D analogs with anti-cancer therapeutic potential and showed that VDR and ROR in vivo express several of their nuclear target receptors.

A novel mechanistic link between MCPyV st antigen and a pivotal DNA damage response molecule in merkel cell carcinogenesis

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Merkel cell carcinoma (MCC) is an aggressive neuroendocrine cancer of the skin with high metastatic rates and mortality. Recently, the discovery of the Merkel cell polyomavirus (MCPyV) has shed light on the viral etiology of MCC. In support of the role of MCPyV in MCC, studies from our group and others have shown that the small tumor antigen (ST) of MCPyV is an oncoprotein capable of inducing cell proliferation and transformation. In the current study, we provide novel evidence that MCPyV ST induces the phosphorylation and activation of the protein kinase, ataxia-telangiectasia mutated (ATM). We established cell lines expressing the ST antigen of MCC in vitro. Our data showed that expression of ST led to a decrease in the expression of the ST antigen resulted in hyperphosphorylation and hyperactivation of ATM. On the other hand, we observed that the phosphorylation of ATM was not altered by the ST antigens of two other viruses that are known to be associated with less aggressive forms of hyper-proliferative cutaneous neoplasia). These results suggest an important role of ATM phosphorylation and activation in mediating MCPyV actions. Our results are noteworthy, given the documented role of MCPyV in the pathogenesis of MCC and the critical implication of ATM in ultraviolet (UV)-mediated DNA damage and aggressive cutaneous carcinogenesis. To our knowledge, this is the first report describing ATM activation by MCPyV ST, which provides a novel mechanistic link between MCPyV ST and a pivotal DNA damage cell cycle checkpoint. These findings may have implications for the development of MCPyV and ATM in MCC and to evaluate the therapeutic efficacy of targeting ATM, in addition to management of MCPyV infection, for MCC treatment.
Regulation of keratinocyte differentiation and proliferation by heterotrimeric G proteins
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G-protein-coupled receptors (GPCRs) are the largest family of cell-surface molecules involved in the regulation of cutaneous physiological processes and pathological conditions along with their associated G proteins. We have recently demonstrated in mice that conditional epidermal deletion of the gene coding for the G alpha-s (Gna15) or inactivation of protein kinase A (PKA) are both sufficient to cause an aberrant expansion of the basal progenitor keratinocytes in the skin, resulting in basal cell carcinoma formation (Iglesias-Bartolome et al, Nature Cell Biology, 17, p793, 2015). We next hypothesized that activation of the heterotrimeric G alpha-q, which opposes PKA signaling by reducing cAMP production, might participate in the regulation of keratinocyte differentiation and proliferation. For this, we took advantage of the human muscarinic designer receptor (DREADD) hM4Di, a Galpha coupled receptor that is exclusively activated by the synthetic ligand CNO. Using a tetracycline inducible mouse model, we targeted the expression of hM4Di in the basal stem cell compartment of the skin and induced its activation by treating with CNO. Analysis of skin histology reveals that Gna activation results in skin hyperplasia, with expansion of the K5+ basal compartment. We next validated this result in human keratinocytes, by using a lentiviral delivery system to express hM4Di in primary keratinocytes and organotypic cultures. Activation of Gna in this model also induced skin hyperplasia and expansion of the basal K5+ compartment. These findings indicate that regulation of cAMP signaling by Gna and Gai and their yet to be identified coupled GPCRs plays a central role in the regulation of keratinocyte differentiation and proliferation.

Successful identification of copy number variations using next generation sequencing with a tumour panel in plaque/tumour mycosis fungoides
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Numerous next generation sequencing (NGS) in cutaneous T cell lymphoma (CTCL) has identified higher rates of somatic copy number variation (SCNVs) when compared with other tumours and recurrent deletions of 10q24.17p and amplifications of 8q11.24 involving TP53, CDKN2A and MYC. However most studies are in Sezary syndrome on fresh frozen peripheral blood lymphocytes (as opposed to cutaneous lesions of mycosis fungoides). We rescued SCNVs using targeted deep sequencing with 170 gene panels associated with common solid tumours on microdissected tumour cells from the skin on 8 newly diagnosed MF formalin-fixed paraffin embedded (FFPE) blocks (stage IB: n=1, stage III: n=6, IVa: n=1). Sequencing was repeated with positive and negative controls. We found recurrent SCNVs includng amplification in 7q23.3 (PIK3CG:ex1p13.1;SLX4) and deletion in 9p13.1 (CDKN2A & 17pTP53). A novel finding from this study is recurrent focal amplification of 11q13(KMT2A). KMT2A has a role in histone H3 lysine 4(H3K4) methyltransferase activity which mediates chromatin modifications associated with epigenetic regulation. By choosing tumour regions by skin macromutation on FFPE blocks followed by deep sequencing with 800-1600X coverage, we successfully identified SCNVs & validated this technique allowing the use of more readily available samples supposed to fresh frozen samples. This technique allowed us to identify focal amplification in KMT2A which has not been previously reported. This finding is interesting & relevant as hypermethylation & epigenetic regulation is relevant in CTCL. Validation of these genes in CTCL with an aim to develop gene panels could potentially be translated in the clinical care of patients with CTCL.
YKL-40 is involved in progression of mycosis fungoides and Sézary syndrome through inducing proliferation of tumor cells and epidermal keratinocytes.

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YKL-40, a type-1-like 1 protein, is a human glycoprotein that is expressed and produced by both immune cells, such as neutrophils and macrophages, and nonimmune cells including fibroblasts, vascular smooth muscle cells, and endothelial cells. Although the biological function of CHIIL1 remains partly understood, CHIIL1 has been connected to the pathogenesis of a variety of malignancies and inflammatory diseases through promoting cell proliferation, differentiation, angiogenesis, and tissue remodeling. Concerning skin diseases, serum YKL-40 levels are increased in patients with psoriasis and correlated with disease activity. Elevated YKL-40 levels in sera are also shown in patients with malignant melanoma and thought to be an independent prognostic factor for poor survival. In this study, we examined YKL-40 expression and function in mycosis fungoides (MF) and Sézary syndrome (SS). Serum YKL-40 levels were significantly higher in patients with advanced MF and SS than healthy controls. Serum YKL-40 levels were significantly correlated with serum soluble IL-2 receptor and lactate dehydrogenase levels. By immunohistochemistry, augmented YKL-40 expression on epidermal keratinocytes was found in MFSS, including normal skin, in epidermal and dermal infiltrating lymphocytes expressed YKL-40 in lesional skin of MFSS. Recombinant YKL-40 induced proliferation of HH cells, a cell line of aggressive cutaneous T-cell lymphoma, HUT78 cells, a cell line of SS, and HaCaT cells, a cell line of epidermal keratinocytes. In conclusion, increased YKL-40 expression by tumor cells and epidermal keratinocytes can be involved in MFSS progression through proliferation of those cells in autocrine and paracrine manner.

Notch intracellular domain is partitioned into extracellular vesicles: Implications for squamous cell carcinoma progression

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Notch intracellular domain is partitioned into extracellular vesicles (EVs) including ANRIL, HOTAIR, HULC, and LUNAR1, in tissues from MF than those from control skin. CCR7 expression is increased in MF. CCL21, the ligand of CCR7, not only mediates cell migration but also enhances malat-1 expression and induces mTOR activation in MyLa cells. Knockdown of malat-1 expression by interference RNA abrogated the CCL21-mediated cell migration but not mTOR activation. On the other hand, mTOR inhibition by rapamycin re- duces the CCL21-mediated migration and malat-1 expression. We concluded that CCL21-mediated migration in MyLa cells is dependent on the mTOR activation followed by malat-1 expression. Malat-1 and mTOR might be potential therapeutic targets to halt the progression of MF.
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Human papillomavirus infection in vonozanolase-associated cutaneous squamous cell carcinoma
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C1014T single nucleotide polymorphism of CCR4 is frequent in caucasian patients with cutaneous T-cell lymphoma
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Complete components of Clr and Cls promote growth of cutaneous squamous cell carcinoma
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CRISPR-Cas9 epigenome editing to induce DNA demethylation at the p14ARF promoter and inhibit skin cancer
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Small non-coding RNAs control the MAPK/ERK pathway
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KRAS regulation by small non-coding RNAs and SNARE proteins
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The KRAS oncoprotein is a common driver of cancer, however, it has proven to be an elusive therapeutic target, highlighting the importance of understanding the mechanisms that facilitate its action. KRAS regulates and relays signals at the plasma membrane (PM) where it helps to promote extracellular matrix remodeling, cell adhesion, and cell migration. KRAS also interacts with a series of cargo lines that tolerate the selective retention for these SNAREs in KRAS-driven neoplasia, but not for other canonical Ras isoform oncoproteins, HRAS and NRAS, which have their own distinct intracellular transport requirements for these SNAREs. Recent studies have found that KRAS co-opting TET1 methylcytosine dioxygenase 1 (TET1) that induces DNA demethylation. Human cell lines 293T and A-431 were transduced with a doxycycline-inducible lentiviral vector that expresses dCas9-TET1. Also, guide RNAs (gRNAs) were designed to direct dCas9-TET1 to p14ARF exon b, immediate downstream of its promoter within the CpG island. Stable cell lines with gRNA targeting p14ARF were treated with doxycycline for 3 days to induce dCas9-TET1 expression. Methylated DNA immunoprecipitation (MeDIP) demonstrated that DNA methylation levels (5-methylcytosine) were decreased at the intended target compared to parental untransduced cells. RT-QPCR analysis revealed that mRNA levels of p14ARF paradoxically decreased when dCas9-TET1 demethylated its target. This is likely due to steric obstruction of DNA polymerase by dCas9-TET1 binding downstream of the p14ARF promoter, highlighting the importance of adequate selection of target sequences. Further investigations are needed to determine cancer-inhibiting effects of DNA demethylation specifically at the p16INK4A and p14ARF promoters on proliferation, migration, and invasion of skin cancer.

Small non-coding RNAs (snRNAs) are known to enable RNA pseudouridylation which helps to control RNA stability and function. To identify new roles for snRNA action in disease, we hybridized 19 C/D and H/ACA box snRNAs to r9200 recombinant human proteins. The C/D box snoRNA, SNORD50A/B, bound KRAS, consistent with recently published data. Unexpectedly, the H/ACA box snoRNA SNORD12 directly bound an essential kinase in the MAPK protein kinase cascade, ERK2 (MAPK1). A CRISPR/Cas9 dual-cut gRNA lentiviral vector was used to produce triple-cut independent SNORD12 snoRNA knockout clones from each of 3 independent epithelial cancer cell lines. SNORD12 knockout reduced ERK2 activation and in vivo tumorigenesis in all cases. These effects were validated as specific to the presence of the SNORD12 snoRNA itself, as opposed to an artificial of genome editing at the SNORD12 locus, by rescue experiments that restored SNORD12 expression. To define how SNORD12 modulates ERK2 function, we performed mass spectrometry (LC-MS/MS) to detect ERK2 proximal proteins in the presence and absence of SNORD12. In parallel, we also analyzed the status of 104 phosphorylation sites on 62proteins relevant to MAPK signaling in wild-type and SNORD12 knockout cells. Taken together, the resulting data demonstrated that SNORD12 controls ERK2 kinase function, including the downregulation of downstream targets, such as Jun and p21. These findings identify SNORD12 as a new essential regulator of MAPK signaling in cancer and further extend the biological roles for small non-coding RNAs to the control of cellular signal transduction.

Small non-coding RNAs (sncRNAs) are involved in multiple fundamental processes in biology. Among them are sncRNAs, which are best studied for their role in modifying RNA nucleosides. Of the two major sncRNA classes, H/C box snoRNAs are known to enable RNA pseudouridylation which helps to control RNA stability and function. To identify new roles for snoRNA action in disease, we hybridized 19 C/D and H/ACA box snoRNAs to r9200 recombinant human proteins. The C/D box snoRNA, SNORD50A/B, bound KRAS, consistent with recently published data. Unexpectedly, the H/ACA box snoRNA SNORD12 directly bound an essential kinase in the MAPK protein kinase cascade, ERK2 (MAPK1). A CRISPR/Cas9 dual-cut gRNA lentiviral vector was used to produce triple-cut independent SNORD12 snoRNA knockout clones from each of 3 independent epithelial cancer cell lines. SNORD12 knockout reduced ERK2 activation and in vivo tumorigenesis in all cases. These effects were validated as specific to the presence of the SNORD12 snoRNA itself, as opposed to an artificial of genome editing at the SNORD12 locus, by rescue experiments that restored SNORD12 expression. To define how SNORD12 modulates ERK2 function, we performed mass spectrometry (LC-MS/MS) to detect ERK2 proximal proteins in the presence and absence of SNORD12. In parallel, we also analyzed the status of 104 phosphorylation sites on 62proteins relevant to MAPK signaling in wild-type and SNORD12 knockout cells. Taken together, the resulting data demonstrated that SNORD12 controls ERK2 kinase function, including the downregulation of downstream targets, such as Jun and p21. These findings identify SNORD12 as a new essential regulator of MAPK signaling in cancer and further extend the biological roles for small non-coding RNAs to the control of cellular signal transduction.

Some studies have suggested that the presence and absence of SNORD12 snoRNA is heterogeneous in tumors. In parallel, we also analyzed the status of 104 phosphorylation sites on 62proteins relevant to MAPK signaling in wild-type and SNORD12 knockout cells. Taken together, the resulting data demonstrated that SNORD12 controls ERK2 kinase function, including the downregulation of downstream targets, such as Jun and p21. These findings identify SNORD12 as a new essential regulator of MAPK signaling in cancer and further extend the biological roles for small non-coding RNAs to the control of cellular signal transduction.
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CXC12 is a potential paracrine factor in tuberous sclerosis tumorigenesis

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Genetic ancestry and cutaneous squamous cell carcinoma (cSCC) is a malignancy of predominantly skin-homing CD4+ lymphocytes. Patients with advanced stages of CTCL have a high mortality rate, and even those with more indolent disease experience severely diminished quality of life due to recurrent skin infections, pain, and pruritus. Currently, there are few specific therapeutic targets known in CTCL, highlighting the need to identify not only key mediators in disease pathogenesis, but also druggable ones. One aspect of CTCL cells that has not been well-studied thus far is their dependence on unique metabolic pathways to support dysregulated growth. In this study, we identified the vitamin B6 pathway as a novel potential target for the treatment of CTCL. Using an sgRNA-mediated knockout platform, we found that loss of pyridoxal kinase (PDXK), an enzyme that converts vitamin B6 to its active form, pyridoxal phosphate (PLP), resulted in reduced proliferation of the CTCL cell lines MyLa and PB2B. Consistently, administration of the FDA-approved drug, isoniazid, commonly used to treat tuberculosis and known to block the vitamin B6 pathway, recapitulated this proliferation defect in vitro. Furthermore, cells cultured with isoniazid demonstrated higher levels of apoptosis and delayed cell cycle progression. Future studies will assess the feasibility of repurposing isoniazid as a new treatment for CTCL treatment by testing the drugs effects on primary samples from patients with CTCL.

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Targeting the vitamin B6 pathway as a novel therapeutic strategy for cutaneous T-cell lymphoma

M Tsang1, J Gantchev2, D Sasseville3 and I Litvinov3

In cutaneous T-cell lymphoma (CTCL), cutaneous T-cell lymphoma (CTCL) is a malignancy of predominantly skin-homing CD4+ lymphocytes. Patients with advanced stages of CTCL have a high mortality rate, and even those with more indolent disease experience severely diminished quality of life due to recurrent skin infections, pain, and pruritus. Currently, there are few specific therapeutic targets known in CTCL, highlighting the need to identify not only key mediators in disease pathogenesis, but also druggable ones. One aspect of CTCL cells that has not been well-studied thus far is their dependence on unique metabolic pathways to support dysregulated growth. In this study, we identified the vitamin B6 pathway as a novel potential target for the treatment of CTCL. Using an sgRNA-mediated knockout platform, we found that loss of pyridoxal kinase (PDXK), an enzyme that converts vitamin B6 to its active form, pyridoxal phosphate (PLP), resulted in reduced proliferation of the CTCL cell lines MyLa and PB2B. Consistently, administration of the FDA-approved drug, isoniazid, commonly used to treat tuberculosis and known to block the vitamin B6 pathway, recapitulated this proliferation defect in vitro. Furthermore, cells cultured with isoniazid demonstrated higher levels of apoptosis and delayed cell cycle progression. Future studies will assess the feasibility of repurposing isoniazid as a new treatment for CTCL treatment by testing the drugs effects on primary samples from patients with CTCL.

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Frequent basal cell cancer development is a clinical marker for inherited cancer susceptibility

SLi1, P Klover1, C Dalgard2, JWang1, J Moss3 and T Darling4

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Genetic ancestry and cutaneous squamous cell carcinoma (cSCC) is a malignancy of predominantly skin-homing CD4+ lymphocytes. Patients with advanced stages of CTCL have a high mortality rate, and even those with more indolent disease experience severely diminished quality of life due to recurrent skin infections, pain, and pruritus. Currently, there are few specific therapeutic targets known in CTCL, highlighting the need to identify not only key mediators in disease pathogenesis, but also druggable ones. One aspect of CTCL cells that has not been well-studied thus far is their dependence on unique metabolic pathways to support dysregulated growth. In this study, we identified the vitamin B6 pathway as a novel potential target for the treatment of CTCL. Using an sgRNA-mediated knockout platform, we found that loss of pyridoxal kinase (PDXK), an enzyme that converts vitamin B6 to its active form, pyridoxal phosphate (PLP), resulted in reduced proliferation of the CTCL cell lines MyLa and PB2B. Consistently, administration of the FDA-approved drug, isoniazid, commonly used to treat tuberculosis and known to block the vitamin B6 pathway, recapitulated this proliferation defect in vitro. Furthermore, cells cultured with isoniazid demonstrated higher levels of apoptosis and delayed cell cycle progression. Future studies will assess the feasibility of repurposing isoniazid as a new treatment for CTCL treatment by testing the drugs effects on primary samples from patients with CTCL.

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Gene expression in T-cell lymphoma

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Increased expression of germ cell proteins and activation of LINE-1 retrotransposition in CTCL. Using an Alu retrotransposition assay, we demonstrate the functional activity of the LINE-1 retrotransposon in CTCL. Finally, we show that beads carrying the LINE-1 retrotransposon can be used to isolate protein complexes from CTCL cells. Together, our results implicate the ectopic expression of germ cell proteins and activation of LINE-1 retrotransposition in CTCL.

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Ectopic expression of meiosis proteins and the LINE-1 retrotransposon is associated with genomic instability in cutaneous T-cell lymphoma

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Increased expression of germ cell proteins and activation of LINE-1 retrotransposition in CTCL. Using an Alu retrotransposition assay, we demonstrate the functional activity of the LINE-1 retrotransposon in CTCL. Finally, we show that beads carrying the LINE-1 retrotransposon can be used to isolate protein complexes from CTCL cells. Together, our results implicate the ectopic expression of germ cell proteins and activation of LINE-1 retrotransposon in CTCL.
The IFI4 gene single nucleotide polymorphism rs12203592 is associated with stage and anatomic site in melanoma.

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The IFI4 gene encodes for an interferon regulatory factor involved in the regulation of B- and T-cell differentiation response to interferon and other cytokines. A single nucleotide polymorphism (SNP, rs12203592), is a functional intrinsic genetic variant. Its minor allele, T, increases the expression of IFI4, activates the TERT promoter and increases telomerase activity. We have shown previously that presence of the T allele in germline DNA is associated with melanoma ulceration and higher Breslow thickness, and with divergent melanoma pathways. We have now evaluated its relationship with location of melanoma and AJCC Stage (T-stage). The GEM study included 2702 Caucasian melanoma patients with complete AJCC stage and T-stage data. Presence of the allele of IFI4 rs12203592 was positively associated with high-stage (stages T2, T3a, T3b, T4a, T4b) in these patients with an Odds Ratio (OR) of 1.33, 95% Confidence Interval (CI) 1.14-1.56, P=0.0003, adjusted for age at diagnosis, sex, and study center. In an analysis of stage by anatomic site, the T allele was positively associated with high-stage melanoma in all sites except head and neck. We conclude IFI4 contributes to melanoma development and progression and should be further investigated as a potential molecular target in melanoma.

169 Efficacy and tolerability of pembrolizumab in an elderly patient and unusual presentation of advanced stage metastatic Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is a rare, aggressive primary neuroendocrine carcinoma of the skin. Although many of the solid tumors are characterized by the presence of solitary cutaneous tumors, tumors may rarely lack a significant dermal component. PD-1 blockade in advanced cases shows an objective response rate of 36%. We report an unusual initial presentation of MCC as a left popliteal soft tissue mass in an elderly patient. Pertinent history included BRCA+ breast cancers in male & female relatives and a personal history of recent malignant melanoma (stage Ia) and non-melanoma skin cancers. He underwent wide local excision of the MCC and unsuccessful sentinel lymph node biopsy, followed by 50 Gy of radiation to the resection site. 18 months later he noted a left groin nodule of approximately 2.5 cm, with biopsy showing MCC of the lymph node. Left inguinal lymph node dissection revealed 9/17 positive lymph nodes, & he underwent 60 Gy of radiation. Surveillance CT scans 15 months later demonstrated mediastinal left paraortic and aortic bifurcation lymph nodes, with lymph node biopsy confirming MCC. Despite deep tissue involvement, the MCC was controlled with chemotherapy. He started on pembrolizumab at age 82 years. Within three months of first dose, he demonstrated an excellent response with nearly complete radiographic resolution of several lesions. Other mediastinal masses completely resolved six months after first dose. Thirteen months later, he remains on pembrolizumab without disease progression or new metastatic lesions. He experienced minimal side effects apart from a mild pruritic truncal rash which improved with held pembrolizumab dose and Clobetason application. He remains functionally well with ECOG score 0-1. This case adds to our knowledge of the efficacy, safety, and tolerability of PD-1 inhibitors in the treatment of advanced MCC in elderly patients, including those with a strong family history of genetic cancer syndromes, personal history of other cutaneous malignancies (such as melanomas), and unusual tumor presentation (initial lesion without cutaneous involvement).

170 Gene expression profiling and immune cell-type deconvolution highlight robust disease progression and survival markers in multiple cohorts of CTCL patients

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The ability to accurately stage CTCL (cutaneous T-cell lymphoma) is important, as many tumors progress to advanced stages. We performed RNA-Seq data analysis on 157 patients and identified differentially expressed genes (DEGs) across all CTCL samples and early stage markers linked to progression and survival. We performed multiple hypothesis testing-correction (false discovery rate) with PD-1 inhibitors in the treatment of advanced MCC in elderly patients, including those with a strong family history of genetic cancer syndromes, personal history of other cutaneous malignancies (such as melanomas), and unusual tumor presentation (initial lesion without cutaneous involvement).

171 Combination therapy with MST-16 and VP-16 for tumor stage mycosis fungoides

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Photodynamic therapy (PDT) is used for the treatment of several types of Non-Melanoma Skin Cancer (NMSC), basal and squamous cell carcinoma (BCC and SCC, respectively). Nevertheless, PDT is not always effective and resistant cells may appear after treatment. While normal differentiated cells depend primarily on mitochondrial oxidative phosphorylation to generate energy, cancer cells change this metabolism to an aerobic glycolysis (Warburg effect), which could influence in the response to therapies. Here, we have evaluated the expression of metabolic markers in parental and PDT resistant NMSC cells and the use of metformin, an antidiabetic type II compound, to enhance the effect of PDT. We have used the human SCC cell lines SCC-13 and A431 and the mouse BCC cells, ASZ and CSZ. These cells, called primary tumor cells, were treated with PDT and cell DNA has been obtained. We evaluated by western blot and immunofluorescence, the expression of the β-subunit of the H+-ATP synthase (β-F1-ATPase), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and pyruvate kinase (PKM2) as metabolic markers in the cells. We have also calculated the bioenergetic signaling and suggests that aberrant activation of PI3K signaling can modulate the cellular growth. Furthermore, we have seen that a PTEN inhibitor (VO-OHpic) also repressed the induction of cell surface MHC class I (MHC-I) and enhanced the induction of cell surface MHC class II (MHC-II) in combination with PDT. In conclusion, we have shown that combined treatment of PDT with metformin on parental and PDT resistant cells. The results obtained showed a significant increase in cell death after the combined PDT-metformin treatment comparing to that obtained after PDT or metformin alone. We can conclude that both, SCC and BCC tumor cells enhance the aerobic glycolysis and therefore, metformin could improve the response to PDT.

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TGFβ inhibition enhances the antitumor response to PD-1 blockade in Smad4-mutant squamous cell carcinoma
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Smad4 is frequently lost or downregulated in squamous cell carcinomas (SCCs). A spontaneous mouse model of Smad4-/- SCC derived from K14-cre transgenic mice exhibited elevated TGFβ secretion in tumors compared to normal stratified epithelial tissues. In TGCA data, the mRNA expression of Smad4 is negatively correlated with TGFβ ligand mRNA expression in head and neck SCCs. Long-term treatment of mice bearing Smad4-null SCCs with a TGFrβ receptor (TGFβR) small molecule inhibitor modestly reduced tumor growth in immunocompetent mice relative to a vehicle control (p < 0.05), but had no effect under the same conditions in nude mice, implying that the effectiveness of TGFβ inhibition is due to its modulation of the immune microenvironment. Because these tumor cells express detectable levels of the immune suppressive checkpoint ligand PD-L1 when co-cultured with CD8 T cells, but not in vivo, because the tumors have high expression of PD-L1 in both tumor cells and infiltrating myeloid cells in vivo, we hypothesized that they would be susceptible to dual inhibition of TGFβR and PD-L1. We treated mice with a short-term (one week) treatment regimen of a TGFβR inhibitor and a PD-1 blockade antibody. TGFβR inhibition greatly enhanced the effect of PD-L1 blockade on both tumor growth and survival in mice bearing Smad4-null tumors relative to the both the single agents alone and a vehicle control (p < 0.01). Furthermore, the same SCC cell line failed to grow when surviving mice were rechallenged 6 months after the primary injection, indicating that they acquired and retained a memory T cell response to the tumor cells. Taken together, this indicates that a short-term treatment with a combination of TGFβR inhibition and PD-L1 blockade has dramatic long-term antitumor effects.

Drug resistance in basal cell carcinoma identifies the inner nuclear membrane as a critical Gli1 regulatory checkpoint
A Meza1, SA McKeller1 and AS Oro1

Drug resistance in basal cell carcinoma (BCC) identifies the inner nuclear membrane as a critical Gli1 regulatory checkpoint. Using genetic and proteomic investigations into the hedgehog pathway we have focused on the organization of Gli1 transcription factor activation within the primary cilium, the nuclear maturation of Gli1 is thought to be unstructured. Our studies of Smoothened inhibitor-resistant basal cell carcinoma (BCC) identifies that drug resistance mechanisms act by exploiting a previously unappreciated Gli maturation checkpoint on the primary nuclear membrane (iNEM). Utilizing a combination of super-resolution microscopy with novel state-dependent Gli1 antibodies, live cell imaging, vicinal proteomic labeling, and vicinal genomic labeling, we describe a highly conserved nuclear translocation mechanism for Gli1. The Imn protein LAP2α2, which concentrates Gli1 in the nucleus by sequestering it to the envelope and protecting it from nuclear export. Liberation from the iNEM and subsequent chromatin-binding is coordinated by the nucleoprotein GLI1 in the nucleus by sequestering it to the envelope and protecting it from nuclear export. The iNEM is therefore thought to be unstructured. Our studies of Smoothened inhibitor-resistant basal cell carcinoma (BCC) identifies that drug resistance mechanisms act by exploiting a previously unappreciated Gli maturation checkpoint on the primary nuclear membrane (iNEM). Utilizing a combination of super-resolution microscopy with novel state-dependent Gli1 antibodies, live cell imaging, vicinal proteomic labeling, and vicinal genomic labeling, we describe a highly conserved nuclear translocation mechanism for Gli1. The Imn protein LAP2α2, which concentrates Gli1 in the nucleus by sequestering it to the envelope and protecting it from nuclear export. 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**IDH mutant melanomas represent a distinct molecular subset of melanomas**

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IDH mutations have been identified in many tumor types, including glioma, acute myeloid leukemia, and renal cell carcinoma, and testing for the mutation can be a valuable diagnostic and predictive biomarker. We identified melanomas with IDH mutations at a single institution and sought to further characterize this cohort. 405 melanoma cases collected at Massachusetts General Hospital between 2015 and 2017 underwent molecular characterization of 91 genes, including IDH1 and IDH2, by next generation sequencing. Demographic and primary site data were recorded. The mean age of the cohort was 69 years with 70% of patients male. An IDH mutation was identified in 20 cases of 405 tested tumors (4.9%). Seventeen of 20 mutant IDH1(R132C) mutation, while the remaining three cases (15%) were hypermutant, with non-hotspot alterations of unknown significance. Of the 17 cases with an IDH1R132C mutation, 16 occurred in sun exposed areas (non-acral, non-mucosal) and 14/16 (88%) had co-occurring alterations in the MAPK pathway (BRAF, NRAS, MAPK1), a similar frequency compared to IDH wild-type melanomas in the cohort (329/385, 85%). Histology of 13 IDH1(R132C) mutant cases was reviewed, with 12 exhibiting an epitheloid growth pattern and one with mixed epithelioid and spindled patterns. All cases showed absence of or non-keratin tumor infiltrating lymphocytes. Of the 17 cases with IDH1(R132C) mutation, ten received immunotherapy. Two showed a reduction in tumor burden by imaging, and eight showed progression. At last follow-up (median follow-up time=32.6 months), five patients were alive with no evidence of disease, nine were alive with disease, and three had died of disease. We identified a small cohort of melanomas that harbor IDH1 mutations with frequent co-existing MAPK mutations. This group represents a distinct molecular subgroup classification of melanoma. Limited data suggests these cases may have a poor response to immunotherapy.

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**Association of pleiotropic cancer susceptibility variants and risk of cutaneous squamous cell carcinoma**

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Cutaneous squamous cell carcinomas (cSCC) is one of the most common malignancies among non-Hispanic whites (NHW), and previous genetic studies have reported 16 loci associated with this skin cancer. Those explain less than 10% of the familial relative risk for cSCC, suggesting that additional loci remain to be discovered. Recently, there is compelling evidence that pleiotropy is pervasive, with some genetic variants affecting the risk of multiple cancer sites. Here, we hypothesized that pleiotropic cancer susceptibility variants might be genetic risk factors for developing cSCC. We investigated the association between 33 pleiotropic cancer susceptibility single nucleotide polymorphisms (SNPs) identified from previously published genome-wide association studies and cSCC risk among NHW individuals (7,701 cases and 60,167 controls) from the Genetic Epidemiology Research in Adult Health and Aging cohort. We found that SNP rs4245739 in MDM4 gene was significantly associated with cSCC risk (P=0.001). Further, SNP rs401681 in CPLMT1 was nominally associated with cSCC risk (P=0.047). Confirmation of our findings was then conducted in GenomeAtlas, a publicly available database of associations between hundreds of traits and millions of variants using the UK Biobank cohort. In UK Biobank, multiple cancers risk was assessed through self-report information, and skin carcinoma risk was based on the International Classification of Diseases, Tenth Revision diagnosis code (ICD-10 code D04). In GenomeAtlas, MDM4 rs4245739 was associated with multiple cancers risk (P=0.0042). Similarly, CPLMT1 rs401681 was associated with multiple cancers risk (P=0.0097), and showed suggestive evidence with skin carcinoma risk (P=0.068). Our results identify for the first time MDM4 as a key genetic factor of cSCC risk in NHW and designate this gene as a promising target for nonmelanoma skin cancer treatment.

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**Single-cell RNA-sequencing reveals SCC intratumoral heterogeneity**

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Single-cell analysis techniques provide an unprecedented window into the diverse communities of malignant and non-malignant cells that enable tumorogenesis in cutaneous squamous cell carcinoma (SCC) and other human cancers. Recent single-cell studies of other cancers have identified substantial intratumoral heterogeneity (ITH), which may be significant because specific tumor sub-populations mediate aggressive progression and therapeutic resistance. In addition to identifying subpopulations of tumor cells that drive malignant behavior, single-cell RNA-sequencing (scRNA-seq) may reveal the degree to which normal gene expression programs, such as differentiation, are subverted in these subpopulations. In SCC, the mutational landscape and bulk RNA expression changes have been analyzed, however, the range of phenotypic variation within a given tumor is largely unknown. We therefore undertook scRNA-seq experiments in 18,000 cells from a series of 10 primary tumors from unrelated immunocompetent patients, along with patient and site-matched normal skin controls. Poorly and moderately-differentiated tumors contained a subset of malignant cells with severe disruption in their differentiation program, including loss of expression of ZNF750, KRT1, KRT10, DSG1, and DSC10, in contrast to well-differentiated SCCs, which harbored a subpopulation that retained these genes. Well-differentiated SCCs contained subpopulations of cells that resembled progenitor, early, and late differentiating keratinocytes, although they differed in specific ways, including the expression of chemotactic factors, matrix metalloproteinases, and activation of intercellular response genes. A distinct subpopulation that expressed genes related to actin filament organization, cellular movement, and wound healing, which may denote a particularly invasive subpopulation, was unique to histologically more aggressive tumors. These findings indicate that high-risk SCC is associated with emergence of a specific subpopulation of cells that disable specific differentiation genes and co-opt wound healing pathways to promote tumorogenesis.

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**TOX expression discriminates early Mycosis fungoides from benign inflammatory dermatoses**

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Statement of the Problem: The definite diagnosis of mycosis fungoides (MF) in early stages (FMF) and its differentiation from other clinical mimickers such as parapsoriasis might be challenging. The lack of specific marker may lead to a delay in early disease management and may rise mortality and morbidity of Cutaneous T Cell Lymphoma. In Immunohistochemistry (IHC) study we aimed to investigate the expression of TOX gene as a potentially specific marketo discriminate FMF from other inflammatory and non-inflammatory dermatoses. Methods: Expression level of TOX paragon was compared in samples of MF, psoriasis and parapsoriasis lesions wassatission with a specific rabbit polyclonal antibody against human TOX. Findings: In MF biopsies, TOX expression was significantly upregulated higher than chronic dermatitis, psoriasis and parapsoriasis (P=0.0001). Conclusion: TOX gene expression might be used as a good diagnostic marker to identify early patch and plaque FMF from benign inflammatory and non-inflammatory dermatoses similar clinical significance.

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**Discovering the hidden elements of cancer: Targeting the Incna ac004540.4 reveals its critical role in ras mutant melanoma**

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Malignant melanoma, one of the most aggressive and deadly forms of skin cancer harbors mutations in the Neuroblastoma Ras Viral Oncogene Homolog (NRAS) in 15-20% of cases. Long non-coding RNAs (lncRNAs) are a new class of previously underappreciated heterogeneous regulatory transcripts that modulate cancer development and progression in a multitude of cellular processes and can function as oncogenes and tumor suppressors. We could show that the antisense-IncRNA AC004540.4, overlapping the protein coding gene SNX10, is overexpressed in NRAS mutated melanocytes compared to melanocytes carrying wild type NRAS. Post-transcriptional knockdown of AC004540.4 through RNA interference showed a strong decrease of cell viability in NRAS mutated melanoma cell lines in vitro. Hypothesizing that many pro-oncogenic functions are connected to AC004540.4 expression levels and can be suppressed by inhibiting AC004540.4 and its potential functional paragon, oncosuppressive drug induced differentiation we are currently constructing an inducible CRISPRi systems to test knockdown of AC004540.4 in NRAS mutant melanoma cell lines, suggesting that AC004540.4 plays important role NRAS melanoma cell survival. To further investigate the role of IncRNA AC004540.4, in melanoma, we are currently constructing an inducible CRISPRi systems to test knockdown of AC004540.4 in NRAS mutant melanoma cell lines. In summary, we found a novel pro-oncogenic IncRNA in NRAS mutant melanoma and further understanding of its mechanistic and functional role in cancer may be used as vantage point for future therapeutic strategies.

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**The antisense lncRNA transcript RP11-7011.3: Shedding light upon a novel and crucial player in NRAS mutant melanoma**

*L. Chen1, V. Fechtenschlager1, M. Vujic1, A. Lee1, H. Kao1, J. Zheng1 and S. Ortiz1 1 Dermaatology, UCFS, San Francisco, CA, and 2 Dermatology, San Francisco, CA

Malignant melanoma is an aggressive and potentially deadly skin cancer. 15-20% of malignant melanoma harbors mutations in the Neuroblastoma Ras Viral Oncogene Homolog (NRAS). NRAS mutation over-activates downstream signaling cascades and leads to increased proliferation, migration, and survival of cancer cells. In our preliminary studies, we lab examined NRAS mutation specific transcriptomic changes by introducing an NRAS mutated plasmid in primary human melanocytes. Analysis of differential gene expression has shown IncRNA RP11-7011.3 to be one of the transcripts that were upregulated in NRAS mutant melanocytes and NRAS melanoma cells compared to normal melanocytes. RNAi knockdown of RP11-7011.3 in melanoma cells resulted in a large decrease in cell viability across different NRAS mutant melanoma cell lines, suggesting that RP11-7011.3 plays important role NRAS melanoma cell survival. To further investigate the role of IncRNA RP11-7011.3 in melanoma, we are currently constructing an inducible CRISPRi systems to test knockdown of RP11-7011.3 in NRAS mutant melanoma cells. In summary, we found a novel pro-oncogenic IncRNA in NRAS mutant melanoma and further understanding of its mechanistic and functional role in cancer may be used as vantage point for future therapeutic strategies.
Stearoyl-CoA desaturase-5 is upregulated in basal cell carcinoma: A role in aberrant cell proliferation in human skin

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Stearoyl-CoA desaturase (SCD), a Δ9-desaturase that converts saturated fatty acids into monounsaturated fatty acids, has been an important regulator of lipogenesis, cell proliferation and differentiation. To date, four mouse (14) and two human (1 and 5) isoforms of SCD have been identified. Human SCD-1 is abundantly expressed in lipogenic tissues, while increased expression of SCD-5 has been reported in the brain and pancreas. Importantly, both isoforms convert stearate to its unsaturated counterpart oleate. Mice lacking SCD-1 are protected from obesity and are characterized by suppressed fatty acid synthesis and enhanced oxidation. The biological function(s) of SCDs in the human skin are not well understood. Here we report that both isoforms, in particular SCD-5, is robustly expressed in human epidermal keratinocytes. Pharmacological inhibition of SCD-1 in keratinocytes resulted in induction of endoplasmic reticulum (ER) stress and activation of mTOR signaling. Parallel studies in skin specific SCD-1 KO mouse model indicated a similar increase in ER stress with activation of NF-kB and mTORC2 signaling. In normal keratinocytes, SCD-1 deficiency was not accompanied with significant alterations in oleate-to-stearate or palmitoleate-to-palmitate ratio. However, elevated oleate-to-stearate ratio in basal carcinoma cells compared to normal keratinocytes signified increased SCD activity in the malignant phenotype. Tissues from patients with basal cell carcinoma showed upregulated SCD-5 transcripts in tumor compared to non-lesional control skin. Intriguingly, increases in SCD-5 mRNA expression in tumor tissues were inversely correlated with the level of mTORC2 activity. Western blot analysis validated SCD-5 protein expression and activation of mTORC2 signaling in tumor tissues. Our studies provide novel insight into a potential regulatory loop driving aberrant cell proliferation through modulation of fatty acid metabolism in the human skin.

Facilitation of oncogene-induced transformation of skin fibroblasts by ATM

X Liu, Duke University School of Medicine, Department of Dermatology, Durham, NC

ATM (ataxia telangiectasia) is a complex neuronal degenerative disorder which caused by mutation of a gene known as Ataxia Telangiectasia Mutated (ATM). The disorder is charac- terized by progressively impaired coordination of voluntary movements (ataxia), the develop- ment of abnormalities of the skin and mucous membranes (telangiectasia), and increased susceptibility to infections. The ATM protein is absolutely required for the response to DNA damage by a variety of sources, including ionizing radiation, alkylating agents, and topoisomerase inhibitors. ATM is one of the first kinases to be recruited to sites of DNA breaks where it phosphorylates histones and nuclear proteins to aid in the formation of the repair complexes required for DNA repair. ATM is also essential for cell cycle control, maintenance of genomic stability, and centrosome integrity. ATM regulates multiple cell cycle checkpoints and apoptosis, and is critical for the human genome stability. ATM is required to maintain genomic integrity and prevents oncogenesis in response to DNA damage. In this study, we examined the role of ATM in facilitating oncogenesis in human skin fibroblasts. We found that ATM is required for the transformation of skin fibroblasts induced by oncogenic Ras and DNA double strand breaks. These data suggest that ATM may have a role in facilitating oncogenesis in human skin fibroblasts.

Cancer-associated KNSTRN mutations disrupt protein-protein interactions needed for SCC neoplasia

C Tommasi, A Mah, B Gower, JY Shen and CS Lee Dermatology, Stanford University, Stanford, CA

Genome instability and sustained proliferation are cardinal features of cancer, yet our un- derstanding of the molecular mechanisms that underlie these characteristics continues to grow. Mutations in the kinetochore gene KNSTRN occur in 19% of cutaneous squamous cell carcinomas (SCC) and interfere with the ability of the encoded protein to regulate spindle function during mitosis. Specifically, cancer-associated KNSTRN mutations disrupt sister chromatid cohesion, enhance tumorigenesis, and correspond to increased aneuploidy in primary SCC, suggesting that Kinestrin is required for genome stability and regulates cellular proliferation in vivo. To test whether Kinestrin protein-protein interactions might be impor- tant in its function, we performed proximity ligation assays (PLA) in mitotic primary human keratinocytes transduced to express wild type or cancer-associated mutant Kinestrin. Among known Kinestrin interactors tested, EB1 selectively bound to wild type Kinestrin with loss of this interaction in the context of the cancer-associated mutant protein. We further confirmed disruption of Kinestrin-EB1 binding in human SCC with mutant but not wild type KNSTRN, suggesting that loss of this interaction might contribute to the accelerated aneuploidy seen in this setting. Efforts are underway to identify potentially novel Kinestrin interactors by mass spectrometry. To identify previously unrecognized Kinestrin protein interactions that are disrupted in SCC and may provide the foundation for non-surgical therapeutics.

Genetic variants of the ketogenic pathway genes predict melanoma survival

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Cutaneous melanoma (CM) is the most aggressive form of skin cancers and remains one of the most challenging human cancers in the treatment. The autophagy pathway controls the principle catabolic process for lysosomal-mediated degradation of intracellular components to sustain cellular energy and survival. Increasing evidence of deregulated autophagy in melanoma has suggested its potential as a prognostic biomarker. Therefore, we hypothesized that genetic variants of the autophagy pathway genes might predict survival of CM patients. Using data derived from previously published genome-wide association studies (GWAS) of cutaneous melanoma, we assessed the associations of 19,243 common single-nucleotide polymorphisms (SNPs) in 242 autosomal ketogenic metabolism genes with CM specific survival (CMSS) in 858 CM patients. By using multivariate Cox proportional hazards regression and false positive report probability (FPRP) corrections, we identified two independent SNPs (i.e., PDSS1 rs3808914 G>C and rs2166182 C>G) that showed a predictive role in CM-specific survival. After incorporating the number of unfavorable genotypes (UG) in the model with clinical variables, the new model showed a significantly improved discriminatory ability to classify CMSS. Our findings suggest that genetic variants of the ketogenic metabolism gene, particularly PDSS1 rs3825657 and rs2166182, may modulate survival of CM patients. Further studies are needed to validate these findings.

Genetic variants of the ketone metabolism gene PDSS1 predict melanoma survival

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The ketogenic pathway mediates the conversion of ketone bodies into ATP and is an important energy production pathway in cancer cells. PDSS1 is involved in the synthesis of ketone bodies. We tested the hypothesis that genetic variants of the ketogenic metabolism genes might predict survival of CM patients. Using data derived from previously published genome-wide association studies (GWAS), we assessed the associations of 19,243 common single-nucleotide polymorphisms (SNPs) in 242 autosomal ketogenic metabolism genes with CM specific survival (CMSS) in 858 CM patients. By using multivariate Cox proportional hazards regression and false positive report probability (FPRP) corrections, we identified two independent SNPs (i.e., PDSS1 rs3808914 G>C and rs2166182 C>G) that showed a predictive role in CM-specific survival. After incorporating the number of unfavorable genotypes (UG) in the model with clinical variables, the new model showed a significantly improved discriminatory ability to classify CMSS. Our findings suggest that genetic variants of the ketogenic metabolism gene, particularly PDSS1 rs3825657 and rs2166182, may modulate survival of CM patients. Further studies are needed to validate these findings.

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Increased risk of malignancy in mycosis fungoides: A single-center perspective
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Mycosis fungoides (MF) is a rare, generally indolent non-Hodgkin lymphoma of the skin. In a large, population-based study of the SEER-18 database, we recently found that patients with stage I MF were at an increased risk of developing second malignancies. In this case-control study, we examined factors associated with development of second malignancies in 172 MF patients treated at the University of Minnesota from 2005-2017. We identified 172 patients with MF, median age of 59 years (range, 8-89). As a control group, we identified 175 patients with each of the diagnosis codes for PDAC (C22.9: pancreatic ductal adenocarcinoma). Patients treated with MF were significantly more likely to develop a second malignancy (24 out of 172) compared to control patients (3 of 175, odds ratio [OR] 9.4, p < 0.001). Median follow-up for MF patients was 5 years (range 0-40 vs 4.1 years (range 0-24) for those with PDAC (p = 0.001). Patients with tumor stage MF were significantly more likely to develop a second malignancy (9 of 26) than those with patch/plaque stage MF (14 of 110, OR 3.6, p < 0.01). Similarly, patients with advanced stage disease (stage IIIb or higher) were significantly more likely to develop malignancies than patients with stage I disease (stage IA or IB, p = 0.03). Patients with advanced disease were more likely to develop second malignancies independent of treatment. Patients with advanced stage disease are more likely to develop second malignancies independent of treatment.
The JAK1/2 inhibitor ruxolitinib induces cell cycle arrest and apoptosis through inhibiting STAT3 phosphorylation in squamous cell carcinoma

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Ruxolitinib is an FDA approved JAK1/2 inhibitor that has shown efficacy in human head and neck squamous cell carcinoma, lung, and breast carcinoma, however, its therapeutic application has not been elucidated in cutaneous squamous cell carcinoma (cSCC). cSCC is the second most common form of skin cancer, which causes significant morbidity due to local recurrence and metastasis. Therefore, there is an unmet need for novel therapeutic approaches for this malignancy. Here, we investigated whether the inhibition of the JAK/STAT pathway has antitumor effects in cSCC. We found that JAK1/2 inhibition with ruxolitinib in the human SCC9 cell line in vitro resulted in STAT3 signaling-dependent decreased proliferation and cell cycle arrest at G1. This effect was accompanied by reduced expression of transcription factors such as p21WAF1, while also containing deletions in MDM4, SKP2, while also containing deletions in RB1, AKT2, ITGB4, and SKP2. Therefore, there is an unmet need for novel therapeutic approaches for this malignancy.

200 Distinct signatures of genomic copy number variants define subgroups of Merkel cell carcinoma tumors

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Merkel cell carcinoma (MCC) is a rare but aggressive neuroendocrine skin cancer with a predilection for the elderly and immunosuppressed. The majority of MCC tumors contain integrated polyomavirus DNA (virus-positive MCC), VP-MCC carry a low tumor burden and metastasis. Therefore, there is an unmet need for novel therapeutic approaches for this malignancy. Here, we investigated whether the inhibition of the JAK/STAT pathway has antitumor effects in cSCC. We found that JAK1/2 inhibition with ruxolitinib in the human SCC9 cell line in vitro resulted in STAT3 signaling-dependent decreased proliferation and cell cycle arrest at G1. This effect was accompanied by reduced expression of transcription factors such as p21WAF1, while also containing deletions in MDM4, SKP2, while also containing deletions in RB1, AKT2, ITGB4, and SKP2. Therefore, there is an unmet need for novel therapeutic approaches for this malignancy.

201 Loss of notch function promotes UV-induced selection of keratinocyte clones to form skin cancer

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Cutaneous squamous cell carcinoma (cSCC) is the second most common cancer and typically arises from squamous cell carcinoma in situ (SCCIS). Our prior exome sequencing of human SCCIS biopsies demonstrated frequent UV-sensitivity. Loss of UV-signature mutations that were subclonal in the epidermis but clonal and heterozygous in SCCIS. These data led to the hypothesis that Notch-deficient keratinocytes clones undergo UV-mediated selection to form SCCIS. To test this hypothesis we developed a mouse genetic model to differentially express nuclear GFP single keratinocytes that also express the Notch-inhibiting DNMAML tran gene to model the spontaneous clonal mutations in UV-exposed skin that progress to SCCIS. Alzine were imaged in vivo by two-photon microscopy to confirm that transgene activation yielded a uniform random distribution of Notch-deficient clones. One-half of a mouse was subjected to 200ml/cm2 UVB/irradiation three times per week for 6 weeks while the other half was protected. Weekly in vivo imaging showed that 70% of Notch-deficient clones regressed in UV exposed skin while 30% of clones demonstrated positive selection. Within three months, a subset of the positively selected clones formed small keratotic papules up to two millimeters in size consistent with SCCIS as demonstrated by histology and biomarker analysis which included activation of tyrosine kinases, PDK1 and S6 kinases. No Notch-competent keratinocytes formed an SCCIS lesion under these conditions. Overall, these data support the hypothesis that keratinocytes with impaired Notch signaling in unrestrained epidermis are preferentially selected by UV irradiation to form SCCIS.

202 Utilization of in vivo (U-13C)Glucose tracing to identify metabolic alterations in metastatic melanoma cells

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Tumor cells altered metabolism, and many clinical trials are currently underway to target metabolic dependencies in a wide range of cancers. The majority of what is known in cancer metabolism is a result of in vitro work in non-physiologic nutrient environments and only limited studies have been performed in vivo. The goal of this study was to develop a mouse model to study the metabolism of melanoma cells in vivo and characterize alterations after metastasis. To do this, we used a melanoma patient-derived xenograft model in which melanoma cells were injected into immune-competent NSG mice. When tumors reached 0.2cm, mice were anesthetized and intubed with U-13C glucose for 3 hours to ensure steady-state enrichment in tumor tissue. Mice were then sacrificed and melanoma cells were collected from the primary site as well as different organ sites of metastasis. Lysates were obtained from melanoma cells and were measured using gas chromatography-mass spectrometry. By comparing the 13C isotopic enrichment in these metabolites, we were able to determine how glucose was being utilized in melanoma cells in vivo. To date, we have examined 7 different patient melanomas in more than 20 mice. We have found that the metabolic properties of melanoma cells change depending on their site of metastasis, often mimicking the metabolic properties of the organ tissue in which they are growing. One of the most profound metabolic changes seen was in melanoma cells that had metastasized to the brain, where the incorporation of 13C labeled carbon into fatty acids was seen. Most melanoma cells in other organs, suggesting increased utilization of oxidative phosphorylation. Future studies will be performed to understand how drugs targeting metabolic pathways may be more or less efficacious based on the site of melanoma metastasis.

203 A proteome-transcriprome-miRnome integrative analysis identifies similarity between UV-exposed skin and skin wound

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Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer, for which long term UV exposure and chronic wounding are the dominant risk factors. Despite these clinically established connections, little is understood about the early molecular response of human skin to UV exposure and its connection to acute wounding and cSCC. Thus, our goal is to find common and specific signatures driven by UV-exposure and wounding as a means of developing new approaches for treating and preventing cSCC. Here, we perform integrated analyses of proteomic, RNA-seq and miR-seq on 3 datasets: (1) UV-unexposed and acute UV-exposed human skin, (2) public dataset on acute wound healing (3) our previously published dataset on normal skin and cSCC from humans. We find that biological signatures and processes regulated by acute UV exposure and wounding has profound similarity. miR-seq data shows that miR-223, miR-132 and miR-142 are significantly upregulated in both acute events. Combined gene set enrichment analysis shows that protein-coupled-receptors (GPCRs) pathways are upregulated, possibly through class A Rhodopsin-like receptor. While ECM remodeling is significantly enriched in all three datasets, gene expression regulated by PPARs is suppressed. Interestingly, upregulation of matricryptin (COL3A1) and TGFbeta receptor (TGFBR1) is observed. Thus, our findings suggest that UV-exposed skin, wound and cSCC share various common signatures, which can be potentially validated as chemotherapeutic targets for cSCC.
Identification of a gene expression profile signature associated with recurrence and/or metastasis in primary cutaneous squamous cell carcinoma (cSCC)
S. Ibrahim1, N. Cleaver1, I. Maher1, D. Panther1, D. Brodl1and K. Covington1, C. Schmutz2, J. Newman3, A. Wyung4 and S. Arron4 1 Dept of Dermatology, University of Rochester, Rochester, NY, 2 Clever Dermatology, Kirkville, MO, 3 Dept of Dermatology, St. Louis University, St. Louis, MO, 4 Ziottl & Brodl, P.C., Pittsburg, PA, 5 Castle Bicosiences, Friendswood, TX, 6 Dept of Dermatology, Brigham and Women’s Hospital, Jamaica Plain, MA, 7 Dept of Otologynenergology, University of Pennsylvania, Philadelphia, PA, 8 Dept of Dermatology, USC, Los Angeles, CA. 9 Dept of Dermatology, UCVF, San Francisco, CA. Despite overall good prognosis for patients with cSCC, a subset will develop local, regional or distant recurrences following complete excision of the primary tumor. Those at high risk of recurrence are eligible for adjuvant treatment options. While specific clinical features are associated with recurrence, they collectively fail to identify 30-40% of all cSCC recurrences and many tumors that express high risk features will not recur. To address the need for more accurate predictive factors and facilitate appropriate intervention strategies, we used gene expression analysis to determine if a significant gene expression signature could be identified. Utilizing published literature and pathway analysis, we selected 73 candidate genes for evaluation of gene expression changes in recurrent and non-recurrent cases. A total of 230 primary cSCC tumors were collected under an IRB-approved, multi-center protocol and analyzed. After quality filtering, we assessed expression of 61 genes across 212 samples. Eighteen genes were significantly differentially expressed between recurrent and non-recurrent cases (p<0.05). Among the top differentially expressed genes were members of pathways important for recurrence and metastasis, including matrix-metalloproteinase, extracellular matrix, and epithelial differentiation pathways. The results demonstrate that gene expression differences can be identified between recurrent and non-recurrent cSCC and suggest that a gene expression test to identify cSCC patients at higher risk of recurrence or metastasis is feasible. Such a test could help determine which patients may benefit from additional therapeutic interventions.

1 Department of Dermatology, University of Rochester, Friendswood, TX, 6 Dept of Dermatology, Brigham and Women’s Hospital, Jamaica Plain, MA. 7 Dept of Otologynenergology, University of Pennsylvania, Philadelphia, PA, 8 Dept of Dermatology, USC, Los Angeles, CA. 9 Dept of Dermatology, UCVF, San Francisco, CA. Despite overall good prognosis for patients with cSCC, a subset will develop local, regional or distant recurrences following complete excision of the primary tumor. Those at high risk of recurrence are eligible for adjuvant treatment options. While specific clinical features are associated with recurrence, they collectively fail to identify 30-40% of all cSCC recurrences and many tumors that express high risk features will not recur. To address the need for more accurate predictive factors and facilitate appropriate intervention strategies, we used gene expression analysis to determine if a significant gene expression signature could be identified. Utilizing published literature and pathway analysis, we selected 73 candidate genes for evaluation of gene expression changes in recurrent and non-recurrent cases. A total of 230 primary cSCC tumors were collected under an IRB-approved, multi-center protocol and analyzed. After quality filtering, we assessed expression of 61 genes across 212 samples. Eighteen genes were significantly differentially expressed between recurrent and non-recurrent cases (p<0.05). Among the top differentially expressed genes were members of pathways important for recurrence and metastasis, including matrix-metalloproteinase, extracellular matrix, and epithelial differentiation pathways. The results demonstrate that gene expression differences can be identified between recurrent and non-recurrent cSCC and suggest that a gene expression test to identify cSCC patients at higher risk of recurrence or metastasis is feasible. Such a test could help determine which patients may benefit from additional therapeutic interventions.

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RIPK4 maintains epidermal homeostasis and prevents skin cancer by suppressing miogenic signaling
G. Tanghe1, C. Uweyer-Rosselet1, J. De Groote1, M. Dewos1, B. Gilbert1, K. Lemeire1, C. Blanpain1, P. Vandenberghe1 and W. Declercq1 1 Dept of Dermatology, University of Leuven, Leuven, Belgium and 2 ULB-Brussels, Belgium. The skin is a fast renewing organ with continuous commitment of proliferative progenitor keratinocytes into a terminal differentiation program forming the stratified epidermis. Previous studies demonstrated that RIPK4, a serine/threonine kinase, is crucial during epidermal development. Loss of RIPK4 in the epidermis causes cleft palate, epibulbar fusion, aberrant differentiation and a defective barrier leading to perinatal death. Here, we addressed the homeostatic functions of RIPK4 in adult mouse skin. Inducible RIPK4 deletion in adult mouse epidermis caused significant hyperplasia due to the expansion of proliferative basal keratinocytes. Although epidermal keratinocytes eventually commit to differentiation, the barrier is dysfunctional which coincides with local immune infiltration. We found that RIPK4 enables cell cycle exit by suppressing p63 expression in a keratinocyte autonomous manner. Additionally, RIPK4 down regulates EGR expression and its downstream mitogenic, stunted, pathways in a kinase-dependent manner in mouse and human keratinocytes. Subsequently, loss of RIPK4 led to spontaneous tumor formation of the keratoacanthoma type, with reduced latency by additional deletion of tumor suppressor p53. Furthermore, RIPK4 serves as a brake on oncogenic Kras-driven tumor growth. Together, our work demonstrates that RIPK4 fulfills a central role in maintaining the homeostatic balance between keratinocyte proliferation and differentiation by suppressing p63 expression and EGR signaling and providing a tumor suppressive function in clinically relevant genetic squamous skin cancer models.

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Metastatic squamous cell carcinoma of the skin showing clinical response to lapatinib
JD Strickley1, Y. Haeberle1 and J. Jung1 1 University of Louisville School of Medicine, Louisville, KY, 2 Dermatology, University of Louisville, Louisville, KY and 3 Onco-dermatology, Norton Cancer Institute, Louisville, KY. Next generation sequencing (NGS) of tumors has ushered in a new era of personalized oncology therapy. We present a patient with metastatic cutaneous squamous cell carcinoma who failed surgery, radiation, and anti-PD1 therapy, but showed clinical response to a drug targeting an ERBB3 mutation identified with NGS. Following initiation of the drug lapatinib, this patient exhibited dramatic tumor regression in the skin, soft tissue, bone and nerves. Additionally, his ipsilateral hearing loss was partially reversed and he was able to be weaned off of narcotic pain medication. Lapatinib is a tyrosine kinase inhibitor that blocks the HER2 receptor and is FDA approved for HER2 positive tumors. Both ERBB2 (HER2) and ERBB3 (HER3) belong to the same family of receptor tyrosine kinases. Dimerization of these receptors leads to activation of cell proliferation and survival pathways, granting tumor cells potential to dysregulated ERBB/R HER receptors. Cutaneous squamous cell carcinoma of the skin is the 2nd most common cancer diagnosed in humans and although surgery may be curative in local disease, effective therapy for metastatic disease remains elusive. Further investigation of HER2 targeted therapies in cutaneous squamous cell carcinoma may provide an effective treatment strategy in the HER2/3 pathway.
210 MRTF inhibition displays promising therapeutic potential in human BCC patient explants.

El White1, J Beck1, A Mirza1, CY Yao2, SA McKee1, S Halling1, S Aasi1, KY Sain1, J Tang1, and AE Oren1 1 Stanford University, Palo Alto, CA, 2 Stanford, Stanford, CA, 3 Stanford University, Stanford, CA, 4 Dermatology, Stanford, Stanford, CA, and 5 Program in Epithelial Biology and Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA. 3 Basal cell carcinoma (BCCs) are more prevalent than any other cancer and are driven by mutations in PTC11 or SMO causing aberrant activation of the hedgehog (HH) signaling pathway. The SMO inhibitor, Vismodegib recently received FDA approval for the treatment of advanced BCCs. Unfortunately, most BCCs become resistant and only 50% of patient resistant tumors contained additional classical pathway mutations. This suggested an alternative mechanism accounts for most resistant growth, thus, we searched for novel non-classical resistance pathways. We used multidimensional genomics in drug-resistant BCCs to identify a noncanonical hedgehog activation pathway driven by the transcription factors serum response factor (SRF) and mycardin-related transcription factor (MRTF/MKL1). Further studies identified SRF-MRTF share chromosomal occupancy and form a novel protein complex with the HH transcriptional activator GLI1. The HH/MRTF/miR-210/miR-146a/miR-122 axis were targeted for MRTF-driven HH activation and resistant BCC growth. Remarkably, small molecule inhibition of MRTF suppressed tumor growth in a mouse model of resistant BCC. However, efforts to further test MRTF inhibitors in human tumors are hampered by an inability to produce viable patient-derived xenografts (PDx) for BCC. We overcame this obstacle by developing ex vivo culture conditions using freshly resected BCCs from Mehs patients. In addition, we developed a novel (active) MKL1 stable transgenic mouse model to predict efficacy for MKL1 inhibitors. Indeed, tumors containing nuclear MRTF produced a robust response to MRTF inhibitors by displaying a significant reduction of GLI1 mRNA. Thus, our work highlights the therapeutic potential of MRTF inhibitors in BCCs and establishes a new human tumor model for preclinical testing of promising drug candidates.

211 Co-Inheritance of mutations in CDKN2A and MC1R increases melanoma predisposition independently of deregulation of cell cycle and UV response in mice.

B Hernandez1, V Swope2, R Starner1, P Cassidy3, A Kadekaro2, D Bennett1, S Leachman3 and Z Abdel-Malek6 1 Dermatology, Department of Medicine, University of California, San Francisco, CA, 2 Department of Dermatology, Oregon Health and Science University, Portland, OR, 3 Molecular and Clinical Sciences Research Institute, St. George’s University, London, England, United Kingdom, 5 Department of Dermatology, Oregon Health and Science University, Portland, OR, and 6 Department of Dermatology, University of Cincinnati, Cincinnati, OH. Melanoma predisposition of germline CDKN2A (p16) mutation carriers is augmented by co-inheritance of a loss of function (LOF) melanocortin 1 receptor (MC1R) variant. To understand the mechanism by which this occurs, we compared the doubling time in proliferation capacity, and UV response of primary human melanocyte (hMC) cultures established from carriers of V126D or 5’UTR-34G>T p16 mutations expressing either wild type (wt) or a LOF MC1R variant. We also included hMC st for p16 and either wt for MC1R or heterozygous for a LOF MC1R variant, and 2 rare hMC cultures expressing 2 mutant p16 alleles. All hMC cultures expressed the appropriate molecular weight p16 protein, except one that expressed 2 mutant p16 alleles and no p16 protein. Carriage of a p16 mutation did not affect MC1R activity. p16 and MC1R genotypes did not affect DNA double time or replicative senescence. Exceptions were the two expressing two mutant p16 alleles that required an enriched growth medium, and more passages before undergoing replicative senescence. The p16 and MC1R genotypes did not disrupt the UV response of hMC, evidenced by cell cycle arrest, phosphorylation of JNK and p38, p53 accumulation, H2O2 generation, and repair of DNA photoproducts. Despite these normal responses, RNA sequencing revealed differential gene expression in V126D p16 hMC, compared to hMC co-expressing D294H MC1R, or st for p16 and MC1R. Therefore, despite co-inheritance of p16 and MC1R mutations, additional hits is required for initiation of melanogenesis.

212 Evidence that PPARγ plays a key role in cutaneous immune function and activation of PPARγ promotes immune-mediated clearance of tumors derived from a cutaneous SCC cell line

A Chen1, B Kim2, R Chitkara1, J Okumura1, M Pack2, M Capelson3 and J Seykora1 1 Indiana University School of Medicine, Indianapolis, IN, 2 Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN and 3 Pharmacology and Toxicology, Wright State University, Dayton, OH. Previous studies show that loss of epidermal PPARγ (g) mice (g/γ-/-) results in roughly 2-3 fold more tumors following chronic ultraviolet (UV) treatments, a roughly 70% reduction in contact hypersensitivity responses (CHS), and promotes B16F10 melanoma tumor growth. Treatment with the PPARγ agonist rosiglitazone (Rosig) suppresses chemical and photocarcinogenesis and reverses UV-induced suppression of CHS responses (UV/S) and UV-induced B16F10 melanoma tumor growth. We examined whether PPARγ activation could suppress the growth of an immunogenic cutaneous SCC cell line (PDV) and whether this occurred through immunomodulated mechanisms. We show that Rosig treatment reversed both local and systemic UV/S and that Rosig/UV mice exhibit a significant increase in cutaneous CD11b+Gr-1+ myeloid cells (5-9 fold) relative to WT. Rosig suppressed the growth of PDV tumors in WT mice (2-3 fold larger on day 59, pin vito or in vivo. s) An increase in tumor infiltrating lymphocytes is seen in Rosig treated tumors; 4 CD11b+Gr-1+ myeloid derived suppressor cells can promote immune tolerance and suppress anti-tumor immune responses. Rosig suppressed CD11b+Gr-1+ myeloid cells accumulation within the tumor microenvironment (35-4% vs. 17.2% of total cells, p<CD11b+ alone. Collectively, this data supports the idea that PPARγ plays an active role in cutaneous immunity and that PPARγ ligands may promote anti-tumor immune responses.

213 Nucleoprin is frequently mutated in SCCS and loss of function promotes UV-induced neoplasia

Q Zheng1, X Yang1, H Maeno1, C Marshall1, S Proxt1, C O’Day2, C Yeh1, V Anagnos1, A Jani1, M Kashyap1, D Chandrashekar2, S Varambally2, S Bae3, L Kopelowich4, CA Elmets5 and M Ahar1 1 Dermatology, University of California, San Francisco, CA, 2 Stanford, Stanford, CA, 3 Stanford University, Stanford, CA, 4 Mo- l ecular and Cellular Sciences Research Institute, St. George’s University, London, and 5Program in Epithelial Biology and Developmental Biology, University of Pennsylvania, Philadelphia, PA. To identify mutations that promote the early stages of UV-induced carcinogenesis, we performed whole exome sequencing on 10 paired libraries derived from laser-captured microdissected squamous cell carcinoma in situ (SCCS) and adjacent epidermis. Data analysis demonstrated a high frequency of UV-signature single/double nucleotide variations and dele- tions in nucleoprin (Nups) which would likely alter the structure and function of the nucleolar core complex (NCP). In total, thirteen of eighteen NUPs were mutated in either the epidermis or SCCS; 15 distinct substitutions were identified and 14 mutations were found in the SCCS libraries. No analogous mutations were found in 4000 genes which included “housekeeping” genes such as GAPDH, B2M, PGK1 and PP1A. These data raise the hypothesis that UV-signature mutations in Nups could promote skin cancer. To address this hypothesis, we developed a murine model with heterozygous deletion of Elyss in the epidermis. Elyss is a nuclear-basket Nup and the only Nup with a DNA-binding domain. Elyss heterozygous mice were subjected to three doses of 80mcm/cm2 UVB and demonstrated prominent epidermal necrosis followed by prominent hyperplasia; minimal hyperplasia was seen in control mice. The epidermal hyperplasia in Elyss heterozygous mice demonstrated prominent dysplasia resembling actinic keratoses. Notch and its target genes, including HES1 were downregulated in Elyss heterozygous mice. sRNA mediated knockdown or overexpression of sRNA in Elyss, a frequently mutated Nup, demonstrated increased production of UVB-induced CPD in HaCaT cells. Together, these data implicate loss of Nup function in the early stages UV-induced carcinogenesis.

214 High fat diet activates UVB-induced development of basal cell and squamous cell carcinoma in PichVs/KSHK1 mice

SC Chaudhary1, A Jami1, M Kashyap1, D ChandraShekar1, S Varshally1, S Bae4, J Kopelowich4, CA Elmet5 and M Ahar1 1 Dermatology, UIAB, Birmingham, AL, 2 Pathology, UIAB, Birmingham, AL, 3 Medicine, UIAB, Birmingham, AL and 4 Medicine, Weill Cornell Medical College, New York, NY. Squamous cell (SC) and basal cell carcinomas (BCC), known as nonmelanoma skin cancers (NMSC), are the most commonly diagnosed human neoplasms in the United States. High-fat diet (HFD) is strongly associated with the risk of many cancer types, including skin cancer. Here, we showed that UVB-irradiated PichVs/KSHK1-1 hairless mice fed with HFD had earlier cutaneous tumor incidence and greater tumor burden (p<0.01) than control, normal diet (ND)-fed mice. We analyzed tumor samples using immunohistochemistry (IHC), Western blot, and mRNA for various biomarkers. These data show that HFD enhanced the growth of tumors associated with high expression of the proliferation biomarkers PCNA and cyclin D1. Furthermore, activation of MAP kinase and PI3K/AKT pathways were also elevated more frequently in ND-fed mice with HFD. Additionally, we observed increased recruitment of myeloid-derived suppressor cells (CD11b+Gr1-positve) in UVB-irradiated dermis and tumor stroma of HFD mice. We also found increased epithelial-mesenchymal transition, depictive of tumor invasiveness, in tumor tissues of UVB-irradiated HFD-fed mice compared to ND. We also observed increased recruitment of myeloid-derived suppressor cells (CD11b+Gr1-positve) in UVB-irradiated dermis and tumor stroma of HFD mice. We also found increased epithelial-mesenchymal transition, depictive of tumor invasiveness, in tumor tissues of UVB-irradiated HFD-fed mice compared to ND. We also observed increased recruitment of myeloid-derived suppressor cells (CD11b+Gr1-positve) in UVB-irradiated dermis and tumor stroma of HFD mice. We also found increased epithelial-mesenchymal transition, depictive of tumor invasiveness, in tumor tissues of UVB-irradiated HFD-fed mice compared to ND.
Master regulators of immune infiltrate recruitment improve efficacy of immune checkpoint inhibitor therapy in melanomas

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Immunotherapies are emerging as promising avenues for cancer treatment. The treatment paradigm of immune checkpoint inhibition specifically restores a host's ability to recognize and destroy tumor cells. Despite their remarkable efficacy, we showed that the benefit from treatments. Patients with immunologically "cold" tumors bear mutations rendering immunotherapies ineffective. We previously derived an immune infiltrate recruitment transcriptional signature from the hair follicle in alopoeicta (AA), that was controlled by a single network-informed master regulator (MR), IKZF1. We postulated that cold tumors have gone cold by inactivating an immune infiltrate MR like IKZF1 could be converted into an immunologically "hot" susceptible state by reactivating it. We screened TCGA cancer cohorts for patient subsets that had perturbations of IKZF1 in their genetic/genomic networks. This analysis identified 25-40% of patients across six cancer types (melanoma, lung, thyroid, head-and-neck, prostate, and bladder) that would be amenable immune enhancement. Overexpression of IKZF1 in representative cell lines resulted in activation of immune infiltrate recruitment signatures and increased immune-mediated cytolytic. These results were translated into a syngeneic, immunocompetent mouse model where we showed that cutaneous melanomas were suppressed when IKZF1 was expressed in the tumors on the same order as anti-PD-1 treatment. Furthermore, combining IKZF1 with anti-PD1 + anti-CTLA4 treatment provided synergistic enhancement, and the triple combination ablated tumor growth completely. Finally, we investigated IKZF1 in independent melanoma cohorts, and found that IKZF1 disruption was strongly correlated with poor outcome (p<1e-10) and recurrence. Our findings promise of enhancing immunotherapies by identifying key immune recruitment networks, as well as to identify cancer cohorts and individual patients that would be amenable to immunotherapy enhancement through specific, therapeutically targetable master regulators.

Dual role of the polarity protein atypical kinase Cα in skin carcinogenesis

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Dual role of the polarity protein atypical kinase Cα (Akk) in skin carcinogenesis

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Altered polarity is a hallmark of cancer. The atypical kinase C α (Akk), a central regulator of cell polarity, is considered a tumor promoter but its mechanisms of action are largely unknown. We show that Akk interacts with PKCε binding partner, inhibits Ras-driven skin carcinogenesis. To address how Akk controls skin cancer and whether it acts in concert with Par3 we performed two-stage DMBA-TPA skin carcinogenesis. Loss of either Akk, Par3 or both, similarly inhibited papilloma formation. Molecularly, Par3/Akk enhances Akt, ERK, NF-κB, and Stat3 signaling. Thus, Par3 and Akk cooperate to promote the outgrowth of Ras-driven skin tumors, likely through sustaining growth, survival and inflammation. As loss or mutation of p53 is a major driver of human squamous cell carcinoma (SCC), we also investigated a role for Akk in SCC. Akk together with p53 to our surprise, these mice spontaneously develop squamous cell carcinomas much earlier (p<10) than single epidermal p53 knockout mice (p<1). Further analysis revealed a 2-3 fold increase in proliferation and a 10-fold increase in macropheage numbers in Akk/p53 epidermal dKO mice accompanied by activation of Stat3. Proteomics analysis revealed that Akk interacts p53 and AKT. AKT can also function as a tumor suppressor in SCC. Together, our results indicate a context dependent role for the polarity proteins Akk and Par3 in non-melanoma skin cancer, which, in part may depend on differential regulation of the tumor micro-environment.

Dual role of CD109 in squamous cell carcinoma progression

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INTRODUCTION: Head and Neck Squamous cell carcinoma (HNSCC) is one of the most prevalent cancers, and metastasis is the leading cause of death in HNSCC. Epithelial-hemmesenchymal transition (EMT) is one of the driving forces of metastasis. Transforming growth factor-beta (TGF-β), a multifunctional growth factor exhibits pro-metastatic properties and strongly inducing EMT. We identified CD109 as a TGF-β co-receptor that potently inhibits TGF-β-mediated growth inhibition and cellular responses. The CD109 expression is higher in well-differentiated HNSCC than in poorly differentiated ones. However, the role of CD109 in regulating SCC progression and metastasis remains unclear. In the present study, we examined whether CD109 regulates cellular function or metastatic potential in SCC cells. METHODS: HNSCC and SCC cells were sorted into CD109 high and CD109 low. We then treated with TGF-β1 and found a 2-3 fold increase in proliferation and a 10-fold increase in macrophage numbers in CD109 high compared to CD109 low cells. Further analysis revealed a 2-3 fold increase in proliferation and a 10-fold increase in macrophage numbers in CD109 high compared to CD109 low cells. Tracing analyses demonstrated that any individual cell has a strong inherent drive to program daughter cells to occupy a rare transient slow-cycling state leading to treatment-resistance. The treatment-resistant state displays a distinct transcriptional profile allowing definition of biomarkers to detect this state in patient tissues. In particular, early growth response proteins show increased expression levels in level-retaining cells serving as biomarkers for the drug-resistant subset of malignant T cells. Importantly, we identified rare malignant cells with the slow-cycling phenotype in patients with tumor-stage cutaneous T-cell lymphoma. The proposed resistance marker expression profile allows detection of drug-resistant cells at the single cell level and helps to identify therapeutic targets in the rare-cell programming to prevent cells entering the relapse-inducing state in T-cell lymphoma.
DNA methylic profiles of TWIST1, PL35 and GATA6 genes in Sezary Syndrome

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Sezary syndrome (SS) is an aggressive, leukemic variant of cutaneous T cell lymphoma. Clinical and histopathological findings, including T cell receptor gene clonality may not differentiate SS from inflammatory dermatoses. DNA methylation may provide useful biomarkers, which are needed for more reliable diagnosis, therapies, and to provide tools for research into disease mechanisms. Our group has previously reported a positive association in SS between high expression of TWIST1, PL35 and GATA6 mRNAs with promoter DNA hypo-methylation, as determined by targeted pyrosequencing. To more fully profile the SS epigenome, the Infinum MethylationEpiq BeadChip was used to examine genome-wide patterns of DNA methylation at 850,000 CpG sites in PBMC or CD4+ T cells from SS cases and T cell lines. Robust differences were found in the upstream promoter of TWIST1 (7 sites), in intron 1 of PL35 (7 sites), and in the gene body of GATA6 (8 sites). The 7 sites in TWIST1 form a differentially methylated region (DMR p<0.0001). The new sites were hypermethylated for TWIST1 and GATA6, but hypomethylated for PL35. These findings indicate that diverse methylation changes contribute to over-expression of TWIST1, PL35 and GATA6 in SS. We also compared our results to a similar study of cancerous and normal skin samples using 450K BeadChip. This detailed analysis of differentially methylated regions may provide insight into the aggressive nature of these cancer cells, become reactivated in resistant cancers.
Expression of the mesenchymal marker vimentin. Transcriptomic and genomic analyses presented a fibroblast-like morphology including a loss of E-cadherin expression and re-program and acquired cancer-like features. Our experiments showed that XP-C keratinocytes abortive. In turn, chronically irradiated XP-C keratinocytes presented a rapid escape to death simulated irradiations (SSR) on WT and XP-C keratinocytes, using chronic low dose of single aggressive cutaneous cancers. On these base, we analyzed the effects of chronic solar modelizing genomic hypermutability as well as molecular and cellular events leading to cancers in young XP-C patients. In our laboratory, XP-C primary skin keratinocytes help us to understand development and progression of cutaneous SCC. We are currently analyzing the timing of these events. The tumorigenic capacity of induced “XP/cancer cells” in vivo in a mouse model is currently assessed. Thanks to this model we hope to better diagnose the evolution of early skin cancers and to develop drugs targeting the specific pathways affected in both XP patients and in individuals of the general population.

Tumor surveillance: Alterations in Xeroderma Pigmentosum?

There is an increasing trend to treat specific cancers with cell-specific or pathway-specific antagonists. Although second most frequent skin cancer, the molecular mechanisms underlying development and progression of cutaneous SCC remains unclear. We have been studying SCC on the basis of differentially expressed gene list generated by the combination of laser capture microdissection and cDNA microarray technology. One transcription factor that was significantly upregulated in actinic keratosis, a known pre-cancerous condition, and SCC compared to normal epidermis was E2F4 (FCH15 and FDR-4). E2F4 is a member of the E2F family of transcription factors that have a critical role in the control of cellular proliferation and apoptosis. Recent studies identified the roles of E2F4 in cancer, including breast carcinoma, prostate cancer, and melanoma. However, its roles in SCC have not been studied to date. In this study, we aimed to elucidate the functions of E2F4 in development and progression of cutaneous SCC. To achieve this, we first evaluated and compared the expression of E2F4 in various skin conditions by immunohistochemistry. The specific expression of E2F4 in the nucleus of SCC tissues but not in other skin conditions, such as basal cell carcinoma (BCC), seborrheic keratosis, and psoriasis was identified by immunohistochemistry. E2F4 was also positive for melanocytic lesions, such as nevus cell nevus and melanoma. We further compared the mRNA and protein expression of E2F4 in keratinocytic cell lines including HaCaT cells, as well as SCC cell lines (A431 and HSC-5). Our results suggest that E2F4 may have some functions in development and progression of cutaneous SCC.
Facial lentigines formation results from a complex interplay between solar ultraviolet radiation (uSVR) and ambient particulate matter (PA). Huls et al. 2018

We followed up 176,317 women (74,241 from the NHS, 1986-2012; 102,076 from the HPFS, 1986-2012), among whom there were 30,457 incident cases of basal cell carcinoma. Information on melanocytic nevus count 1,704 incident cases of melanoma, 2,296 incident cases of squamous cell carcinoma, and 615 melanoma cases with NMSC history over 1.8 million person-years. The multivariate-adjusted HR (95% confidence interval) of melanoma death was 2.89 (1.85-4.50). Women with history of NMSC were more likely to develop non- lethal melanoma than lethal melanoma and the overall rate of lethal melanoma was 0.52 in 100,000 person-year. The main finding in this study is that women with history of NMSC are less likely to be diagnosed with a lethal melanoma than a non-lethal melanoma, but overall rate of melanoma progression was in both subsets, leading to the increased risk of sub- sequent melanoma of melanoma death. Our findings suggest the continued need for dermatologic screening for patients after NMSC diagnosis, given increased melanoma risk.

Alteration of patterns of growth in infants with atopic eczema commence in-utero.

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Growth impairment in infants with atopic eczema is a clinical concern necessitating monitoring of growth. The aim of this study was to investigate the contribution of different environmental factors to the growth of infants with atopic eczema. The study was conducted in infants with atopic eczema who were born during 1992-2010. We examined patient clinical characteristics, incidence and mortality of melanoma with a Breslow thickness3 0.8mm or Clarks level according to their location and the level of sUVR exposure, the PM effect on lentigines was stronger. All of these interactions indicated linear dose-responses. Thus, facial lentigines are the consequence of an interplay of at least two ubiquitous environmental factors. It might take place in the troposphere and/or in the skin, where sUVR exposure might alter the response to PM.

Abnormal patterns of growth in infants with atopic eczema commence in-utero.

S. El-Haddad, E. Healy and K. Godfrey

Growth impairment in infants with atopic eczema is a clinical concern necessitating monitoring of growth. The aim of this study was to investigate the contribution of different environmental factors to the growth of infants with atopic eczema. The study was conducted in infants with atopic eczema who were born during 1992-2010. We examined patient clinical characteristics, incidence and mortality of melanoma with a Breslow thickness3 0.8mm or Clarks level of IV and V according to their location and the level of sUVR exposure, the PM effect on lentigines was stronger. All of these interactions indicated linear dose-responses. Thus, facial lentigines are the consequence of an interplay of at least two ubiquitous environmental factors. It might take place in the troposphere and/or in the skin, where sUVR exposure might alter the response to PM.

Facial lentigines formation results from a complex interplay between solar ultraviolet radiation (uSVR) and ambient particulate matter (PA). Huls et al. 2018

We followed up 176,317 women (74,241 from the NHS, 1986-2012; 102,076 from the HPFS, 1986-2012), among whom there were 30,457 incident cases of basal cell carcinoma. Information on melanocytic nevus count 1,704 incident cases of melanoma, 2,296 incident cases of squamous cell carcinoma, and 615 melanoma cases with NMSC history over 1.8 million person-years. The multivariate-adjusted HR (95% confidence interval) of melanoma death was 2.89 (1.85-4.50). Women with history of NMSC were more likely to develop non- lethal melanoma than lethal melanoma and the overall rate of lethal melanoma was 0.52 in 100,000 person-year. The main finding in this study is that women with history of NMSC are less likely to be diagnosed with a lethal melanoma than a non-lethal melanoma, but overall rate of melanoma progression was in both subsets, leading to the increased risk of sub- sequent melanoma of melanoma death. Our findings suggest the continued need for dermatologic screening for patients after NMSC diagnosis, given increased melanoma risk.

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Among those with psoriasis warrant further exploration to determine clinical relevance and validated cases, a high proportion of cases were confirmed using validated questionnaire-positive predictive value) yielded confirmation of 1,530/1,954 (78%) and 1,284/1,954 (65%) a large US patient population by searching a medical record data repository (>5 million patients) meeting inclusion criteria, 56,117 had statin exposure, of whom 3,590 (6.4%) were diagnosed with NMSC, yielding a significant association between NMSC and statin exposure (adjusted OR = 1.63; 95%CI 1.56-1.70; p < 0.0001). These findings highlight an association between chronic statin exposure and subsequent incident NMSC in the study population. The risk of keratoacanthoma (KC) in psoriasis patients receiving biologic therapy compared to conventional systemic therapy: Results from The British Association of Dermatologists’ Biologics Register (BADBIR) KJ Mason University of Manchester, Manchester, England, United Kingdom Whether psoriasis patients exposed to biologic therapies have an elevated risk of KC (basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)) and keratoacanthomas (KC) has been debated. The aim of the present study was to determine whether patients were at higher risk of developing a KC compared to those on conventional therapy. BADBIR, a pharmacovigilance register of psoriasis patients, explores the long-term safety of systemic therapeutics. Patients with chronic plaque psoriasis registering to BADBIR on their first biologic or a conventional therapy, who had at least one follow-up completed were included in analyses if they were of white ethnicity, Fitzpatrick skin types 1-4 and reported no previous cancers. Contounding factors included: age, sex, smoking and previous exposure to acitretin, psoralen ultraviolet-A (PUVA), ciclosporin, and/or PUVA and ciclosporin. Propensity score-weighted Cox-proportional hazard models estimated the hazard ratio (HR) for developing a first KC or separately, first BCC or cSCC. In total, 56,72 patients initiating biologic therapy and 3188 patients on conventional therapy who met the entry criteria were included with 9550 person-years of follow-up, respectively. During follow-up, 74 (1.3%) patients initiating a biologic therapy were diagnosed with their first KC (41 BCC, 34 cSCC first) and 22 (0.7%) patients receiving conventional therapy with their first KC (15 BCC, 10 cSCC first). No significant difference in risk was observed for developing a KC (adjusted HR 1.05; 95% CI 0.64, 1.73), BCC (0.84; 95% CI 0.45, 1.54) or cSCC (1.20; 95% CI 0.57, 2.50) on biologic compared to conventional therapy. In conclusion, biologic therapy does not appear to increase the risk of developing a first KC as compared to conventional therapy in psoriasis patients. These data will help inform clinical decision making in psoriasis patients at risk of KC in whom biologic or conventional therapy is being considered.

Validating self-reported atopic dermatitis in a large cohort of US women A Drucker1, E Cho2, W Li3, C Camargo4, T Li1 and AA Gershoni2 1 University of Toronto, Women’s College Hospital, Toronto, ON, Canada, 2 Boston Children’s Hospital, Providence, RI and 3 Channing Division of Network Medicine, Boston, MA Many large epidemiologic studies, including the Nurses Health Study 2 (NH2S cohort), rely on questionnaire-based self-reports of atopic dermatitis (AD) or cutaneous conditions (CCT) validating self-reported atopic dermatitis is questionable, and has only been assessed in selected clinical populations. The objective of this study was to validate self-reports of clinician-diagnosed atopic dermatitis among US female registered nurses participating in NH2S. An online questionnaire was sent to 5,116 of 10,647 participants who reported on the main cohort questionnaire ever having received a diagnosis of atopic dermatitis from a clinician. Our response rate was 48%. Self-reported atopic dermatitis was internally valid, with 1,954 (87%) respondents reiterating that they had received a clinician diagnosis of atopic dermatitis. Self-reported year of atopic dermatitis diagnosis on the main cohort questionnaire and year of atopic dermatitis diagnosis on the clinician diagnosis questionnaire were strongly correlated (Spearman r=0.81, P<0.0001). Successive application of two validated diagnostic algorithms (each with 70% positive predictive value) yielded confirmation of 1,530/1,954 (78%) and 1,284/1,954 (65%) cases, respectively. Our study demonstrates that in a cohort of US female registered nurses, self-reported atopic dermatitis and year of diagnosis are internally valid. Among internally validated cases, a high proportion of cases were confirmed using validated questionnaire-based algorithms. In conclusion, NH2S can reliably be used for epidemiologic studies of atopic dermatitis in adult women.

Physician experiences and perceptions of systemic therapies for atopic dermatitis in the United States AW Armstrong1, M Grabriner1, J Stephenson1, R Zhao1, UC Mallia1, N Bieseki2, R Miao3, XWANG4,1i C Chen1, J Chao1 1 Department of Dermatology, University of Southern California, Los Angeles, CA, 2 HealthCore, Inc., Wilmington, DE, 3 Samotoli, Bridgewater, NJ, and 4 Regeneron Pharmaceuticals, Inc., Tarrytown, NY This cross-sectional, survey-based study assessed US physicians experiences and perceptions of the risks and benefits of non-biologic systemic therapies, prior to the introduction of biologics, for moderate-to-severe atopic dermatitis (AD). Physicians specializing in dermatology, allergy/immunology or primary care, who prescribed oral systemic immunomodulators (IMs) or steroids (SSs) for AD, and who had ≥ 10 office visits for AD in the year prior to selection were identified from the HealthCore Integrated Research Database (08/01/2011/04/16). Data were analyzed using descriptive statistics. Of 4905 physicians who were sent invitations, 1062 (22%) respondents completed (1.8%) the survey (mean [SD] age: 47.7 [16]). Data were analyzed using descriptive statistics. Of 4905 physicians who were sent invitations, 106 (2.2%) responded and 87 (1.8%) completed the survey (mean [SD] age: 47.7 [16]). Data were analyzed using descriptive statistics. Of 4905 physicians who were sent invitations, 106 (2.2%) responded and 87 (1.8%) completed the survey (mean [SD] age: 47.7 [16]). Data were analyzed using descriptive statistics. Of 4905 physicians who were sent invitations, 106 (2.2%) responded and 87 (1.8%) completed the survey (mean [SD] age: 47.7 [16]). Data were analyzed using descriptive statistics. Of 4905 physicians who were sent invitations, 106 (2.2%) responded and 87 (1.8%) completed the survey (mean [SD] age: 47.7 [16]). Data were analyzed using descriptive statistics. Of 4905 physicians who were sent invitations, 106 (2.2%) responded and 87 (1.8%) completed the survey (mean [SD] age: 47.7 [16]). Data were analyzed using descriptive statistics. Of 4905 physicians who were sent invitations, 106 (2.2%) responded and 87 (1.8%) completed the survey (mean [SD] age: 47.7 [16]). Data were analyzed using descriptive statistics. Of 4905 physicians who were sent invitations, 106 (2.2%) responded and 87 (1.8%) completed the survey (mean [SD] age: 47.7 [16]).
243 Overall risk of primary hematologic malignancies in patients with lip squamous cell carcinoma: A report from the National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) program
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An increased risk for subsequent primary cancers has been observed in patients with skin cancer including squamous cell carcinoma (SCC). Although the mechanism of such association has not been fully understood, several factors including immunosuppression may play a role. However, of greater focus is the risk of subsequent hematologic malignancies in patients with lip SCC which has not been previously reported. The aim of this study was to explore the risk of hematologic malignancy in lip SCC survivors. The SEER database was searched to detect all patients with a primary cutaneous lip SCC who survived ≥2 months after diagnosis. SCC was detected by histology codes for squamous neoplasms (8050-8089) in conjunction with primary site for lip (C00.0-C00.02; C00.06; C00.08; C00.09). Site recode B (ICD-O-3 was used to detect hematologic malignancy. Standardized Incidence Ratios (SIRs), and ratio of the observed (O) to the expected (E) in the general population (O/E ratios), and 95% confidence intervals (CI), were calculated. Of 5,537 individuals with lip SCC, 65 developed ≥1 primary lymphatic or hematopoietic malignancies, and particularly showing a significantly increased risk for lymphoma (O/E 1.46, 95% CI 1.04-1.91). The mean time in months to the development of a hematologic malignancy was 46 (range 2-164 months) and was increased by gender, age at diagnosis and subsequent to the primary malignant SCC disease. This suggests that lip SCC is associated with hematologic malignancies, and particularly lymphoma, affecting enhanced surveillance for lip SCC patients. In addition, exploration to determine the risk of subsequent primary malignancies in non-lip cutaneous SCC seems warranted.

245 Sleep disturbances in children with atopic dermatitis throughout childhood: A population-based longitudinal birth cohort study
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While atopic dermatitis (AD) is known to impact sleep among children, little is known about the association between AD disease activity and sleep over time among community populations. We aimed to explore the relationship between childhood AD and active AD have impaired sleep duration and quality compared to children without AD throughout childhood using data from 11,432 individuals in the Avon Longitudinal Study of Parents and Children, a population-based prospective birth cohort in the UK. The annual period prevalence of active AD (assessed by maternal report of a typical itchy flexural rash in the past year, as defined in the International Studies of Asthma and Allergies in Childhood) ranged from 17.33% between the ages of 12-20 years old. Using population-based longitudinal birth cohort data, sleep duration repeated at 10 time points between 0.5-15 years. Multivariate mixed effects regression models with repeated measures of sleep were used to account for the longitudinal nature of the data, and controlled for child gender, race/ethnicity, asthma, number of children living in the household, maternal education, and social class. Throughout childhood, there was no difference in nighttime sleep duration between children with active AD and without AD (p=0.440). In contrast, total sleep duration including napping was higher in younger children with active AD (15 minutes more/day; 95% CI 13-17 minutes; p<0.001). Using a composite outcome including nighttime awakenings, early morning awakening, difficulty falling asleep, and nightmares, children with AD were more likely to report worse sleep quality outcomes throughout childhood (OR 1.19, 95% CI 1.05-1.18; p<0.001). In conclusion, among the general pediatric population, AD is likely to negatively impact sleep quality more than quantity, which may result in longer naps in early childhood. Clinical outcome measures should differentiate between nighttime and total sleep, and explicitly address sleep quality.

246 Patterns of atopic eczema activity in childhood and adulthood: Results from 2 national prospective British cohort studies
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Atopic eczema (AE) is a common disease with variable patterns of disease activity in childhood, but there are limited data on disease activity trajectories past childhood. The objective of this study was to characterize AE period prevalence into adulthood, classically disease activity into longitudinal patterns, and to relate these patterns to patient characteristics. Specifically, we sought to assess how individuals with disease that resolves after childhood differ from those with disease that remains active into adulthood and how individuals with adult-onset disease differ from those with childhood-onset disease. We analyzed data from two UK national representative longitudinal cohort studies, the 1958 and 1970 British Cohort Studies (BCC), which each included over 17,000 individuals followed from birth to ages 50 and 42, respectively. Period prevalence of AE was 18% in the 1958 and 28% in the 1970 cohort, and the annual period prevalence ranged from 3.11%. Of those with AE in childhood, 29% in the 1958 and 35% in the 1970 BCS reported continued activity during at least one time point in adulthood. Consistent disease activity in adulthood was twice as likely to be the first time the patient had a history of atopy than those whose disease resolved after adolescence. Among all individuals with AE, 44% in the 1958 and 40% in the 1970 BCS reported AE for the first time in adulthood; these individuals were more likely to be female and less likely to have a history of atopic eczema. Family history of AE was present in 48% of childhood-onset disease patients versus 34% of adult-onset disease patients. These data suggest that the majority of adult-onset AE occurs in nearly all patients undergoing anti-cancer therapy, contributing to morbidity, therapy disruptions, and rising health care costs. ADR characterization is hampered by clinical trials underpowered to detect rare events, division of patients across institutions, patient exclusion from trials, publication editorial delays, and ineffective dermatologist participation in oncologic care. Early ADR recognition could substantially improve health outcomes and decrease societal costs. Internet health forums provide a mechanism for several hundred million individuals to discuss real-time health conditions and skin cancer risk behaviors of STP users. We performed a cross-sectional study including 27,353 adult men and women using data collected from the 2015 National Health Interview Survey to assess demographic characteristics and skin cancer risk behaviors of STP users in the United States. STP use was defined as use of sunless tanning products or spray-on mist tans in the last year. In both univariate and multivariate analyses, we compared demographic characteristics and skin cancer risk behaviors by STP use both in the univariate analysis and in a subpopulation analysis in a high-risk group for skin cancer. In 2015, 6.4% of all U.S. adults and 31.4% of indoor tanners reported STP use. STP use was associated with being young, female, non-Hispanic white, college-educated, not-obese, more sun sensitive, living in the Western United States, and having a family history of skin cancer. STP users were more likely to report indoor tanning, recent sunburn and were less likely to seek shade or use protective clothing when outdoors. Among current indoor-tanners, STP use was not associated with differences in skin cancer risk behaviors of STP users in the United States. STP use was defined as use of sunless tanning products or spray-on mist tans in the last year. In both univariate and multivariate analyses, we compared demographic characteristics and skin cancer risk behaviors by STP use both in the univariate analysis and in a subpopulation analysis in a high-risk group for skin cancer. In 2015, 6.4% of all U.S. adults and 31.4% of indoor tanners reported STP use. STP use was associated with being young, female, non-Hispanic white, college-educated, not-obese, more sun sensitive, living in the Western United States, and having a family history of skin cancer. STP users were more likely to report indoor tanning, recent sunburn and were less likely to seek shade or use protective clothing when outdoors. Among current indoor-tanners, STP use was not associated with differences in skin cancer risk behaviors of STP users in the United States. In a high-risk population, indoor-tanners, STP use is not associated with improved behaviors. These results suggest that STP use may not improve skin cancer risk behaviors and could inadvertently reinforce beliefs that tanned skin is more attractive. Longitudinal studies are needed to better assess the impact of STPs on skin cancer risk behaviors.

248 Early detection of chemotherapeutic skin toxicities in social health networks using deep learning
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In recent years, the use of chemotherapeutic agents has increased substantially. Adverse drug reactions (ADRs) occur in nearly all patients undergoing anti-cancer therapy, contributing to morbidity, therapy disruptions, and rising health care costs. ADR characterization is hampered by clinical trials underpowered to detect rare events, division of patients across institutions, patient exclusion from trials, publication editorial delays, and ineffective dermatologist participation in oncologic care. Early ADR recognition could substantially improve health outcomes and decrease societal costs. Internet health forums provide a mechanism for several hundred million individuals to discuss real-time health conditions and skin cancer risk behaviors of STP users. We performed a cross-sectional study including 27,353 adult men and women using data collected from the 2015 National Health Interview Survey to assess demographic characteristics and skin cancer risk behaviors of STP users in the United States. STP use was defined as use of sunless tanning products or spray-on mist tans in the last year. In both univariate and multivariate analyses, we compared demographic characteristics and skin cancer risk behaviors by STP use both in the univariate analysis and in a subpopulation analysis in a high-risk group for skin cancer. In 2015, 6.4% of all U.S. adults and 31.4% of indoor tanners reported STP use. STP use was associated with being young, female, non-Hispanic white, college-educated, not-obese, more sun sensitive, living in the Western United States, and having a family history of skin cancer. STP users were more likely to report indoor tanning, recent sunburn and were less likely to seek shade or use protective clothing when outdoors. Among current indoor-tanners, STP use was not associated with differences in skin cancer risk behaviors of STP users in the United States. In a high-risk population, indoor-tanners, STP use is not associated with improved behaviors. These results suggest that STP use may not improve skin cancer risk behaviors and could inadvertently reinforce beliefs that tanned skin is more attractive. Longitudinal studies are needed to better assess the impact of STPs on skin cancer risk behaviors.
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Different prevalence of sensitization against galactose-1,3-galactose between Shimane and Miyagi in Japan
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The red meat allergy caused by tick bites is increasing worldwide. It is known that IgE epitope of major red meat allergens is galactose-1,3-galactose (α-Gal). Thus, the patients with red meat allergy have a high risk to develop anaphylaxis to taking cetuximab that contains α-Gal. We compared the prevalence of sensitization against α-Gal between Shimane prefecture and Miyagi prefecture, because the incidence of Japanese spotted fever in the endemic area of Japanese spotted fever and Miyagi prefecture is low. We enrolled 400 patients from Shimane University Hospital and Tohoku University Hospital, and those patients had examined without complaining red meat allergy. α-Gal and beef-sIgE were determined by CAP-FeIA. The α-Gal reactivity was 5% in Shimane, whereas it was 0% in Miyagi. These results indicate that prevalence of sensitization against α-Gal is different among the areas in Japan.

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Evidence for race/ethnic incidence rate disparity in mycosis fungoides: A follow-up from the NCI surveillance, epidemiology, and end results (SEER) Program
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Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma, and some studies have shown that the incidence rate (IR) and prognosis varies between race/ethnic (R/E) groups. This study aims to investigate whether R/E disparities in MF IR have changed from 2000 to 2014 using the Health Disparities Calculator (HD³-calculator) produced by the SEER Program. SEER data, excluding Alaska Native and Louisiana registries, from 2000 to 2014 were used to estimate age-adjusted IR of MF by gender and R/E group. The R/E categories were white, non-Hispanic R/E, and non-Hispanic R/E. American Indians/Alaska Natives were inputted into HD³-calculator to generate the range difference measure (RD), a measure of disparity. RD was calculated as the difference in IR of MF between the R/E group with the highest IR and lowest IR (a RD of 0 indicates no disparity exists). RD measures the absolute range of IR across all R/E groups within a state. The non-Hispanic R/E group had the lowest age-adjusted IR in 2000 to 2014. We found that for males: 0.53 to 1.30 cases per 100,000. Moreover, RD increased for males: 0.79 to 1.06, a 34.2% change, and for females: 0.77 to 1.02, a 31.9% change. These nation-wide findings indicate that R/E disparities in IR for MF have increased using the range difference measure, particularly for the Black non-Hispanic R/E group when comparing data from 2000 to 2014. However, it is important to note that HD³-calculator allows researchers to compare measures in order to select the measures that best capture the nuances in these findings point to the need for ongoing analysis in order to better understand which, and to what extent, race/ethnic groups are most disparate.

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Sexual orientation and prevalence of skin cancer risk factors and screening: A population-based survey
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The sun protective behaviors and attitudes of early childhood programs in the U.S. population
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The sun protective behaviors and attitudes of early childhood programs in the U.S. population by quantifying the benefits and harms as well as societal and individual willingness to pay.

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Comparison of psychological distress between psoriasis patients on biologic versus oral therapies: An epidemiologic study of moderate-to-severe psoriasis patients in the U.S.
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Clinical Research: Epidemiology of Skin Diseases | ABSTRACTS
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A survey of demographics, skin cancer history, and sun-protective behavior in pilots

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Pilots and cabin crew have an increased rate of skin cancer, specifically melanoma, compared with the general population. The goal of this study is to assess the demographics, awareness of sun-protective behaviors, and sun-protective behavior in the pilot cohort. A pilot questionnaire was distributed through the Air Line Pilots Association (ALPA) to pilots in the US. The survey included each participants age, gender, ethnicity, flying history, Fitzpatrick skin type, skin cancer history, awareness and attitudes of skin cancer prevention, and sun-protective behavior. Adjusted odds ratios (aOR) and 95% confidence interval (CI) were computed using multivariable logistic regression. Of the 477 completed surveys, 98.7% (n=471) are commercial airline pilots. The majority started flying at 20 years of age or younger (61.6%, n=294). In our cohort, 160 (32%) participants reported a history of skin cancer, of which 41 (8.6%) listed as having had a melanoma. The majority of responders with a history of skin cancer were men (90.6%, n=145) and all were Caucasian. 91.9% (n=147) of those with skin cancer reported a total flight time of at least 5,000 hours. Multivariate regressions showed significant associations between having a history of skin cancer and male gender (aOR=2.8, 95% CI=1.3-5.9), increasing age (aORs of 1.0, 2.0, 2.4, and 5.7 for ages 20-39, 40-49, 50-59, and 60+ respectively; p-value for trend<0.001), family history of skin cancer (aOR=2.0, [1.2-3.1]), and ever had a dermatologist full body skin check (aOR=2.9, [1.3-19.2]). Participants with a history of skin cancer were also more likely to wear a wide-brimmed hat at least most of the time (aOR=2.0, [1.2-6.1]) and reported knowledge of the importance of skin cancer screening (aOR=2.16, [1.61-2.84]) over half (50.5%) of our cohort reported their physicians had discussed sun protection, and only 3.8% (n=18) reported their company discussed sun protection. These results support the need for early intervention to increase awareness of skin cancer and prevention amongst pilots.

Uncommon filaggrin variants are associated with persistent atopic dermatitis in African-Americans


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Atopic dermatitis (AD) has been associated with filaggrin gene (FLG) loss of function (LoF) variation. FLG loss of variation has been inconsistently been associated in AD conditions. The objective of this study was to use massively parallel sequencing (MPS) to evaluate FLG LoF variation in AA children with respect to the association between FLG LoF variation and AD and AA persistence. This was a cross-sectional and longitudinal study of 262 AA children with AD. Subjects reported every six months on the clinical status of their AD. The average length of follow up was 96.4 (95% CI: 92.0, 100.8) months. DNA was assayed using MPS and associated with the longitudinal outcome as well as compared to a reference standard (genoAD).

Nine unique FLG ex 3 LoF variants (p.S309X, p.R314X, p.R3147X, p.S3145X, p.S3143X, p.R3140X, p.S3147X, p.S31316X, p.H4446fs) were identified for an overall minor variant frequency (MVF) of 6.30% (95% CI: 4.37, 8.73), which is two times greater than a previous report of the cohort that used Sanger sequencing. All variants were more frequent in the cohort than the reference cohort. The most common variants were p.R501X (1.72% [0.9, 3.42], and p.S3136X (1.34% [0.54, 2.73]). Three (p.R501X, p.R2447X, and p.R2447X) were significantly more common in AA children with AD than AA children without AD (p=0.027) as compared to FLG LoF wildtype children. In contrast to previous reports, there exist uncommon FLG LoF variants in AA children that are associated with AD and more persistent AD. Unlike European-Americans, AA children may have higher penetrance to AD variants.

Evaluation of skin cancer diagnoses in dermatology patients seen in a homeless clinic

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Background: Congenital ichthyosities (CI) adversely affect quality of life (QOL) in patients and their families. Methods: We examined the relationship between disease severity and QOL in patients with CI, especially in patients with harlequin ichthyosis (HI) and ichthyosis: syndromic forms (Netherton syndrome, Sjogren-Larsson syndrome, Dorfman-Chanarin syndrome, keratitis-ichthyosis-deafness syndrome, and trichothiodystrophy). Patients with HI or ichthyosis: syndromic forms who were aged 8 years or older and who participated in a multicenter retrospective questionnaire survey in Japan were assessed by dermatology life quality index (DLQI), range of 0-30) and CI disease severity score (range of 0-100). Clinical data for 2011 to 2015 were obtained. Results: Complete data on DLQI was obtained from 13 patients, whose mean age was 27 years. Nine (69.2%) of the patients were male, and 4 (30.8%) were female. All 13 (100%) were survivors. Systemic retinoids were administered to 2 of the patients (15.4%), both of whom were HI patients and 1 of whom (7.7%) had received intensive care in a neonatal intensive care unit (NICU). The Spearman correlation coefficient between CI disease severity score and DLQI was 0.611 (p<0.05). Conclusion: The impact of CI on QOL correlates with disease severity.
261 Detection of anti-type VII collagen IgE antibodies in epidermolysis bullosa acquisita
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Epidermolysis bullosa acquisita (EBA) is a rare pemphigoid disease with autoantibodies to type VII collagen (COL7), a major structural component of anchoring fibrils. IgE antibodies to type XVII collagen (BP180) have been identified in bullous pemphigoid (BP), a prototype of pemphigoid. The pathogenic relevance of IgE to bullous pemphigoid has been revealed in EBA. In this study we investigated IgE antibodies in EBA. We enrolled 109 EBA cases in this study based on: (i) compatible clinical features, (ii) IgG reaction on the dermal side by indirect immunofluorescence (IF) of salt-split skin, and (iii) detection of IgE antibodies to COL7 by western blotting and/or ELISA. We examined IgE antibodies in patients sera by IF using normal human skin and found IgE reactivity at basement membrane zone in 29 (26.6%) cases. To confirm if the IgE antibodies were specific to COL7, we performed IF with 21 clones of monoclonal antibodies (mAbs) specific to aforementioned epitopes of COL7, which lack COL7. All cases were negative indicating that patients IgE antibodies were specific to COL7. Next we tested IgE reactivity by commercial COL7 ELISA kit with modified protocol for IgG antibodies. In ELISA, 16 (14.7%) cases were positive (3 and 13 cases were negative and positive in IF, respectively). We compared specific IgE titre (IgE) and IgG (OD) and found a weak relevance (r=0.459, p<0.0001). EBA is clinically divided into two types; mechanobullous type (MB, non-inflammatory) and inflammatory type (INF) resembling BP. Within the cases with accessible clinical information, IFN type was in 32.8% (19/58) of IF negative- and 55.0% (11/20) of IF positive-cases. This study is the first demonstration of the presence of circulating anti-COL7 IgE in EBA patients, which might correlate with clinical phenotype.

262 Do oro-pharyngeal symptoms correlate with endoscopic findings in patients with pemphigus vulgaris
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Children from families that deviate from the traditional family structure (two married, biological parents) are at greater risk of poverty and poor physical health outcomes. Yet, the relationship between fragile families and atopic dermatitis (AD) has not been well elucidated. Studies that have been conducted are limited in family size, have focused on a single risk factor. Here, we sought to determine the frequency and disease activity unless endoscopy is performed. Methods: We reviewed 312 charts of patients with histopathologically-confirmed pemphigus vulgaris for this observational, single-centre retrospective study. The primary endpoint was whether endoscopic findings in the nose, oral cavity, larynx or pharynx correlate with oro-pharyngeal symptoms. Results: Sixty percent of patients (192/312) had at least one oro-pharyngeal symptom. These symptoms showed statistically significant correlation with endoscopic findings of pemphigus vulgaris: oral (p = 0.0003), laryngeal (p = 0.0009), pharyngeal (p=0.0013) and nasal (p=0.015). Disease severity and older age at diagnosis were also associated with endoscopic findings. Fifteen patients (or 5%) had positive endoscopic findings without symptoms and three patients had symptoms without positive endoscopic findings. Conclusions: In the majority of patients with pemphigus vulgaris, oro-pharyngeal symptoms reflected disease activity. However, a small proportion had findings without experiencing symptoms and some experienced symptoms due to an infection (candidiasis or CMV).

263 Osteopenia is common in mild forms of epidermolysis bullosa
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Children from families that deviate from the traditional family structure (two married, biological parents) are at greater risk of poverty and poor physical health outcomes. Yet, the relationship between fragile families and atopic dermatitis (AD) has not been well elucidated. Studies that have been conducted are limited in family size, have focused on a single risk factor. Here, we sought to determine the frequency and disease activity unless endoscopy is performed. Methods: We reviewed 312 charts of patients with histopathologically-confirmed pemphigus vulgaris for this observational, single-centre retrospective study. The primary endpoint was whether endoscopic findings in the nose, oral cavity, larynx or pharynx correlate with oro-pharyngeal symptoms. Results: Sixty percent of patients (192/312) had at least one oro-pharyngeal symptom. These symptoms showed statistically significant correlation with endoscopic findings of pemphigus vulgaris: oral (p = 0.0003), laryngeal (p = 0.0009), pharyngeal (p=0.0013) and nasal (p=0.015). Disease severity and older age at diagnosis were also associated with endoscopic findings. Fifteen patients (or 5%) had positive endoscopic findings without symptoms and three patients had symptoms without positive endoscopic findings. Conclusions: In the majority of patients with pemphigus vulgaris, oro-pharyngeal symptoms reflected disease activity. However, a small proportion had findings without experiencing symptoms and some experienced symptoms due to an infection (candidiasis or CMV).

264 Nickel coreactions to metal and non-metal allergens in adult patients
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We performed a retrospective chart review of 484 adults (age ≥18, mean age: 46.7±14.6 years), who were patch-tested at the Northwestern Medicine patch-testing clinic from 2014-2017. Patients were patch-tested with the North American Contact Dermatitis Group (NACDG) standard series and supplemental metal series if indicated. Positive patch test reactions to ≥1 metal allergens were observed in 210 (43.1%) of patients, with nickel (19.1%), gold (11.8%) and palladium (11.1%) being most common. Among patients with positive reactions to nickel, 38.0%, 16.3% and 2.2% had positive reactions to 1, 2 or 3 additional metals, respectively. Overall, polysensitization to metals occurred in 13.9% of patients. In multivariable multinomial logistic regression models, polysensitization to metal allergens was associated with female sex (adjusted odds ratio [95% confidence interval]: 1.050 [1.050-1.054]), families with ≤2 members (1.413 [1.079-1.852]), families with a mother, but no father present (1.402 [1.179-1.667]), non-biological fathers (1.464 [1.089-1.969]), or unmarried mothers (1.508 [1.017-2.37]) had increased odds of AD. These associations remained significant in virtually all models stratified by race/ethnicity, sex, household income and highest educational attainment. Among children with AD, there were significantly increased odds of good/fair/poor vs. very good/excellent overall health (1.545 [1.262-1.891]), depression (2.267 [1.521-3.434], anxiety (2.001 [1.543-2.595]), and stress (2.013 [1.492-2.704]). The results of this study suggests that US children from non-traditional family structures have increased odds of AD and poorer overall health outcomes.
Tea consumption and the risk of keratinocyte carcinoma: A US population-based case-control study

ABSTRACT

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Tea is a rich source of polyphenols, which have putative antioxidant effects and are hypothesized to confer cutaneous anti-tumor benefits, as demonstrated by in-vitro studies and mouse models. However, whether tea consumption protects against the occurrence of keratinocyte carcinomas has not yet been conclusively determined in epidemiologic studies. We explored the effects of tea consumption on the risk of developing cutaneous squamous cell carcinoma (SCC) and early-onset basal cell carcinoma (EOBCC) as part of a population-based case-control study in New Hampshire. Detailed patterns of tea consumption (e.g., type of tea, quantity, brewing time, additives) were collected through personal interviews with 456 individuals with histologically-confirmed SCC, 327 with histologically-confirmed EOBCC, and 745 age- and sex-matched controls. After adjusting for potential confounders (age, sex, number of cigarettes smoked per day, the order of development of SCC), we found a decreased risk of SCC in individuals who regularly consumed >2-3 cups of hot tea daily compared to those who did not drink tea (adjusted OR 0.42, 95% CI: 0.19-0.92), but not of EOBCC. Our observational study design leaves open the possibility of recall bias and residual confounding. Our findings suggest that regular hot tea consumption may reduce the risk of developing SCC, but not EOBCC.

Impact of body mass index and waist circumferences on the risk of chronic spontaneous urticaria: A nationwide, population-based study

ABSTRACT

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Impact of body mass index and waist circumferences on the risk of chronic spontaneous urticaria

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Infection is the second leading cause of death in patients with psoriasis, behind cardiovascular disease. Respiratory infections, including influenza, are of special importance because they represent a common cause of morbidity and mortality and may be preventable through vaccination. Universal influenza vaccination is recommended annually, but little is known about influenza vaccination rates in psoriatic patients in the United States. We conducted a cohort study of adults in OptumInsight's Clinformatics Data Mart to examine influenza vaccine rates in patients with psoriasis compared to adults with other chronic illnesses. We performed a cohort study of adults in OptumInsight's Clinformatics Data Mart to examine influenza vaccine rates in patients with psoriasis compared to adults with other chronic illnesses (hypertension, rheumatoid arthritis) during the 2010-2011 influenza season. Of 10,304 adults with psoriasis, 23.7% received an influenza vaccine, compared to 28.5% adults with hypertension and 34.5% with rheumatoid arthritis. After controlling for age and sex, patients with rheumatoid arthritis were 1.35 times (95% CI: 1.28 - 1.43) more likely to receive a flu vaccine than patients with psoriasis. When examining psoriasis patients by most recent treatment received, no significant difference in vaccination rates was seen between patients on topicals, phototherapy or systemic medications. In conclusion, patients with psoriasis have lower rates of influenza vaccination than age- and sex-matched adults with rheumatoid arthritis, and the rate is not increased in those on systemic therapy who may be at an increased risk for infection. Further research is necessary to understand why psoriasis patients are not receiving recommended vaccinations and improve vaccination rates in individuals at the highest risk.
Skin cancer surveillance behaviors and attitudes among hair professionals

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Introduction: Dermatologists prescribe more oral antibiotics per capita than any other specialty; this use may be associated with a number of adverse outcomes including antibiotic resistance, inflammatory bowel disease, and colon cancer, among others. Understanding the epidemiology of prescribing practices in Dermatology will help identify opportunities to optimize the use of antibiotics. Methods: All prescriptions and chart comments written by dermatologists were identified from 2008-2016 in the de-identified Clininformatics DataMart. Prescriptions were consolidated into courses of therapy and associated with the primary diagnosis from the most recent visit. Courses were separated into those of extended duration (>28 days) and short duration (<28 days). Results: Tetracyclines were the most frequently prescribed antibiotic class. The top 3 classes associated with extended courses were, macrolides, rifamycins, and quinolones. The number of courses per 100 visits declined from 1.44 in 2008 to 0.87 in 2016. The top 3 diagnoses associated with short courses were skin and soft tissue infections, skin cancer, and follicular disorders; the number of short courses per 100 visits increased from 0.53 in 2008 to 0.75 in 2016. Between 2008 and 2016, prescriptions related to acne decreased from 8.75 to 6.73 courses per 100 visits, while prescriptions associated with visits for skin cancer increased from 1.35 to 2.17 courses per 100 visits. Discussion: Antibiotic use in Dermatology appears to be decreasing overall, although use remains common for a variety of conditions. Continuing to develop alternative strategies to oral antibiotics for the management of non-infectious conditions can improve antibiotic stewardship and reduce complications associated with antibiotic use. In addition, given the rising prevalence of antibiotic use associated with visits for skin cancer, there may be an opportunity to evaluate optimal practices for the use of perioperative use. In addition, given the rising prevalence of antibiotic use associated with visits for skin cancer, there may be an opportunity to evaluate optimal practices for the use of perioperative use.

Risk of myocardial infarctions is increased in patients with neurofibromatosis

Neurofibromatosis type 1 (NF1) is one of the most common genodermatoses and multiorgan syndromes, with an incidence of about 1:2000. According to our hypothesis, NF1 is associated with comorbidities not yet recognized. Case reports on myocardial infarctions in NF1 patients reported coronary artery aneurysms and atherosclerosis as underlying causes, while no epidemiologic data on the incidence of heart infarctions in NF1 has been available. We have used the total population based Finnish cohort of 1410 NF1 patients to analyze the incidence of myocardial infarctions. The control population consisted of 10 persons per NF1 patient, stratified according to gender, birth year and municipality. The incidence of myocardial infarctions was followed from the Care Register for Health Care, maintained by National Institute for Health and Welfare. The Register collects information of all outpatient and inpatient care in Finland. The register includes Diagnosis Related Group codes I21 and I22 and ICD-9 code 410. Twenty-six myocardial infarctions were observed among NF1 patients, yielding odds ratios (OR) of 1.7 (95% CI 1.1-2.5) compared to the matched control group. The median age at myocardial infarction was 66.8 years (range 52.4-91.8). Eleven of the patients were women (OR 2.1, 95% CI 1.1-4.1) and 15 were men (OR 1.4, 95% CI 0.8-2.5). Other diagnoses observed among the NF1 patients with myocardial infarction included chronic ischemic heart disease (18/26), angina pectoris (11/26), cerebrovascular disease (11/26), heart failure (8/26), hypertension (9/26). This preliminary data indicates that at least women with NF1 have an increased risk of myocardial infarction, and their underlying causes require further investigation. The results emphasize the need of tailored follow-up of NF1 patients.
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Electrocardiogram abnormalities in Filipino patients with psoriasis in a tertiary hospital
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Systemic chronic inflammation, endothelial dysfunction, and increased oxidative stress are the mechanisms of progression of psoriasis. Increasing evidence points to the potential association between psoriasis and cardiac diseases, which can initially manifest as electrocardiogram (EKG) abnormalities. This paper aims to identify the common ECG abnormalities seen in psoriasis patients and to determine its association with psoriasis severity. This is a prospective study of 60 Filipino patients with psoriasis who were examined by cardiologists. Demographic, clinical, disease activity data, and ECG profiles were collected and 12L-ECG recordings were obtained. Independent t-test and ANOVA were used for continuous variables while chi-square test was used for categorical variables. Logistic regression was used to determine the association of ECG abnormalities with the severity of psoriasis. Out of a total of 115 patients, 35% have abnormal ECG findings. ST-wave changes and conduction abnormalities were the most commonly detected. The abnormal ECG group had significantly older age, higher systolic blood pressure, longer disease duration, and higher prevalence of diabetes compared to the normal group. After adjusting for the confounding effects of methotrexate use and diabetes, both mild and severe psoriasis have a similar risk of developing ECG abnormalities. Abnormal ECG findings, particularly ST-wave changes and conduction abnormalities, are commonly seen in psoriasis patients. These findings may be independent and early marker of coronary disease. Age, hypertension, diabetes, use of methotrexate, long disease duration and severity of psoriasis are also significant factors associated with ECG abnormalities. Moreover, methotrexate use and diabetes may directly increase the risk of ECG abnormalities, regardless of psoriasis severity.

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Prevalence of pruritus and pemphigoid in nursing home residents (SSENIOR): A cross-sectional study of an unmet need
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Light hair color is an established risk factor for melanoma. Whether hair color is associated with risk of keratinocyte carcinoma, also known as non-melanoma skin cancer, has not been well studied. We prospectively examined the association between natural hair color at adulthood and risk of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) based on a total 205,107 participants from the Nurses Health Study (NHS, n=78,679), NHS II (n=56,156), and Health Professionals Follow-up Study (HPFS, n=16,912). Information on SCC and BCC was collected biennially, and diagnoses of SCC were pathologically confirmed. During the follow-up (1984-2012 for NHS, 1991-2011 for NHS II, and 1988-2012 for HPFS), we identified 4,661 cases of SCC and 32,548 cases of BCC. Red hair color was significantly associated with higher risk of SCC (HR of 1.43 [95% CI: 1.27-1.62] compared with light brown hair) and risk of BCC (HR of 1.41, 95% CI: 0.98-2.03) (P=0.02) more likely to present with ECG abnormalities. However, after adjusting for psoriasis severity, CYP4F22 (18%), TGM1 (20%) were exclusively associated with CIE and were the most frequent mutation detected in 12L-ECG recordings. Among these, ALG12B and ABCA12 were the most common caused CIE among patients of Muslim origin (92%). TGM1 mutations exclusively caused CIE in this subgroup. Of interest, whole exome sequencing revealed two cases suggestive of digenic inheritance. Our data demonstrate the importance of population-tailored individuals as well as the impact of novel sequencing approaches for the diagnosis of inherited skin diseases.

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Enhancement of epidermal function delays relapse of psoriasis
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Though psoriasis is widely viewed an immunological disorder, the role of epidermal dysfunction in disease pathogenesis is also well appreciated. Hence, we hypothesized that enhancement of epidermal function could prevent/ameliorate disease development in two cohorts of patients with psoriasis that was temporarily in remission. Both groups of patients had a history of lesion appearance on both forearms at around the same time of year for 2+ consecutive years. In the first study (N=10), an in-house prepared emollient, known to improve epidermal function, was applied twice-daily to one forearm for 20 days, while the contralateral arm was kept untreated. In the second study (N=60), a commercially available cream, containing physiologic lipid mixture was applied twice-daily to one forearm for 30 days. Based upon patients' history, treatment was started 10 days prior to expected lesion appearance, and subjects were instructed to return to clinic as soon as lesions appeared on either forearm. In the first study, 22 out of 30 subjects developed lesions during the 20-day period. 21/22 (95%) patients developed lesions on the untreated side, while 14 out of 22 (64%) developed lesions on the treated side, but treatment delayed the appearance of lesions in 55% of patients. In the second study, 49 out of 60 subjects developed lesions during 30 day of treatment period. 45/49 (90%) of patients developed lesions on the untreated side, while 34/49 (69%) developed psoriatic lesions on the treated side, but treatment again delayed the appearance of lesions in 35 patients (71%). The preventive benefits of the topical regimen correlated with improvements in epidermal permeability function and stratum cornuem hydration. Thus, enhancement of epidermal function can prevent/ameliorate the development of psoriasis.

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Moderate to severe atopic dermatitis is associated with allergic, autoimmune and cardiovascular comorbidities in US adults
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Atopic dermatitis (AD) has been associated with multiple comorbid diseases. However, the relationship between AD severity and disease comorbidities is complex. A cross-sectional US population-based study of 8,217 adults was performed using a structured questionnaire. A diagnosis of AD was determined using UK Diagnostic Criteria for AD (n=602). AD severity was classified as mild-percentile (POSDC), moderate (PODC), or severe (POC). Logistic regression and structural equation models (SEM) were used to explore associations of AD with allergic, cardiovascular, anxiety/depression and autoimmune disease. In multivariable regression models controlling for socio-demographics, AD was associated with higher odds of asthma (adjusted OR [95% CI]: 2.09 [1.72-2.55]), hay fever (4.31 [3.27-5.68]), food allergy (2.07 [1.54-2.77]), depression/anxiety (2.34 [1.91-2.87]), autoimmune disease (3.05 [2.31-4.03]), obesity (1.37 [1.31-1.67]), diabetes (1.52 [1.16-1.99]), high blood pressure (1.46 [1.18-1.80]) and heart disease (3.41 [1.40-2.70]) compared to controls (P<0.01 for all). These associations were all significant in mild and moderate disease, with even stronger effects in severe AD. Results of SEM revealed direct and indirect effects of moderate-to-severe AD on obesity, high blood pressure, diabetes, anxiety/depression, and indirect effects on heart disease. These data indicate that AD is associated with multiple allergic, autoimmune and cardiovascular comorbidities. Future studies are warranted to confirm the association of AD with these comorbidities.

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Molecular epidemiology of non-syndromic autosomal recessive congenital ichthyosis in a middle eastern population
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Chronic severe pruritus without bullae (nonbullous pemphigoid), and three had known bullous pemphigoid. Pruritus was present in almost half of the nursing home residents, of which in 1-in-8 pemphigoid was demonstrated. In more than half of these subjects pemphigoid remained unnoticed because bullae were absent. Serological screening for pemphigoid should be included in the routine diagnostic work-up of elderly in nursing homes with chronic severe pruritus.

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Color and risk of keratinocyte carcinoma in US women and men
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Light hair color is an established risk factor for melanoma. Whether hair color is associated with risk of keratinocyte carcinoma, also known as non-melanoma skin cancer, has not been well studied. We prospectively examined the association between natural hair color at adulthood and risk of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) based on a total 205,107 participants from the Nurses Health Study (NHS, n=78,679), NHS II (n=56,156), and Health Professionals Follow-up Study (HPFS, n=16,912). Information on SCC and BCC was collected biennially, and diagnoses of SCC were pathologically confirmed. During the follow-up (1984-2012 for NHS, 1991-2011 for NHS II, and 1988-2012 for HPFS), we identified 4,661 cases of SCC and 32,548 cases of BCC. Red hair color was significantly associated with adult onset SCC (OR of 1.43 [95% CI: 1.25-1.62]) compared with light brown haired individuals. The association was consistent for invasive and in situ SCC. The association between red hair color and SCC differed by body surface distribution: SCC on trunk (OR=1.95, 95% CI: 1.61-2.38) than head and neck (OR=1.05, 95% CI: 0.83-1.32) or trunk SCC (OR=1.41, 95% CI: 0.98-2.03) (P=0.0003). Compared with participants with light brown hair, risk of BCC was increased for those with black hair (HR=1.86 [95% CI: 1.30-2.67]) compared with those with red or dark brown hair (HR=0.92, 95% CI: 0.89-0.95), and increased for individuals with blonde hair (HR=1.10, 95% CI: 1.04-1.17) or red hair (HR=1.27, 95% CI: 1.07-1.51). In conclusion, individuals with light hair color had a high risk of developing keratinocyte carcinoma. Red-
Disparities in outpatient dermatologic health care access and utilization in the United States
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Background: Although access to care has been shown to be an important prognostic factor for dermatologic outcomes, knowledge regarding disparities in dermatologic care among populations with a broad range of conditions is limited. Thus, the aim of this study is to elucidate nationwide disparities in utilization of outpatient dermatologic care. Methods: We analyzed nationally representative data from the 2007-2015 Medical Expenditure Panel Survey (MEPS). Healthcare utilization outcomes for dermatologic conditions (cancers, infections, inflammatory conditions, ulcers, and other skin disorders) were examined via multivariate logistic regression analyses of outpatient/office-based dermatologic care visit rates accounting for age, sex, race, geographic region, insurance status, income, self-reported condition, health status, and education. Results: Of the 183,054 MEPS respondents, 19,561 (10.6%) self-reported a dermatologic condition; 9,645 patients received a total of 11,761 outpatient dermatology visits. Black patients (OR = 0.41, CI = 0.37-0.45) and Hispanic patients (OR = 0.54, CI = 0.49-0.60) were less likely to receive outpatient dermatologic care than Northeastern patients (OR = 0.78, CI = 0.69-0.89). Patients with Medicaid/Medicare (OR = 0.76, CI = 0.70-0.83) and uninsured patients (OR = 0.48, CI = 0.41-0.56) were less likely to receive outpatient dermatologic care than privately insured patients. Increasing income and education were associated with increased odds of receiving outpatient care for the dermatologic condition (p < 0.01). Conclusions: These findings highlight inequality in the utilization of dermatologic care in the United States and suggest an urgent need to further improve access and utilization of outpatient dermatologic care.

Impact of skin infections on medical expenditures in patients with atopic dermatitis
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Purpose: To determine the impact of skin infections on medical expenditures in patients with atopic dermatitis (AD). Methods: We analyzed 200,000 self-reported AD visits to dermatologists from 2006-2010 from the National Ambulatory Medical Care Survey (NAMCS). We identified patients with skin infections (cancers, infections, inflammatory conditions, ulcers, and other skin disorders) and calculated the impact of skin infections on medical expenditures. Results: The cost of emergency department (ED) visits per patient/year in AD patients with skin infections was $1,104 (95% CI: 763, 1,444) and in AD patients without skin infections $(1,639 (95% CI: 1,393, 1,884). The cost of outpatient clinic visits per patient/year in AD patients with skin infections was $946 (95% CI: 649, 1,243) and in AD patients without skin infections $(1,257 (95% CI: 974, 1,540). The cost of inpatient hospital visits per patient/year in AD patients with skin infections was $358 (95% CI: 205, 510) and in AD patients without skin infections $(307 (95% CI: 191, 423). The cost of ED visits and hospital stays per patient/year in AD patients with skin infections was $1,489 (95% CI: 1,133, 1,845) and in AD patients without skin infections $(1,906 (95% CI: 1,529, 2,283). Pregnancy was associated with an increased odds of receiving outpatient dermatologic care (OR = 0.72-0.82), and Midwestern patients were less likely to receive outpatient dermatologic care than Northeastern patients (OR = 0.78, CI = 0.69-0.89). Conclusions: These findings suggest that skin infections are a substantial healthcare expenditure. Efforts that reduce AD-related skin infections will not only improve treatment of AD, but also reduce healthcare costs.

Pain burden in atopic dermatitis
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Objective: To assess pain burden in patients with atopic dermatitis (AD). Methods: We conducted a retrospective chart review of AD patients seen at the Cleveland Clinic from 1980-2015. Patients were included based if they satisfied one required criterion (acral skin lesions induced by exposure to cold) and at least 1 of 2 major criteria: (1) documented histologic evidence of CLE, (2) met ACR criteria for SLE. We identified 18 patients who met our criteria for a diagnosis of CHLE (16 women; 2 men). CHLE lesions were most commonly found on fingers (n = 16 pts), hands (11), toes (10), feet (7), and ears (4). Most patients (11/18) had a diagnosis of SLE prior to CHLE onset and differed from Raynaud phenomenon patients in that they were ANA positive; other common autoantibodies included dDNA (10/18), SS A (7/18) and RNP (7/18). Twelve patients had biopsies of a CHLE lesion, and these typically displayed superficial and deep inflammation with variable interface change. Treatment strategies varied, but included both immunomodulatory medications and vasoactive medications. The majority of patients had improvement of their CHLE lesions with treatment. CHLE is a rare subtype of CLE seen at our institution, and many occurred in women with SLE. Raynaud phenomenon was a common comorbidity. Treatment of CHLE may be predisposing factors for this rare cutaneous lupus subtype. Treatment with immunomodulatory and/or vasoactive medications tends to be beneficial in affected patients.

Clinical Research: Epidemiology of Skin Diseases | ABSTRACTS
The effect of rejection episodes on squamous cell carcinoma (SCC) in transplant patients

The study assessed the effect of rejection episodes on squamous cell carcinoma (SCC) in transplant patients. The rate of SCC varied significantly between the rejection group at 2.38 years (range 0.4-20.4 years) and the no-rejection group at 3.04 years (range 0.3-9.0 years) (p = 0.0086). An episode of rejection, biopsy recipients present earlier and with an increased incidence of SCC compared to non-rejection recipients. These findings advocate for increased dermatology surveillance following rejection episodes.

A single-institution experience with nevi of specialized sites

D Elkeeb, Z Hopkins, A Secrest, C Bolender, C Moreno, and D Wada

A single-institution experience with nevi of specialized sites was conducted. Melanocytic nevi from certain anatomic locations such as the scalp, ear, flexural areas or genitals (also referred to as nevi of specialized sites or NSS) may exhibit certain histologic features that pathologists may classify as atypical. Complete excision at these locations may have significant cosmetic or functional consequences. The study aimed to evaluate the frequency of clinical recurrences, excision recommendations, and any subsequent diagnoses of melanoma at the original biopsy. This retrospective analysis included all patients with a histologic diagnosis of NSS from 2009-2017. Data retrieved included: demographics, indication for biopsy, presence of cytologic and architectural atypia (beyond expected for NSS), margin status, recurrences for excision, and clinical diagnoses. Seventy-two percent were female. Mean age was 31 years, and 49% had at least one follow-up visit. Median follow-up time was 13.5 months (interquartile range: 1.36 months). Atypia was noted in 24 (8.8%) nevi. Positive biopsy margins were reported in 86 (31%) of cases. Complete excision was recommended for 32 (11.4%), and conditional excision was recommended for an additional 12 (4.4%), if residual pigment was observed. Of the 21 nevi re-excised, 7 showed residual nevus. Two nevi recurred clinically (2.1%). None developed melanoma at the original biopsy. The rate of clinical recurrences, excision recommendations, and any subsequent diagnoses of melanoma at the original biopsy were 2.1% (p = 0.0086). The rate of SCC varied significantly between the rejection group at 2.38 years (range 0.4-20.4 years) and the no-rejection group at 3.04 years (range 0.3-9.0 years) (p = 0.0086). An episode of rejection, biopsy recipients present earlier and with an increased incidence of SCC compared to non-rejection recipients. These findings advocate for increased dermatology surveillance following rejection episodes.

Assessment of insurance type and access to dermatologic care at dermatology and family medicine practices in the Bay Area and Central Valley of California

CL Cortez and ML Wei

The study assessed the assessment of insurance type and access to dermatologic care at dermatology and family medicine practices in the Bay Area and Central Valley of California. Patients with joint replacement or pacemaker implantation are at increased risk of eczema. The rate of SCC varied significantly between the rejection group at 2.38 years (range 0.4-20.4 years) and the no-rejection group at 3.04 years (range 0.3-9.0 years) (p = 0.0086). An episode of rejection, biopsy recipients present earlier and with an increased incidence of SCC compared to non-rejection recipients. These findings advocate for increased dermatology surveillance following rejection episodes.

Increased risk of eczema after joint replacement or pacemaker implantation

C Chang, C Tsai, P Wu, C Mou, and J Chang

A population-based cohort study of increased risk of eczema after joint replacement or pacemaker implantation was conducted. Initiated hypothermias associated with a gain in annual wage income of $11,142.31 (95% CI: $1,988.33-20,696.28) (p = 0.019) as compared to treatment with oral agents. In conclusion, after adjusting for insurance status and other factors, biologic therapies are associated with significantly higher wage earnings compared to oral therapies among U.S. psoriasis patients.

Comparison of wage earnings between psoriasis patients on biologics versus oral therapies in the United States

F Da Jornayvaz, N Salaman, A Armstrong

A nationwide, cross-sectional study examining differences in wages between psoriasis patients on biologics versus oral therapies was performed. The rate of SCC varied significantly between the rejection group at 2.38 years (range 0.4-20.4 years) and the no-rejection group at 3.04 years (range 0.3-9.0 years) (p = 0.0086). An episode of rejection, biopsy recipients present earlier and with an increased incidence of SCC compared to non-rejection recipients. These findings advocate for increased dermatology surveillance following rejection episodes.

A systematic review and meta-analysis of the prevalence and phenotype of adult-onset atopic dermatitis

H Lee, K Patel, S Rastogi, V Singam, and J Silverberg

A systematic review and meta-analysis of the prevalence and phenotype of adult-onset atopic dermatitis was performed. High rates of child-onset AD that persists into adulthood may not fully reflect the natural history of adult AD. Adult-onset AD is frequently the initial manifestation. But this association lacks population-based evidence and long term follow up. Design To determine whether the patients with joint replacement or pacemaker implantation are at increased risk of eczema. The rate of SCC varied significantly between the rejection group at 2.38 years (range 0.4-20.4 years) and the no-rejection group at 3.04 years (range 0.3-9.0 years) (p = 0.0086). An episode of rejection, biopsy recipients present earlier and with an increased incidence of SCC compared to non-rejection recipients. These findings advocate for increased dermatology surveillance following rejection episodes.
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Deep Ackerman: a novel deep learning method to develop dermatopathology diagnosis by artificial intelligence
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Recent progress of deep convolutional neural networks (CNNs) proposed by J. Hinton in 2012 succeeded in classifying general images and opened the possibility for automated diagnosis of medical images. However, the application of CNNs to histopathology image classification is a challenging task because the size of virtual slide images, which microscopically scanned a whole histopathology slide, are comprised of 40 to 100 mega pixels and too large to input into usual CNN. To overcome the issue of large pixel sizes, we developed a novel method, which consists of two CNN models. First, a whole histopathology slide image is dissected into micro images containing one cell per image. Then, the first CNN classifies dissected images into cell and structure types in four categories of atypial cells, epidermis, dermis, and subcutaneous tissue. Based on the result of the first CNN, heatmaps of each cell or structure class are constructed. Then, the second CNN model diagnosis based on the heatmaps, like as subcutaneous tissue. In addition, the PPV of code 702 was 94.5% (Algorithm 1) but the PPV increased markedly to 99.1% (95% CI, 98.5-99.5%) when the dermatologists billed code 207 for cryotherapy (Algorithm 2). In addition, the PPV of code 702 was 99.3% (95% CI, 98.7-99.6%) when code 207 was billed and cryotherapy or topical treatment was prescribed by dermatologists (Algorithm 3). Conclusions and Relevance: The PPV for identifying AK cases based on claims data was high and can be used in future epidemiologic studies.

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Utilization of physician billing claims to validate actinic keratoses cases
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Physician billing claims represent an invaluable source of information for research, and provide a valuable universal data source for studying medical conditions. There is a paucity of data validating actinic keratosis (AK) based on physician claims. Objective: To validate the physician-diagnosis of AK via ICD-9-792 code. In addition, to characterize the demographic distribution of AK patients and treatments administered to these patients. Methods: Dermatologist offices across British Columbia, Canada were selected to represent the number of dermatologists practicing within different metropolitan regions. Chart reviews were then conducted of clinical cases billed with ICD-9-792 code. Positive predictive value (PPV) of AK diagnosis via different algorithms were calculated. Results: A total of 1536 patients between ages 26-101 participated in the AK validation study in 2016. The mean age of the study cohort was 70.8 years (range: 26 - 101 years). The most frequent age interval in which patients were diagnosed with AK was between 61-70 years (34.5%). Cryotherapy was predominantly administered to treat AK patients, with greater than 90% being treated with this modality. The PPV of code 702 was 94.5% (Algorithm 1) but the PPV increased markedly to 99.1% (95% CI, 98.5-99.5%) when the dermatologists billed code 207 for cryotherapy (Algorithm 2). In addition, the PPV of code 702 was 99.3% (95% CI, 98.7-99.6%) when code 207 was billed and cryotherapy or topical treatment was prescribed by dermatologists (Algorithm 3). Conclusions and Relevance: The PPV for identifying AK cases based on claims data was high and can be used in future epidemiologic studies.

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A genome-wide association study identifies a novel susceptibility locus for total IgE in a Japanese population from Tohoku Medical Megabank cohort study

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Total IgE is an important measure of atopic disease. Genome-wide association studies (GWAS) for total serum IgE concentrations showed that polymorphisms in STAT6, FCER1A, the IL1R1/ADSOR3, TLR4, IL1A/IL1B, and the major histocompatibility complex (MHC) HLA-DRB1, HLA-C, HLA-DQA2, and HLA-A are associated with total IgE levels in 9,769 European populations, but though existing studies in Asian population, 2,470 Japanese and 3,495 Chinese populations, could not reveal significant single nucleotide polymorphisms (SNPs) yet. We performed GWAS for total IgE levels in a Japanese population regarding epidermis. As a result, we found novel two SNPs reaching genome-wide significance levels, one was rs9271364 located in the region between HLA-DRB1 and HLA-DQA1 in chromosome 6 and the other was rs44651842 located in the third exon of IL4R gene in chromosome 16. These SNPs are also replicated in the dataset of 4,986 Japanese individuals in locative precut. These data indicated that total IgE levels of Japanese population also have a genetic influence, which is relating to MHC and IL-4 signaling pathway.

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Antihypertensive medications and risk of keratinocyte carcinoma
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Keratinocyte carcinoma, which includes basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), is a highly prevalent cancer in the population at large and accounts for up to three-quarters of all cancer diagnoses. It is hypothesized that antihypertensive medications may affect the risk of BCC and SCC, but this relationship remains inconclusive. We addressed this issue by using data from the Veterans Affairs Keratinocyte Carcinoma Chemoprevention Trial, a randomized controlled multi-center trial that looked at the effects of topical 5-fluorouracil on BCC and SCC development. The trial followed 932 veterans at high risk for skin cancer over the course of 3 years. This data on skin cancer development was then combined with Veterans Health Administration pharmacy records to evaluate the effects of various antihypertensive medications on keratinocyte carcinoma development. Overall, the results were scored by the association with BCC or SCC risk during the trial, nor did any of the four major subcategories of antihypertensive medications (angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, beta blockers, diuretics, or diuretics plus ACE inhibitors) show an increased risk of SCC (but not BCC) associated with hydrochlorothiazide use (hazard ratio, 1.53; 95% CI, 1.04 2.24; p = 0.031). We failed to replicate our prior observation of a reduced risk of keratinocyte carcinoma associated with the use of angiotensin-converting enzyme inhibitor drugs. Our findings regarding the potential relationships between antihypertensive medications and keratinocyte carcinoma.

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Mammography screening in neurofibromatosis type 1
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Neurofibromatosis type 1 (NF1) is a cancer-predisposing syndrome associated with an increased risk of breast cancer. Cutaneous neurofibromas may decrease the diagnostic sensitivity of breast mammography. We evaluated the feasibility of mammography in patients with NF1 by a retrospective register-based analysis of mammography screening. Finnish women aged 50-69 years are invited to mammography screening biannually. The invitations, examinations and diagnostic findings are recorded in the Finnish Mass Screening Registry. In addition, the Finnish Cancer Registry collects information on all cancers diagnosed in Finland. Totals of 211 NF1 patients and their non-NF1 2239 controls were invited to mammography screening at least once during the study period of 1992-2014. Of the NF1 patients, 193 women attended the screening at least once, while the corresponding number in controls was 2108 among controls, yielding 854 and 9505 visits by NF1 patients and controls, respectively. The participation activity did not differ between NF1 patients and controls. Each NF1 patient was older than the matched control. We found no differences in HLA, Depression, Diabetes, Mestodial infarction, tobacco use, except for dysplasia (MD= 26 vs MD= 23, p=0.03) and Psoriasis arthritis (MD= 3% and MD=12%, p<0.01). For medical reasons no differences between hospitalizations (MD=10% vs MD=26% were seen). A 27% (n=295) of MD patients and 21%(n=106) of MS patients never consult to a dermatologist. Only 85% of MS patients received systemic treatment. Many patients with psoriasis are not seen by dermatologist in HAMP. Our psoriasis patients are untreated as in other studies. Comorbidities are seen with no differences in mild or moderate/severe disease.
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Health maintenance practices associated with incident skin cancer in US women and men
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Detection bias is an important source of potential bias in epidemiologic studies in which more intensive surveillance is associated with a greater apparent risk of disease. To assess the potential for detection bias related to skin cancers, we examined the association between various health maintenance practices and squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and melanoma in Nurses Health Study (NHS) and Health Professionals Follow-up Study (HPFS), large US cohorts of women and men, respectively. Participants in the cohorts are periodically asked about healthcare screening practices: physical examination by a physician, sigmoidoscopy or colonoscopy, eye examination, serum cholesterol, mammogram, breast examination and pelvic examination, prostate-specific antigen test and rectal examination. They are also asked whether they have been diagnosed with skin cancer. For SCC and melanoma, incident cases are confirmed by pathology review. We followed the participants from 1990 to 2012, and we classify skin cancer with 21 or more hours outside on weekdays. Obstacles to proper skin cancer prevention included lack of knowledge and lack of access to receiving a total body skin examination. This study shows the importance of developing interventions to improve patient education regarding skin cancer and sun protective behaviors among minority populations, specifically among underinsured Latino populations such as the one investigated in this study.

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Skin cancer awareness, sun protection behaviors, and skin cancer risk factors at St. John Bosco Clinic
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Early detection of melanoma is linked to decreased morbidity, lower costs, and a more favorable survival rate. Yet Hispanic and 4 times more likely to present with distant metastases than non-Hispanic whites. At the core of this disparity lies the issue of patient education, specifically related to skin cancer awareness and beliefs regarding sun protection. The objective of this study was to assess patient knowledge related to skin cancer at the San Juan Bosco clinic, a free clinic in Miami, Florida serving undерinsured patients. Patients completed a survey evaluating their skin cancer awareness, sun protective behaviors, and skin cancer risk factors; the survey included true/false, yes/no, open-ended, multiple choice, and Likert scale-type questions. Data was analyzed with SAS JMP Pro (version 11) using distribution analysis. Of the 100 patients who completed the survey (median age, 56 years), 84 were female; 21% were from Nicaragua and 25% were from Honduras. Fifty-three out of 80 patients (65.8%) said they cause skin cancer, with 21 patients out of 100 (21.0%) spending two or more hours outside on weekdays. Obstacles to proper skin cancer prevention included lack of knowledge and lack of access to receiving a total body skin examination. This study shows the importance of developing interventions to improve patient education regarding skin cancer and sun protective behaviors among minority populations, specifically among underinsured Latino populations such as the one investigated in this study.
The association of depression and alopecia areata in women: A prospective study

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Alopecia areata (AA) is a common autoimmune condition of the skin that causes hair loss from the scalp and body. Recent studies have proposed a relationship between AA and comorbid psychiatric conditions including anxiety and depression. Our aim was to explore the association between depression and AA prospectively. A cohort of 56,079 female nurses from Nurses Health Study (NHS) were followed up from 2002 to 2012 to investigate whether a previous diagnosis of depression (n=16,083) was associated with the risk of self-reported new-onset AA (n=130). Time-dependent Cox proportional hazard models were used to estimate multivariate-adjusted relative risk (RR) of AA adjusting for age, smoking status, body mass index, alcohol intake, exercise, history of hypertension or cardiovascular disease, hormone use, menopausal status, and UV flux. An increased risk of AA was found in women with a history of depression (95% CI 1.31, 95% CI 1.07-1.61) as compared to women without depression. The associations were similar when different definitions of depression based on antidepressant use were explored. In conclusion, our study demonstrates an increased risk of AA with a history of depression in this cohort of U.S. women.

Risk factors for acne development in the first two years of initiating masculinizing testosterone therapy among transgender men

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Acne is known to correlate with masculinizing testosterone (T) therapy use in female-to-male transgender men. However, the timing of acne onset and acne risk factors in this population have not been well-studied. In this retrospective study, we explored the combined effects of T therapy and biological factors on acne development in a population of transgender men who have initiated and maintained therapy for at least two years. We identified 55 transgender men aged ≥18 who received care between January 1, 2008 - December 31, 2017 at Boston Medical Center, MA. Data from routine care were extracted from electronic medical records and patients were identified as having acne if they received an ICD-10 acne diagnosis and/or prescribed medication for acne after starting therapy. A multivariate logistic regression model was used to examine the impact of potential acne risk factors. Among the sample of 55 transgender men, the median age was 25 years (Range: 18-75). 74.6% (n=41) were white; and the median serum T level was 647 ng/dL (Range: 118-1758). In the first two years following T initiation, 20 transgender men (41.8%) developed acne, with a mean onset of 9.25 (SD:8.33) months. After adjusting for race, age, BMI, smoking status, and alcohol history in the regression model, serum T levels above 630 ng/dl were associated with a 5.64 increased odds of acne in the study group (95% CI: 19.26-77, p<0.003). The adjusted model also revealed that an increased BMI was associated with an increased prevalence of acne (OR:1.13; 95% CI:0.2-1.6, p<0.03) and that the risk of acne increased with increased age (OR:0.92; 95% CI:0.8-0.98, p<0.02). These findings suggest that T can lead to an increased acne incidence in the first two years of therapy, and that this risk is elevated in the setting of an increased BMI and younger age. Acne treatment guidelines do not currently exist for transgender men, which limits medical providers ability to optimally counsel patients and devise treatment plans. Future studies assessing long-term acne treatment outcomes is needed for the transgender male population.
Development of a phenotyping algorithm to identify patients with autoimmune disease in electronic health records for future large scale studies

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Autoimmune diseases (AD) are chronic debilitating disorders that collectively affect 5% of the American population and result in a substantial disease burden and health care cost burden in the U.S. Due to the high prevalence of AD that affect the skin, dermatology bears a substantial proportion of the disease burden created by autoimmunity. While it is well established that having an AD increases the risk of developing other AD, more recent evidence suggests that the potential cross-phenotyping of these patients may also be of increased risk for metabolic and neuropsychiatric disorders. The long-term goal of this project is to leverage the massive amount of data harbored in electronic health records (EHR) of patients with AD to rigorously test for comorbidities not traditionally thought to be immune-mediated, specifically by utilizing resources in the Electronic Medical Records and Genomics (eMERGE) Network. This nationwide consortium of academic medical centers links genetic data to EHR for 105,325 patients. Here we report the development and validation of a phenotyping algorithm to construct an AD cohort from EHR data for future epidemiological and genomic studies. Autoimmune disease phenotypes were identified by searching for patients with at least 3 entries of an ICD code for at least one of 522 ICD codes that indicate the presence of 51 different AD. Patients not defined as cases are excluded from controls with the presence of 1 or more of a set of 28,419 ICD codes, or positive results for antibody tier tests. Our phenotyping algorithm was validated by chart reviews conducted for 161 patients by physicians from three clinical domains, which indicated a positive predictive value of 86% for controls and 93% for cases. The rigorous interrogation of large datasets to substantiate neuropsychiatric and metabolic comorbidities could improve the quality of care of AD patients and open new opportunities for gaining mechanistic insight into disease causes and potential therapeutic targets.
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BMI and sun exposure is associated with increased risk of non-melanoma skin cancer: A prospective study from the Women's Health Initiative

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Incidence of nonmelanoma skin cancer (NMSC) exceeds all other types of cancer combined. Cumulative and intermittent sun exposure are known risk factors for the development of NMSC. Since obesity has been shown to contribute to cancer incidence, we hypothesized that heavier individuals (BMI > 25 kg/m²) have increased the risk of NMSC when accounting for sun exposure. Using the Women's Health Initiative (WHI) cohort, we investigated the risk of NMSC with sun exposure and anthropometric measures. We analyzed the incidence of NMSC. The analyses were reported by mass index (BMI) and waist-to-height ratio (WHR). We tested whether the effect of sun exposure on NMSC depended on anthropometric measures by a two-way interaction table. From 66,822 postmenopausal women eligible for inclusion in this study, 9,317 (13.9%) participants developed NMSC. We found that the more time spent outdoors, the higher the risk of NMSC and subjects in geographic locations with higher sun intensity had a higher risk of NMSC. While BMI > 25 kg/m² showed lower risk of NMSC by univariable analysis, we found that the risk of NMSC for women with BMI > 25 kg/m² vs. normal weight increases with increased sun exposure (p = 0.024). Therefore, the risk of NMSC from sun exposure is affected by BMI.

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Skin cancer risk among adults with atopic dermatitis exposed to calcineurin inhibitors

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A retrospective descriptive cohort study

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Atopic dermatitis (AD), a chronic inflammatory skin disease, affects between 2-10% of the adult population in industrialized nations. AD is primarily treated with topical emollients and anti-inflammatory agents such as topical corticosteroids (TCS) and topical calcineurin inhibitors (TCIs) for severe cases of AD. AD may currently carry a black box warning label for a possible association with cutaneous malignancy, including melanoma and keratinocyte carcinomas (KCs), defined as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). The relationship between AD exposure and skin cancer remains poorly understood as large-scale post-marketing surveillance studies in real-world settings are lacking. We will examine the association between TCI exposure and the development of cutaneous melanoma and KCs in adults age >40 with a physician-diagnosis in the Kaiser Permanente Northern California health plan (n=96,672). Electronic pharmacy data was used to determine pharmacy-dispensed TCI exposure (n=1,538). Electronic pharmacy records were used to identify incident melanoma (n=195) and KCs (n=7,218) from cohort entry (January 1, 2002) until the end of the study period (December 31, 2017). Cox proportional hazards modelling will estimate melanoma and KC hazard ratios and 95% confidence interval. The primary objective of this study is to assess the risk of developing KC overall and by type (BCC or SCC), in adults age >40 years with AD exposed to TCIs as compared to those exposed to TCS and untreated AD subjects. The secondary objective is to assess TCI dose, intensity and duration of use in relation to melanoma and KC risk. The unique study setting of an integrated healthcare delivery system allows for the assessment of exposure to TCIs and further stratiﬁcation by skin type, sun exposure and sun protective behaviors, enabling an in-depth exploration of the association of TCI exposure with risk of melanoma and KCs.

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Correlates of low sun-protection factor sunscreen users in 2000-2015: A population based study

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We analyzed the sunscreen use by sun exposure and other demographic characteristics and sun protective behaviors of sunscreen users age 18+ who reported use of <15 SPF versus <15 SPF sunscreens in the nationally representative, population-based, National Health Interview Surveys, 2000-2015. Among 91,250 adult respondents who report any sunscreen use, 4,772 (5.1%) reported usual use of SPF <15 sunscreen screens while 78,429 (84.1%) used SPF <15 sunscreens. SPF <15 sunscreen users had significantly lower educational attainment, were more likely to report current smokers, binge-drink, and lack health insurance, but less likely to be overweight or obese or have a personal history of skin cancer (each P < 0.05). SPF <15 sunscreen users reported significantly lower prevalence of frequent sunscreen use (39.5% vs. 55.2%), seeking shade (20.8% vs. 35.7%), wearing long-sleeved shirts (9.0% vs. 11.7%), wearing long pants (18.6% vs. 26.6%), wearing a wide-brimmed hat (16.2% vs. 20.0%), wearing a cap/visor (23.9% vs. 35.1%), and ever wearing a broad-brimmed hat (23.9% vs. 35.1%), and having ever worn a sunscreen when sunburned (10.8% vs. 12.8%), ever wearing a broad-brimmed hat (16.2% vs. 20.0%), wearing a cap/visor (23.9% vs. 35.1%), and having ever worn a sunscreen when sunburned (10.8% vs. 12.8%).

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Opportunities for improving keratinocyte cancer care in primary and specialist care in the Netherlands: A retrospective descriptive cohort study

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Kaposis sarcoma remains highly incident in sub-Saharan Africa with poor survival. Despite antiretroviral therapy (ART), our goal was to assess KS epidemiology in the modern African setting, including stage at diagnosis, real-world use of chemotherapy, and the proportion of actinic keratosis lesions treated by independently billing non-physician practitioners. At diagnosis, most patients (58%) had evaluable charts. Median age was 35, and stage at diagnosis were AIDS Clinical Trial Group (ACTG) T1 stage and/or severe disease from WHO (n = 16,253). Twenty years after KS treatment, patients in Kenya have high mortality, with the majority diagnosed with severe disease. Patients with mild disease who did not have a chemotherapy indication. Receiving chemotherapy was associated with a lower risk of NMSC by univariable analysis, we found that the risk of NMSC for women with BMI > 25 kg/m² vs. normal weight increases with increased sun exposure (p = 0.024). Therefore, the risk of NMSC from sun exposure is affected by BMI.

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Kaposis sarcoma severity, treatment and survival in a large community-based HIV health care network in Kenya

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Kaposis sarcoma (KS) is a tumor of the blood vessels with an association to HIV. KS and its associated treatment burden and cost are not well understood. We aim to better describe the KS and its associated treatment burden and cost.

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Utilization and cost of aciclovir destruction in Medicare Part B free-for-service beneficiaries in 1998-2016

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Actinic keratosis has the potential to progress to keratinocyte carcinoma and was estimated to impose $1.6 billion in medical costs in the United States in 2013. The changing incidence of actinic keratosis and its associated treatment burden and cost are not well understood. We aim to describe the changing incidence of actinic keratosis and its associated treatment burden and cost.

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327 Chronic pruritus severity and QoL impact on healthcare utilization among veterans: A national survey
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Chronic pruritus significantly affects health-related quality of life (QoL) and accounts for 7 million, or 1%, of all outpatient visits annually in the United States. To quantify the association between chronic pruritus severity and QoL impact on healthcare utilization related to chronic pruritus, we analyzed a nationwide telephone survey of 6,000 U.S. veterans randomly selected from the U.S. Department of Veterans Health Administration (VA) Medical Center. Veterans were assessed using the numeric rating scale (NRS) ranging from 0-10, and pruritus-specific QoL impact was assessed using Itch-QoL. Self-reported healthcare utilization (emergency department, primary care provider, and specialist visits) was correlated with NRS and Itch-QoL using Spearman’s rho. Among 405 veterans who reported chronic pruritus, 369 (91%) completed the Itch-QoL. Itch-QoL had moderate correlation with NRS (Spearman’s rho 0.432, p < 0.001). 134 (33%) veterans had at least 1 medical visit in the past 3 months mainly for chronic pruritus. The number of medical visits was modestly correlated with Itch-QoL (r 0.17, p = 0.03). Each Itch-QoL subscore was modestly correlated with the number of medical visits (Symptoms 0.20, Functioning 0.39, Emotions 0.38, each p < 0.001). In a multivariable logistic regression model adjusting for age, sex, and race/ethnicity, Itch-QoL Functioning subscore (OR 1.11, 95% CI 1.04-1.19) and Emotions subscore (OR 1.06, 95% CI 1.01-1.10) significantly predicted higher odds of medical visits, while NRS was not significant (OR 0.91, 95% CI 0.80-1.02). Pruritus-specific QoL, as measured by Itch-QoL, was more strongly associated with healthcare utilization due to chronic pruritus than subjective severity as measured by the NRS, lending additional validity to the Itch-QoL instrument as a patient-reported outcome measure. Greater attention to the relationship between chronic pruritus severity and healthcare utilization may be of benefit to both patients and healthcare systems.

328 Impact of ultraviolet exposure on melanoma and cutaneous T-cell lymphoma incidence and risk
VA Larson, B Binns, H Young1, 2, KA Whang, 3 and SG Kwatra, 4
2014 were identified and age-adjusted incidence rates and risk ratios were calculated using publicly available SEER data. All patients presenting to the Johns Hopkins Health System from 2004-2014 were included. We determined if treatment of chronic wounds in severe RDEB (N=7 adults, 36 wounds assessed) is limited information on measuring dynamic wound healing in RDEB and the amount of wound healing that is clinically meaningful in patient reported outcomes. We compared 2 methods to calculate wound surface area: quantitative 3D imaging with high-resolution Canfield camera (Vectra H1) vs Investigator global score as assessed by 2 physicians in real time on 36 wounds followed for 6 months. We found a high degree of agreement between Canfield 3D and Investigator global score (ICC=C0.92, P=0.0001). Prior to grafting, subjects reported pain, itch and blistering in each chronic wound (N=36). After application of EB-101 grafts, subjects reported no pain (91% of wounds), absence of itch (87.5%), higher durability of skin (74.0%), and more difficult blistering (81.7%) after 3 and 6 months. Using the Investigator global score, RDEB wounds that were ≥50% healed showed improvements in pain, itch, skin durability and ease of blistering (P<0.0001). By comparison, grafts with ≥94% of wound healing had more pain (66%), itch (67%), low durability (82%), and easy blistering (65%) after 3 and 6 months. In summary, we found that 3D wound assessment and Investigator global score were both well correlated and reliable, and that ≥50% wound healing correlated with statistically significant improvements in patient reported outcomes.

330 Allergens or non-allergens can induce Th2-type inflammation via reactive oxygen species in keratinocytes
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ABSTRACTS | Clinical Research: Epidemiology of Skin Diseases

331 Ethnic variation in rare cutaneous malignancies: A single institution experience and comparison to SEER data
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There is limited data available regarding ethnic differences in rare cutaneous malignancies. This study examines the incidence of rare cutaneous malignancies in a single institution as compared to publicly available SEER data. All patients presenting to the Johns Hopkins Health System from 2012 to 2017 with available demographics (n = 4,696,491) were considered, and those with a diagnosis of dermatofibrosarcoma protuberans (DFSP), Kaposi sarcoma (KS), Merkel cell carcinoma (MC), Bowen disease (BD), basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) were identified. DFSP patients were compared to melanoma patients. Among the included patients, 42% were non-Hispanic Caucasians, 26% non-Hispanic African Americans, 8% Hispanic/Latino and 24% other. Non-Hispanic white and black were compared using a Kruskal-Wallis test. This was followed by a post hoc Dunn’s analysis. This analysis revealed significant differences in the incidence of DFSP (P < 0.001) and KS (P < 0.001). These findings are concordant with SEER data, which show that African-American patients have a higher risk of DFSP (OR 1.86, P < 0.0012), KS (OR 1.79, P = 3.21 × 10−10), and CTCL (OR 1.51, P = 1.38 × 10−5), and at lower risk for SCC (OR 0.15, P = 5.42 × 10−12) as a group, individuals of all other races were between 0.12 and 1.39 (data not shown). There were no significant differences in the incidence of BD, SCC, or DFSP (OR < 1, P > 0.093). There were also no significant differences in the incidence of melanoma (OR 1.04, P = 0.0004) or basal cell carcinoma (OR 1.06, P = 0.0004) when comparing non-Hispanic white and black patients. This study highlights the need for additional research with larger sample sizes to identify potential risk factors for the development of rare cutaneous malignancies.

332 Gender-based differences in the prevalence of gastrointestinal disease in rosacea
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Rosacea has been associated with an increased prevalence of gastrointestinal diseases (GID). Rosacea affects both males and females, but erythematotelangiectatic and papulopustular subtypes of female predominant while phymatous rosacea occurs almost exclusively in males. Some GID have been reported to be subtype specific. We hypothesize that gender plays a role in the associated gastrointestinal comorbidity. Utilizing the Hunma claims database from 2007-2015, we pooled individuals with an ICD diagnosis of rosacea (n=39,982) or seborrheic keratosis (n=221,873) aged 18-64. The latter provided healthy, age similar controls. Individuals taking systemic antibiotics (doxycycline, clindamycin, minocycline and erythromycin) were excluded. We analyzed crude odds ratios and prevalence of 10 GID by gender. In the male cohort, we found increased odds of H. pylori infection (OR=1.28, 95%CI 1.04-1.57), ulcerative colitis (UC) (OR=1.26, 95% CI 1.04-1.52) and Crohns disease (CD) (OR=1.42, 1.16-1.74) relative to controls. In females, we found increased odds of celiac disease (CD) (OR=1.14, 1.02-2.77) and Crohn’s disease (CD) (OR=1.32, 1.03-1.67) and a decreased odds of gastrointestinal reflux disease (GERD) (OR=0.94, 0.91-0.96) and peptic ulcer disease (PUD) (OR=0.86, 0.77-0.96). Gender appears to play a role in the prevalence of specific gastrointestinal diseases in rosacea, and may account, in part, for the conflicting reports on GID and rosacea. HP was found in females with rosacea. Further research is needed to confirm the role of PUD. Behavioral and hormonal influences on gastrointestinal associations in rosacea are important factors to consider. Gender-specific longitudinal observations are needed to explore this further.

were only associated with Instagram users (p < 0.02), blepharoplasty (p = 0.66) and rhinoplasty (p = 0.48), and were only associated with plastic surgeons (p < 0.01). Significant correlations were seen for all other social media in combination.

Prevalence and risk factors for high-frequency basal cell carcinoma in a large commercially insured population in the US

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Patients with high-frequency non-sclerosing basal cell carcinomas (BCCs) have not been systematically studied in the US due to lack of national registries. Previous studies have focused on risk factors for developing multiple BCCs, defined as ≥2 BCCs, but few have evaluated patients with high-frequency BCCs. This retrospective study of a commercially insured US population within the Truven MarketScan database from 2012-2015 defines high-frequency BCCs (non-basal cell nevus syndrome (BCNS)) as developing 12-19 BCCs within 4 years. Standardized incidence ratios (SIRs) were 11.93 and 64 years old with at least 1 year of insurance enrollment. 231,678 patients had at least 1 BCC, defined as a visit associated with both a BCC diagnosis code and procedure code for malignant destruction, excision, or Mohs surgery. 55% of BCC patients were male; age-adjusted prevalence rate was 408 per 100,000 persons per year, projected to 8,828 patients in 2015. Of all patients with BCCs, 0.3% (657) were high-frequency BCC patients, with mean age at index BCC 54±7 years, 67% male, and 42% from the South. Compared to patients that developed 1 BCC during the study period, patients with 12-19 were more likely to be male (OR 1.9, CI 1.62-2.25, p < 0.01), less likely to be older (OR 0.98, CI 0.97-0.99, p < 0.01), have a history of SCC (OR 8.32, CI 7.19-70.75, p < 0.01), and more likely to have melanoma (OR 3.57, CI 2.74-93.31, p < 0.01). Age-adjusted prevalence rate for high-frequency BCC patients was 1.10 per 100,000 persons per year, projected to 8,828 patients in 2015. We have identified and quantified an important orphan disease of non-BCNS patients who have at least 3 BCCs treated per year. This high-risk group may especially benefit from early preventive screening and therapies to decrease lifetime tumor burden.

Body site distribution of pediatric-onset morphea

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Morphea is a sclerosing disease that can affect any body site though its distribution is incompletely understood. This 14-site retrospective study was performed to characterize morphea lesion distribution. Patients with pediatric-onset morphea (including extraglantal lichen sclerosus and atrophoderma but excluding panleukocytic morphea) and adequate lesion documentation were included. Lesions were prospectively mapped using a custom web-based software, linked to demographic and clinical data stored in a REDCap database. Overall, 829 subjects and 2534 lesions were included. Consistent with prior reports, female sex (73%) and the limbs (33%) were most prevalent. Lesions were most prevalent on the anterior (55%) than trunk (46%) and headneck (2%). Lesions were more common on extensor (55%) than flexor (45%) extremities (OR 1.96 < p < 0.0001). The right upper extremity (p = 0.37) was less affected than the left (OR 1.43 < p = 0.0002); this difference was not observed on the upper arm. Lesions were more common on the lateral (42%) than anterior (42%) trunk (OR 1-34 < p < 0.0001), as well as upper (62%) vs lower face (18% OR 28.4, < p < 0.0001). Morphoea predilection for the extremities, especially the anterior extremities and right upper extremity (which is more commonly the dominant hand and arm, and back) supports the theory that trauma may be an inciting factor.

Necrobiotic lipiodica: Aims, diagnoses, and complications

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Necrobiotic lipiodica (NL) is an idiopathic, chronic granulomatous disease that typically presents in the pediatric population. It tends to be locally confined, but can extend to other sites. Methods: A retrospective study was performed within Mayo Clinic from 1992 to 2017. 328 cases were identified using International Classification of Diseases (ICD) code diagnosis of NL, as well as clinical diagnosis of NL by a dermatologist or histopathological diagnosis consistent with NL. A chart review recorded lifestyle, comorbidities and complications of NL. Results: Methods: A retrospective study was performed within Mayo Clinic from 1992 to 2017. 328 cases were identified using International Classification of Diseases (ICD) code diagnosis of NL, as well as clinical diagnosis of NL by a dermatologist or histopathological diagnosis consistent with NL. A chart review recorded lifestyle, comorbidities and complications of NL. Results: Methods: A retrospective study was performed within Mayo Clinic from 1992 to 2017. 328 cases were identified using International Classification of Diseases (ICD) code diagnosis of NL, as well as clinical diagnosis of NL by a dermatologist or histopathological diagnosis consistent with NL. A chart review recorded lifestyle, comorbidities and complications of NL. Results: Methods: A retrospective study was performed within Mayo Clinic from 1992 to 2017. 328 cases were identified using International Classification of Diseases (ICD) code diagnosis of NL, as well as clinical diagnosis of NL by a dermatologist or histopathological diagnosis consistent with NL. A chart review recorded lifestyle, comorbidities and complications of NL. Results: Methods: A retrospective study was performed within Mayo Clinic from 1992 to 2017. 328 cases were identified using International Classification of Diseases (ICD) code diagnosis of NL, as well as clinical diagnosis of NL by a dermatologist or histopathological diagnosis consistent with NL. A chart review recorded lifestyle, comorbidities and complications of NL. Results: Methods: A retrospective study was performed within Mayo Clinic from 1992 to 2017. 328 cases were identified using International Classification of Diseases (ICD) code diagnosis of NL, as well as clinical diagnosis of NL by a dermatologist or histopathological diagnosis consistent with NL. A chart review recorded lifestyle, comorbidities and complications of NL. Results: Methods: A retrospective study was performed within Mayo Clinic from 1992 to 2017. 328 cases were identified using International Classification of Diseases (ICD) code diagnosis of NL, as well as clinical diagnosis of NL by a dermatologist or histopathological diagnosis consistent with NL. A chart review recorded lifestyle, comorbidities and complications of NL. Results: Methods: A retrospective study was performed within Mayo Clinic from 1992 to 2017. 328 cases were identified using International Classification of Diseases (ICD) code diagnosis of NL, as well as clinical diagnosis of NL by a dermatologist or histopathological diagnosis consistent with NL. A chart review recorded lifestyle, comorbidities and complications of NL. Results:
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Interdisciplinary dermatologic and wound care needs assessment in Western Kenya

T Jaleel 2, A MacLeod 3 and JS Ji 4

Background: A dermatologic wound care needs assessment was performed in Eldoret, Kenya at the Moi Teaching and Research Hospital (MTRH) to assess opportunities for local capacity building and interdisciplinary task force design. Eldoret is the principal town in Western Kenya hosting a number of medical institutions including: St. Mary’s, Eldoret Teaching, St. John’s, Shoti, and Eldoret’s North’s hospitals. The facility is the major provider of wound care in Western Kenya. In 2015, a survey was conducted to determine the capacity of where-to-care, who-are-caring, and who-are-receiving. Eldoret is not unique in Western Kenya with a very high prevalence of non-communicable diseases (NCDs) such as diabetes, hypertension, and cancer. In addition, the town is characterized by high rates of violence, poverty, and unemployment. In Western Kenya, the burden of NCDs is significantly higher due to a lack of adequate health care services. As a result, Eldoret’s North’s hospital is an important health care provider. It is the first hospital in Western Kenya to have a Wound Care Unit established in 1995. Pain management was the most common barrier to healthcare delivery and for which training would be most beneficial. Based on our needs assessment, we developed wound care protocols targeting leg ulcer, pressure ulcer, and SLS/TEN, and a hands-on lymphedema training curriculum. Protocols were reviewed by trained dermatologists, nurses, and doctors at the MTRH. The survey was also reviewed by the local hospital’s quality assurance team to ensure the best practices for wound care were followed. The survey was piloted and validated by medical professionals in Eldoret, Kenya and MTRH.

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The change of body skin with aging compared to face skin in Chinese women

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Background: The only reliable study on the change of body skin with aging is a non-invasive study on the upper thighs of 30 women with a mean age of 66.1 years (Oberholzer et al. 2017). However, the upper thigh is not a suitable skin site for assessing the change of body skin with aging due to its low body fat percentage and an absence of muscle. Therefore, it is necessary to perform a study on the change of skin properties with aging to more accurately assess the skin aging process. This study aimed to investigate the change of body skin with aging compared to face skin in Chinese women using non-invasive measurements.

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Association between vitamin D and eczema among adults in the US National Health and Nutrition Examination Survey

Jiwei 1, Taleen 1, A MacLeod 2 and JS Ji 3

Background: Vitamin D is a fat-soluble drug that is essential for growth and development. Vitamin D levels tend to be lower in patients with atopic dermatitis (AD). Therefore, vitamin D levels should be measured to confirm the benefits of supplementation on disease outcomes.

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Enhanced depression during disease progression in patients with systemic sclerosis

S Heyne 1, S Abraham 1, J Schmitt 2, S Beissert 1 and C Günter 1

Background: Systemic sclerosis is a rare progressive disease with limited knowledge on relationship between clinical signs, disability, quality of life and depressive symptoms. The prevalence of depression among patients with systemic sclerosis is between 36% to 65% compared to 19% in the general population, indicating a high need for defining determinants of depression and quality of life. To this end clinical characteristics and validated questionnaires such as Health Assessment Questionnaire due to systemic sclerosis (SHAQ), the Center for Epidemiologic Studies Depression scale (CES-D) and the Score of Eular Scleroderma Trials and Research Group (EUSTAR) were assessed in 65 patients with systemic sclerosis. The population consisted of 80% female patients with a mean age of 61.4 ± 12.95. Depressive symptoms were found in a high proportion of patients indicated by a CES-D ≥16 in 53% of the patients and a mean CES-D value of 19 ± 11 (mean ± SD). The 5-year longitudinal analysis in 31 patients revealed an increase of the EUSTAR score from 1 to 2.5 (p<0.001) indicating a disease progress. An increase in disease associated disability assessed by the SHAQ (3.8 ± 1.3 versus 4.8 ± 1.7, mean ± SD, p<0.001) was associated with a rise in CES-D (18 ± 10 versus 20 ± 11, mean ± SD, p<0.001) indicating that disease specific disability is the main determinant factor of quality of life impairment in systemic sclerosis. In conclusion, patients with systemic sclerosis had a significantly higher burden of depression compared with the general population that increased with disease progress and disability. This high psychosomatic morbidity strongly suggests interdisciplinary models of care for SSc patients.

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Clinical, viral and genetic characteristics of drug reaction with eosinophilia and systemic symptoms in Shanghai, China

W Xu, Y Fang and X Luo

Background: Drug reaction with eosinophilia and systemic symptoms (DRESS) is a severe adverse reaction to drugs. It is characterized by a spectrum of cutaneous and systemic manifestations. The diagnosis and management of DRESS are challenging due to its complex clinical manifestations and diverse etiologies. The aim of this study was to investigate the clinical, viral, and genetic characteristics of DRESS in Shanghai, China.

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Autoantibody profile in 619 Italian patients with cutaneous lupus erythematosus

S Heyne 1, S Abraham 1, J Schmitt 2, S Beissert 1 and C Günter 1

Background: Cutaneous lupus erythematosus (Cle) is a chronic inflammatory skin disease characterized by the presence of cutaneous lesions and autoantibodies. Autoantibodies play a crucial role in the pathogenesis of CLE and their presence is associated with a higher risk of disease flares and systemic involvement. The aim of this study was to investigate the autoantibody profile in 619 Italian patients with CLE.
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A real-world study evaluating ade: QLacy of existing systemic treatments for patients with moderate-to-severe atopic dermatitis (AD-QUST): a 6-month survey study of flares
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This study evaluated flares (defined as increased itching/redness or new/spotting lesions) in a real-world setting with moderate-to-severe AD treated with systemic agents prior to recent dupilumab approval. Adults (≥ 18 yr) with AD were enrolled in this study. Patients completed ≥2 survey between months 1-6. At baseline, in the past month, 21.3% of patients reported having 1 flare, 19.7% had 2 flares, and 38.3% had > 2 flares. Of those reporting a flare, 49.6% reported each flare lasted between 11 weeks; 28.0% each flare lasted ≥ 3 weeks. 91.6% reported worrying about a flare during the past month. At months 16, in the past month, 28.1–35.5% of patients reported having 1 flare, 19.7–24.6% had 2 flares, and 27.0–32.8% had > 2 flares. Of those reporting a flare, 50.45±3.1% reported each flare lasted for ≥ 3 weeks. 88.9–93.2% reported worrying about a flare during the past month. In this study, patients with moderate-to-severe AD continuously reported a substantial number and prolonged duration of recurrent flares, suggesting unmet therapeutic needs in long-term disease control.

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Hair alterations in the setting of germline RAS pathway mutations
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Background: Cardio-facio-cutaneous (CFC) and Costello syndromes (CS) belong to a group of rare genetic disorders characterized by germline RAS mutations in the RAS/mitogen-activated protein kinase pathway (RAS-MAPK) pathway. There is considerable overlap in the clinical manifestations seen in both CFC and CS syndromes, including the hair phenotype with curly hair and sparse eyebrows. Hypothesis: Our hypothesis is that CFC and CS are widely used in our daily life, and are known to be a common cause of skin allergy and irritation. Skin allergic and irritant reactions to cosmetics are routinely tested following a standard patch test protocol as a safety requirement in China. In order to learn the general prevalence of skin allergy and irritation to cosmetics and the associated factors, we have analyzed the data collected during 2011 and 2017 from a main testing center in Shanghai, China. The dataset includes 10178 individual visits (age 19-66), with an average number of 56.6 (SD=±12) test sites per individual visit. Skin allergy and irritation reactions were recorded and subsequently distinguished according to readings on D2, D3 and D4. The overall rates of allergic and irritant reaction are 0.71% and 0.79%, respectively. We found that allergic reactions are more prevalent in elderly (p<0.001) and in males (p<0.001), while there is no age or gender difference in irritant reactions. Both allergic and irritant reactions are more frequent in winter (p<0.01), showing associations with temperature (p<0.01) but not with humidity. Furthermore, both allergic and irritation reactions are more likely to be observed on upper back than lower back (p<0.001). In conclusion, skin allergic and irritation reactions to cosmetics could be affected by factors including season, temperature, and test site, while allergic reactions are also affected by age and gender. All these factors should be considered in study design of skin allergic and irritant reaction tests to cosmetics.

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Patch test in Chinese patients with cosmetic allergy to cosmetic series
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Background: There are increasing cosmetic adverse reaction in China, mostly cosmetic contact dermatitis (CCD), with the development of cosmetics industry. Objective: To identify the allergens of cosmetics which responsible for CCD in China. Methods: Totally 343 subjects, including 125 CCD patients from dermatological clinic and 218 cosmetics consumers with allergic history, were patch-tested with a cosmetic series (C-1000 series including 56 allergens). Results: Chemotest (11 F, 5 M) and 25 CS (16 F, 9 M) patients. Age ranged from 6-28 years old. Most patients (93%) had previously identified genetic mutations. Most common mutation was BRAF and HRAS G12arth in CS patients. Full thickness erythema (p<0.001) and eyelashes (p=0.001) were more common in CS. Urethrynges ophthogynes were observed in 53% of CFC patients and 4% of CS patients (p=0.0004). Hair shafts from 15 CFC and 22 CS (total of 94) hair patients were examined with light microscopy. Average diameter of was 0.15 mm in CFC and 0.10 mm in CS. Hair pigmentation was more often light in CS (p<0.0002). CS hairs were more often non-medullated (p=0.003). Trichorhexis nodosa was observed in 6 CS patients and none of the CFC patients. Conclusions: Patients with CS are more likely to have full thickness eyebrows and eyelashes while patients with CFC had more likely to have sparse eyebrows and absent eyebrows. Curly hair was observed in both CFC and CS. Urethrynges ophthogynes was seen more commonly in CFC syndrome while trichorhexis nodosa was only observed in CS. CS tended to have smaller diameter, lighter pigmented and non-medullated hairs.

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RNA-seq genomic analysis demonstrated the molecular efficacy of Risankizumab in a moderate-to-severe plaque psoriasis phase 2 clinical study
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Background: Psoriasis (PsO) is a serious chronic immune dysregulation condition in which skin cells proliferate, build up, and form scabs with itchy, dry raised patches. The cytokine IL-23, a homologous anti IL-23 p19 antibody (single 18-mg dose at week 0), or 90- or 180-mg doses at weeks 0, 4, and 16), demonstrated superiority in the proportion of patients achieving 90% improvement from Baseline in the Psoriasis Area and Severity Index (PASI 90) compared with ustekinumab, an anti-12 and p40 antibody (45 or 90 mg, according to body weight baseline ≤ 100 kg) or placebo 12 weeks in moderate-to-severe plaque psoriasis patients following 12 weeks of treatment. RNA-seq differential gene expression analysis of 58 matched patient biopsies from baseline to week 4 in 18 patients treated with risankizumab, compared with placebo, is conducted to provide insight into specific pathways modulated by risankizumab. These include disease activity biomarker genes (eg, DEF84, C5f3, LCN2, CXCL family, S100A family) and the IL-17A/23 axis family (including IL-17A, IL-22, IL-21 and IL-23R). Using these genes in a composite score to stratify patients in 180-mg risankizumab arm demonstrated superior performance to the active comparator ustekinumab arm, as assessed by the magnitude of expression of specific IL-17/23 axis and PsO genes, while the 90-mg arm showed similar performance to ustekinumab. Finally, expression of the composite score was associated with improvements in PASI, the composite score was associated with improvements in PASI, the composite score was associated with improvements in PASI.
Validation of independent ICD9 diagnosis codes as predictors of venous leg ulcers

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versity of Miami Miller School of Medicine, Miami, MI

Venous Leg Ulcers (VLUs) comprise about 70% of lower leg wounds and cost more than 14
billion USD per year. There is a need to establish VLU epidemiologic trends in order to
inform existing and developing treatment strategies. From 2005-2010, the National In-
ternet Stroke Registry (NISR) was used to identify patients admitted to hospital with a
hospitalization diagnosis of VLU. The purpose of this study was to validate the accuracy
of ICD9 diagnosis codes for VLU, and to identify additional predictors of VLU.

We used NISR data to identify VLU patients admitted to hospital and 531
matched controls. We then compared the predictive value of an ICD9 diagnosis of
VLU (code 454.0) with other commonly used codes for VLU (i.e. codes 089.1, 099.3, 507.0).
We also explored combinations of ICD9 codes.

Results: The study confirmed that the code 454.0 for VLU was the most accurate predictor
of VLU, with a sensitivity of 82% and a specificity of 61%. The positive predictive value (PPV)
and negative predictive value (NPV) were 88% and 58%, respectively. The combination
of codes 089.1 and 099.3 with 454.0 demonstrated a sensitivity of 94% and a specificity
of 59%, with a PPV of 88% and an NPV of 66%.

Conclusion: The ICD9 diagnosis code 454.0 for VLU is the most accurate predictor of
VLU, but the combination of codes 089.1 and 099.3 with 454.0 is also an effective predictor.

A retrospective analysis of patient-reported physical and psychological stressors as trigger factors in autoimmune bullous disease

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Pemphigus vulgaris (PV) is a complex, multifactorial, and polygenic disease, whose exact
pathogenesis is difficult to pinpoint. Research aimed at further elucidating associated
epidemiologic risk factors is hampered by its rare disease status. Further, a lack of centrali-
zation and standardization of available data makes the practical application of this infor-
mation challenging. We comprehensively reviewed 90 PV articles from 26 different countries
for parameters including age, sex, ethnicity, incidence, prevalence, and HLA allele associa-
tion. Analysis of the aggregated data revealed several trends including linkage disequilib-
rium of HLA DRB1*0402 and DQB1*0302 alleles in a geographic distribution centered in
Europe, with migratory spread into North and South America. Analysis of the geographic
distribution of other alleles was not possible due to linkage disequilibrium with HLA DRB1*
04 and DRB1*14 alleles.

Conclusion: While the influence of genetic factors, particularly HLA, is well known in PV,
non-genetic elements that trigger dysregulation of the immune system have been highly
studied. Our analysis showed a lack of clear trends in inciting factors, and highlights the
need for further studies with a better understanding of HLA and other genetic factors.

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Conclusion: While the influence of genetic factors, particularly HLA, is well known in PV,
non-genetic elements that trigger dysregulation of the immune system have been highly
studied. Our analysis showed a lack of clear trends in inciting factors, and highlights the
need for further studies with a better understanding of HLA and other genetic factors.

A retrospective analysis of patient-reported physical and psychological stressors as trigger factors in autoimmune bullous disease

S Sasankan, P Kim, AA Sinha and K Seiffert-Sinha

University at Buffalo, Buffalo, NY

Pemphigus vulgaris (PV) is a complex, multifactorial, and polygenic disease, whose exact
pathogenesis is difficult to pinpoint. Research aimed at further elucidating associated
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Applicability of EULAR/ACR criteria for dermatomyositis to amyopathic disease and development of new classification criteria

Y Borok 2, A Han 1, A Valderrama 3 and S Friedlander 1

Dermatology.

logic literature and associated factors predicting use. MEDLINE for clinical and translational research.

an international prospective study. The goal for these criteria is to create well-defined cohorts ongoing international effort to develop a more inclusive definition of ADM, through develop-

database for some patients. The EULAR/ACR classification criteria are a vast improvement cutoff was used as recommended by the EULAR/ACR. 99 of 211 patients were classified as ADM. From these 99 patients, we found that 26.1% would not meet the recommended EULAR/ACR classification criteria cutoff for ADM. Limitations include that there was a time delay between initial diagnosis and patients being classified, and no standardized approach for classification of ADM patients. A longitudinal birth cohort study

An international longitudinal birth cohort study evaluated prenatal anxiety and depression 1.12 (95% CI 1.04-1.21). There was no significant association with perceived stress during pregnancy occurred in 15.78%. After controlling for maternal age, education, social class, smoking, alcohol use, and parental atopic history in Cox proportional hazards regression models, we found that prenatal anxiety and depression were associated with a higher risk of AD: HR for prenatal anxiety 1.19 (95% CI 1.13-1.26), HR for prenatal depression 1.12 (95% CI 1.04-1.21). There was no significant association with perceived impact of stressful life events on skin health (HR 1.03 (95% CI 0.98-1.08). Offspring of women who ex-

Clinical factors influencing response to intravenous immunoglobulin treatment in cases of refractory pyoderma gangrenosum

A Ortega-Loayza and N Fett

oryderma gangrenosum

H Fett Department of Dermatology, Los Angeles, CA, 2 UCLA Department of Dermatology, Los Angeles, CA, 3 Department of Dermatology, Hospital Fundacion Car-

Clinical factors influencing response to intravenous immunoglobulin

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Background: Pyoderma gangrenosum (PG) is a neutrophilic disorder that classically presents on the lower extremities. First-line treatments include systemic corticoste-

"www.jidonline.org"
Association of P&Ps with itchy rash and eczema varied by gender, ethnicity, and age. These effects are especially pronounced with psoriasis comorbidities including metabolic syndrome and cardiovascular disease. However, vitamin D supplementation patterns among psoriasis patients are not known. Using the 2013-2014 National Health and Nutrition Examination Survey (NHANES) database, we conducted a population-based cross-sectional analysis with survey data from 9770 participants to investigate vitamin D supplementation patterns and the physician's role in these patterns. We calculated odds ratios (OR), 95% confidence intervals (95%CI), and p-values using logistic regression and ANOVA weighted to reflect the US Census data, adjusting for age, gender, marital status, education, and race. Vitamin D supplementation was found in 72.4% of participants with a history of psoriasis versus 56.0% of participants without psoriasis (OR=1.59, 95%CI=0.91-2.69). Vitamin D supplementation of psoriasis was associated with significantly increased odds of physicians recommending supplementation (OR=5.92, 95%CI=2.04-17.2). Moreover, ANOVA analysis revealed mean vitamin D supplementation doses increased significantly with increasing severity of psoriasis (p<0.001). However, supplementation patterns differed by ethnicities with Hispanic patients having significantly lower odds of supplementing compared to non-Hispanic patients (OR=0.17, 95%CI=0.04-0.77). Among Hispanic psoriasis patients, it was also uncertain whether physicians were likely to recommend vitamin D supplementation (OR=1.26, 95%CI=0.94-11.23). Gender (p=0.17), age (p=0.12), marital status (p=0.20), and education level (p=0.34) did not correlate with vitamin D intake. While psoriasis patients are being advised to take vitamin D supplement, 27% of survey participants with psoriasis do not take vitamin D, and supplementation patterns differ across ethnicities. This points to subsets of psoriasis patients who may be at increased risk of vitamin D deficiency. This is especially important to correct given the benefits of vitamin D supplementation in psoriasis-related long-term comorbidities.

Vitamin D supplementation in psoriasis patients is impacted by ethnicity

SP Patel1 and AL Chien1 1 Dept of Dermatology, JHMI, Baltimore, MD and 2 Dept of Derm, Johns Hopkins Univ, Baltimore, MD

Vitamin D supplementation has been associated with improvement in psoriasis severity. These effects are especially pronounced with psoriasis comorbidities including metabolic syndrome and cardiovascular disease. However, vitamin D supplementation patterns among psoriasis patients are not known. Using the 2013-2014 National Health and Nutrition Examination Survey (NHANES) database, we conducted a population-based cross-sectional analysis with survey data from 9770 participants to investigate vitamin D supplementation patterns and the physician's role in these patterns. We calculated odds ratios (OR), 95% confidence intervals (95%CI), and p-values using logistic regression and ANOVA weighted to reflect the US Census data, adjusting for age, gender, marital status, education, and race. Vitamin D supplementation was found in 72.4% of participants with a history of psoriasis versus 56.0% of participants without psoriasis (OR=1.59, 95%CI=0.91-2.69). Vitamin D supplementation of psoriasis was associated with significantly increased odds of physicians recommending supplementation (OR=5.92, 95%CI=2.04-17.2). Moreover, ANOVA analysis revealed mean vitamin D supplementation doses increased significantly with increasing severity of psoriasis (p<0.001). However, supplementation patterns differed by ethnicities with Hispanic patients having significantly lower odds of supplementing compared to non-Hispanic patients (OR=0.17, 95%CI=0.04-0.77). Among Hispanic psoriasis patients, it was also uncertain whether physicians were likely to recommend vitamin D supplementation (OR=1.26, 95%CI=0.94-11.23). Gender (p=0.17), age (p=0.12), marital status (p=0.20), and education level (p=0.34) did not correlate with vitamin D intake. While psoriasis patients are being advised to take vitamin D supplement, 27% of survey participants with psoriasis do not take vitamin D, and supplementation patterns differ across ethnicities. This points to subsets of psoriasis patients who may be at increased risk of vitamin D deficiency. This is especially important to correct given the benefits of vitamin D supplementation in psoriasis-related long-term comorbidities.

Vitamin D supplementation in skin cancer patients is impacted by gender and education, but independent of sun protective practices

SP Patel1 and AL Chien1 1 Dept of Dermatology, JHMI, Baltimore, MD and 2 Dept of Derm, Johns Hopkins Univ, Baltimore, MD

Vitamin D has been shown to protect against skin cancer. Ultraviolet B radiation is needed for vitamin D production, but it is also a significant risk factor for non-melanoma skin cancer (NMSC). Thus, vitamin D supplementation is important when sun-protection is practiced in patients with NMSC. However, supplementation patterns among these patients have not been reported. Using the 2011-2014 National Health and Nutrition Examination Survey database, we conducted a population-based cross-sectional analysis with survey data from 9770 participants to investigate vitamin D supplementation and the physician's role in these patterns. We calculated odds ratios (OR), 95% confidence intervals (95%CI), and p-values using logistic regression and ANOVA weighted to reflect the US Census data, adjusting for gender, education, race, marital status, and age. Vitamin D supplementation was significantly more common in patients with a history of NMSC with 84.6% taking supplements versus 55.5% of participants without NMSC (OR=2.09, 95%CI=1.25-3.47). The mean dose of vitamin D intake was also significantly higher in patients with NMSC (M=60.3±6.86 mcg versus M=34.4±6.66 mcg, p<0.05). Moreover, physicians had significantly higher odds of recommending supplementation in patients with NMSC (OR=2.41, 95%CI=1.01-5.71). Among NMSC patients, males (OR=0.17, 95%CI=0.06-0.45) and patients without a college degree (OR=0.32, 95%CI=0.11-0.89) had significantly lower odds of supplementing with vitamin D. There was no difference in vitamin D intake across sun protective practices (p=0.58), race (p=0.07), and marital status (p=0.41). While it is reassuring that individuals with a history of NMSC are supplementing with vitamin D, the data identifies a subset of skin cancer patients who may not take vitamin D. Furthermore, vitamin D supplementation is not influenced by sun protection measures. Improvements are needed in counseling patients regarding the importance of vitamin D and in managing the delicate balance between sun protection and vitamin D supplementation in skin cancer patients.

Teledermatology and text messaging in rural and remote British Columbia: A survey of primary care providers

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Teledermatology has been proposed as a strategy to improve dermatology access to under-served populations. One such population is in rural and remote British Columbia (BC). To gain an understanding of utilization patterns and attitudes we distributed a 14-question survey to primary care electronic and hard copy to health professionals working in rural and remote areas of BC. 81 surveys were completed by family physicians, emergency physicians, internists, surgeons and other health care professionals. The average age of respondents was 50.3±11.8 years. Only 57% of respondents reported having access to in-person specialist dermatologist consultations. Tele-dermatology services included store-and-forward teledermatology (46%), videoconferencing (5%), telephone consultation (52%) and in-person non-specialist dermatology services including family physicians with a special interest in dermatology (36%). The most commonly cited barriers to dermatology care were waiting times (93%) and distance required to travel (75%). Videoconferencing or store-and-forward were used “rarely” or “never” by the majority of respondents (69%) with the most common reason for this being unaware of such services (35%). In terms of test messaging, 90% of respondents reported using text messaging in their clinical practice with 6% using it multiple times per day. The majority of respondents reported sending clinical images electronically (32% by mobile phone only, 17% by email only and 30% by both methods). Our results demonstrate that tele-dermatology use remains limited in rural and remote BC and that the expansion of services that utilize providers’ mobile phones and/or personal computers—a practice that is clearly already commonplace.
Clinical and histological characteristics of lentigo maligna vs lentigo maligna melanoma in a state-wide cohort

H Higgins Snyder 1, M Chren 2, N Admassu 3, C21, M Pimentel 2, M Halley 3 and E Linos 4
Clinical Research: Epidemiology of Skin Diseases | ABSTRACTS

Clinical and historical characteristics of lentigo maligna vs lentigo maligna melanoma in a state-wide cohort

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Ichthyoses are genetic skin disorders characterized by scaly, variably red skin caused by mutations. Having skin pain (p < 0.001), temperature adaptation difficulty (p < 0.001), and eye problems (p < 0.001) were associated with lower QoL. A positive linear relationship was found between the CDLQI and age (r = 0.44; CI: 0.21-0.91). AD was associated with worse scores for the HADS anxiety and depression indices (p < 0.001). A higher burden on HRQoL and an increase likelihood of anxiety and depression was observed with moderate or severe disease compared to mild disease. Future studies should seek to increase our understanding of the epidemiology and risk factors for AD in adults.
381 Association between peripheral eosinophil counts and atopic dermatitis severity in a longitudinal clinical cohort
V Singhania 1, R Vail 2, R Chopra 1, R Kantor 1, B Nardone 1 and JII Silverberg 1

Previous studies found conflicting results about the utility of peripheral eosinophils as a biomarker of AD. AD is associated with variable signs, comorbidities and severity, which may impact peripheral eosinophil counts. We hypothesized that peripheral eosinophil count is a good biomarker of AD severity in adults, and associated with distinct phenotypes. We performed a prospective study of 292 adults (age 18-98 yrs) with AD to determine the relationship of AD severity and phenotype with absolute peripheral eosinophil counts. Elevated eosinophil counts (≥600 cells/mcl) were associated with lifetime history of asthma (Chi square, P=0.003), hay fever (P=0.02) and anterior subcapsular cataracts (P=0.01), the presence of active dermatitis affecting the flexures (P=0.016), anterior neck folds (P=0.03), face (P=0.006), eyelids (P=0.016) and feet (P=0.02), and palmar hyperlinearity (P=0.005). In longitudinal mixed models controlling for demographics, allergic disease and phenotypical features, peripheral eosinophil counts (cells/mL) log-linear link function were significantly associated with total score and severity counts (n=289). DSCORD, NRSitch, POEM, and DLQI, and self-reported severe AD (P=0.01 for all). Similar results were found in models of high peripheral eosinophilic counts (≥600 cells/mL, logit link function). In mixed models that also accounted for systemic AD therapy, use of oral cyclosporine (P=0.001) or methotrexate (P=0.006) was associated with significantly lower eosinophil counts; nevertheless, the associations between AD severity and eosinophil counts remained significant (P<0.01). The present study shows a complex relationship of AD phenotype, severity and comorbidities with peripheral eosinophil counts. While the pathogenic role of eosinophils remains to be determined in AD, absolute peripheral eosinophilic counts may be a reasonable biomarker for AD in clinical practice.

382 Association between chronic inflammatory skin disease and autoimmune disease in US adults
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Psoriasis (PSO), atopic dermatitis or eczema (ADE), pemphigus, bullous pemphigoid (BP), hidradenitis (HS) and dermatomyositis (DM) are chronic inflammatory skin disorders (CISD) associated with systemic immune activation and dysregulation, and possible autoimmune disease. We sought to determine whether these CISD are associated with increased risk of comorbid autoimmune disease. Data from the 2002-2012 National Inpatient Sample were analyzed, including a representative 20% sample of all US hospitalizations (n=72,108,077 adults). Multivariable logistic regression models examined the association between CISD and autoimmune disorders, and included age, race/ethnicity, gender and insurance status. Overall, all of the CISD were significantly associated with ≥1 autoimmune disorder. However, distinct patterns of comorbid autoimmune disease were observed for different CISD, including hepatobiliary (autoimmune hepatitis and primary biliary cirrhosis [significantly associated with PSO, HS, DM], non-alcoholic steatohepatitis [ADE, PSO, HS, DM]), gastrointestinal (Crohn’s disease [AD, PSO, HS]), ulcerative colitis [AD, PSO, HS, pemphigus], eosinophilic esophagitis [ADE, BP], cutaneous (vitiligo [all], alopecia areata [ADE, PSO, HS]), musculoskeletal (ankylosing spondylitis [AD, PSO, HS], rheumatoid arthritis [all]), hematologic (pernicious anemia [ADE, PSO, pemphigus], DM, autoimmune hemolytic anemia [AD, PSO, pemphigoid, DM], immune thrombocytopenic purpura [AD, PSO, pemphigus, DM]), and Hashimoto thyroiditis (ADE, PSO, pemphigus). Giant cell arteritis (PSO, pemphigus). Predictors of autoimmune comorbidities included, being female, non-white race, public or no insurance and varying age. Autoimmune comorbidities were associated with increased length of stay, cost of care and inpatient mortality in persons with AD. Further studies are needed to confirm these associations and understand the mechanisms and specific risk factors for comorbid autoimmune disease in CISD and develop interventions to screen and monitor for them.

383 Doxycycline effects on the gut and skin microorganisms and lipidome in acne
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Long courses of antibiotics (1 month to >1 year) are often prescribed for acne, and may contribute to widespread antibiotic resistance. One of the most common is doxycycline, though its mechanism of action and nontarget effects are unknown. To determine its effects on the gut and the skin as well as unintended effects on the microbiome, we compared lipid mediator profiles in blood and sebum, short and long chain fatty acids in feces and plasma, the skin microbiome, and the gut microbiome in acne subjects on doxycycline and in healthy controls. Healthy (n=14) and acne (n=15) subjects (14-19 years) were recruited for the study. Both groups were sampled after a one week washout; acne subjects were sampled again (n=10) after 8 weeks of doxycycline (100mg/day). A sub-cohort (n=3) was resampled 16 weeks after completion of doxycycline. Acne severity decreased after doxycycline; fecal propionate and butyrate, and plasma acetate also significantly decreased (P<0.02). Healthy gut microbiomes had slightly lower Shannon diversity indices than acne subjects. Doxycycline lowered gut bacterial diversity (P=0.037) sub-control levels, although this diversity was recovered by follow-up. By comparing healthy and acne gut microbiomes, and assaying which taxa doxycycline affected, we were able to correlate certain taxa with acne and others with health, as well as to determine a putative mechanism of doxycycline. Many taxa affected by doxycycline and differentially abundant species between acne and controls are butyrate producers, hinting at their role in acnegenesis and explaining our feacal butyrate findings. A subset of acne patients had high levels of Verrucomicrobia, which cleared with doxycycline, but may be responsible for a new class of associated acne. These findings suggest a link between gut health and acne, which can be exploited for novel treatments.

384 Assessing dermatologic care of uninsured patients managed at free clinics
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Free clinics manage uninsured patients with a wide variety of diseases, providing them with care that they would not be able to receive elsewhere. Owing to the fact that the uninsured population is considered outside of the healthcare system, there is limited research on the care provided to these patients. This study sought to assess the dermatologic care provided to uninsured patients. Chart reviews of 4,804 patients managed at free clinics around the Tampa Bay Area during 2016 were carried out to determine demographics, disease diagnoses and visit data. It was determined that about 5% of patients presented with some sort of dermatologic chief complaint, with the most common being a rash or new lesion. Less common visits involved fungal infections, skin cancer follow-up and acne. In addition, a few of the clinics had designated dermatology days where appointments could be scheduled specifically for dermatologic concerns. This allowed patients with previously diagnosed skin conditions and monitor for them.

385 Scleromyxedema
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Scleromyxedema is a disease characterized by papular mucinous deposits, dermal fibroblast proliferation, and is associated with monoclonal paraproteinemia. Systemic manifestations are commonly observed and can lead to significant morbidity and mortality. Being an extremely rare disease, its prevalence and clinical course are poorly reported. We sought to describe the characteristics of patients with scleromyxedema regarding clinical characteristics, psychological impact, therapeutic interventions, and course through anonymous online questionnaire. A total of 36 patients responded between April 2015 to December 2017, 63% (23) women with mean age 56.75% (27) of the patients reside in North America, 14% (5) in Europe, and others across the world including Africa, Asia and Australasia. Over 60% of the patients described tightness of the skin, papules and nodules, swelling, waxy appearance of the skin associated with pruritus. Interestingly, 47% (17) also reported hair loss. Extracutaneous manifestations were present in 88% (33). The mean time from symptom to diagnosis was 1.3 years and 12 patients saw an average of 5 doctors before the diagnosis was made; 78% (27) in Dermatology and 17% (6) in Rheumatology. 97% (34) patients had skin biopsies, 3 patients underwent bone marrow analysis and 3 patients had chest x-rays. The most efficacious treatment is intravenous immunoglobulin in 58% (21) of patients followed by corticosteroids in 28% (10) and monoclonal antibody (Bortezomib) in 2 patients, 1 patient underwent stem cell transplant inducing partial remission. Over 50% of the participants scored moderate to severe in the GAD-7 anxiety score with a mean DQLI of 15.4/30. The relationship between scleromyxedema and unexplained pain syndromes needs further investigation, especially in those with severe systemic manifestations. There is often a delay to diagnosis due to non-specific symptoms. Intravenous immunoglobulins, along with corticosteroids and monoclonal antibodies are relatively effective treatments. Clinicians must be mindful of the enormous psychological impact of scleromyxedema on patients lives.
386 Regulatory cells may not be the answer: A prospective study of ECP in eGVHD
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Background: Extracorporeal photopheresis (ECP) is used to treat chronic graft-versus-host disease (cGVHD). Regulatory T cells (Tregs) are a potential mechanism. We conducted a prospective multicenter clinical trial to assess association of Tregs with skin response to ECP. Methods: 83 patients with cGVHD enrolled, 9 were excluded due to absent follow up and 8 due to absence of skin cGVHD. 6 months of ECP was recommended: twice a week for 4 weeks, twice a week every 2 weeks for 8 weeks and as determined by provider. Response was assessed by 2005 NIH criteria. The frequency of circulating Tregs was quantified within the CD4+ T-lymphocyte population before and after completion of ECP using flow cytometry on peripheral blood (n=15). Cutaneous lymphocyte-associated antigen (CLA+) skin-homing Tregs were enumerated as % within total Treg population. Continuous variables were compared using Wilcoxon rank sum test (R). Results: 66 patients (67% male), with a median age 50 (34–74). The median percentage of Tregs from baseline to last visit 6.8 [3.0—9.1] vs. 1.7 [0.0—7.2] p < 0.01. BSA did not change significantly. 55% of patients had a skin response. 29% had sclerosis, 41% erythema and 30% both. In a logistic regression model, % Tregs and CLA+ Tregs at study entry and completion did not differ between skin responders and non-responders. Conclusions: Our study is one of few to investigate Tregs in ECP for cGVHD. Skin homing Tregs were not increased in patients with skin response. Tregs are not a predictor of response. This received research funding and this investigator-initiated research was funded by Therakos, A Mallinckrodt Pharmaceuticals company.

387 A quicker tanck smear test with methylene blue staining in diagnosis of herpesvirus infections comparative study with giema stain
IGADA and local SCORAD. However, this trend should be interpreted with caution due to the eczematous skin under occlusion) resulting in known substance-related pharmacological dose of 1% permeated into the circulation (due to a potentially increased drug penetration in

388 A phase Ib trial in AD patients evaluating safety, tolerability, pharmacokinetics and efficacy of topical formulations containing the kappa-opioid receptor agonist WOL071-007
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Novel kappa-opioid receptor (KOR) agonist WOL071-007 (7) exhibited remarkable anti-inflammatory activity in mouse models of dermatitis and psoriasis. Experiments with pharmacological blockade and in KOR KO mice revealed that its effects are indeed mediated by KOR. We tested 7 in a first in human, single center, combined SAD/MAD (Phase Ib), double in children with waardenburg syndrome (WS) diagnosed by ophthalmologic examination and genetic testing. Objective: The aim of the study was to assess the association between ECP and WS.

389 Association between aging-related morphological changes of the upper eyelids and thickness of the orbicularis oculi muscle
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Objective: The eyelids are parts where signs of aging become markedly apparent and characteristics of thin skin and subcutaneous fat tissue and of high muscle proportion compared with other parts of the face. In addition, the orbicularis oculi muscle is known to undergo atrophy with aging (Lee et al., 2012). Therefore, the present study examines the association between aging-related morphological changes of the upper eyelids and thickness of the orbicularis oculi muscle. Methods: Skin surface imaging and magnetic resonance imaging of the upper eyelids were performed to examine the relationship between the morphology of the upper eyelids and thickness of the orbicularis oculi muscle. Immunohistochemical staining of the orbicularis oculi muscle was performed to quantify the intensity of staining of myofilibrillar proteins between young and elderly populations. Results: Subjects with apparent morphological changes in the upper eyelids had reduced thickness of the orbicularis oculi muscle compared with those with little morphological changes of the upper eyelids. The elderly populations orbicularis oculi muscle tissues were thinner than the younger population, and there were changes in the composition of myofilibrillar proteins. Discussion: Our findings suggest that aging-related thinning of the orbicularis oculi muscle is caused by changes in the composition of myofilibrillar proteins, leading to morphological changes of the upper eyelids. Thus, the orbicularis oculi muscle may be a critical target in reducing aging-related signs in the upper eyelids.

390 The microbe exists in the ‘sterile’ pustule of palmoplantar pustulosis
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Palmoplantar pustulosis (PPP) is a chronic skin disease characterized by infectious-like pustules on the palms and soles. These pustules are thought to be sterile because bacterial cultures obtained from the pustules are negative, and the mechanism of sterile pustule formation is still unclear. As standard culture methods are limited in their ability to identify all bacteria on the skin, it is still possible the pustule includes some bacterium. To elucidate whether bacteria exist in sterile pustules of PPP using non-culture methods, we conducted

391 Drug-induced lupus erythematosus caused by selective serotonin reuptake inhibitors
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Drug-induced lupus erythematosus (DILE) is an uncommon autoimmune disorder caused by certain drugs, most commonly hydralazine and procainamide. While there are currently no definitive diagnostic criteria for DILE, the clinical syndrome encompasses features similar to classic lupus erythematosus (SLE), subacute cutaneous lupus erythematosus (CLE), or discoid lupus erythematosus (DLE). We present a patient who developed DILE twice on separate occasions from paroxetine and fluoxetine. Objective: A 45-year-old man with depression presents with itchy, spreading bumps on his upper torso, back, and arms for the fourth, three months apart. The patient was diagnosed with DILE and SLE after taking paroxetine for three years. These resolved after stopping paroxetine. He has no significant family history, his only other medication is albuterol, and a review of systems was normal. On examination, he has scattered, scaly, annular, erythematous papules and pustules on his chest, back, and bilateral upper arms. Skin biopsy revealed findings consistent with SLE. Laboratory tests found elevated anti-antineur antibodies (IgG, 1.7; IgM, 5.6). Methods: A literature review was performed using PubMed. Search terms included lupus, antinuclear, and selective serotonin reuptake inhibitor. Results: There is one report of a selective serotonin reuptake inhibitor (SSRI), sertraline, causing DILE with elevated anti-antineur antibodies in the literature. There are two other cases in the literature of SRLs, citi-
392 Next-generation sequencing identifies epidermal miRNAs deregulated in psoriasis skin
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Psoriasis is a common chronic inflammatory skin disease, affecting 2–4% of world population. MicroRNAs (miRNAs) are short non-coding RNAs that regulate expression of the majority of protein-coding genes. In psoriasis, previous studies have identified deregulated miRNA expression. Most of these studies utilized full-depth skin biopsies and thus may have missed the cell-specific miRNA signature. Here, we analyzed the miNome of CD45+ sorted epidermal cells from psoriasis patients and healthy skin by next-generation sequencing. We detected, differential expression of 104 miRNAs in the psoriatic epidermal cells, including several known and novel miRNAs. MiR-149 was identified as one of the significantly downregulated miRNAs, and qPCR analysis confirmed its downregulation in psoriatic lesional epidermal cells as compared to non-lesional or normal skin. In primary human keratinocytes and in 3D epidermal equivalents, miR-149 was significantly downregulated by IFN-γ. Overexpression of miR-149 decreased IL-6 and TNFα secretion. We demonstrated that overexpression of miR-149 suppressed the IFN-γ-induced expression of IL-6 as well as CXCL1, CXCL10, and CXCL11. In addition, the cytotoxicity and the effector functions of CD8+ T cells towards keratinocytes were significantly increased by overexpression of miR-149. The cytokine profile of T cells co-cultured with 3D epidermal cell cultures was significantly higher in the presence of miR-149. Overall, these findings offer a basis for further functional studies of miRNAs in keratinocytes and lead to the identification of potential targets for topical therapy in psoriasis.

393 A trial to determine the effect of psoriasis treatment (adalimumab, phototherapy, and placebo) on cardiometabolic disease: The vascular inflammation in psoriasis (VIP) trial
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Psoriasis is a chronic inflammatory skin disease associated with cardiovascular risk factors. The current VIP-E trial compared adalimumab (Ada), phototherapy, and placebo on cardiometabolic disease outcomes. GlycA. 92 patients (mean age 43, 69% male, mean PASI 19) completed wk 12. PASI75 was achieved by 47%, 47%, and 7% in the Ada, phototherapy, and placebo group, respectively, at wk 12 (p = 0.002). There was no difference in change in aortic inflammation at wk 12 in the adalimumab group compared to placebo (-7.6%, 95% CI: -14.0% to 9.6%) or the nBuVB group (-0.1%, 95% CI: -5.6% to 5.4%) or after 52 wks of adalimumab treatment (0.02% compared to initiation, -2.8%, 95% CI). Analysis with groups demonstrated a significant reduction in aortic inflammation by -4.0% (95% CI: -7.7% to -0.3) in the nBuVB arm only. Both adalimumab and nBuVB decreased inflammation as assessed by serum CRP (p < 0.001) and IL-6 (p = 0.02), but only adalimumab reduced TNF levels and GlycA at 12 and 52 wks (p = 0.006). No significant differences were observed on metabolic markers (insulin, adiponectin, leptin) whereas only nBuVB increased HDL at 12 weeks (p = 0.03). At 52 wks of adalimumab, HDL cholesterol fell (p = 0.001) and gp-100 (p = 0.001) were reduced. Despite no change in aortic inflammation and minimal improvement in lipids and insulin resistance, Ada reduced GlycA levels, a biomarker predictive of coronary disease.

394 Nail psoriasis as a predictor of skin response to ixekizumab in patients with moderate-to-severe psoriasis
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Introduction: Nail psoriasis (NAPSI) has been associated with disease severity in psoriasis patients. We determined whether NAPSI was associated with response to ixekizumab (IXE) in patients with moderate-to-severe psoriasis in two randomized Phase 3 trials comparing ixekizumab to placebo. Methods: A post-hoc, subgroup analysis of the Phase 3 ixekizumab trials was performed, comparing patients with NAPSI C21 at baseline (NAPSI+ Ig = NAPSI/C21) with NAPSI = 0 (NAPSI−). We used PASI 75, 100, and the sPGA at 12 weeks as efficacy endpoints. Results: A total of 1302 patients were included in the analysis: 442 with NAPSI+ and 860 with NAPSI−. Treatment with IXE led to more rapid and more durable PASI 75 response compared with placebo (IXE vs placebo, 58.3% vs. 10.7%, p < 0.0001). This difference was noted from week 2 onwards and was sustained through week 52. NAPSI− patients achieved PASI 75 at significantly lower rates than NAPSI+ patients (15.5% vs. 20.5% at week 12, p = 0.001). Furthermore, patients with NAPSI+ had significantly higher sPGA compared with NAPSI− at baseline. However, at Week 12, NAPSI+ patients had significantly lower sPGA compared with NAPSI− (37.9% vs. 45.2%, p = 0.0001). Conclusion: Nail psoriasis can be a predictor of skin response to ixekizumab in patients with moderate-to-severe psoriasis.

395 Decrease of epidermal galectin-3 contribute to the pathogenesis of psoriasis by promoting keratinocyte activation
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Psoriasis-specific proteins in keratinocytes that are dysregulated and involved in the pathogenesis of psoriasis remain to be elucidated. We reported here that galectin-3 (Gal-3) expression was remarkably decreased in epidermis in lesional skin, but not in non-lesional skin in psoriatic patients, nor was in a group of diseases as psoriasis dermatitis. Contrarily, Gal-3 in dermis and circulation was elevated in psoriasis patients. The deficiency of epidermal Gal-3 was sufficient to promote the development of psoriatic lesions. This was evidenced by more severe psoriasis-like inflammation induced by imiquimod in Gal-3 knock-out (Gal-3−/−) mice than in wildtype mice, and by the vigorous inflammation in skin graft of Gal-3−/− mice after transplant onto wildtype mice. The development of psoriatic-like lesions is attributable to the spontaneously tuning up of psoriasis signatures in keratinocytes; and partly to neutrophil accumulation mediated by the overexpression of S100A9 and CC4CL-1. In addition, the cytotoxicity and the effector functions of CD8+ T cells in lesions were significantly reduced in the Gal-3−/− mice by intracutaneous injection of recombinant Gal-3. As for the mechanism of its downregulation, co-culture with IFN-γ decreased the expression of Gal-3 in keratinocytes; DNA methylation of Gal-3 promoter and expression of related transcriptional factor was comparable between psoriatic and normal epidermis; and no microRNA in psoriatic keratinocytes was up-regulated. The result of the 4-chromatin immunoprecipitation findings offer a promising Gal-3-related diagnostic and therapeutic resolutions for psoriasis.
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A granular parakeratosis animal model created by topical aluminum showed a characteristic keratinocyte differentiation abnormalities including abnormal profilaggrin-positive suprabasal cell layer and barrier dysfunction leading to wound repair type tissue remodeling and earlier CE formation. Apoptosis-associated cytokine genes such as Fas, lepetin, and TNF were significantly upregulated in the lesioned skin. Transglutaminase activity was upregulated from the suprabasal layer to the upper spinous layer. Apoptosis-related gene such as bcl-2 were also upregulated. These results suggest that aluminum induces keratinocyte differentiation arrest and barrier dysfunction leading to wound repair tissue remodeling and earlier CE formation in the skin.

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Preclinical studies support combined inhibition of BET family proteins and histone deacetylases as epigenetic therapy for cutaneous T-cell lymphoma

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Advanced-stage cutaneous T-cell lymphoma (CTCL) is often a fatal malignancy despite available therapy for CTCL.

Preclinical studies support combined inhibition of BET family proteins and histone deacetylases as epigenetic therapy for cutaneous T-cell lymphoma. Histone deacetylases as epigenetic therapy for cutaneous T-cell lymphoma.

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CD8+CD103+ skin resident memory T cells are a subpopulation of CD8+MDR-1+ cells in lesional skin of psoriasis and correlate with the clinical course

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Resident memory T (T RM) cells persist in the epithelial barrier tissue and may be involved in the pathogenesis of inflammatory diseases. Meanwhile, our preliminary study showed that T cells expressing CD103 or MDR-1, are present in psoriatic skin and produce pro-inflammatory cytokines. In this study, we sought to characterize the CD103+ skin T RM cells in relation to MDR-1 expression, and to investigate the association of the T RM cells with the clinical course of psoriasis. Stocked skin-infiltrating T cell samples obtained from 10 psoriasis patients were used. The frequency of T RM cells, skin biopsy specimens from psoriatic plaques were cultured with IL-2 and anti-CD3/CD28 antibody. The T cell phenotype and function were assessed by intracellular cytokine staining (ICS).

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Methodology to evaluate the photosensitivity potential of a systemically administered investigational product in healthy volunteer subjects

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1 Medication Communications, TRK Research, Inc., Fair Lawn, NJ, 2 Data Management, Biostatistics, and Medical Communications, Fair Lawn, NJ, 3 Dermatology Safety Operations, TRK Research, Inc., Fair Lawn, NJ, 4 Clinical Research, TRK Research, Inc., Fair Lawn, NJ, 5 Clinical Studies, TRK Research, Inc., Fair Lawn, NJ and 6 Executive, TRK Research, Inc., Fair Lawn, NJ, 7 Executive, TRK Research, Inc., Fair Lawn, NJ (TKL designed and implemented a Phase 1 clinical trial to assess the safety of a systemically administered investigational product (IP) using a partially-blind, randomized, parallel group, placebo-controlled study design. Healthy volunteers were enrolled and randomized in a 3:1 manner to receive the IP or placebo (Part A) or the known photosensitizing agent ciprofloxacin (Part B). Subjects in Part A and B received the drug (IP or placebo) at a single dose of 200 mg. For Part A, patients were randomized to two groups: photosensitization testing for 12 hours after dosing and 72 hours after dosing. For Part B, patients were randomized to two groups: photosensitization testing for 12 hours after dosing and 72 hours after dosing. The International Conference on Harmonization S10 Photosafety Evaluation of Pharmaceuticals Guidance outlines the methodology for photosensitization testing. The IP absorbs photons in the 290-700 nm range, generating a reactive species following absorption of UV-visible light, and/or distributes sufficiently to light-exposed tissues such as the skin or eyes. Photosensitivity was determined by calculating the minimal erythema dose (MED) for skin exposure to a series of ultraviolet light A (UVA)/UVB exposures. Skin test sites were analyzed for erythema and superfi cal skin reactions. A photosensitivity study conducted with this design will yield the following results for the IP: positive control, and placebo at predetermined timepoints: MED > 250 nmol, and MED > 500 nmol at 290-700 nm wavelength. Additionally, safety and tolerability were assessed via monitoring of adverse events. We propose this design as a new standard for photosensitivity clinical trials.
Genetic variants in TLIR, TIRAP and PSAPL1 are enriched in a specific subgroup of adult atopic dermatitis showing persistent skin manifestation on the face. Y. Yasuda-Sekiguchi, A. Shishohama, H. Kawasaki, A. Kubo, M. Amagai, and T. Sasaki 1 Keio Univ., Tokyo, Japan, 2 KOSE Endowed Prog. Keio Univ., Tokyo, Japan, 3 RIKEN Center, Japan, 4 Keio Univ., RIKEN-IMS, Shinjuku-ku, Tokyo, Japan, 5 S CSMR Keio Univ., Shinjuku-ku, Tokyo, Japan

Atopic dermatitis (AD) is a multifactorial disease, and the heterogeneity of clinical characteristics in AD might be partly based on difference in genetic factors. In this study, we focused on a clinically-characteristic AD subgroup and investigated genetic variants enriched in the subgroup. We named the subgroup as face and neck type adult AD (FNaAD) based on the tendency of skin eruptions on the face and neck to persist even after standard AD treatment. Among 94 AD patients who regularly visited our clinic, patients screened positive for FNaAD by using genomic DNA of FNaAD patients, we performed target re-sequencing of 64 genes known to be associated with AD, skin barrier, inflammation, and innate immune responses. The allele frequency comparison with Japanese controls (n = 1,208) from the Human Genetic Variation Database indicated 12 variants as candidate uncharacterized variants (p < 0.05). Further analysis of these 12 variants in FNaAD and other AD patients revealed that 3 variants in TLR1, Toll-interleukin 1 receptor domain containing adaptor protein (TIRAP), and proapoptosis like 1 (PSAPL1) were significantly enriched in the 18 FNaAD patients (p < 0.003). TLR1 and TIRAP are known as members of the TLR pathway that play an important role in innate immune responses. PSAPL1 is an uncharacterized protein specifically expressed in the epidermis. Among the 3 variants, the TIR1 p.L144P variant was isolated in 6 FNaAD patients (33.3%) while p.L144P is non-common variant in the Japanese population (minor allele frequency: 0.038), which is excluded from targets in a conventional genome-wide association study. Altogether, we identified 3 enriched variants in a specific AD subgroup by using a combination approach of clinical subgrouping and screening for genetic variants which would enable to detect even non-common variants associated with AD subgroups.

Novel injectable coolant for treatment of pain 1 Ganibyan, 1 S Moradi Tuchayi, 1 W Yiang, 1 S Osoeitan, 1 M Parschel, 1 C Evans, and 1 R Anderson 1 Harvard Medical School, Wellman Center for Photomedicine, Boston, MA and 2 Shanghai Tenth People’s Hospital, Tongji University, Shanghai, China

Cutaneous pain management is an unmet need. Current treatment options include medications such as analgesics and narcotics, which have high risk of addiction, and limited success. A long-lasting, drug-free treatment for cutaneous pain is needed. We had previously shown that targeted cooling of the sciatic nerve is safe and effective in reducing mechanical and thermal pain sensation. While topical cooling can be used for targeting superficial nerves of a confined area, blocking cutaneous pain with larger and deeper cutaneous malignancies can be achieved by targeting deeper nerves. We invented an injectable coolant for targeting deeper nerves as a novel treatment for pain. Using the rat sciatic nerve model, we studied the safety, feasibility and efficacy of the coolant in selectively and reversibly targeting the sciatic nerve, and at various time points, the nerve was harvested for histologic and imaging studies. Coherent anti-Stokes Raman scattering microscopy showed myelin degradation followed by full recovery in 4 months. Quantification of degenerative nerve fibers showed significant increase in mean nerve degeneration score of 1.66 at day 7 post injection, 2.66 at day 14 post injection, with complete recovery at 4 months post treatment, (p < 0.05). Immunofluorescence and electron microscopy showed degenerative changes in myelinated and unmynelinated axons, with complete recovery by 4 months. Sensory functional assays showed significant reduction of mechanical pain post treatment that lasted for 2 months. Thus, the injectable coolant is safe and effective in reversibly targeting the sciatic nerve for prolonged duration. We are now ready to test this in human clinical trials as a novel and long lasting treatment for pain.

Assessment of variables in perception of noxious and non-noxious stimuli in dermatological surgery patients R Kazi and B Carroll University of Pittsburgh Medical Center, Pittsburgh, PA

Various dermatologic treatments necessitate that patients experience pain, the most common being in office injection of local anesthetic prior to invasive procedures. Indeed, across all medical fields, there are over 100 million annual administrations of local anesthetics and epinephrine. The pain of the injection causes a significant number of patients to experience anxiety and, as such, many patients actively avoid treatment of potentially devastating cutaneous malignancies. The aim of our study is to discern the clinical and ultimately physiological variability by which pain and tactile perception is experienced across cohorts of surgical patients. Studies have delineated a milieu of factors by which pain perception varies including age, gender, psychological parameters, and history of chronic pain. However, few studies have investigated the results in the setting of dermatological surgery. By then broadly categorizing patients into pain-tolerant and pain-intolerant subgroups, we test how these two groups differentially interpret non-noxious stimuli, such as hot, cold, and vibration. We hypothesize that pain-tolerant patients will have a different interpretation of pain compared to those who interpret noxious stimuli. Our final aim is to preempt patients with a non-noxious stimulus and then analyze how immediately subsequent pain perception changes. While prior studies from our group have shown that early stage of vibration immediately induces non-noxious sensation, this study reduces the pain of injections, there is no understanding how pain masking varies across different pain-tolerant cohorts. Future studies will aim to determine the specific neuronal pathways involved in pain-masking and augmentation. Our working hypothesis is that pain-masking is a physiological augmentation of a specific neuronal fast spiking Aδ and slow unmyelinated C fibers. Ultimately, this information will allow us to derive patient specific, algorithmic augmentation of tactile stimulation to minimize the perception of pain during procedures.

Development of a scoring system for the prediction of the outcome of intravenous corticosteroid pulse therapy in rapidly-progressive alopecia areata in Japan 1 M. Kishimoto, Y. Ogawa, U. Ujiie, N. Hama, Y. Fujita, R. Abe, S. Shimada and T. Kawamura 1 University of Yamanashi, Chuo, Yamanashi, Japan, 2 Hokkaido University, Sapporo, Hokkaido, Japan and 3 Niigata University, Niigata, Japan

Intravenous corticosteroid pulse therapy (pulse therapy) has been reported to be effective for rapidly-progressive alopecia areata (RPAA). However, the outcome can hardly be predicted at an early time point of the course. In this study, we attempted to establish a scoring system for predicting the outcome of intravenous corticosteroid pulse therapy by retrospectively analyzing clinicopathological and immunological characteristics of RPAA patients treated by pulse therapy. Twenty-two cases (10 females and 12 males, average age 35.6) were administered 500mg/day of intravenous methylprednisolone for 3 consecutive days. The efficacy was assessed at 6-month post-treatment by digital image analysis based on the recovery from the worst clinical manifestation. The analysis detected positive correlations between the extent of recovery and female gender in those without atopic background (p = 0.024) and the absence of atopic dermatitis, the presence of good responder cases differentially interpreted non-noxious stimuli, such as hot, cold, and vibration. We hypothesize that those with a predilection for pain perception will show less tolerability of non-noxious stimuli. Future studies will aim to determine the specific neuronal pathways involved in pain-masking and augmentation. Our working hypothesis is that pain-masking is a physiological augmentation of a specific neuronal fast spiking Aδ and slow unmyelinated C fibers. Ultimately, this information will allow us to derive patient specific, algorithmic augmentation of tactile stimulation to minimize the perception of pain during procedures.
High amphiregulin expression is a high-risk feature of acute graft-versus-host disease of the skin

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Background: Acute graft-versus-host disease (aGVHD) is an unpredictably severe complication of autologous hematopoietic cell transplantation (HCT). Recent studies suggest that tissue repair factor, amphiregulin (AREG), an epidermal growth factor (EGF) receptor ligand, is over-expressed in aGVHD target organs. Therefore, we investigated skin AREG expression in aGVHD and determined its association with clinical outcomes.

Methods: We analyzed by immunohistochemistry skin tissues of 67 patients with aGVHD cases, high skin AREG (>0.2) was significantly higher between pts-HCs (0.42) was significantly higher than HCs-HCs (0.38) and pts-pts (0.37) and between pts-HCs (0.42) was significantly higher than HCs-HCs (0.38) and pts-pts (0.37) (P<0.05), indicating oral dysbiosis in pts. A principle coordinate analysis (PCoA) plot also showed clearly different distribution of pts. Next, we analyzed the Unifrac distance among the pts with different skin AREG expression. If AREG was not associated with oral microbiota with smoking habits, periodontitis and putative oral-asthro-osteo (PAO) showed significant difference. The analysis of bacterial abundance in pts and HCs demonstrated lower Proteobacteria (pts-24%, HCs-17%, P<0.05) at the phyla level, and lower Haemophilus (pts-10%, HCs-17%, P<0.05) and higher Prevotella (pts-17%, HCs-10%, P<0.05) at the genus level in pts. Interestingly, these differences were observed in pts with PAO vs. HCs (pts-PAO-21%, HCs-PAO-8%, HCs-17%, P<0.05); prevotella: pts-PAO-20%, HCs-10%, P<0.05), while not in pts without PAO (n=5). Taken together, these results suggested oral dysbiosis in pts, particularly in the patients with PAO.

The efficacy of 1550-nm erbium-glass fractional laser treatment and its effect on the expression of insulin-like growth factor 1 and Wnt/b-catenin in androgenetic alopecia

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ABSTRACTS | Clinical Research: Pathophysiology and Therapeutics

Background: Hair loss is a common problem that affects about 30% of the adult population in the world. Hair is believed to be due to the stimulation of hair regrowth, but the mechanism of healing is still unclear. With the Wingless-related integration site (Wnt) proteins and insulin-like growth factor 1 (IGF-1) is a new and effective treatment for androgenetic alopecia (AGA) is 1550-nm erbium-glass (Er-Glass) fractional laser treatment. The wound healing process associated with this treatment is believed to be due to the stimulation of hair regrowth, but the mechanism of healing is still unclear. Both the Wingless-related integration site (Wnt) proteins and insulin-like growth factor 1 (IGF-1) are important molecules that promote new hair growth. The aim of this study was to evaluate the efficacy of 1550-nm Er-Glass fractional laser treatment and determine the gene expression of IGF-1 and Wnt/b-catenin in female patients with AGA.

Methods: Forty AGA patients (n=20) were recruited and enrolled in this prospective randomized, double-blind, placebo-controlled study. The patients were randomly divided into two groups: 1. Control group (n=10) patients received placebo treatment, and 2. Treatment group (n=10) patients received 1550-nm Er-Glass fractional laser treatment with power of 12W, 14 passes, and 0.2mm spot size. The safety and efficacy of this treatment were evaluated at 6 weeks by Trichoscopy and Hair Counting with a hair counting device. The measurement of hair count was performed at three regions: frontal, fronto-vertex, and vertex of the head. The hair count was performed 3 times, and the average was recorded. The hair density was calculated by the following formula: Hair density = hair count / (Area of scalp/10000). The safety of this treatment was evaluated by assessing the adverse effects were noted and recorded. The statistical analysis was performed using the Student’s t-test and the chi-square test. Significant differences were noted at P<0.05.

Results: The mean hair density in the frontal region increased significantly after 6 weeks of treatment (P<0.05). The mean hair density in the fronto-vertex and vertex regions also increased significantly after 6 weeks of treatment (P<0.05). The safety of this treatment was evaluated by assessing the adverse effects. No significant adverse effects were observed in all groups. The mean hair density in the frontal region increased significantly after 6 weeks of treatment (P<0.05). The mean hair density in the fronto-vertex and vertex regions also increased significantly after 6 weeks of treatment (P<0.05). The safety of this treatment was evaluated by assessing the adverse effects. No significant adverse effects were observed in all groups.

Conclusion: The results of this study suggest that 1550-nm Er-Glass fractional laser treatment is safe and effective for the treatment of AGA. The mechanism by which 1550-nm Er-Glass fractional laser treatment increases hair growth may not be limited to Wnt10A/b-catenin or IGF-1 expression.
417 Immunotherapeutic options for skin cancer prevention in xeroderma pigmentosum

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A 17 yo woman from the Dominican Republic with xeroderma pigmentosum (XP), multiple primary skin cancers, and multiple uncontrolled squamous cell carcinomas. She has been assessed for resection of new and recurrent skin cancers. Her physical exam was notable for a tender, clean-based ulcer on her left upper forehead, which she had first noticed three months prior. Biopsy showed invasive squamous cell carcinoma and she was referred to Head and Neck Surgery. CT of the head and neck was significant for an irregularly enhancing mass of the left frontal scalp, involving the full thickness of the scalp. Involvement extended to the outer table of the calvarium and CT of the neck showed no evidence of regional lymphadenopathy. Given the presence of multiple primary skin cancers, she underwent partial resection of her frontal scalp SCC, in addition to sentinel lymph node (LN) biopsy of the jugular chain LNs and of parotid LNs, which showed metastasis to the left preauricular parotid LN. These procedures were followed by resection with full thickness skin graft. In addition to her ulcer, the patient also complained of severe vision. Ophthalmology exam was unremarkable. Biopsy of the right lateral limbus demonstrated a micronodular SCC that was not amenable to complete resection. Since malignancies associated with XP are predicted to have a high tumor mutational burden, which often predicts response to immune checkpoint inhibitors, she was started on pembrolizumab and 5-FU/calcipotriol combination therapy. During the time between her initial presentation and her start date for combination therapy, she had developed seven invasive SCC’s, one metastatic SCC, and one unresectable SCC. Since starting combination therapy, she has not developed a single new invasive SCC and her metastatic disease has cleared. These data demonstrate the efficacy of pembrolizumab and 5-FU/calcipotriol combination therapy for aggressive skin cancer prevention in this XP patient. Similar trends have been noted in two additional XP patients followed in MGH clinics.

416 Effectiveness and safety of rituximab in recalcitrant pemphigoid diseases

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Effectiveness and safety of rituximab in recalcitrant pemphigoid diseases. The medical records of patients with pemphigoid diseases that were treated between 2010 and 2017 with RTX were reviewed. Early and late endpoints, defined according to international consensus, were disease activity (DA), disease remission (DR), partial remission (PR), complete remission (CR) and relapses. Safety was measured by reported adverse events. Patients (n=6) were treated with a median of 1.5 doses of RTX (n=6). The mean age was 61.7 years (28-80). The disease duration was 15.4 years (0.1-43). One patient had a history of relapsing and remitting disease. The disease activity in these patients evolved into classic morphea. The histologic and clinical follow-up showed 5 patients remission (CR or PR) and 1 patient relapse. Repeated treatment with RTX led to remission (PR or CR) in 85.7% of the retreated cases. Some patients could not achieve remission, but the cumulative response for all patients was 70.5% (95% CI 51.8-87.9%). Our data suggest that rituximab has potential as an effective treatment of pemphigoid diseases. Further studies are needed to determine the optimal treatment regimen.

415 Isotretinoin safety in patients with acne and inflammatory bowel disease?

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The association between isotretinoin use in acne and inflammatory bowel disease (IBD) is unclear. Isotretinoin associations to IBD, from exaggerated attorney-initiated reports and inflammatory bowel disease (IBD) may be uncertain. Isotretinoin has been shown to improve inflammation and to reduce the risk of developing IBD. We aimed to determine if isotretinoin has an anti-inflammatory effect in patients with acne and IBD. We performed a retrospective chart review of 23 patients who were diagnosed with acne and IBD. Comparing patients who received isotretinoin therapy after a diagnosis of IBD with patients who did not receive isotretinoin therapy, IBD disease severity in both isotretinoin and non-isotretinoin groups was similar with 33% having severe disease and 66% having mild disease. Isotretinoin therapy has been shown to improve inflammation and to reduce the risk of developing IBD. We found that isotretinoin has anti-inflammatory effects which may be helpful in IBD. We aimed to study the safety of isotretinoin in IBD patients who received isotretinioin therapy for acne. We performed a retrospective chart review of 23 patients who were diagnosed with acne and IBD. Comparing patients who received isotretinoin therapy after a diagnosis of IBD with patients who did not receive isotretinoin therapy, IBD disease severity in both isotretinoin and non-isotretinoin groups was similar with 33% having severe disease and 66% having mild disease. Further studies are needed to determine if isotretinoin has an anti-inflammatory effect in patients with acne and IBD. Further studies are needed to strengthen these claims.
422 Defining the gene expression signature for human facial rejuvenation

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Techniques such as laser rejuvenation and resurfacing are commonly used to induce a more youthful appearance to the skin, yet the molecular mechanism of these treatments need further investigation. To define the mechanism and potentially identify biomarkers for efficacy, we conducted a 14-week open-label, single-arm study in 16 patients (n=17, age range 44-55 yrs old) with 12.8% ± 4% deep wrinkles. All subjects were treated with fractional ablative laser therapy (10.64 nm CO2 fractional laser). The skin biopsies were performed pre and post-treatment. Gene expression analyses were performed at baseline and at 2, 4, 8, and 12 weeks post-treatment. We found that the combination of wound healing pathways, stem cells, ECM remodeling and extracellular matrix (ECM) production were significantly differentially expressed after fractional laser treatment. Most of these pathways were upregulated after treatment and included basement membrane proteins, growth factors, and extracellular matrix proteins. These results suggest that the diverse gene expression changes associated with laser treatment can be used to identify potential biomarkers for efficacy and to optimize treatment regimens.

423 Gentian violet induces pro-apoptotic and anti-proliferative effects in cutaneous T-cell lymphoma (CTCL): Preclinical studies

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Gentian violet (GV) is a well-known antifungal agent that has been extensively used in the management of cutaneous T-cell lymphoma (CTCL). However, the precise mechanism of action of GV is not fully understood. In this study, we aimed to investigate the pro-apoptotic and anti-proliferative effects of GV in CTCL. We used CTCL cell lines, human skin organotypic cultures, and murine cutaneous T-cell lymphoma model.

Results: We found that GV induced significant apoptosis in CTCL cell lines, as evidenced by increased caspase 3/7 activity and decreased Bcl-2 expression. GV also inhibited proliferation of CTCL cell lines, as evidenced by decreased Ki67 expression. We also found that GV downregulated several pro-inflammatory cytokines, including TNF-alpha and IL-6.

Conclusion: Our findings suggest that GV has pro-apoptotic and anti-proliferative effects in CTCL, which may contribute to its clinical efficacy in the treatment of this disease.

424 Immune response of T cells in plaque psoriasis to secukinumab

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Objectives: To assess the efficacy and safety of the topical application of collagen-derived dipeptide and grifola frondosa extract for treating atopic dermatitis (AD).

Methods: This open-label single-center trial was conducted for 8 weeks, comprising 4-week treatments with a lotion containing CDP and GF extract (CDPGF treatment) and a placebo. Initially, 24 patients (20 females and 4 males) with moderate AD were treated with a placebo for 4 weeks. Subsequently, these patients received CDPGF treatment for 4 weeks. Diabetic treatment and clinical AD-related tests, including transsiderminal water loss (TIWL), visual analogue scale (VAS) of pruritus, and the observation of the stratum corneum (SC), were performed.

Results: TIWL revealed a significant decrease in transepidermal water loss (p<0.05). Pruritus significantly decreased during both treatments (p<0.05). The stratum corneum thickness of patients receiving CDPGF treatment was significantly lower than that of patients receiving placebo (p<0.05). It was concluded that CDPGF treatment was effective in improving the symptoms of AD.

Conclusion: Our findings suggest that CDP and GF are effective for treating mild or moderate AD.
A small molecule CCR2 antagonist delays tumor macrophages and stimulates CD8 T cell accumulation in a murine model of cutaneous T cell lymphoma (CTCL) 

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We screened a panel of small-molecule compounds identified from biological screening campaigns for pharmacological inhibitors of CCR2, a chemokine receptor expressed by tumor macrophages (M2). Using a preclinical model of CTCL, we found that treatment with CCX598, a potent CCR2 antagonist, significantly delayed tumor growth and increased tumor-infiltrating CD8+ T cells. CCX598 was also shown to be a potent inhibitor of CCR2 in vitro, with IC50 values measured in the low nanomolar range. These findings suggest that blocking CCR2 may have therapeutic potential for the treatment of CTCL and other CD8+ T cell-deficient conditions.

428 Characteristic of circulating dendritic cells in patients with cutaneous adverse drug reaction 
N Takamatsu, T Kanaguchi, T Watanabe, Y Watanabe and M Aihara Dept of Dermatology, Yokohama City University, Yokohama, Kanagawa, Japan

Drug eruptions (DE) are thought to be induced by T cells that have been sensitized to drug antigen. T cells from DE patients have been reported to display a macrophage-like phenotype (M1). Subsets of M1 DCs have been identified in the peripheral blood, myeloid DCs (mDC1 and mDC2) and plasmacytoid DCs (pDCs). However, the behavior of DCs in the pathogenesis of severe DE remains unclear. In this study, we determined circulating and skin DCs in patients with DE to clarify whether they differ depends on the disease types. Seven cases of toxic epidermal necrolysis (TEN), 5 of Stevens-Johnson syndrome, 5 of drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms, 8 of psoriasis with generalized maculopapulovesicular rash and 6 of healthy volunteers were determined as cases or controls, respectively.

Peripheral blood was obtained from patients at 3 times points: at first visit, during systemic therapy, and after remission. Flow cytometric analysis was used to enumerate the population of mDCs and pDCs. The proportions of circulating mDCs and pDCs at first visit decreased in all disease types compared to controls, and a significant decrease at first visit tended to be lower and slowly recovered in patients with TEN compared to that in other types, although no significant differences were found. Infiltrated mDCs were further identified by immunohistochemistry. mDCs as detected CD11c+ cells were clearly increased in some cases. However, no obvious association was observed between the number of infiltrated CD11c+ cells and disease severity or types. In conclusion, circulating mDCs and pDCs were decreased in the acute phase of DE and they possibly migrated to the dermis. The prominent reduced degree of circulating mDC1 in acute phase might be a predictor of development to TEN.

430 Thyroid function abnormalities and autoimmunity in chronic spontaneous urticaria 
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Background: The pathogenesis of chronic spontaneous urticaria (CSU) is thought to be associated with autoimmune mechanisms. The linkage of thyroid autoimmunity with the pathogenesis of CSU has been investigated by comparing the CSU patients with healthy subjects. However, they have not been compared with those with other urticaria subtypes (non-CSU). We aimed to compare the prevalence of thyroid function abnormalities and autoimmunity between CSU and non-CSU patients. Method: This is a hospital-based, one-year retrospective study at a tertiary level hospital in Japan. We explored the frequency of thyroid function abnormalities and measured levels of auto-IgG antibodies to thyroglobulin (anti-Tg) and thyroid peroxidase (anti-TPO) in patients with urticaria. Thyroid function was assessed using the thyroid stimulating hormone assay (TSH, 0.5-5 mIU/L) and free thyroxine (FT4, 0.9-1.79ng/mL). Results: The 60 patients enrolled were divided into two groups: the CSU group (30 patients, M/F=11:19, mean age 44.8 yrs) and non-CSU group (30 patients, M/F=9:21, mean age 46.5 yrs). In the non-CSU group, the most frequent phenotype was acute spontaneous urticaria (73.3%). In the CSU group, the median TSH was 1.56 mIU/L (0.9-10.6). The CSU group had more frequent elevation of TSH values compared to the non-CSU group (13.3% vs 0.0%, p<0.05). The prevalence of decreased levels of TSH is 6.7% in both groups. The CSU group had more frequently elevated levels of FT4 compared to the non-CSU group (23.3% vs 11.3%, p=0.12). Although the presence of anti-Tg and anti-TPO was more frequent in the CSU group (11.3% vs 0.0%, p<0.05), there was no significant association of the prevalence of anti-Tg and anti-TPO with CSU compared with non-CSU (anti-Tg, 23.3% vs 12.0%, p=0.50; anti-TPO, 13.3% vs 8.9%, p=0.72). Conclusion: This study showed a significantly higher frequency of thyroid function abnormalities, but not that of thyroid autoimmunity in CSU patients, compared with those with other phenotypes of urticaria.

432 Investigating a causal relationship between body mass index and inflammatory skin disease using mendelian randomization 
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To identify causal relationships between body mass index (BMI) and psoriasis or obesity, we performed a Mendelian Randomization (MR) study of BMI in 150,508 individuals from the UK and Norway who were studied using a sample MR and a genetic instrument to define BMI created using 97 polymorphisms from the most recent genome-wide association studies. The majority of these biomarkers were not associated with BMI (p > 10-5), but of 505 BMI quantitative trait loci (BMI-QTL) and 505 BMI-QTL loci, 6 were BMI adjusting factors for BMI in the meta-analysis data for 731,021 individuals. The 1- and 2 sample MR estimates were meta-analysed. A causal effect in the reverse direction - of psoriasis or adiposity on BMI - was also investigated. These MR analyses have shown that BMI increases risk of psoriasis by 9% per kg/m2 (OR 1.09, 95% CI 1.06-1.12) and AD by 2% per unit increase in BMI (OR 1.02, 1.00-1.04). Conversely, there is little evidence for either psoriasis or AD influencing BMI. The finding that increased BMI has a causal effect upon both psoriasis and AD raises the possibility of a common mechanism by which obesity contributes to the development of AD and obesity, which may be implicated in a causal role in establishing an immunosuppressive tumor microenvironment (TME) that supports tumor growth. We have reported the establishment of high grade skin tumor lymphoma in syngeneic mice by injection of MBL T lymphoma cells in ear skin following the delivery of D pare. In this model, macrophages play a key role in sustaining tumor growth. Thus, we hypothesize that blocking monocyte trafficking (through inhibition of specific chemokine receptors) into skin can influence tumor development. Herein, we maculopapulovesicular rash and 6 of healthy volunteers were determined as cases or controls, respectively.

Peripheral blood was obtained from patients at 3 times points: at first visit, during systemic therapy, and after remission. Flow cytometric analysis was used to enumerate the population of mDCs and pDCs. The proportions of circulating mDCs and pDCs at first visit decreased in all disease types compared to controls, and a significant decrease at first visit tended to be lower and slowly recovered in patients with TEN compared to that in other types, although no significant differences were found. Infiltrated mDCs were further identified by immunohistochemistry. mDCs as detected CD11c+ cells were clearly increased in some cases. However, no obvious association was observed between the number of infiltrated CD11c+ cells and disease severity or types. In conclusion, circulating mDCs and pDCs were decreased in the acute phase of DE and they possibly migrated to the dermis. The prominent reduced degree of circulating mDC1 in acute phase might be a predictor of development to TEN.

428 Characteristic of circulating dendritic cells in patients with cutaneous adverse drug reaction 
N Takamatsu, T Kanaguchi, T Watanabe, Y Watanabe and M Aihara Dept of Dermatology, Yokohama City University, Yokohama, Kanagawa, Japan

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These results suggest serlopitant may be a potentially useful treatment for EB itch and provide score relative to placebo (−0.015 point/day reduction, p < 0.02), corresponding to an 0.88 point improvement greater than placebo. Significant downregulations of key inflammatory axes, including Th1/CXCL9, Th2/CC18, IL-23R, DFFBA, and TGFβ1, were restricted to the high IL-22 group (p < 0.05). We also defined a set of baseline predictors of response, mostly involving T-cell and dendritic-cell genes. This is the first human study showing profound effects of IL-22 blockade in AD skin, particularly in patients with high-IL-22 responses to fezakinumab.
Clinical Research: Pathophysiology and Therapeutics

ABSTRACTS

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FAS-ligand (FASL) expression in cutaneous T-cell lymphoma (CTCL) is regulated by promoter methylation and can be derepressed by methotrexate

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One of the major mechanisms of T-cell apoptosis is activation-induced cell death (AICD) – a process by which upregulation of FASL results in apoptosis via the extrinsic (caspase 8-mediated) pathway. Because it is so potentially lethal to T cells, expression of FASL is very tightly controlled. We hypothesized that like many other tumor suppressor genes, FASL expression is at least partially regulated by promoter methylation. Using hRNA technology in-vitro, we show that knockdown of DNA methyltransferase (DNMT) -1 and -3A result in upregulation of FASL mRNA and protein in CTCL lines. We also demonstrate a dose-response relationship between reduction of DNMT knockdown and FASL expression using western blot. We showed previously that MTX has the ability to inhibit DNA methylation by depleting cellular S-adenosylmethionine, the main methyl donor for DNMTs. Treatment of CTCL lines with MTX increased FASL expression and further enhanced the FASL upregulation induced by DNMT knockdown andexpression was mediated mainly by keratinocytes. Treatment with reagents that inhibit demethylation, propidium iodide/Annexin V flow cytometry as well as activation of caspases. These studies document epigenetic regulation of FASL expression by DNMTs and show that MTX can inhibit the suppressive effects of DNMTs resulting in increased CTCL cell death. We know from our prior studies that MTX also increases expression of FAS (the death receptor for FAS). In aggregate, these findings provide a rationale for including MTX in combination therapies designed to enhance CTCL apoptosis. This could include other drugs (e.g. histone deacetylase inhibitors, retinoids, interferons) or physical modalities (e.g. phototherapy or ionizing radiation).

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Sezary syndrome patient-derived xenografts for 21-color flow cytometry immunophenotyping and CART cell therapeutic testing

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Epigenetic modulation including DNA methylation and histone post-translational regulations play an important role in genome regulation, cellular proliferation and differentiation in almost all tissues and its deviation accounts for most inflammatory diseases. However, whether these processes implicate in hyperproliferation of postrheumatic epithelial has yet been investigated. Here we observed that in psoriatic lesions from both patients with psoriasis and imiquimod (IMQ)-induced mice, bistone H3 lysine 27 (H3K27me3) and its methyltransferase enhancer of zeste homolog 2 (EZH2) were co-located and parallelly over-expressed by keratinocytes in all layer of skin barrier (basal, spinous and suprabasal), epidermal cells. Moreover, aberrantly expressed EZH2, targeting keratinocytes reversed the skin phenotype of IMQ-induced mice by attenuating epidermal thickness of psoriatic lesions. As in vitro, knockdown of EZH2 expression in human keratinocytes with lentivirus caused subdued methylation of H3K27me3 companied with expression downregulation, the knockdown of EZH2 by siRNA in keratinocytes suppressed cell cycle of keratinocytes, which aggravates epithelium hyperplasia in psoriatic lesions and may be an alternative therapeutic target for psoriasis.

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Acute onset/exacerbation of dermatomyositis and Sjogren Syndrome following ingestion of IsaLean® herbal supplement: Clinical and immunostimulatory findings

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The use of complementary and alternative medicine has gained popularity in the US over the last few decades. However, herbal supplements are known to have adverse medical effects. We observed two patients with acute onset of their classic dermatomyositis (DM) and one patient with acute onset of Sjogren’s syndrome (SS) after the ingestion of IsaLean®, a herb-based weight-loss product. The purpose of this study was to investigate and characterize the immunostimulatory properties of the herbal and dietary supplement IsaLean®. Peripheral blood mononuclear cells were isolated from five DM patients and five control subjects stimulated with increasing concentrations of IsaLean®, 0.05, 0.5, and 5 μg/ml to evaluate the cellular production of tumor necrosis factor (TNFα), interferon alpha (IFN-α), and interferon beta (IFN-β), which are key pathogenic cytokines in DM and SS. The cells were also incubated with IsaLean® and lipopolysaccharide (LPS), and the effect of neutralizing anti-TLR4, quinacrine (QC), and hydroxycytrosine (HCQ) on the cellular production of TNFα was examined. Cytokine production was measured by enzyme-linked immunosorbent assay. The one-way analysis of variance with Dunnet’s multiple comparison test was used to compare the level of TNFα, IFN-α, and IFN-β after stimulation with IsaLean and treatment with anti-TLR4, QC, and HCQ. IsaLean® increased cellular secretion of TNFα at 0.5 μg/ml (p<0.01) and 5 μg/ml (p<0.001). Anti-TLR4 suppressed cellular secretion of TNFα from IsaLean® (p<0.001) and LPS-stimulated cells (p<0.01). QC significantly reduced the production of TNFα from IsaLean® (p<0.001) and LPS-stimulated cells (p<0.05) compared to HCQ. Ultimately, IsaLean® induced secretion of key immunostimulatory cytokines (TNFα, IFN-α, and IFN-β) from immune cells of DM patients in vitro. Further, the results suggest the pro-inflammatory effects of IsaLean® are mediated through TLR4.

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EZH2-dependent epigenetic modulation of histone H3 lysine-27 contributes to psoriasis by promoting keratinocyte proliferation

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Histones are highly conserved nuclear proteins that play important roles in gene expression, cellular proliferation and differentiation in almost all tissues and its deviation accounts for most inflammatory diseases. However, whether these processes implicate in hyperproliferation of postrheumatic epithelial has yet been investigated. Here we observed that in psoriatic lesions from both patients with psoriasis and imiquimod (IMQ)-induced mice, bistone H3 lysine 27 (H3K27me3) and its methyltransferase enhancer of zeste homolog 2 (EZH2) were co-located and parallelly over-expressed by keratinocytes in all layer of skin barrier (basal, spinous and suprabasal), epidermal cells. Moreover, aberrantly expressed EZH2, targeting keratinocytes reversed the skin phenotype of IMQ-induced mice by attenuating epidermal thickness of psoriatic lesions. As in vitro, knockdown of EZH2 expression in human keratinocytes with lentivirus caused subdued methylation of H3K27me3 companied with expression downregulation, the knockdown of EZH2 by siRNA in keratinocytes suppressed cell cycle of keratinocytes, which aggravates epithelium hyperplasia in psoriatic lesions and may be an alternative therapeutic target for psoriasis.

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Dithranol in psoriasis: Keratinocyte-neutrophil crosstalk and TREM1 signaling

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Dithranol has been one of the most potent topical treatment options for psoriasis, yet its precise mechanism of action is still not fully understood. In this study, we analyzed the effects of dithranol between 15 psoriasis patients were taken before, during and at the end of treatment (week 2-3), when topical dithranol administration had reduced PASI by a mean of 57%. Gene expression analysis performed by using microarray, differentially expressed genes were identified and Ingenuity Pathway Analysis (IPA) was used to predict pathways and upstream regulators. Comparing lesional skin after 4 days of treatment to baseline, we found a significant upregulation of genes involved in keratinization and skin barrier (e.g. PSORS1, PSORS3, KRT), extracellular matrix (e.g. COL4A1, COL4A2) and immunomodulatory functions (e.g. CXCL5, CXCL8) caused upregulation of cytokines (e.g. IL1B, IL6) and chemokines (e.g. CXCL5, CXCL8). IPA predicted TNF, IL1B and IL-22 caused upregulation of IL17 cytokine expression which is enhanced by TNFalpha, IL6, IL1B and IL-22. Treatment together. Our data suggest that keratinocytes and their crosstalk with neutrophils (and not cells of the acquired immune system) are the primary targets of dithranol. TREM1 gene expression was downregulated both at the early and late timepoint suggesting an anti-inflammatory role of dithranol and the cell-mediated macrophage inhibition and to a lesser extent on B and T cells; dithranol may act in psoriasis by interfering monocytes and to a lesser extent on B and T cells; dithranol may act in psoriasis by interfering
446 Exploring molecular transformation in psoriatic patients during 84 days of anti-IL-17A treatment

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Antagonists of IL-17 have proven to be highly effective in the treatment of psoriasis. However, many of the involved molecular mechanisms involved are still to be determined. Moreover, the molecular remission of psoriasis remains elusive. The purpose of this study was to investigate the molecular complexity in psoriatic patients during anti-IL-17A treatment. 14 patients treated with secukinumab (anti-IL-17A) were included in the study. Skin biopsies and blood samples were collected at days 0, 4, 14, 28, 56 and 84. Clinical scores such as PASI, PGA and BSA were registered at each visit. Samples were then processed for microarray, qPCR and IHC. Inflammatory mediators such as CXCL8, IL-19, DEF4 and IL-36A were downregulated early at day 4 after start of treatment, whereas other mediators such as S100A7, IL-20 and CCL20 were downregulated at day 14. Moreover, when comparing nonlesional skin with lesional skin at day 84 the expression of several molecular markers were altered even though the psoriasis had cleared. IB, encoded by the NFKBIZ gene, is a key regulator in psoriasis. Anti-IL-17A treatment was morphologically similar to MADISH, the absence of CYP1A1 expression in the skin lesions under vemurafenib following dioxin-like exposure, was not observed in these lesions. Vemurafenib, erlotinib and gefitinib did not induce CYP1A1 activity. Although the skin lesions under vemurafenib treatment were morphologically similar to MADISH, the absence of CYP1A1 expression in the cysts and the absence of CYP1A2 activation by vemurafenib led us to consider that these lesions were different from true MADISH and not mediated by a cross-talk of AHR signaling, but rather to a hyperactivation of PI3K-Akt pathway. A strong expression of CYP1A1 in the epidermal wall of cysts must be required for MADISH, the hallmark of an exposure to dioxin-like/chloracne compounds.

447 RAStopic comedo-like lesions induced by vemurafenib: A model of skin lesions similar but not identical to those induced by dioxins (MADISH)

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Patients treated with vemurafenib for metastatic melanoma often develop skin lesions similar to those observed after exposure to dioxin-like compounds, called MADISH (metabolizing acquired dioxin-induced skin hamartoma). We performed a clinical trial aimed at comparing the skin lesions observed under vemurafenib treatment with MADISH. In a prospective case-series study, we explored the histological aspect of skin lesions in 10 cases treated with vemurafenib for malignant melanoma. We also analyzed the ability of vemurafenib and tyrosine kinase inhibitors to induce dioxin-AHR pathway, using EROD assay. All patients had a non-inflammatory acniform eruption of predominantly facial localization with notable retroauricular involvement and clinically compatible with chloracne/MADISH. Histological analysis showed intracellular cysts containing epithelial wall with preserved granular layer, epithelial projections, lamellar keratinocytes, variegated acanthosis and fibroelastic glands in some cases. The expression of CYP1A1, the gene of which is highly induced following dioxin-like exposure, was not observed in these lesions. Vemurafenib, erlotinib and gefitinib did not induce CYP1A1 activity. Although the skin lesions under vemurafenib treatment were morphologically similar to MADISH, the absence of CYP1A1 expression in the cysts and the absence of CYP1A2 activation by vemurafenib led us to consider that these lesions were different from true MADISH and not mediated by a cross-talk of AHR signaling, but rather to a hyperactivation of PI3K-Akt pathway. A strong expression of CYP1A1 in the epidermal wall of cysts must be required for MADISH, the hallmark of an exposure to dioxin-like/chloracne compounds.
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BET inhibition markedly potentiates CTCL cell viability and is synergistically potentiated by BCL2 or HDAC inhibition

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Sequence-selective BET inhibitors have shown therapeutic targeting of BET proteins is a small molecule targeting approach that selectively disrupts bromodomain and extrahistidinal domain (BET) proteins that regulate the transcription of genes, such as MYC, that are commonly altered in CTCL and critical for cell cycle regulation. We examined the effect of BET inhibition on CTCL cells in vitro and evaluated the potential synergy of BET inhibition with BCL2 or HDAC inhibition. Sensitivities were evaluated in established CTCL cell lines (HL-60, Myla, Hut78 and Sez4) and 12 advanced CTCL patient-derived samples. Malignant cells were purified from patients peripheral blood and exposed to BET inhibition with BCL2 or HDAC inhibitors. Cytoxic effects were evaluated as apoptosis induction via caspase-3/7 activation at 24h and cell viability via ATP quantitation at 72h. CTCL cells showed decreasing viability with increasing dose of JQ1 (mean IC50: patient cells=6.05 µM, cell lines=6.1 µM). All samples showed striking synergy, as assessed by the Chou-Talalay method, when JQ1 was combined with either a BCL2 inhibitor (ABT-199) or HDAC inhibitor (romidepsin or vorinostat). The combination index at 10% cell viability ranged from 0.08-0.49 for ABT-199, 0.21-0.61 for romidepsin and 0.17-0.28 for vorinostat. Cytotoxic effects and synergy were also seen with another BET inhibitor, mibremib (mean IC50: 2.09 µM). MYC gene expression after 24h drug exposure decreased by 25% and 145 fold for JQ1 combined with vorinostat or romidepsin, respectively. In summary, BET targeting substantially inhibits CTCL cell viability and this effect can be synergistically potentiated by BCL2 or HDAC inhibition. Thus, BET inhibitors, alone and in combination with other agents, may represent a novel therapeutic strategy in the treatment of CTCL.

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G8R 930 induces progressive and sustained changes in atopic dermatitis biomarkers in patient skin lesions

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G8R 930 is a first-in-class, humanized monoclonal IgG1 antibody specific for inhibiting OX40, a costimulatory receptor on activated T-cells. This proof-of-concept study (NCT02683928) investigated the effects of G8R 930 on atopic dermatitis (AD) biomarkers and generated the first clinical evidence of biological activity. Adults with BSA ≥10%, EASI ≥12, SCORAD ≥20, IGA ≥3, and history of inadequate response to topical treatments were randomized 1:1 to G8R 930 (10 mg/kg IV, at baseline [BL] and Day 29) or placebo (PBO). Inclusion criteria were moderate-to-severe AD with a ≥7% TEAE from baseline to Day 29. 139 subjects were randomized to G8R 930 (n=70) or PBO (n=69). Subjects were treated for 12 weeks. Safety and tolerability were assessed. The primary outcome was reduction in body surface area (BSA) involvement, as assessed by the PASI-75 response. Secondary outcomes included a 25% reduction in BSA involvement (PASI-25), a ≥50% reduction from baseline in ADActivity Investigator Global Score (ASI) and EASI, and a decrease in the percentage of patients with CR/VR. A significant reduction from BL to Day 29 was observed in 3 AD biomarkers: CR/VR (p<0.01), PASI-25 (p<0.01), and EASI (p<0.001). G8R 930 induced progressive and sustained changes in AD biomarkers and may represent a novel therapeutic strategy in AD.

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Ruxolitinib inhibits cyclosporine a (CSA) induced proliferation of squamous cell carcinoma (SCC): Implications for treating catastrophic SCC in organ transplant recipients (OTR)

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Intermittent-irrepressible SSs are prone to potentially deadly catastrophic SCC. Curative options that target cancer yet spare the allograft are unavailable. We have shown increased risk for catastrophic SCC in our OTR cohort via CSA-mediated induction of IL-22. Herein, we found that CSA drives SCC cell proliferation and tumor growth through IL-22 via JAK/STAT induction. We in turn inhibited that growth in vitro and in vivo via JAK 1/2 blockade with Ruxolitinib. We studied IL-22 and CSA induced proliferation in five SCC cell lines derived from patient pancanceras: four SCC and one teratocarcinoma. IL-22 induced SCC proliferation was ~60% increased in the cell lines that metastasized, greatest proliferative response occurred in the least aggressive lines. IL-22 treated A431 SCC cells showed rapid STAT3 phosphorylation and increased STAT3, JAK1 and JAK 3 expression on qPCR. We also found IL-22 and downstream JAK/STAT pathway related genes were highly expressed in CSA exposed patient transplanted and in SCCs with perineural invasion. In nude mice engineered with human SCC, IL-22 (4ug/d M-F, n=9) and CSA (20mg/kg d, n=9) increased tumor growth compared to vehicle (1.8 and 1.7 fold respectively, p<0.05) and upregulated IL-22 receptor, JAK1 and STAT3 expression. Ruxolitinib treatment (2.5g/d, n=8) reduced tumor volume by 44% (p<0.05) and reversed the accelerated tumor growth induced by CSA (53% volume reduction, p<0.05). IL-22 receptor expression was reduced in tumors exposed to Ruxolitinib. CSA and IL-22 hasten aggressive behavior in SCC. Targeting the IL-22 axis via selective JAK1/3 inhibition may reduce the progression of aggressive SCC in immunosuppressed OTRs without compromising tolerance of the transplanted organ.

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Molecular correlation with clinical outcomes in an open label clinical trial of oral tofacitinib in patients with alopecia areata

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Alopecia areata (AA) is a common autoimmune disease in which immune system targets the follicular matrix, resulting in non-scarring alopecia. Our previous studies in mice revealed that CD8+ T-cells were necessary and sufficient to induce AA. We identified the γ- chain cytokine and IFN pathways as key molecular circuits. We showed that tofacitinib successfully abrogated γ-chain cytokine and IFN pathways, prevented the onset of AA, and reversed established disease in the C3H/HeJ model. Extending these observations to the clinic, we here conducted a small open label study of oral tofacitinib in 12 patients with moderate to severe AA. Following limited response to the initial dose (5mg BID), the dose was escalated (10mg single daily) in 8 patients. Eight of 12 patients demonstrated partial (<50%) hair regrowth, 3 patients demonstrated partial (<50%) hair regrowth, and 1 patient demonstrated no regrowth. Gene expression profiling of scalp tissue and ALADIN scores correlated with clinical response. To investigate T-cell dynamics in response to tofacitinib treatment, we performed high throughput TCR chain sequencing of scalp biopsy tissue, and found a marked decrease in TCR clonality after 24 weeks of treatment. Many of the T-cell clones that were expanded at baseline were either decreased in frequency or disappeared from the scalp after treatment. All responders showed a reduction in expanded scalp CD8+ T-cell clones, and one who did not respond showed increased TEAEs. Our open label study of oral tofacitinib showed dramatic clinical responses in moderate-to-severe AA, which correlate with normalization of gene expression profiles and a decrease in TCR clonality in scalp biopsy tissue. These mechanistic findings provide a strong rationale for larger placebo-controlled clinical trials using JAK inhibitors in AA.
CD8+ T cell skin infiltration predicts disease progression in CTCL patients

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CD8+ T cell infiltration predicts disease progression in CTCL patients. In summary, CD8+ T cell infiltration in the initial CTCL skin biopsy could be predictive of disease progression. To examine this, a cohort of 33 CTCL patients (9, 4.7) with a median follow-up of 78 months were divided into two groups depending on whether they developed progressive disease (n=13). We studied their skin biopsy specimens by multiplexed tyramide signal amplification based staining for CD4, CD8, FoxP3, PD-1 and DAPI expression, followed by image deconvolution and automated cell analysis. Patients with greater CD8+ T cell infiltration had significantly improved progression-free survival in univariate (HR=0.6, CI 0.4-0.97, p=0.04) and multivariate analysis (HR=0.34, CI 0.2-0.68, p=0.01). When we restricted the analysis to early stage patients, this finding remained significant (HR=0.6, CI 0.4-0.98, p=0.04) and did not correlate with malignant clone frequency or age. The number of FoxP3+ regulatory T cells, PD-1+ T cells and total CD4+ T cells were not associated with improved survival. Ratios between these cell types were also examined for potential associations with prognosis. We found that an increased ratio of CD4+ T cells/FoxP3+ T cells was associated with worse survival (HR=1.0, CI 1.01-1.03, p=0.003). We did not find a benefit of treatment related to CD8+ T cell density in the lesional skin of CTCL patients is predictive of disease progression and may be a useful adjunct in risk-stratifying early stage patients.

Non-histaminergic itch mediators elevated in the skin of human scabies patients and a porcine model of scabies

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Scabies affects more than 100 million people worldwide. However, the underlying pruritic mechanism is not well understood. The expression levels of several known itch mediators (histamine, TRPV1, TRPA1, tryptase, PAR2, and B-tubulin for epidermal nerve quantification) were examined in scabies-infested control and human scabies subjects and pig model. Skin biopsies from scabies patients, 4 healthy volunteers, and 3 pigs (one infected ear and contralateral control ear) underwent immunohistochemistry. In both human and pig skin, TRPA1 and PAR2 were statistically elevated in the epidermis (p<0.001) of scabies-infested tissue compared to control. Also, the number of tryptase+ mast cells was 2-fold higher (p=0.01) in the dermal-epidermal junction of infested skin of both species. Interestingly, epidermal nerve fibers were increased (p<0.05) in human scabies tissue, but not in the pig model. Histamine and TRPV1 levels did not statistically differ between scabies and control. This study suggests that pruritus induced by scabies infestation is non-histaminergic and that these mediators could serve as potential antipruritic targets.

Systemic fluorouracil causing subacute cutaneous lupus erythematosus

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Case History: A 67-year-old woman with pancreatic adenocarcinoma presented with photosensitivity and pruritic photodistributed annular plaques over the bilateral extensor forearms and dorsal hands after her fourth cycle of FOLFIRINOX (folinic acid, fluorouracil, irinotecan, oxaliplatin). Dermatopathology demonstrated diffuse perivascular lymphocytic infiltrate and dermal mucin, suggestive of lupus erythematosus. Laboratory evaluation demonstrated an antinuclear antibody level of 8.0 U, anti-Ro IgG > 8.0 U, and anti-La IgG > 8.0 U. Systemic lupus was excluded given the lack of systemic symptoms, normal renal function, and normal urine studies. She was diagnosed with subacute cutaneous lupus erythematosus (SCLE) thought to be induced by systemic fluorouracil. Treatment and Follow-up: Photoprotection was advised, and she was treated with topical steroids while receiving additional chemotherapy. She tolerated therapy well, with no evidence of photosensitivity or exacerbation of the skin condition.

Safety of thalidomide-sulfasalazine for PIP prophylaxis in patients taking methotrexate for chronic inflammatory or autoimmune diseases

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Introduction: Methotrexate (MTX) is a potent immunomodulator and a key component of combination therapy (CT) in the treatment of rheumatoid arthritis (RA). Despite superior efficacy compared to prednisolone or sulfasalazine (SSZ), CT represents the most effective means of preventing complications of MTX therapy. We aimed to evaluate the safety of thalidomide (Thal) + sulfasalazine (SSZ) in patients taking MTX for RA.

Methods: All patients on Thal + SSZ CT for RA were prospectively evaluated for adverse events (AEs). AEs were assessed at each visit. Discontinuation of MTX and Thal + SSZ CT was considered if AEs were severe or if the patient requested discontinuation. The primary endpoint was the incidence of serious AEs (SAEs). Two hundred and twenty-four patients were enrolled in the study. AEs were reported in 142 patients (63.3%). MTX was discontinued in 10 patients (4.5%) due to SAEs. The most common AEs were gastrointestinal and infection-related.

Results: The incidence of SAEs was 15.9% (35/219) and the most common SAEs were neutropenia (4.6%), pneumonia (3.8%), and bacteremia (3.8%). The incidence of AEs was 65.9% (142/219) and the most common AEs were gastrointestinal (24.3%), infection-related (22.4%), and dermatological (15.8%). There were no cases of MTX-induced skin toxicity or pustular drug reaction.

Conclusion: Thal + SSZ CT is well tolerated in patients taking MTX for RA. Further studies are needed to evaluate the long-term safety and efficacy of Thal + SSZ CT in this population.
A comparison of the efficacy and safety of fractional CO2 laser and fractional Er:Yag laser for the treatment of xanthelasma palpebrarum: A randomized split-face controlled trial

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Xanthelasma is a common cutaneous xanthoma occurring on or around the eyelid. In recent years, laser therapy has been well-recognized for its efficacy and safety for the treatments. 30 patients with bilateral xanthelasma were recruited. The two sides of each patients face were randomly divided into the fractional CO2 laser therapy group or the fractional Er:Yag laser therapy group. Fractional CO2 laser therapy was used with the DeepFx mode, energy of 15 ml, 15 % power density, 0% overlapping, and 1-3 passes. Fractional Er:Yag laser therapy was used with the MOP mode, energy of 20 ml, 20% power density, 0% overlapping, and 1-3 passes. Therapeutic patients received up to 5 treatments, with a 4-week interval. Efficacy and safety of each laser therapy were evaluated based on the degrees of lesion improvement. The percentages of patients who achieved 75% lesion clearance rate or above after five treatments of fractional CO2 laser and fractional Er:Yag laser were 82% and 52%, respectively. Percentage of patients who achieved 75% lesion clearance rate or above after 3 or 4 treatments of fractional CO2 laser was also higher (61 vs. 48%) than those who received the same number of treatments of fractional Er:Yag laser. No serious adverse events were reported during either type of laser treatment. Fractional CO2 laser therapy and fractional Er:Yag laser therapy are both effective and safe in treating xanthelasma, it requires fewer numbers of fractional CO2 laser therapy to achieve the same lesion clearance rate.

Usefulness of ulceration and hyperkeratosis as clinical predictors of Merkel cell polyomavirus-negative and combined Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is a rare cutaneous neuroendocrine carcinoma, typically associated with Merkel cell polyomavirus (MCPyV). Combined non-neuroendocrine components, particularly squamous cell carcinoma, and thought to represent a distinct disease process from pure MCC, and the diagnosis of combined MCC is not highly-reproducible based only on histologic assessment. This study analyzed the predictive clinical factors for MCCpV status and its morphological variants. We aimed to evaluate the significance of clinical parameters of ulceration/hyperkeratosis as useful novel non-invasive MCCpV markers. In our study, 20 MCC specimens from 20 patients were examined. Patients were divided into two groups based on MCCpV status and morphological subsets among those diagnosed with MCC: MCCpV- positive versus MCCpV-negative MCC patients. We performed a multivariate logistic regression analysis using stepwise forward selection to predict the presence of MCCpV-negative MCC and combined MCC were evaluated. Of the 20 patients studied, 10 cases (50%) were immunohistochemically identified as showing keratin 20+ and Cytokeratin 7+ using the MM10 antibody (C2M2), whereas 5 patients showed ulceration/hyperkeratosis was a predictive factor for detection of MCCpV-negative and combined MCC, being observed in 80%/50% of cases in which MCCpV was not identified. Among 10 MCCpV- positive MCCs, absence of ulceration and hyperkeratosis was found in 10 cases demonstrating smooth surface. Clinically, ulceration/hyperkeratosis may be a useful factor for diagnosing MCCpV-negative MCC and combined MCC. Cases presenting with clinical evidence of ulceration/MCPV-negative MCC possibly suggest the presence of MCCpV-negative and combined MCC.
A study of skin characteristics according to humidity during sleep
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During sleep, the skin is exposed to various environments for example low or high humidity and the average of 7 to 8 hours of sleeping in those situations can affect skin condition. Therefore, the objective of this study is to determine skin characteristics according to humidity during sleep. Eleven healthy women in their ages of 20s and 30s were controlled. They slept more than 7 hours at lower than 30% relative humidity (RH) environment on the first day and at higher than 70% on the second day. The room temperature were controlled 22±2°C. (Three measurement points) 1) before for sleep (after wash), 2) after 7 hours sleep (before wash), 3) after wash. Skin hydration, skin color, sebum secretion and trans-epidermal water loss (TEWL) were measured. The statistical significance was determined at P < 0.05. After 7 hours of sleep in 30% RH condition, skin hydration decreased by 24.23% significantly, but there was no significant difference after sleeping in 70% RH. The sebum level was increased after sleep at 30% RH. The TEWL did not show differences according to the humidity during sleep but significantly increased after facial cleansing in 30% RH sleeping condition. The skin brightness and yellowness were increased and redness was decreased after sleep at 30% RH condition. The skin brightness and yellowness were increased and redness was decreased after sleep in 70% RH. Where as no significant change were found in 30%RH. In this study, we confirmed that the changes in skin characteristics may be affected by humidity during sleep. When sleeping in dry environment, skin hydration decreases but the amount of sebum increases to compensate for skin dryness. In humid environment, skin color is brightened by hydration of the stratum corneum. Therefore, this study might suggest how to care the skin before sleep depending on the room humidity.

CARD14 variants in pityriasis rubra pilaris
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We screened 15 sporadic and 1 familial PRP patients for SNVs in the CARD14 gene. In the familial PRP patient we identified three genetic variants (rs117918077, rs2066964 and rs28674001), while control individuals (3 healthy and 2 psoriatic volunteers) carried the wild-type alleles. Of the 15 sporadic PRP patients 8 patients carried SNVs, in all of them only 2 types of polymorphisms were detected either alone or in combination (rs2066964, rs28674001) additionally, in 3 patients we detected mutations in the CARD14 gene (rs2289541, rs34367357, c.1198_1199CG/TA). Further in vitro and in situ functional studies were carried out in the familial PRP patient to examine the functional relevance of the genetic variants. Immunofluorescent staining revealed nuclear localization of the NF-κB p65 subunit in the PRP skin specimen, indicating activating NF-κB allele, in contrast to healthy and psoriatic skin sections where only cytoplasmic staining of inactivated NF-κB was shown. NF-κB-luciferase reporter assay demonstrated significantly increased NF-κB activity in keratinocytes from the PRP patient compared to healthy keratinocytes. Characterization of the cytokine profile of the keratinocytes and PBMCs demonstrated that the higher NF-κB activation in PRP cells induced higher responses to inflammatory stimuli compared to healthy cells. Our study indicate the importance of CARD14 in PRP patients and highlights that functional characterization of rare and common variants of the CARD14 gene can bring us closer to understanding the role of genetic variants in disease pathogenesis.

Efficient cancer control with immune checkpoint blockade requires cytokine-induced senescence
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Immunotherapy with monoclonal antibodies against exhaustion-associated surface molecules reactivates T cells and induces durable therapeutic stability in a variety of metastatic cancers. Cancer regression with this immune checkpoint blockade (ICB) requires cancer cell killing but the mechanisms causing the long-lasting tumor dormancy remain unknown. To analyze cancer cell dormancy induced by ICB we used a model of adoptive transfer of tumor-associated antigen (TA-Age) specific T helper cells (Th1) in transgenic tumor-bearing mice. Th1 immunotherapy prolongs the life of mice by induction tumor cell senescence. Combining adoptive transfer of Th1 cells with ICB, PD-L1/LAG-3 (Programmed-Death-Ligand-1/Lymphocyte-Activation Gene 3) significantly increased life time, even when started with advanced stage of disease, or 4 weeks prior to cancer-induced death. The therapy restored a normal health status in the mice, destroyed the large cancers partly and induced a p16Ink4a and Ki67 senescent phenotype in the remaining cancer cells. The therapeutic effect was strictly defined on an intact interferon Stat1-signalling pathway in the cancer cells. Tag2 driven cancers of Stat1a knockout mice did not respond to the therapy. While TA-Age specific Th1 cells were found in all treatment groups, p16Ink4a and Ki67 senescent tumor cells were absent from the Stat1-deficient cancer. Moreover, Stat1a knock-out cancer cells were resistant to interferon-induced senescence. The long-term therapy with interferon-signaling via Stat1 contributes to cancer control by ICB through the induction of cytokine-induced senescence in cancer cells.

Conserved overall efficacy of systemic therapies in elderly psoriasis patients - a two-country, multi-center, prospective, non-interventional study
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Therapeutic Psoriasis is a chronic systemic disease causing erythrospausaneous plaques on the skin and is associated with a number of internal co-morbidities. As the aging population is usually not specifically studied in clinical trials, it remains unclear whether current drugs demonstrate equal efficacy in the elderly. Methods: The data from 5345 patients with moderate to severe psoriasis, which are included in the German (Pro-Best) and Swiss (SDNTT) psoriasis networks, were collected. The cohort was stratified into a group ≥65 years old (controls) and another for patients below age 65 (elderly). Results: Response rates were equal between younger and older psoriasis patients with the exception of methotrexate, which was more effective in older patients until month 6 Discussion: This study showed that patients ≥65 years have a comparable treatment response to patients below this age threshold.
Microvesicles induce pro-inflammatory cytokines in dermatomyositis
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Microvesicles (MVs) are micro-scale bilayer membrane vesicles released from almost all cell types under activation or apoptosis. They have been detected in various bodily fluids, organs, and immune and non-immune settings. MVs harbor molecules from their parental cells, which may mediate intercellular communications, and are thought to play an important role in autoimmune inflammation as they have been shown to induce the synthesis of various pro-inflammatory cytokines in several immune cell types. Previous studies have demonstrated that circulating microvesicles are increased in a variety of autoimmune diseases, including dermatomyositis (DM). In the current study, we aim to characterize the induction of pro-inflammatory cytokines by MVs isolated from patients with dermatomyositis. Peripheral blood mononuclear cells (PBMCs) were isolated from eight DM patients and five healthy controls and subsequently stimulated with either no treatment, MVs isolated from the homologous plasma, or homologous MV-free plasma. The levels of TNFα, IFN-γ, IL-6, and IL-8 secreted by the cells in the conditioned medium were then quantified with an enzyme-linked immunosorbent assay (ELISA). In DM patients (n=8), significantly increased cellular secretion of IFN-γ, IL-6, and IL-8 compared to no treatment (p<0.05) and MV-free plasma (p<0.05). In the healthy controls, MVs did not significantly increase TNFα secretion from the homologous BMCs compared to no treatment and MV-free plasma. MVs from DM patients significantly increased pro-inflammatory cytokines from their homologous immune cells, suggesting that MVs likely play a role in the pathogenesis of DM. In conclusion, our preliminary studies indicate that circulating MVs are important pro-inflammatory mediators that amplify pro-inflammatory responses in patients with DM.

Comparison of two generation photosensitizers of PsD-007 and HMME photodynamic therapy for treatment of port-wine stain: A retrospective study
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Positive effects of hydrogen-water bathing in patients of psoriasis and parapsoriasis
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Positively, the photosensitizers contained in 1/34 achieved at least 50% improvement in PASI score compared with only 16% (n=20) of patients who received bathing. Our findings suggested that hydrogen-water bathing therapy could fulfill the unmet need for chronic inflammatory skin diseases.

Benign T cells drive visible inflammation in cutaneous T cell lymphoma
P Vives-Garcia1, J O Malley1, J Crouch1, E Seger1, J Truage1, E Lowery1, A Geha2, T Kupper2, P Wolff3 and R Clark1 1 Medical University of Graz, Graz, Austria, 2 Brigham and Women’s Hospital, Harvard Medical School, Boston, MA and 3 Austrian PUVA Study Group, Graz, Austria

Skin lesions of mycosis fungoides (MF) contain both malignant & benign infiltrating T cells. We studied MF skin before & after PUVA using high throughput sequencing (HTS), gene expression profiling & immunostaining. Though all patients improved clinically, 30% of patients experienced improvement of clinical inflammation (CAILS, mRNAV; despite the continued presence of many malignant T cells in skin. Improved CAILS scores correlated with turnover of benign T cell clones as measured by HTS (R^2=0.3). Nanostring profiling demonstrated that benign T cell associated genes were markedly different before & after treatment. Benign T cells were strongly associated with a CD4+/CD8+ Th2 signature (CCL18, CSF1) before but a CD4+/CD8+ Th1 signature after treatment. 25/29 of the downregulated genes in untreated MF vs healthy skin mapped to the post-translational modification of keratin 16 (KRT16) in normal skin but absent from untreated MF. The CD8 signature also correlated with clearance of malignant cells. Change scores in CAILS were correlated to change scores across the dataset to identify the gene set that drive clinical inflammation. Reduced clinical inflammation is linked to loss of the detrimental benign T cell signature & acquisition of the posttreatment benign T cell signature but was unrelated to the malignant T cell signature, suggesting benign T cells drive visible inflammation in MF. OK-432/OK-4M, interactions are known to drive chronic T cell inflammation.

We found that hydrogen-water bathing therapy could fulfill the unmet need for these chronic inflammatory skin diseases.

Positive effects of hydrogen-water bathing in patients of psoriasis and parapsoriasis
Q Zhu1, Y Wu1, Y Li2, Z Chen1, L Wang1, H Xiong1, E Dai3 and X Lu1 1 Department of Dermatology, Huashan Hospital Affiliated to Fudan University, Shanghai, China, 2 Department of Dermatology, Huadong Hospital, Fudan University, Shanghai, China and 3 Department of Dermatology, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China and 4 Department of Dermatology, Shanghai Dermatology Hospital, Shanghai, China

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We found that hydrogen-water bathing therapy could fulfill the unmet need for these chronic inflammatory skin diseases.
482 Itch and pain mediators in non-melanoma skin cancer J Rosen1, J Natkemper2, J Sanders, T Hashimoto3, F Halez4, O Sanguereva5 and G Younes6 1 Department of Dermatology, Miami University, Ohio, USA 2 School of Medicine, Miami, FL 3 Department of Pathology, Wake Forest University Baptist Medical Center, Winston Salem, NC

Itch and pain are symptoms often experienced by patients with non-melanoma skin cancers (NMSC) and are associated with poor quality of life. However, the pathophysiology underlying itch and pain in NMSC is poorly understood. Biopsies of skin cancer lesions (n=50) underwent immunohistochemistry for PAR2, tryptase, substance P and β-tubulin for nerve fibers. At time of biopsy collection, patients ranked their itch and pain intensity at the biopsy site on an 11-point (0-10) numerical visual analog scale. Lesions that were rated to be more itchy than painful had increased (p<0.001) expression of PAR2 in the epidermis and tryptase at the dermal-epidermal junction. Lesions that were rated more painful than itchy had elevated (p<0.01) substance P in the epidermis. The expression level of PAR2 significantly correlated (r²=0.78) with itch ratings, while the expression level of substance P significantly correlated (r²=0.81) with pain ratings. Overall, these findings provide evidence that itch markers are more associated with BCC lesions while pain markers are more associated with SCC lesions.

483 Oliber, freedom in your hands F Palacios1, C Vivallo1, B Lopez1, J Calaf1, M Castro1, P Guiraldes2, M Aedo1, D Andreis1,2 and G Osio3 1 Dermatología, UCSD, San Diego, CA 2 Clinical Oficina Clínica Española, Santiago de Chile

Nowadays there are several diseases leading to partial or total atrophy of the hands, such as Epimyosistem Bolusus (EB), arthritis, osteoarthritis, burns or hand amputations. Patients from different backgrounds are affected by their daily activities which include eating, brushing his teeth or drawing. Overall, these patients have their autonomy highly compromised. Current solutions for people with atrophied hands are very basic, mainly because the available options are prosthesis for people who lack a whole hand from the wrist up. Thus, a solution for atrophied or partially functional hands is of urgent need. We have created Oliber, an orthosis with a mechanism of magnets that by means of metal plates allows to attach any object the user wishes to use, providing autonomy during daily activities. Some of Oliber’s advantages are: easy posture, softness of the material, it can hold up to 1 kg, and the user can pick their favorite color. The product has been tested and validated with users and experts like dermatologists, physiatrists, kinesiologist and nurses. It also has been tested with users with Epimyosistem Bolusus, demonstrating the functionality, ergonomics and freedom. The idea behind Oliber is to improve the quality of life for people suffering from different diseases, like Arthritis, Arthrosis, burned hands, and any person with difficulties to manipulate objects with their hands.

484 Mechanisms in residual plaques in patients with an overall good response to biologics KM Smith1, S Mashiko2, R Edelmayer3, YB 1, V Kaimal1, L Olson3, S Huang3, J Wetter1, K Sheller1,2,3, G Singhal3, A Karmann3, S Cao4, C Maazi4, E St-Cyr Proulx2, Z Liu, J Knueger1, M Saraf1 and R Blissomme1 1 AbbVie, Worcester, MA, 2 Univers de Montreal, Montreal, QCA, 3 AbbVie, North Chicago, IL, 4 Rockefeller Univ, New York, NY and 5 Innovagemont, Montreal, QCA, Canada

The majority of psoriasis patients treated with biologics respond with 75-90% skin clearance. These patients possess "residual plaques" which can cover 1-5% of body surface area. To elucidate mechanisms of plaque persistence despite overall good response to drug, we studied fifty subjects: psoriasis patients treated with three different biologics with refractory plaques, untreated patients, and healthy controls. All subjects had skin biopsies collected during acute cellular rejection (n=25) and non-rejection (n=25) underwent immunohistochemistry with at least mild erythema. Lesion biopsies from the largest cohort of face transplant patients at a single center worldwide, we characterized the molecular changes specific for rejection compared to inflammatory dermatoses or healthy facial skin. In pathway analysis, the top canonical pathways included Th1 associated with itch linked with more superficial basal cell carcinoma (BCC) lesions and pain associated with itch and with more superficial BCC lesions. In face transplant recipients, 90% of rejection biopsies had increased (p<0.001) expression of PAR2, tryptase, substance P and β-tubulin for nerve fibers. At time of biopsy collection, poorly understood. Biopsies of skin cancer lesions. The mediators underlying itch and pain of non-melanoma skin cancers are poorly understood. Biopsies of skin cancer lesions (n=50) underwent immunohistochemistry for PAR2, tryptase, substance P and β-tubulin for nerve fibers. At time of biopsy collection, patients ranked their itch and pain intensity at the biopsy site on an 11-point (0-10) numerical visual analog scale. Lesions that were rated to be more itchy than painful had increased (p<0.001) expression of PAR2 in the epidermis and tryptase at the dermal-epidermal junction. Lesions that were rated more painful than itchy had elevated (p<0.01) substance P in the epidermis. The expression level of PAR2 significantly correlated (r²=0.78) with itch ratings, while the expression level of substance P significantly correlated (r²=0.81) with pain ratings. Overall, these findings provide evidence that itch markers are more associated with BCC lesions while pain markers are more associated with SCC lesions.

485 Topical ivermectin decreases serine protease activity in individuals with rosacea T Yu1, F Shalni1, C Wu2, A Di Nardo3 and T Hata1 1 Dermatology, UCSD, San Diego, CA 2 Ivermectin Research, New York, NY 3 Ivermectin Research, New York, NY

Increased levels of the antimicrobial peptide cathelicidin and kalikrein (KLK) the trypsin-like serine protease that cleaves cathelicidin into its active form (LL-37), have been shown to play a role in the pathogenesis of rosacea. Increased levels of Toll-like receptor 2 (TLR2), which play a role in recognizing patterns associated molecular patterns, have also been found in lesional skin of subjects with rosacea. Activation of TLR2 on keratinocytes leads to higher expression and activity levels of KLK5, leading to increased expression of LL-37 and its fragments. Topical ivermectin, an antiparasitic agent has been FDA-approved for the treatment of inflammatory lesions of rosacea, however, mechanisms behind this medication are still not completely understood. The purpose of this study is to determine whether the use of topical ivermectin may improve rosacea symptoms by decreasing mediators of the cathelicidin pathway through a reduction in serine protease activity, thus normalizing LL-37 expression on human skin. This was a single-site, 16-week open-label study of up to 15 subjects with an Investigators Global Assessment (IGA) ≥ 1 and at least mild erythema who received topical ivermectin daily for 12 weeks, with 4 in-office visits (screening, then monthly follow-ups for 3 months and one follow-up telephone visit one month after study medication completion). Four tape strip samples were obtained at each visit to determine LL-37 expression, and serine protease activity was measured at baseline and weeks 4, 8 and 12. Preliminary data of 6 subjects in the ivermectin group showed serine protease activity to be significantly lower in subjects at week 12 in comparison to baseline (p<0.002 by two-way ANOVA for multiple comparisons). Daily application of ivermectin appears to decrease serine protease activity, and may be one of the contributing factors in the improvement of clinical symptoms associated with papulopustular rosacea.

486 Molecular analysis differentiates inflammatory dermatoses from skin rejection in face transplant recipients T Win, B Dying-Anderen, J Traeger, L Riella, B Pominach and R Clark Brigham and Women’s Hospital, Harvard Medical School, Boston, MA

Face transplantation is a life-transforming procedure for severely disfigured patients. Clinical challenges include early skin rejection from inflammatory dermatoses and infections caused by biopsies from the largest cohort of face transplant patients at a single center worldwide, we characterized the molecular changes specific for rejection compared to inflammatory dermatoses. Using NanoString gene expression profiling, we analyzed 36 human face transplant skin biopsies collected during acute cellular rejection (n=25) and non-rejection (n=11), and compared with biopsies from non-transplanted patients with rosacea (n=3) and compared with biopsies from non-transplanted patients with rosacea (n=3) and delayed type hypersensitivity reaction (DTH; n=4), or healthy skin (n=4). Gene expression findings were validated with the protein level using immunofluorescence staining of biopsies. Genes over-expressed in rejection compared to non-rejection included ones involved in T cell signaling, interferon-γ (IFNγ) expression, and cytotoxicity. Strikingly, rejection was associated with overexpression of IFNγ inhibitors such as CTLA4 and PD1. A set of 142 genes was differentially expressed (adjusted p value <0.05) exclusively in rejection but not in inflammatory dermatoses or healthy facial skin. In pathway analysis, the top canonical pathways included Th1 and Th2 activation and T helper cell differentiation. Upstream regulator analysis indicates that the genes overexpressed in rejection are potentially activated by IFNγ, while IL17 is implicated for activating genes overexpressed in DTH and rosacea. This is the most comprehensive study to date to identify the molecular signature of face transplant rejection. T cell receptor triggering according to IFNγ expression and IL17 expression with an unexpected co-inhibitory signaling between the effector T cells and antigen presenting cells, raising the possibility of active negative control within the rejecting transplants. Our data indicate that skin rejection, although distinguishable on histology from inflammatory dermatoses, reveals extensive differences at the molecular level.

487 Induction of T cell exhaustion by JAK1/3 inhibition in the treatment of alopecia areata z dai1 and AA Christianso1 1 Dermatology, Columbia University, New York, NY and 2 Dermatology, and Genetics and Development, Columbia University, New York, NY

Alopecia areata (AA) is an autoimmune disease caused by T cell-mediated destruction of the hair follicle. Therefore, approaches that effectively halt the pathogenic T cell response are predicted to have therapeutic benefit for AA treatment. T cells rely on a duality of TCR and gamma chain (γC) cytokine signals for development, activation and peripheral T cell homeostasis. ATI-50002 is a potent, selective next-generation JAK1/3 inhibitor, predicted to disrupt 4C cytokine signalling. We found that CH14.2H6 mice with AA that were fed with ATI-50002 in chow diets robustly induced hair regrowth even after a short 5 week-treatment course in a dose-dependent manner. Immunohistochemistry of treated skin revealed significantly decreased AA-associated inflammation, and CD8+FoxP3+ T cells and CD8+CD44+CD26L effector/memory T cells, known to be associated with the pathogenesis of AA, were markedly reduced in treated mice. Since disruption of the 4C cytokine network in T cells has been shown to induce T cell exhaustion in the setting of chronic viral infection, we postulated that one potential mechanism of action of JAK inhibitors in AA could be via selective induction of T cell exhaustion. Indeed, we observed high expression of the co-inhibitory receptors PD-1 and Tim-3, known to be markers of T cell exhaustion expressed on effector/memory CD8+ T cells, together with decreased IFNγ production, in treated mice. Taken together, our data indicate that selective induction of T cell exhaustion using JAK inhibitors offers a mechanistic explanation for the success of this treatment strategy in the reversal of autoimmune diseases such as AA.
488\textbf{Thermal pattern determination in patients with rosacea using infrared thermography}\footnote{S Ramírez1, T Bierbe, GN Ríos-Ríos, M Arbeau1, E Ginot2, R Aminzadeh2, A Kairbile, C Osornio Martinez2, R Cabrera Alonso3, M Amat-Mas4, B Moncada5, and F González Contreras2, 1Universidad Autónoma de San Luis Potosí, Bonn, Germany, 2CICYTU-UASLP, San Luis Potosi, Mexico, 3CICYTU-UASLP, San Luis Potosi, Mexico and 4Dermatology Department, Hospital Central Ignacio Morones Prieto, UASLP, San Luis Potosi, Mexico.}

Rosacea is a chronic cutaneous inflammatory disease that affects mostly the facial skin, commonly more observed in caucasian population. Although, its pathophysiology is still not clarified it has been theorized to be caused by immunologic alterations and neurovascular dysregulation. Because of the complexity of rosacea, for a diagnosis, it can be categorized into four mainly clinical presentations: papulopustular, erythematotelangiectatic, ocular, and phymatous, but phenotypic presentations of rosacea are more heterogeneous leaving the diagnosis unclear, mistaken or even, and commonly can be confused with another type of disease. Currently, it is suggested, that there is a difference in temperature between the skin with rosacea and the normal skin, but this information is still not confirmed. Our aim is to establish an individual diagnosis criteria based in the difference of thermal patterns of healthy with rosacea and the normal skin, but this information is still not confirmed. Our aim is to establish an individual diagnosis criteria based in the difference of thermal patterns of healthy.

489\textbf{Dupilumab efficacy in atopic dermatitis in four randomized phase 3 trials (liberty ad solo 1&2, chocolates, cafe)}\footnote{S Ramirez Valladolid1, F Chiwo2, C Osornio Martinez2, R Cabrera Alonso3, B Moncada4, and F González Contreras2, 1 Universidad Autónoma de San Luis Potosí, Bonn, Germany, 2CICYTU-UASLP, San Luis Potosi, Mexico, 3CICYTU-UASLP, San Luis Potosi, Mexico and 4Dermatology Department, Hospital Central Ignacio Morones Prieto, UASLP, San Luis Potosi, Mexico.}

Dupilumab (DUP), a fully human IL-4Rα mAb, inhibits signaling of IL-4 and IL-13, key drivers of type 2/T2-mediated inflammation. DUP is approved in the EU, USA, and other countries for the treatment of adults with inadequately controlled moderate-to-severe atopic dermatitis (AD). Our objective is to present efficacy and safety data from four phase 3 trials; pooled SOLO 1&2 (NCT02277744, NCT02277769) - DUP monotherapy vs placebo (PLC) in patients (pts) with moderate-to-severe AD; CHRONOS (NCT02269988) - DUP administered concomitantly with topical corticosteroids (DUP+TCS) vs PLC+TCS in pts with moderate-to-severe AD; and CAFE´ (NCT02755649) - DUP+TCS vs PLC+TCS in pts with severe AD not adequately controlled with, intolerant to, or medically ineligible for oral cyclosporine A (CSA). Pts were randomized to DUP 300 mg SC weekly or PLC 16 wk in all trials. More DUP-treated pts vs PLC achieved a global self-assessment score (0-100) of 15% or more improvement at Wk16 in all trials pooled SOLO (37.5 (SD 22.8) vs 9.5 (SD 21.9), p < 0.001), CHRONOS (43.4 (SD 41.1) vs 12.5 (SD 20.1), p < 0.001), and CAFE´ (40.5 (SD 22.5) vs 14.8 (SD 21.3), p < 0.001). In all trials the most common adverse events (AE) >10% attributable to dupilumab were injection site reactions and conjunctivitis. DUP did not increase overall rate of infections. DUP improved AD signs and symptoms as monotherapy and with TCS, including in pts refractory or intolerant to CSA.

490\textbf{Samycyne (diphenycyprolene ointment) for the treatment of common warts}\footnote{Roman Jakubiec1, BC Carda, M Saurin, B Barefoot, and G DispensyR & K Pharmaceutical, Marlborough, MA.}

Diphenycyprolene (DPCP) is a potent topical immunotherapeutic agent that has been used in a compounded form since the late 1970s by physicians for the treatment of common warts, alopecia areata, and cutaneous metastases of melanoma. Although not approved by the FDA or EMA, physicians continue to use compounded DPCP because of its perceived superior efficacy, ease, and safety when compared to other dermatologic conditions. The use of the drug as reported in the literature is not standardized and widely varying strengths have been suggested for the sensitization and challenge treatments with DPCP, resulting in inconsistent results from a safety and efficacy viewpoint. R&K Pharmaceuticals has developed a proprietary compounding formulation of DPCP called Samycyne that consists of a low sensitization (0.4%) DPCP dose followed by a standardized weekly treatment dose of 0.04% DPCP regimen. Careful attention to the local reactions to be expected from a topical immunomodulator. Results show a statistically different temperature changes on the two kind of face surface, and so, we conclude that the digital infrared technology could be used as an additional noninvasive medical diagnosis tool to rosacea detection.
PD-1-positive tumor-infiltrating lymphocytes are associated with poor clinical outcome after pulmonary metastasectomy for colorectal cancer

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Introduction: The aim of anti-PD-1/PD-L1 immunotherapy is to induce a long-lasting antitumor response. The current study sought to evaluate the clinical and pathological implications of PD-1 expression in tumor infiltrating lymphocytes (TILs) and tumor cells in patients with colorectal cancer treated with PD-1/PD-L1 inhibitors. Patients and Methods: We retrospectively analyzed the medical records of 308 patients who underwent pulmonary metastasectomy for colorectal cancer between 2010 and 2016. PD-1 expression in TILs and tumor cells was determined using immunohistochemistry. Results: PD-1 expression was detected in TILs and tumor cells in 23.3% of patients. High PD-1 expression in TILs was associated with impaired overall survival. Conclusion: PD-1 expression was a strong predictor of survival in patients with colorectal cancer treated with PD-1/PD-L1 inhibitors.

RXI-109 treatment to reduce the formation of hypertrophic dermal scars

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RXI-109 is being developed to target and reduce connective tissue growth factor (CTGF), a key regulator of the dermal scarting pathway, to reduce hypertrophic scarring. RXI-109 is a modified small interfering RNA (siRNA) that is designed to enter cells efficiently and reduce CTGF through RNA interference (RNAi). In the US, there are currently no drugs approved to prevent hypertrophic scar formation. A phase 2a clinical study was conducted with RXI-109 to evaluate its impact on the reduction of hypertrophic scar formation after scar revision surgery. RXI-109-1402 was an open-label, multi-center, prospective, randomized, within-subject controlled study evaluating the effectiveness and safety of RXI-109 on the outcome of scar revision surgery for hypertrophic scars in healthy adults. The study enrolled 4 cohorts to compare the clinical outcome of PD-1/PD-L1 expression in TILs and tumor cells. Expression levels significantly differed between metastases and primary tumors. High PD-1 expression by TILs was associated with impaired overall survival. Additionally, the subgroup of patients, who experienced an upgrading in their TILs/PD-1 status between primary and metastasis had a worse survival outcome compared with patients with the same grade or a downgrading. Thus, PD-1 expression by TILs is a strong prognostic marker in CRC patients with pulmonary spreading.

Metformin exerts anti-neoplastic effects against human and murine squamous cell carcinoma

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Here we investigate the use of metformin against the growth of human and murine SCC both in vitro and in vivo. Metformin inhibits the size of tumor colonies in clonogenic assays and further demonstrated anti-proliferative effects against several human RDEB-SCC lines and the murine SCC VII line in vitro. In vivo, treatment with metformin resulted in a small yet significant delay in the time to development of visible tumors, as well as in a reduced growth rate of the tumors. Thus, metformin significantly increased the efficacy of the murine SCC VII line in vitro and in vivo. Tumor development in mice treated with metformin was significantly delayed compared to controls, indicating that metformin is a promising candidate for the treatment of RDEB-SCC.

Gender stratifies psoriatic patients therapeutic needs - A two-country, multicenter, prospective, non-interventional study

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Introduction: We evaluated the ability of anti-psoriatic therapies to fulfill these patient needs and investigated whether the expectations are stratified by age and gender. Methods: Patients with moderate to severe psoriasis are included in the Psoldest or SINDIT registry when treated with a biologic or other systemic agent that was not used previously. At the first clinical visit, all patients filled out a patient needs questionnaire. Here, data of patients included in Psoldest registry consists of 4894 and the SINDIT of 449 participants from October 2011 until December 2016 were analyzed. Results: 5, 343 patients were included in the analyses, representing patients with a marked disease burden (59.6% male, mean age 47.6 years, mean PASI 14.2, mean BSA 22.7). The most important patient needs were to get better skin quickly and be better at PM effects. Sub group analyses revealed differences in needs, especially higher needs regarding social impairments in patients aged less than 65. Out of 25 items reflecting patient needs, 20 were rated significantly more important by women than men. Discussion: Women showed higher expectations of all psoriasis treatment options than men. The reason is as yet unclear, but could be due to higher perceived importance of women's personal appearance. In the future, the systematic assessment of patient needs and the resulting data matrix will allow to set specific individual treatment goals as well as overall implementation of the patient benefit index.
Lidocaine potentiates thermal injury to proliferating skin and carcinoma cells

All Raff, M Purschke, C Thomas and R Anderson 1 Massachusetts General Hospital, Boston, MA, 2 Beth Israel Deaconess Medical Center, Boston, MA

Background: Hyperproliferative cutaneous lesions, such as non-melanoma and melanoma skin cancers, are the most commonly diagnosed cancers in the United States. While surgery remains the mainstay of treatment, it can be costly and complications can include infection, scarring, infections. Hyperthermia (40-44°C) has been utilized for many years to treat various cancer types as it induces localized cell toxicity. Combining local anesthetics, such as lidocaine, with hyperthermia has been shown in vitro to enhance cell death. Objective: We investigated whether the combination of hyperthermia with lidocaine enhanced cell toxicity in cutaneous cell lines and whether the effect is specific to rapidly proliferating cells. Methods: We exposed normal skin cell lines (fibroblasts, keratinocytes), skin cancer cell lines (melanoma, basal cell carcinoma), and a mucosal cancer cell line (cervical carcinoma) to various concentrations of lidocaine (0.0-0.4%) over a range of temperatures (37-44°C) for 10 minutes. Cell viability was assessed with an MTT assay and cell cycle with propidium iodide and flow cytometry. Results: We demonstrate that cancer cell lines show significantly increased cell death when treated with lidocaine and hyperthermia compared to normal cells (p<0.05). Further, we show that cancer cell lines have a higher percentage of cells in S-phase (28-57%) compared to normal skin cell lines (13-19%), which directly correlated with cell toxicity from combined hyperthermia and lidocaine (R-square = 0.6752). Conclusions: In the future, combined mild hyperthermia with lidocaine may provide a non-invasive alternative to treatment of skin cancer. This treatment demonstrates selectivity for rapidly dividing cancer cells compared to normal skin cells. Further, lidocaine can be applied specifically to cutaneous lesions to potentially minimize unwanted side effects on the surrounding tissue. Additional investigation via clinical trials are needed to confirm these in vitro data.

Cyclosporine vs. IV corticosteroids for the management of SJS/TEN

AB Raff1, M Purschke2, C Thomas2 and R Anderson2

Background: Steven Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN) are rare, but potentially life-threatening, clinical entities. While cutaneous drug-induced lymphocyte transactivation (LDLA) is considered the hallmark of TEN, the cause-and-effect relationship of SJS/TEN remains unknown. Supportive care and intravenous (IV) corticosteroids are the mainstay of therapy given the lack of a validated treatment protocol. Objective: To assess if cyclosporine is a safe and effective alternative to IV corticosteroids for the management of SJS/TEN. Methods: We retrospectively analyzed the medical records of 53 patients with a diagnosis of SJS/TEN admitted to Massachusetts General Hospital over a 5 year period. Patients were divided into two groups (cyclosporine vs. IV corticosteroids) based on the treatment they received. Cyclosporine was not used in all cases due to the lack of insurance coverage, prior use of cyclosporine, and adverse effects of cyclosporine. We assessed the mean duration of 5.95 days until reepithelialization was first noted. There were no incidences of infection, and the most common adverse events were hypertension, acne, and pruritus. Conclusions: Cyclosporine was found to be an effective and well-tolerated treatment choice for severe SJS/TEN. Further prospective randomized clinical trials are necessary to confirm these findings.

Calciphylaxis: important issues in the pathologic diagnosis of a still under-recognized entity

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Calciphylaxis is a critical diagnosis with high associated patient morbidity and mortality. It remains an under-recognized clinicopathologic entity. We sought to characterize calciphylaxis cases seen at our institution and explore challenges in the pathologic diagnosis, with a focus on the use of special stains, subcategory training and awareness of the disease. 41 cases with a clinicopathologic diagnosis of calciphylaxis were collected at Massachusetts General Hospital between 2012 and 2017. Cases included in-house cases (n=22) and outside consults (n=19). Pathology reports and clinical data were reviewed. The mean age of the cohort was 62 years with 61% of patients female. The most common known risk factors in the cohort included end-stage renal disease (n=26) and obesity (n=6). For 12/41 cases (29%), a first false-negative pathologic diagnosis was made. At time of diagnosis, 25/41 of cases were stained for calcium with von Kossa stain. In cases with the correct diagnosis, 22/29 (76%) had a von Kossa stain positive for calcium with a confidence level of 3.5 (0 for unlikely and 10 for certain) compared to normal skin cells (p<0.05). Further, we show that cancer cell lines have a higher percentage of cells in S-phase (28-57%) compared to normal skin cell lines (13-19%), which directly correlated with cell toxicity from combined hyperthermia and lidocaine (R-square = 0.6752). Conclusions: In the future, combined mild hyperthermia with lidocaine may provide a non-invasive alternative to treatment of skin cancer. This treatment demonstrates selectivity for rapidly dividing cancer cells compared to normal skin cells. Further, lidocaine can be applied specifically to cutaneous lesions to potentially minimize unwanted side effects on the surrounding tissue. Additional investigation via clinical trials are needed to confirm these in vitro data.

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Regulatory T cells, Tregs, are increased in multi-parameter flow cytometric profiling of T cell populations in psoriasis

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Psoriasis vulgaris is a T cell mediated skin disease. To study T cells in peripheral blood we compared the number and percentage of regulatory T cells (Tregs) and conventional T-helper cells, including a panel of activation markers in healthy donors (n=7), psoriatic patients (n=19) and between psoriatic patients before (n=19) and after (n=9) a systemic treatment, with (mainly) anti-TNF-alpha biologics. We recruited psoriatic patients with a PASI-score >10 to compare the course of disease with immunological parameters. Including chemokine receptor expression, we characterized different T cell subsets in blood. Within CD3+CD4+ conventional T helper (Th) cells we distinguished in Th1 (CD25^lowCXCR3^highCCR4^negativeCCR6^negative), Th2 (CD25^lowCXCR3^lowCCR4^highCCR6^high), Th17 (CD25^lowCXCR3^highCCR4^highCCR6^high) and Th22 cells (CD25^lowCXCR3^highCCR4^highCCR10^high) as well as in regulatory T cells (CD25^highCD127^lowFoxP3^high Treg), as the latter ones play a role in inhibiting autoreactive T cells. Psoriasis treatment had no significant effect on Th1, Th2 and Th22 cells, but unexpectedly, the number of Th17 cells (in 80mL whole blood) increased from an average of 1.5 million cells before to 3.9 million cells after therapy, which is almost the same level of cells that we observed in healthy donors (3.3 million cells). According to these results, the percentage of Th17 cells slightly rose after treatment of the psoriatic patients. Interestingly, the number of Tregs expressing the skin homing receptor cutaneous lymphocyte antigen (CLA) dropped from 430,000 before therapy to 180,000 cells as well as the percentage of CLA positive Tregs decreased from 27% before to 6% after treatment, reaching similar average values of Tregs in the blood of healthy donors. This flow cytometric study will help to identify a strategy to sort T-helper cells and Treg from patients at different disease stages for RNA sequencing. With this technique we intend to identify molecular targets which are regulated by certain biologics in psoriasis.

Comparing and contrasting molecular and immunohistochemistry outcomes from microbiopsy and conventional biopsy skin and lesion samples from volunteers

T Prow
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We have developed and patented a microneedle-based biopsy device, microbiopsy, for minimally invasive skin sampling. The device takes sub-millimeter skin samples as cores or absorbed lysates depending on the microneedle configuration. Our hope is that this type of sampling can one day be used on cosmetically sensitive areas and in paediatric cases or in cases where a substantial number of biopsies are needed for screening suspicious lesions. To this end we set out to compare molecular outcomes from microbiopsies with conventional biopsies. Data comparing and contrasting gene expression outcomes from topical drug treated human skin sampled with microbiopsy and conventional punch biopsy will be shown. These include the expression of the inflammatory markers IL-1β, IL-8, TNFα and the antioxidant response gene HMO-1. We will present unpublished data comparing and contrasting mRNA expression profiling in microbiopsy and conventional biopsy sampling of non-melanoma skin cancer lesions. We will show data from RNA-Seq outcomes comparing microbiopsy samples to shave biopsy samples from basal cell carcinoma and actinic keratos. In this experiment we took 5 microbiopsies from within lesions and then performed shave biopsy excision. The RNA quality from 4 pooled microbiopsies from an actinic keratosis lesion was 5.9 (649ng total), whereas the shave biopsy had a RIN of 6.7 (31ng total). The Pearson correction coefficient was 0.77 and 0.93 for microbiopsy vs shave biopsy in the actinic keratosis (r²=0.77) and basal cell carcinoma (r²=0.90), respectively. We hypothesize that this is due to increased lesion heterogeneity in the actinic keratosis. Microbiopsy sampling could be valuable to investigate skin lesion heterogeneity. Finally, using microbiopsy does not require local anaesthetic nor sutures. The data we have gathered supports the hypothesis that microsampling has the potential to enable far more clinical dermatology research than currently possible by increasing the number of sampling events, decreasing pain and eliminating disfigurement in volunteers.
508 Impact of teledermatology services at the Atlanta VA Medical Center: Assessing patient satisfaction
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Teledermatology is designed to remotely diagnose and treat dermatologic conditions in patients. This cross-sectional survey-based study (N = 100) aimed to explore patient satisfaction with teledermatology and two distinct teledermatology models at the Atlanta Veterans Affairs Medical Center (AVAMC). Our hypothesis was that mailing medication recommendations along with prescriptions to teledermatology patients (telemedicine mode) results in higher patient satisfaction compared to providing the prescriptions to primary care practitioners (PCPs) (teleconsultative mode). We also hypothesized that increased distance between a patient’s primary care physicians and the AVAMC increases patient satisfaction with teledermatology services at the AVAMC and introduction of the telemedicine model did not significantly impact patient opinions. As the population continues to age, teledermatology will likely continue to be a viable and attractive option for all providers.

509 Association between biologics and major cardiovascular events in adults with plaque psoriasis: A cohort study in the British Association of Dermatologists Biologic Interventions Register Biologic Interventions Register (BADBIR)
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The association between biologics and major cardiovascular events (CVEs) in adults with plaque psoriasis is unclear. We used prospective cohort data from BADBIR to compare CVE risk associated with different therapies in participants recruited 09/2007/10/2016. Anti-interleukin-12/23 agent-ustekinumab (UST) was compared with tumour necrosis factor-α inhibitors (TNFi)-etanercept (ETN) and adalimumab (ADA) in a main analysis and UST, ETN, and ADA in a sensitivity analysis. The primary outcome was fatal or non-fatal major CVEs including acute coronary syndrome, unstable angina, myocardial infarction and stroke occurring on therapy or within 90 days after the last dose. Propensity-score weighted Cox proportional hazards regression models estimated hazard ratios (HRs) with 95% confidence intervals (95% CIs). Safety data were compared in patients from CBOCs (teleconsultative mode) and the AVAMC (telemedicine mode) in seven areas surveyed. Patients from CBOCs further away from the AVAMC were not significantly more likely to agree with the statement “teledermatology is more convenient than face-to-face appointments.” However, 69% of patients at the AVAMC agreed with the statement. Sixty-nine percent (69 out of 100) of all patients surveyed agreed with the statement “I would use the teledermatology service again.” Increased distance between a patient’s primary care physicians and the AVAMC did not significantly impact patient opinions. As the population continues to age, teledermatology is likely to continue to be a viable and attractive option for all providers.
A pre-Delphi consensus exercise to define screening for psoriatic arthritis and measurement of psoriatic arthritis symptoms in psoriatic clinical trials

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The International Dermatology Outcome Measures initiative defined a set of domains to be measured in clinical trials of psoriatic arthritis. Symptoms is part of one of these domains. This work seeks to define PA screening in psoriatic clinical trials and to identify appropriate measures for PA symptoms. The performance of 4 screening tools and the psychometric properties of 3 PA symptom measures (Patient Global (PG)-arthritis, Routine Assessment of Patient Index Data-3 (RAPID-3) and PaAID) were compared by stakeholders in a pre-Delphi, face-to-face meeting. Participants voted on the role of PA screening in psoriatic trials, and on the clinimetric properties of PG-arthritis, RAPID-3 and PaAID. Of the 47 stakeholders who participated in the voting, 91% agreed that all psoriasis trial participants should be screened for PA. Our results showed that 48% of the participants voted that PG-arthritis matched the domain, 62% agreed that it was feasible, and 29% believed that it was sensitive. Results of HH guidelines were translated to RAPID-3. 61% agreed that it was a good measure of the domain, 65% claimed that it was feasible, and 54% voted that it was responsive. Finally, 58% of participants agreed PaAID was a good measure for PA symptoms, 73% voted it was feasible, and 50% claimed it was responsive. This pre-Delphi study showed all psoriasis trial participants should be screened for PA (PaAID and PaAID were preferred over PG-arthritis. Based on these results, PaAID was recommended for use in all future PA trials in psoriasis. This will be followed by a Delphi-survey involving a larger stakeholder group.

Global collaboration for establishment of a prognostic index in mycosis fungoides & Sezary Syndrome

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The PROCIPI Study, a PROspective Cutaneous Lymphoma International Prognostic Index study, launched in 2015 collecting clinico-pathological, genetic, quality of life & treatment data in mycosis fungoides, Sézary syndrome (SyS) to develop a prognostic index. A SyS tissue biobank for translational studies. Central clinico-pathological review is performed to confirm diagnosis & stage. Unique databases were developed with 816 patients registered so far from 46 sites over 5 continents. 600 early stage (IA-IIIa) patients; 381 male/219 female & 236 advanced stage (IIIB/IV) male/94 female. Stage IA-IIIA was an exclusion criterion. With a pre-treatment & post-treatment phase, 86% agreed with the treatment. A 12-mo follow-up phase was maintained through end of treatment (EOT). PRO endpoints were assessed with the Autoimmune Disease Impact Measure (ADcQoL) (range 0-100), Dermatology Life Quality Index (DLQI; range 0-30), Patient-Oriented Eczema Measure (POEM; range 0-28), and change in the patient’s global assessment of disease activity (PtGA; range 0-4) of 0/1 and EOT difference (SE) of 2.79 (0.48), P<0.005, respectively. This was retained in the non-mucosal group (DLQI p<0.05), but not for the mucosal group between the B/PDAI and ABIS. The Skindex-3 and DLQI overall had the strongest correlations with QOL in our cohort and subgroups. There were no significant differences in the subgroups for the B/PDAI (0.2), compared to the ABIS (2). The B/PDAI better correlated with DLQI and Skindex-3 than the ABIS. Assuming QOL worsens with disease severity, the B/PDAI may be a better tool than the ABIS.

Effects of the oral janus kinase 1 (JAK1) inhibitor PF-04965842 on patient-reported outcomes (PROs) in adults with moderate to severe atopic dermatitis (AD)

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We report PROs from the double-blind, Phase 2b study of oral JAK1 inhibitor PF-04965842 for treatment of AD (NCT02780167). 267 adult patients (pts) with moderate to severe AD were randomized 1:1:1:1:1 to 12-wk oral, once-daily PF-04965842 (10mg, n=49; 30mg, n=55; 100mg, n=56; 200mg, n=55) or placebo (PBO, n=56). PRO endpoints were mean change from baseline (BL) in patient-reported outcome change for the post-24h NRS, range 0-10), Dermatology Life Quality Index (DLQI, range 0-30), Patient-Oriented Eczema Measure (POEM, range 0-28), and proportion of pts with Patient Global Assessment (PGA; range 0-4) of 0/1 and ≥2-point improvement from BL. Pruritus severity in the 200mg and 100mg groups was significantly improved vs PBO by day 2 and day 3, respectively, and was maintained through end of treatment (EOT) (EOT difference [SE] of 2.79 [0.482], P<0.005, respectively). The 200mg and 100mg groups also showed significant improvement vs PBO by wk 1, which was maintained through EOT observed response of EOT at 51.9% vs 7.4% (OR 90% CI 4.8, 31.27). PF-04965842 resulted in rapid and lasting improvements in PROs. The 200mg dose of oral PF-04965842 showed significant improvement in all PROs, and the 100mg group exhibited significant improvement in pruritus NRS severity, DLQI, and POEM scores.

A combination of re-exposure and active learning maximizes medical student satisfaction and long-term recognition of skin lesions

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Family physicians (FPs) are often the first medical providers approached when a patient has a skin growth of concern. Numerous studies have shown that dermoscopy improves a clinicians ability to diagnose malignant neoplasms. However, the majority of dermoscopy training has been geared toward dermatologists. The Triage Amalgamated Dermoscopic Algorithm (TADA) is a simplified dermoscopic algorithm for skin cancer detection that may be beneficial for FPs. Prior data showed a sensitivity of 93.7% and specificity of 72.1% for malignant skin growths in a limited-complexity setting. In this study, we aim to 1. validate the ability of FPs to identify dermoscopic images of skin cancer following TADA training 2. Improve the specificity of TADA by adding clinical and dermoscopic training of benign neoplasms to TADA training. Methods: We conducted a pre- and post-test in which FPs were asked to identify 60 unique dermascopic images in which FPs were asked to identify 60 unique dermascopic lesions. This intervention was conducted at three separate sites, and sites were randomized to a variation in the Intervention: All participants completed Clinical decision-making process 1. Cohort 1 was a 2-site v. 2 visits QOL was assessed with the Autimmune Blennous Disease QOL (ABIQO), Dermatology Life Quality Index (DLQI), Skindex (Symptoms [S], Functioning [F], and Emotions [E]), and Short Form Survey 36 (SF-36). Disease severity was scored with the B/PDAI and Psoriasis Disease Activity Index (BPDAI) and Autimmune Blennous Skin Disorder Intensity Score (ABIS). Correlation between QOL scores and disease severity was analyzed using Sperman rank (r). Subgroup analysis was performed on those with mucosal involvement (n=18) and those without (n=16). B/PDAI scores were compared with skin cancer prior to intervention score (out of 30 correct responses) was 17.9 SD 4.5 and increased to 23.5 SD 3.0 on the post-intervention evaluation, p<0.01. For all possible benign diagnoses, the sensitivity and specificity of the diagnosis did increase. For malignant diagnoses, the final sensitivity was 98% and the specific 0.78%. Based on the high sensitivity of adding education on benign skin lesions improves the sensitivity of diagnosing malignant skin lesions, but slightly decreases the specificity. Our results support previously published data showing TADA is an effective dermoscopy teaching algorithm for FPs.
Three-dimensional modeling and comparison of nasal flap designs

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Aims: Three-dimensional (3-D) imaging systems are available for reconstructive surgeons, but the use of these systems in reconstructive nasal surgery is limited.

Methods: The objective of this study was to compare different flap designs using a 3-D imaging system.

Results: The 3-D imaging system was used to capture images of actual post-Mohs nasal defects. Designs were assessed based on the following: tissue undermining area, total incision length, motion (expressed as a length incision/Euclidean signed distance), and undermining efficiency (g/SAtrimmed + SAincision*100). Transposition, rotation, and advancement flap designs were modeled on the 3-D images of twelve consecutive patients with nasal defects. Designs were assessed based on the following: tissue undermining area, total incision length, motion (expressed as a length incision/Euclidean signed distance), and undermining efficiency (g/SAtrimmed + SAincision*100). Rotation flap designs gave the best results as they were the least time-consuming. Advancement flap designs are relative to other flap designs. Transposition designs are the least suitable for repair. In our study, advancement flaps had less total undermined area when compared to the transposition flaps and rotation flaps (although not statistically significant). Advancement flaps are more efficient in terms of 1° and 2° motion (p<0.027). Incision and undermining efficiency is equivalent between all designs (p=0.01, and p<0.18, respectively). While there are some significant differences between the flap designs studied, the clinical significance of these differences is unknown. Consequently, the choice of a repair design should be made based on the ability for the repair option to attain a functionally and aesthetically successful reconstruction.

Improved keratinocyte carcinoma outcomes with annual dermatology assessment after solid organ transplantation: Population-based cohort study

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Aims: The impact of annual dermatology assessment in patients after solid organ transplantation (SOT) on keratinocyte carcinoma (KC) development is unclear.

Methods: A population-based electronic medical record cohort study was conducted in the province of Ontario, Canada. Annual dermatology visits were performed by dermatologists, and the outcome of interest was the diagnosis of KC during the entire observation period. The primary endpoint was the development of at least one KC during the observation period. The secondary endpoint was the development of at least one KC after the first observation visit in patients with no KC at baseline.

Results: A total of 44,132 recipients of solid organ transplantation were included in this study. The incidence of at least one KC was significantly lower in SOT recipients having ever seen a dermatologist (2.1%) compared to those never seeing a dermatologist (7.2%) (HR 0.30 [95% CI 0.27-0.34], p<0.001). Adherence levels were universally low with only 45% of SOT recipients having ever seen a dermatologist and 2.1% being fully adherent during the entire observation period. Annual dermatology assessment may reduce the development of advanced KC in transplant recipients. Strategies are needed to improve adherence rates in order to help decrease long-term morbidity after transplantation.

Cultured epidermal autograft from clinically revertant skin in recessive dystrophic epidermolysis bullosa

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Recent studies have shown epidermolysis bullosa (EB) patients to have sporic autosomal dominant revertant mutations in their skin. Patients with EB lesions that revert spontaneously have often been used to study the potential to realize fundamental treatments for severe EB, very few attempts toward such treatments have been reported. We encountered a case of recessive dystrophic EB (RDEB) with deep skin ulcers and severe deformities. The patient was 16 years old and had been treated with cultured epidermal autografts (CEAs) and dermatological treatments for 8 years. A research trial of CEAs from revertant skin in RDEB patients as a single-center study. CEAs were performed by NGS method. There is no statistically significant difference in distribution of HLA-DRB1 or DPB1 alleles. The haplotype frequency of having any DRB1 alleles in linkage disequilibrium with DQB1*03:02 among patients and controls. In summary, DRB1 is the primary genetic locus contributing to susceptibility to dermatomysitis positive for anti-TIF1 antibody in Japanese patients.

DRB1 is the primary genetic locus contributing to susceptibility to dermatomysitis positive for anti-TIF1 antibody in Japanese patients

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Recent studies have shown epidermolysis bullosa (EB) patients to have sporadic normal-DRB1 alleles. The haplotype frequency of having any DRB1 alleles in linkage disequilibrium with DQB1*03:02 among patients and controls. In summary, DRB1 is the primary genetic locus contributing to susceptibility to dermatomysitis positive for anti-TIF1 antibody in Japanese patients.
Association of tumor response to PD-1/PD-L1 immunotherapy and type of dermatitis that arises after the immunotherapy

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cutaneous adverse events are common with Programmed Death (PD)-1/1 PD-Ligand (L1) in-hibitors. However, the nature of the specific cutaneous adverse event of dermatitis has not been investigated across various PD-1/PD-L1 inhibitors, and the clinical significance of dermatitis is not well characterized. Therefore, we performed a retrospective case-control study at a single academic center using the Stanford Cancer Research Database. Inclusion criteria were patients with a dermatologic study assessed at least at baseline and at 6 months after initiation of safety atezolizumab. Cases were defined as individuals with new dermatitis after initiation of PD-1/ PD-L1 inhibitor and up to three months after drug discontinuation, for which a skin biopsy had been performed. Controls were individuals who did not develop dermatitis. Propensity score matching was used to ensure case and control pairs were comparable. A total of 96 controls for analysis. The most common histologic patterns were lichenoid dermatitis (50%), spongiotic dermatitis (40%), atypical lymphoid infiltrate (5%), and vascular interface dermatitis (5%). The overall response rate was 65.0% for cases and 17.0% for controls (p = 0.0007), odds ratio: 7.3 (95% CI 2.3-24.1), adjusted for PD-1/PD-L1 inhibitor used. PFS and OS times were significantly longer for cases than controls by Kaplan-Meier analysis (p = 0.0006 for OS). Together, these results suggest dermatitis associated with favorable oncologic outcomes, and future studies may identify more specific molecular markers that can predict response to immunotherapy in patients.

Disparities in annual direct and indirect healthcare costs between nonwhite and white patients with chronic pruritus

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Chronic pruritus (CP) significantly impacts quality of life (QoL). We have reported racial dis-parities in QoL impact and medical resource utilization due to CP. Aside from previous studies on individual pruritic diseases, the economic burden of CP from the multitude of possible eti-ologies has not been characterized nor analyzed by race. We investigated the potential disparity in annual direct and indirect healthcare costs between nonwhite (N = 116) and white (N = 271) patients with CP from our VA Pruritus Study, which sampled 2,000 veterans nationally. Surveys assessing the direct costs, including ER and primary care visits, and indirect costs, including time lost from work due to CP, were used to estimate costs via public pricing databases and national work payment estimates, respectively. One-way ANOVA and Kruskal Wallis tests were performed for numerical covariates and chi-square testing for categorical covariates. Most of the CP subjects were male (95%) and reported moderate itch (59%) while 23% reported mild and 19% severe itch. The median annual total direct cost for treating CP was $286. Adjusting for age, itch severity, and education in a multivariate regression model, race significantly predicted annual total direct costs; median annual direct costs significantly differed (p < 0.046) as compared to whites. Total indirect costs also significantly differed between whites and non-whites (p = 0.019), with a statistical trend (p = 0.055) for nonwhites missing more days from work (60 vs. 104); this difference did not remain after adjusting for itch severity and age. Our results illuminate the differential economic burden among nonwhites that spans various aspects of direct and indirect costs and supports the growing literature on the racial disparity in CP.

Factors predicting likelihood of skin improvement in cutaneous lupus erythematosus

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Skin damage manifestations of cutaneous lupus erythematosus (CLE), such as dyspigmenta-tion and scarring, are highly variable, but may improve over time. Little is known on which factors distinguish patients who do and do not experience skin damage improvement. To identify these clinical factors, we analyzed longitudinal observational data collected prospectively from a cohort of patients with CLE enrolled in the University of Texas Southwestern cutaneous Lupus Registry. These data include demographics, diseases history, medication history, autoantibodies, and disease severity scores such as the Cutaneous Lupus Activity and Severity Index (CLASI). Patients with CLASI disease ≥2 scores ≥5 and ≥2 study visits were included in the analysis. Patients with drug-induced CLE were excluded. We designed two response thresholds based on relative decrease in CLASI-D score of ≥10% and ≥30% on consecutive visits. We performed univariable and multivariable logistic regression to identify factors associated with skin damage improvement and non-improvement. 74 patients with variable follow-ups between July 2009 and September 2016 were included in the analysis. At the CLASI-D ≥30% and improvement thresholds, higher initial disease activity as measured by CLASI activity (A)-score (CLASI-D ≥20%: OR 0.91 (95% CI: 0.84-0.98), p = 0.02; CLASI-D ≥40%: 0.88 (0.79-0.97), p < 0.009) and African American race (CLASI-D <20%: OR 0.40 (1.07-1.93), p = 0.03; CLASI-D ≥40%: 0.25 (0.08-0.82), p = 0.02) were associated with decreased likelihood of skin damage improvement. Markov chain Monte Carlo models adjusting for variation in follow-up length and a range of CLASI-D thresholds yielded similar results. Potential explanations for these findings include evolving skin damage in the setting of active inflammation, and increased perceptibility of skin damage in darker skin types. These results will help inform patient and physician expectations for skin damage change in the setting of CLE.

Psoriasis and diet: Evidence synthesis from the Medical Board of the National Psoriasis Foundation

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While psoriasis is a chronic inflammatory disease, dietary interventions have been studied extensively. Of note, an interesting role for diet in psoriatic patients with previous studies showing that dietary patterns, such as the Mediterranean diet, can be associated with lower disease activity. This meta-analysis sought to evaluate the impact of dietary change on psoriatic patients. We performed a meta-analysis of diet interventions in psoriasis that were completed in the last five years. The primary aim of this meta-analysis was to determine if dietary intervention reduced disease activity. The literature search revealed 49 studies that were included in the analysis. The primary outcomes of interest were changes in disease activity using the Psoriasis Area Severity Index (PASI) and Psoriasis Area Index (PAI). The results of the meta-analysis showed that the intervention group had a significantly lower disease activity compared to the control group [mean difference (MD) = 1.65 (95% CI, 0.57-2.72); p = 0.001]. No significant difference was found in the duration of the intervention. Additionally, the results of this meta-analysis suggest that dietary interventions may help improve skin damage in psoriatic patients. Future studies are needed to further investigate the potential of dietary interventions in psoriasis.
Patient-reported anxiety decreases with isotretinoin therapy

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Patient reported outcome (PRO) measures are important in measuring the impact of a given disease state on a person's mental and physical health as well as assessing the quality of health care. The PROMIS (Patient Reported Outcomes Measurement Information System) is a PRO system developed and validated by NIH to assess specific domains within physical, mental, and social health across disease states that can be performed in the clinic. The PROMIS system was initiated at outpatient dermatology clinics in April 2016. Acne vulgaris is an often anxiety inducing disorder that has a significant impact on quality of life. We hypothesized that the anxiety domain would decrease over time in patients treated with isotretinoin, suggesting an improvement is self-reported anxiety. It was found that the anxiety domain decreased with treatment in patients who completed PROMIS Anxiety assessment (n=28, mean difference = 5.6, p=0.00042, paired t test). Of the 8 patients (28%) who initially had clinically significant anxiety, only 4 (14%) remained in the clinically significant range at the final scoring. PROMIS is a convenient and robust system that can validate treatment success in outcomes-based healthcare models.

Perceptions of eczema in social media

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Skin disease causes a significant amount of distress in patients, but feelings of stigmatization or embarrassment may prevent these patients from discussing important issues with their physicians or family. Social media has evolved to serve as a platform for these patients to freely communicate their experiences with skin disease to patients suffering from the same disease, as well as a larger, worldwide audience. Thus, user content on social media reflects what patients consider important about their disease, as well as what they wish others to know about their experiences with their disease. This study aims to identify patterns in content on Instagram posts discussing eczema to better understand patient and public perceptions of eczema. 347,243 public Instagram posts from 2013 to 2017 were identified to contain the hashtag #eczema. Hashtags within each post were compiled and their frequencies calculated for analysis. The most frequently used hashtags were manually filtered, with hashtags advertising commercial products or using foreign language removed from analysis. The five most popular hashtags identified in posts with #eczema were #eczema, #eczemanail, #eczema, #eczema, and #eczema, appearing in 22.0%, 19.7%, 11.9%, 10.4%, and 10.2% of #eczema posts respectively. #naturalskincare and #vegan were the 9th and 10th most popular hashtags, while #sensitive, #eczema, and #eczema appeared 11th, 12th, and 14th spots respectively. This study demonstrates that natural living is of significant interest to patients for the treatment and management of eczema. Additionally, skin appearance is a primary concern of eczema patients, with #beauty being the 8th most popular hashtag, appearing in 8.0% of posts included in our analysis. The trends observed in this study can help dermatologists better understand implicit perceptions and expectations patients have about eczema, to better communicate and develop effective treatment plans for this burdensome skin disease.

Analysis of serum interleukin-10 and interleukin-35 levels in pemphigoid patients

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Pemphigoid is one of main compositions of autoimmune bullous diseases as well as pemphigus, which is characterized histologically by subepidermal blistering and immunologically by autoantibodies against the dermal-epidermal junction. Although interleukin (IL)-10 is an anti-inflammatory cytokine and IL-10 producing B cells, called B10 cells, are reported to regulate some human autoimmune diseases, the association between B10 cells and several autoimmune diseases has not been well-evaluated. We have previously reported decreased level of B10 cells in patients with pemphigus, but not with pemphigoid. On the other hand, according to recent reports, IL-35, which is a novel member of IL-12/23 family, is thought to play an important role in immunoregulatory and autoimmune disease processes. While it has been reported that IL-35 may act as an efficient therapeutic strategy for pemphigus, the association between IL-35 and pemphigoid has not been evaluated. Therefore, we investigated serum IL-10 and IL-35 levels by enzyme-linked Immunosorbent assay using serum samples from 40 untreated pemphigoid patients and 10 healthy individuals in this study. We also conducted an evaluation regarding the association between the serum cytokine levels and disease severity using Bullous Pemphigoid Disease Area Index, and summarized differences in clinical manifestations, such as the number of eosinophils or autoantibodies against BP180-NC16A titers in the peripheral blood, and administration history of dapsone/depolymerase-4 inhibitor.

Hidradenitis suppurativa: Characterization of a dedicated treatment center in the Bronx

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Hidradenitis suppurativa (HS) is an inflammatory disease of recurring abscesses, often considered a chronic disease of the skin. There are dedicated treatment centers to provide care using a patient-centered approach. We postulated that dedicated resources would improve quality of life. A retrospective chart review of HS cases from January 2015 to November 2017 was conducted, including Dermatology Quality of Life Index (DLQI) scores at initial visit and follow-up every 6 months. Non-parametric statistical analysis was performed. Of 375 patients, 74.0% were female; and 26.0% male; 36.3% were Hurley stage I, 25.8% stage II, and 36.9% stage III. HS diagnosis was made by a dermatologist (46.5%), provider (12.5%), local specialist (17.9%), and non-provider (23.1%). At follow-up (8.4%). Median symptom duration prior to initial visit was 6, 8, and 10 years for stage I, II, and III, respectively. Overall, symptom duration was shorter for those diagnosed by a dermatologist. A family history of HS-like symptoms was reported by 35.5% of patients as follows: father (26.7%), mother (28.7%), brother (24.4%), sister (20.7%), and other (30.8%), with 8% patients who had a higher DLQI (16) than those without (12). Of interest, 37.9% of patients reported disease triggers, including perspiration (21.1%), stress (17.6%), mesenchymal imbalance/pregnancy (17.9%), heat (11.2%), food items (10.3%) and physical activity (5.0%). After treatment for 6 months, the median baseline DLQI scores decreased from 11 to 9 with a significant median DLQI change per patient of -2.5 (p<0.05). Before treatment, DLQI scores were 8 (n=63), 13 (n=61) and 19 (n=95) in stages I, II and III respectively; at follow-up, DLQI scores were 11 (n=9), 8 (n=15), and 9 (n=25) change in stage I/II compared to baseline, p<0.016. We found dermatologists most commonly diagnose HS. A third of our patients identified family members with HS-like illness, as well as a variety of disease triggers. Our data shows a dedicated HSCT may have a beneficial impact on quality of life.

The Rivelin® patch: an adhesive patch for targeted treatment of oral lichen planus

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OLP is an inflammatory oral disease where erosive painful lesions impact quality of life. Preclinical data indicate that a cobaltated containing Rivelin® patch has the potential to overcome the current treatment challenges fulfilling an unmet need. Objectives: 1) Investigate adhesive traits and tolerability of OLP lesions patches applied to symptomatic OLP lesions. 2) Evaluate the feasibility to apply patches, Methods: A total of 13 symptomatic OLP were enrolled in an open label study, to confirm the tolerability and safety profile of plain patches in patients. Out of 13 OLP patients did 12 complete the study. A target lesion was identified by the investigator and subjects applied 1-2 patches to the target lesion twice daily for 28 days. Patients were assessed at weekly visits and data on adhesion time and symptoms were collected in daily diaries. Results: Mean adhesion time was ~ 90 mins. An assessment of the tolerability and sensation of the Rivelin plain patches revealed a profound positive feedback. No worsening of symptoms and lesions were observed. All subjects could successfully apply patches. Conclusions: The Rivelin® patch provide a new treatment strategy. Based on these findings the Cts patch will be evaluated in a phase 2b study in 240 symptomatic OLP patients with the assumption of providing longer contact time of Cobaltated at lesion site, securing a unidirectional fixed dose (two-layer-patch) and a high compliance (all patients could apply patches with a favorable tolerability and sensation profile).

Predicting response to ustekinumab in patients with psoriasis: A multicentre prospective observational cohort study

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Biologic therapies have transformed treatment of immune-mediated inflammatory diseases, yet significant numbers of patients experience treatment failure. Although variability in drug response has been explained by the genetic basis of psoriasis, there is limited data in psoriasis. In this prospective observational cohort study (n=60 dermatology specialist centres), we show for the first time that serum levels of the IL-12/23 antagonist ustekinumab can predict subsequent response. We analysed data from 407 patients on ustekinumab (65% male, median age 46 years) recruited to Biomarkers of Systemic Treatment Outcomes in Psoriasis (BSTOP) within the British Association of Dermatologists Biologic Interventions Registry (BADBIR), which records detailed clinical information including demographics, comorbidities, treatments and adverse effects. Serum concentrations of ustekinumab were measured using an enzyme-linked immunosorbent assay at baseline and at 6 months. Response to treatment was defined as PASI 75 (primary outcome). Significant findings related to ustekinumab drug levels taken early in the treatment course (before 12 weeks; n=117 patients) were 33 (9%) made it to PASI 75 at 6 months and 19 (5%) made it to PASI 75 at 12 months. This was a significant predictor of 6-month PASI 75 response (OR(sqrt drug level) 2.42, 95%CI 1.27-4.60, p=0.007). Multivariable modelling showed that baseline PASI was also a key baseline predictor influencing individual-interaction variance. This real-world study with pragmatic drug level sampling provides evidence to support future predictive measures of treatment response, management of psoriasis, highlighting the importance of taking drug levels into account when searching for biomarkers of treatment response.
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Fc\(\gamma\)R blockade with SYNT001 for the treatment of pemphigus

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In pemphigus, immune activity leads to pemphigus vulgaris (PV) or pemphigus foliaceus (PF). SYNT001 is a human IgG1 monoclonal antibody optimized to inhibit Fc\(\gamma\)Rn. SYNT001-103 (NCCT0375904) is an ongoing, open-label phase 1b study evaluating safety and clinical effect of SYNT001 in PV or PF. Up to 3 cohorts were assigned to receive 5 weekly doses of SYNT001 intravenous administration. Primary objective is safety; secondary objectives include pharmacokinetics and clinical response. Follow-up is through Day 112. The study is approved by each site’s IRB; all subjects provide written informed consent. As of 12/31/2017, 3 subjects (2 PV; 1 PF) were enrolled in Cohort 1 (10 mg/kg). All completed 5 doses of SYNT001 and were in various stages of follow-up. Two subjects were retreated using a related mild-to-moderate headache after Dose 1 only. The 3rd subject had a transient urticarial infusion reaction after doses 4 and 5 responsive to diphenhydramine. By Day 30 1 PV subject had decreases of 48% in total IgG, 63% CIC, 30% anti-Dsg1 titer and 40% anti-Dsg3. Pemphigus Disease Area Index (PDAI) was 47 at baseline, 38 on Day 14 and a low of 15 on Day 28. By Day 10 the other PV subject had decreases of 59% in IgG, 48% CIC, 64% anti-Dsg1 and 92% anti-Dsg3. The patient was withdrawn on Day 19 at a baseline PDAI of 54 and a low of 13 by Day 33. By Day 30, the PF subject had decreases of 65% in IgG, 47% CIC and 12% anti-Dsg1. Day 3 was negative. PDAI was at baseline and a low of 7 on Day 21. These interim data show SYNT001 is well tolerated in PV and PF. This first-in-human study in pemphigus provides initial proof-of-concept for anti-Fc\(\gamma\)R treatment with SYNT001 in this disorder.

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Intravenous immunoglobulin is an effective treatment for refractory cutaneous dermatomyositis

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Cutaneous dermatomyositis (DM) is often refractory to multiple systemic medications, suggesting need for effective alternative treatments. We investigated effects of intravenous immunoglobulin (IVIG) on patients with refractory cutaneous DM. Retrospective review of 42 patients treated with IVIG for refractory cutaneous DM at our institution with clinical data available at DM diagnosis. IVIG was initiated for refractory cutaneous DM alone (n=19) or refractory cutaneous and muscular disease (n=27) in patients with various DM subtypes. Overall, 83% of patients had cutaneous DM improvement, including 87% treated for refractory cutaneous DM and 82% treated for refractory cutaneous and muscular disease. Cutaneous DM improvement occurred regardless of DM subtype and was observed on average 1.38 IVIG cycles. No statistically significant clinical predictors of IVIG response/lack of response were detected. IVIG use resulted in decreased systemic glucocorticoid use to manage refractory cutaneous DM in 80% of patients. These findings suggest IVIG can be clinically- and cost-effective refractory cutaneous DM treatment and warrants prospective study.

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Digital assessment of hidradenitis suppurativa cutaneous activity

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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease affecting up to 2% of European adults. Disease activity is commonly assessed by counting of inflammatory nodules, abscesses and fistulas. Thus, it is time consuming and subject to inter-rater variability. We sought to assess HS disease activity by means of automated, digital image analyses. Digital images of axillary and inguinal HS nodules were collected in a clinical routine setting, using smartphones and a CE medical device certified skin imaging platform. Photos were automatically normalized for illumination and color. Image characteristics such as an erythema-score and image complexity were calculated for all photos comparing affected and unaffected skin. Parameters were used to calculate the HS Activity and Score (HiSASS) and correlated with the average Physician Global Assessment (PGA) of each picture provided by 3 independent dermatologists. Follow-up images were used to evaluate disease activity over time. 226 photos of 150 HS-affected skin areas (52% axillary, 48% inguinal) and 33 non-affected controls were analyzed. HiSASS correlated significantly with PGA scores (p<0.000). Further, HiSASS clustered disease activity into three categories similar to the Hurley grading system: HiSASS<5 mild (PGA<0.1); 0.7-1.3, moderate (PGA=2.3); 1.5-1.6 and severe disease (PGA>5). 1.6-1.9. Additionally, the HiSASS allowed for a dynamic assessment of disease activity over time: Increasing HiSASS in the follow-up group (n=10; baseline: HiSASS<5, 1.6, follow-up: HiSASS<5, 1.9) indicated disease worsening and correlated significantly with increasing PGA scores (p<0.009). Normalized mobile phone images could allow a fast, reliable, dynamic and reproducible disease severity assessment in HS patients.

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Changes in blood involvement in Sezary syndrome positively correlate with skin severity but not in mycosis fungoides

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Sezary syndrome (SS) is a neoplastic disorder of T cells. CD4+CD7- and CD4+CD26- where B0 defines SS. We assessed if changes in b-class over time measured by flow correlated with mSWAT score, 41 correlated with mSWAT & 17 had no change in mSWAT. b-class and to mSWAT correlated (71%) vs only 24/52 (46%) in MF, p<0.002. Also 75/168 patients had mSWAT change without b-class change (61 with MF). We show that in SS changes between B0-B1 measured by flow varies significantly with increasing PGA scores (p<0.001). Of SS patients, 13/8 patients had mSWAT change without b-class change involving B2. 104/105 patients had mSWAT change with b-class change. Changes in b-class significantly with increasing PGA scores (p<0.001).

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Evaluating the burden of prior authorization in the treatment of psoriasis

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Psoriasis affects over 7.4 million people in the United States, and many remain untreated or undertreated. We conducted a retrospective review of all dermatology visits in an academic practice from May 1, 2017 to July 31, 2017 to investigate the PA burden for dermatology will likely continue to increase. Further studies are needed to assess the burden of dermatology PAs on patient treatment outcomes and administrative healthcare costs.
544 Adjuvant radiotherapy following resection for non-melanoma skin cancer: A retrospective analysis of recurrence risk at Stanford

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Adjuvant radiotherapy is often employed following resection of high risk non-melanoma skin cancers (NMSCs). However, little is known about either the risk of recurrence or the risk factors for recurrence following adjuvant radiotherapy. We performed a retrospective chart review of all adults with isolated NMSCs. To determine whether an online model provides easier access to care than in-person care, we conducted a 12-month, multi-centered, pragmatic, randomized controlled trial. Psoriasis patients were randomized to intervention or usual care for their psoriasis management. Clinical results emphasized the critical need for additional studies to further describe the measurement properties of treatment satisfaction instruments used in pediatric psoriasis and identify validated, standardized psychometric properties. We present a minimally invasive approach using advanced imaging technologies and microbiopsy sampling that removes significant hurdles when studying cosmeceuticals. We utilized in-depth interventions. Our intervention was one day and the assessment was 5 months afterwards. As our data are promising, a future prospective controlled cohort study may be interesting to determine the role of the capacity model.

545 Online models of specialty-care delivery have the potential to improve access to care in chronic skin diseases. To determine whether an online model provides easier access to care than in-person care, we conducted a 12-month, multi-centered, pragmatic, randomized controlled trial. Psoriasis patients were randomized to intervention or usual care for their psoriasis management. Clinical results emphasized the critical need for additional studies to further describe the measurement properties of treatment satisfaction instruments used in pediatric psoriasis and identify validated, standardized psychometric properties. We present a minimally invasive approach using advanced imaging technologies and microbiopsy sampling that removes significant hurdles when studying cosmeceuticals. We utilized in-depth interventions. Our intervention was one day and the assessment was 5 months afterwards. As our data are promising, a future prospective controlled cohort study may be interesting to determine the role of the capacity model.

546 Testing zinc oxide particle responses in volunteer skin using a microbiopsy based approach in volunteers

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Zinc oxide particle treatment has been shown to be both benign and toxic in different cell and animal systems. We and others have investigated particle penetration in skin after topical treatment. Evaluating sunscreens in volunteers and cell models has significant limitations. We present a minimally invasive approach using advanced imaging technologies and microbiopsy sampling that removes significant hurdles when studying cosmeceuticals. We have found that oxidative stress can be assessed in Zinc oxide particle-treated volunteer skin using conventional dyes in freshly microbiopsied samples. These living, miniature skin samples revealed stable oxidative stress levels in intact and tape-stripped volunteer epidermal tissue after exposure to ZnO-NP in vivo, however, responded with significant oxidative stress in the granulocytes. In vitro, treated with tert-butyl hydrogen peroxide. Multiphoton microscopy of treated volunteer skin showed no penetration of zinc oxide particles in the viable epidermis in intact and barrier disrupted skin. Microbiopsies from zinc oxide particle with and without UV treated volunteer skin were evaluated for Interleukin-8 (IL-8) and hemeoxygenase-1 (HMO1) mRNA expression with real time PCR. These results showed elevated IL-8 and HMO1 gene expression in UV treated skin (3 x minimal erethema dose) induces expression. Zinc oxide particle treatment decreased IL-8 and HMO1 mRNA expression levels over placebo treated skin. Sunscreen pre-treatment decreased UV induced IL-8, but HMO1 expression was slightly increased. We see these data as support for the development of standardized microbiopsy based nanotoxicology testing for topical engineered materials.

547 Gender perceptions of scientists among 5th graders

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An interesting dialogue occurred at the 2017 SIDs annual Irving H. Blank Forum: Building Team Science: Creating a Culture of Diversity and Inclusion; reality and perception may not always match. While scientists come from all gender and racial backgrounds, a child’s perception of a scientist may not accurately reflect this. Previous studies have shown that this mismatch is especially prominent with girls, regardless of age or cultural background, as they pre-dominantly have a mental perception of scientists as men. This is particularly concerning as it may subconsciously influence future career choices and performance in school. Dermatologists may have an opportunity to influence this area since many children visit dermatologists for treatment as they are considered women. In this capacity, we propose that the purpose of this study is to examine the influence of a female scientist role model on children by using the Draw-A-Scientist Test (DAST) methodology, mentioned in last years Irvin H. Blank Forum, which circumvents any verbal communication difficulty by asking children to draw a scientist, and then analyzes the drawings with a checklist of 15 characteristics of a stereotypical scientist. We performed a retrospective analysis on classwork from a group of 5th graders from a local elementary school. Early in the school year, the one of the authors, a female scientist, hosted a day-long interactive botany session. Five months later, the science teacher asked the children to draw a scientist. A total of 70 drawings were collected, 44% from self-identified girls. 77% of the girls and 3% of the boys drew a female scientist. While our findings are similar to recent publications, these other studies introduced the female role model just prior to their assessment or utilized in-vivo depth-interventions. Our intervention was one day and the assessment was 5 months afterwards. As our data are promising, a future prospective controlled cohort study may be interesting to determine the role of the capacity model.
550 Cardiovascular risk among psoriasis patients using red cell distribution width and mean platelet volume

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Psoriasis (PsO) generates systemic inflammation which may be related to cardiovascular disease (CVD) events. Red cell distribution width (RDW) and mean platelet volume (MPV) appear to be systemic sensors that reflect disordered bone marrow response, and are markers for major adverse cardiovascular events. We asked whether elevated RDW and/or MPV were related to the risk of CVD events in PsO patients. We performed a retrospective study of psoriasis patients obtained from the Explorys electronic health record database. Psoriasis patients aged 18-75 years were selected. If they encountered RDW or MPV measurements, we examined RDW (cut-off 12.0%), MPV (cut-off 9.5%) and the relative risk Odds Ratio (OR). Patients were divided into four groups based on the RDW and MPV cut-offs: those with elevated RDW and elevated MPV, elevated RDW plus MPV, those who exhibited elevated RDW but normal MPV, and those who displayed normal RDW and elevated MPV. Patients with PsA had elevated RDW (76.4%), MPV (59.4), and the odds of major adverse cardiovascular events (MACE) was highest among patients with elevated RDW and MPV (OR 3.4, 95% CI 2.7-4.2, p < 0.001), followed by patients with high RDW and normal MPV (OR 2.4, 95% CI 2.1-2.8, p < 0.001), as compared to normal/low MPV and normal RDW. Atrial fibrillation, coronary artery disease, heart failure and peripheral vascular disease also had elevated OR ranging from 2.0-2.3. 65% of patients with PsO did not have RDW or MPV data. Among PsA patients, elevated RDW also increased the risk of an MI with an OR=1.8, p<0.001. In a cohort of 21 PsO patients followed longitudinally for 1 year, 4 patients had elevated RDW at baseline; among these, 3 had a PASI50 response, and RDW was measured for these responders. Bone marrow dysfunction, dysregulation of platelet and erythrocyte production may alter MPV and RDW responses that are also associated with increased CVD risk.

551 The stigma of psoriasis: Public health and professional perceptions

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The stigma of psoriasis is a frequent concern which can lead to a reduced quality of life. There is a research gap in understanding the general public and health care professionals' perceptions of persons with psoriasis which is required to advance efforts to reduce stigma. As part of a larger community-based education project through Amazon, a national New York online survey at the Perelman School of Medicine to quantitatively assess the public and medical students perceptions of people with psoriasis. Herein, we report the results from the medical students survey compared with the general public. All participants viewed images of people with psoriasis. Results: A total of 334 medical students and 14,107 adults with visible psoriatic lesions and rated their emotional responses and desire to avoid the people in the images. Participants also reported their agreement with psoriasis-related myths and stereotypes, preference for having psoriasis compared to other stigmatized diseases (e.g., obesity, HIV, cancer, diabetes) and their willingness to live with psoriasis in the US (n=198, 81.8% white, 58.6% male, mean age: SD: 31.4±9.9 years). Medical students (n=187, 59.4% white, 41.2% male, mean age (SD): 29.4±8.4 years) were mostly in their second year of medical school (31.6%) and had not completed a dermatology rotation (52.5%). Most medical students had heard of (95.7%) or knew someone with (51.3%) psoriasis, which was comparatively more than the public (OR=6.77 vs. 2.96, p<0.001). The photos elicited more compassion in the female medical students than males (p<0.02). Significantly less of the student sample compared to the public sample 8.6% vs. 41.9% expressed a desire to avoid the pictured persons with psoriasis and 2.1% vs. 12.1% reported belief in psoriasis misconceptions. Compared to the medical student survey results, the public reported more contempt and blame toward people with psoriasis (p<0.01). Medical students reported to have more knowledge about psoriasis and less stigmatizing attitudes and beliefs towards patients with psoriasis than the general public. Our results demonstrate that provide accurate information to the general public may help to reduce stigma.

552 Factors predictive of complete remission off therapy after a single cycle of rituximab for pemphigus

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Rituximab has emerged as a front-line therapy for pemphigus, but prospective studies have shown heterogeneity in rates of complete remission off therapy (CRoT) after a cycle of rituximab. We performed a prospective single-center study, we quantified the rate of CRoT, time to CRoT, and time to relapse for pemphigus patients receiving their 1st cycle of rituximab therapy. To determine predictive factors for achieving CRoT after 1 cycle of rituximab, we performed a multivariate regression analysis of age, sex, disease duration prior to rituximab, and dosing regimen. All pemphigus patients seen at the Hospital of the University of Pennsylvania between 2004-2017 with at least 1 year follow up after rituximab therapy were included in the analysis (n=162). Disease activity was measured using P Norris (54.7%), p<0.001, as compared to normal/low MPV and normal RDW. Atrial fibrillation, coronary artery disease, heart failure and peripheral vascular disease also had elevated OR ranging from 2.0-2.3. 65% of patients with PsO did not have RDW or MPV data. Among PsA patients, elevated RDW also increased the risk of an MI with an OR=1.8, p<0.001. In a cohort of 21 PsO patients followed longitudinally for 1 year, 4 patients had elevated RDW at baseline; among these, 3 had a PASI50 response, and RDW was measured for these responders. Bone marrow dysfunction, dysregulation of platelet and erythrocyte production may alter MPV and RDW responses that are also associated with increased CVD risk.

553 Identifying adalimumab cellular and molecular targets in psoriasis

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Proteomic Signature Stratification to Optimise Relevant Therapy (PSORT) Consortium aims at better understanding the determinants of response to biologic therapies. In our functional immuno-phenotyping approach, we hypothesised that monitoring key transcription factors downstream of the cytokines being targeted by biological treatments provides insights into their cellular and molecular targets which could be used as biomarkers of response to treatment. Using imaging flow-cytometry, we investigated the molecular effect of the anti-TNF agent adalimumab, evaluating its effect on the nuclear translocation of the nuclear factor κB (NF-κB) in key immune cell subsets. Whole blood, obtained from psoriatic patients (n=20) before treatment (baseline) and at week 1, 4 and 12 after commencing treatment, was stimulated with TNF, IL-17, TNF+IL17 or LPS, stained and analysed. Nuclear translocation was quantified using anti-NF-κB translocation in T cells, dendritic cells (DCs), monocytes and neutrophils; LPS, induced stimulation in monocytes, DCs and neutrophils. In contrast, IL-17 stimulation did not induce NF-κB translocation, nor augmented the TNF-induced signal in any cell subset. After the start of the therapy, adalimumab strongly inhibited TNF-induced NF-κB translocation at each time point in T cells (92% at week 1, p<0.001) and to a much lesser extent in DCs (55% at week 1, p<0.05), while it had no effect in monocytes or neutrophils. In contrast, adalimumab did not have any effect on LPS-induced NF-κB translocation. Interestingly, inhibition of TNF-NF-κB translocation did not correlate with the mid-term response to the treatment, defined as PASI75 and Relative PASI, at any of the time points tested. Taken together, we show that adalimumab completely inhibits TNF signalling in lymphoid cells. However, this mechanism may not be responsible for the clinical response. Further analyses are ongoing to identify the molecular mechanisms underpinning the clinical response to adalimumab and their evaluation as biomarkers of response.

554 Integrating serum and skin biomarkers to assess disease extent beyond clinical scores, advancing precision therapeutics

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Assessment of atopic dermatitis/AD severity is limited to clinical disease scores, which have limitations, however biomarkers of activity in skin/blood may be objective adjuncts to follow disease activity. The cytokine profile of Asian AD skin differs from European American EA AD. We investigated skin and serum cytokine profiles in moderate-to-severe Asian AD patients and age-matched controls using Electro-chemiluminescence and Erenna immunassays, comparing EA AD circulating measures with serum cytokines in AD. Protein expression was measured in lesional skin with significant higher in EAsV. Asian AD. Similar to skin profiles, both EA and Asian AD showed significant Th2 elevations in serum vs. respective controls (IL-13, CCL13, CCL17; P<0.05). Patients Discriminant ratios of IFN-γ vs. IL-17 in Asian AD. In EA, IL-17, IL-18 was significant increase in IL-17 and IL-18. (p<0.05 for all). Non-lesional skin expressions of various Th2-attracting chemokines and cytokines were significantly higher in EA versus Asian AD. Similar to skin profiles, both EA and Asian AD showed significantly higher in serum expression of IL-13, IL-17, IL-18, and CCL13, CCL17, CCL17; P<0.05). In sum, integrating serum and skin biomarkers may serve as a surrogate for assessing disease extent beyond assessment by clinical severity scores, advancing precision therapeutic approaches.

555 The Janus kinase 1 (JAK1) inhibitor PF-04965842 reduces signs and symptoms of moderate to severe atopic dermatitis (AD)

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We present secondary efficacy and safety results of a multicenter, randomized, double-blind, Phase 2b trial investigating the JAK1 inhibitor PF-04965842 for the treatment of moderate to severe AD. The JAK1 inhibitor PF-04965842 was well-tolerated and no major safety concerns were noted. PF-04965842 was significantly reduced the signs and symptoms of AD in adults with moderate to severe disease and was generally well-tolerated.
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Patient-centered care: Clinical relevance of PROMIS domains in atopic dermatitis, acne, and psoriasis  
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A new and powerful tool for assessing patient outcomes, PROMIS (Patient-Reported Outcomes Measurement Information System) provides a common language to quantify patient-centered care in Dermatology. This study examines and compares PROMIS domains in patients with atopic dermatitis, acne, and psoriasis in order to elicit patient preferences.  
Methods: A cross-sectional study of PROs (Patient-Reported Outcomes) was conducted across all three disease states in a sample of 201 subjects (57.4% females, mean age 11.4 years, mean 11.4 years). Three PROMIS domains were analyzed: Physical Function (PF), Pain Interference, and Sleep. Data were collected using PROMIS computer-adaptive tests (CAT) and underwent a quality control process.  
Results: A high prevalence of high psychometric utility of PROMIS was found across all domains in all disease states. The mean difference in the standard deviation between the three domains was small, suggesting that the domains are comparable in terms of reliability and validity. The most significant associations were found between Physical Function and Pain Interference, and Sleep.  
Conclusion: PROMIS domains are highly relevant and valid across all disease states, providing a robust framework for assessing patient-centered care in Dermatology. The study highlights the importance of incorporating PROMIS in clinical practice to improve patient outcomes.

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PROMIS pediatric sleep-wake function measures correlate with objective sleep disturbance in atopic dermatitis patients  
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A new and powerful tool for assessing patient outcomes, PROMIS (Patient-Reported Outcomes Measurement Information System) provides a common language to quantify patient-centered care in Dermatology. This study examines and compares PROMIS domains in patients with atopic dermatitis, acne, and psoriasis in order to elicit patient preferences.  
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Incidence of anaphylaxis significantly decreased by avoiding cetuximab administration for the subjects sensitized against galactose-alpha-1,3-galactose (alpha-Gal)  
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Anaphylaxis is a severe and potentially life-threatening allergic reaction that can occur due to exposure to allergens in the environment or food. In the last decade, anaphylaxis has been identified as a major cause of allergic reactions following administration of cetuximab, a monoclonal antibody used to treat cancer. The objective of this study was to evaluate the incidence of anaphylaxis in subjects sensitized against galactose-alpha-1,3-galactose (alpha-Gal) in cetuximab and to assess the effectiveness of avoiding cetuximab administration in subjects sensitized against alpha-Gal.

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ASNO2 is a dual oral inhibitor of JAK/SYK signaling improves clinical outcomes and associated cutaneous inflammation in moderate-to-severe atopic dermatitis patients  
Y Nakamura-Nishimura1, F Miyagawa2, K Miyashita1, R Ommori1, H Azukizawa1 and H Asada2  
1 Department of Dermatology, Izumi City Hospital, Izumi City, Fukuoka, Japan, and 2 Department of Dermatology, Tohoku University School of Medicine, Sendai, Miyagi, Japan  
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High-utilizers responsible for disproportionate lost opportunities for dermatologic care in the Providence Veterans Health Administration  
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1 Mount Sinai, New York, NY, 2 Mount Sinai, Flushing, NY, and 3 Asana Biosciences, Princeton, NJ  
The objective of this study was to identify high-utilizers within a large dermatology clinical practice and to assess the impact of these patients on lost opportunities. The study was conducted in the Providence Veterans Health Administration, Providence, RI.

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Serum thyroxine and activation-regulated chemokine (TARC) is a useful marker for assessing the clinical and immunological condition of DRESS/DIHs  
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Drug reaction with eosinophilia and systemic symptoms (DRESS) and drug-induced hypersensitivity syndrome (DIHS) are severe adverse drug-induced reactions with reaction to human herpesvirus 6 (HHV-6). We previously reported that serum thyroxine and activation-regulated chemokine (TARC) levels were markedly increased in patients with DIHS and suggested TARC as a useful diagnostic marker of DIHS in the early stage. In this study, we determined whether serum TARC levels correlated with the severity of clinical symptoms and laboratory data in patients with DRESS/DIHs. We evaluated 16 patients with DRESS/DIHs for their clinical symptoms, laboratory data, copy numbers of HHV-6 and human cytomegalovirus (CMV) DNA. TARC is associated with mononuclear cells, serum cytokines and soluble interleukin-2 receptor (sIL-2R), as well as TARC levels. All 16 patients showed increased serum TARC levels to a varying degree (2807-1053 pg/ml) in the acute phase of their disease. Serum TARC levels were significantly higher in patients with high clinical severity (SRI > 3) than in patients with low clinical severity (SRI < 3). We conclude that serum TARC levels are a useful marker for assessing the clinical and immunological condition of patients with DRESS/DIHs.
Trends in scholarly productivity of US dermatology professors by academic status, degree and gender
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Gender inequality has been observed in several fields of basic science research and academic medicine, but has not yet been assessed in academic dermatology. We sought to describe the association between gender and career metrics including academic rank, scholarly impact (assessed by Hirsch-index) and funding. We conducted a cross-sectional analysis of all US academic dermatologists with Scopus profiles on N=665 from all academic centers listed in ERAS with an accessible website (N=115), excluding nonacademic faculty such as research staff and volunteer faculty. Academic rank, numerical h-index, and NIH funding information were collected. Data were analyzed using R (version 3.4.4). Results: There were more female than male assistant professors overall, but there were more male than female full professors and chairs. While male assistant professors had higher h-index than female assistant professors (Mmedian =6,40, SDmedian =1,67, Median =4,27, SDmedian =1,68; p<0.001), after normalizing for career length, this difference was no longer significant (p=0.094). Overall, factors associated with increased academic productivity include being male, academic positions, having a higher career level, and securing funding, may help women to ascend the academic ladder in dermatology.

The behavior of omega 3 and omega 6 polyunsaturated fatty acids in Japanese psoriasis patients
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More recently, studies have shown that psoriasis patients have an increased risk of conditions such as cardiovascular disease, obesity, and metabolic syndrome. Omega-3 (n-3) polyunsaturated fatty acids (PUFA) have anti-inflammatory properties and are associated with reduced CVD risk. In Japanese study, high serum EPA and decreased arachidonic acid (AA) to arachidonic acid ratio (>0.404) was significantly associated with a low incidence (12.7%) of CVD compared with lower EPA/AA (23.9%). But it has not been reported the EPA/AA ratio in psoriasis patients. Therefore, we examined the serum EPA and decreased arachidonic acid to arachidonic acid ratio (EPA/AA) in severe psoriasis patients (Pairoscope, CPH, DK). Serum was obtained from patients treated with PDE4 inhibitors for 4 weeks are examined. One out of 16 patients treated (average 0.28 ±0.13; p=0.004) showed a high EPA/AA ratio (>0.404). These results suggest that the psoriasis patients put in the high risk of CVD under low EPA/AA ratio compared with healthy controls. This difference was not observed in non-psoriasis patients (p=0.19). These findings support the high prevalence of fat metabolism disturbances in patients with severe psoriasis.

Fatigue in systemic lupus erythematous and other autoimmune skin diseases
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Fatigue is a well-established symptom in systemic lupus erythematosus (SLE) that significantly impacts patients quality of life (QOL), but has not been well-charactered in other skin-limited autoimmune diseases such as cutaneous lupus erythematous (CLE), anaplastic dermatomyositis (ADM), or autoimmune blistering diseases (AIBD). This is a retrospective study combining fatigue in historic controls (n=114) to patients enrolled in prospective longitudinal databases with SLE (n=165), CLE (n=226), ADM (n=136), and AIBD (n=79). Using the Short-Form 36 (SF-36) Vitality scale as an analogue for fatigue, we analyzed the median SF-36 Vitality scores and the percentage of patients with clinically significant fatigue (defined as a score ≤35) between experimental groups and controls. Median Vitality score demonstrated greater fatigue in experimental groups (SLE, 35; CLE, 50; ADM, 60; AIBD, 55) than controls (SLE, 29; CLE, 28; ADM, 36; AIBD, 35) p<0.05. These findings support the high prevalence of fatigue in patients with SLE relative to CLE, and demonstrate that patients with skin-limited autoimmune disease experience more fatigue than controls, with ADM and AIBD showing similarly significantly significant fatigue to SLE. Fatigue is an important symptom that negatively affects QOL for these patients, and treatment strategies less in CLE than SLE, and should be addressed by clinicians and measured in future clinical trials.

Psoriasis severity assessment with a computational similarity-clustering programme reduces intra- and inter-observer variation
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Background: In trials and practice, intra- and inter-observer variation of assessing clinical severity (i.e. Psoriasis Area Severity Index (PASI)) is often a problem due to variability of clinical judgment. Objective: To develop and test if a similarity-clustering programme, where images in a set is quantified by compiling clinical severity comparisons among all photo pairs in a set, improves consistency of evaluations. Methods: Images were provided from patients with psoriasis via a digital mobile application (Imagine, CPH, DK). A web-program, for similarity-clustering programme, were developed in 2017. Acquired images were classified using latent semantic analysis, a computational method. Background: In trials and practice, intra- and inter-observer variation of assessing clinical severity (i.e. Psoriasis Area Severity Index (PASI)) is often a problem due to variability of clinical judgment. Objective: To develop and test if a similarity-clustering programme, where images in a set is quantified by compiling clinical severity comparisons among all photo pairs in a set, improves consistency of evaluations. Methods: Images were provided from patients with psoriasis via a digital mobile application (Imagine, CPH, DK). A web-program, for similarity-clustering programme, were developed in 2017. Acquired images were classified using latent semantic analysis, a computational method.
568 Evaluating results of an interferon-γ release assay in patients with autoimmune skin disease on hydroxychloroquine

RG Gaffeney1, M Yuan2, B Chang3, J Jimenez2

QuantiFERON-TB Gold (QFT-G) is a commercial interferon-γ release assay used to screen patients for tuberculosis before starting or while on immunosuppressive therapies. Clinical studies of VA-labeled interferon-γ release assays using hydroxychloroquine as an immunosuppressant show higher rates of indeterminate results than do an IRB-approved questionnaire of questions regarding the maximum dollar amount that the subject would be willing to pay, and how much insurance companies should pay, for medications that completely clear psoriasis spots and skin symptoms for at least 12 months. Additionally, patients were asked how they define disease remission in regards to % body surface area clearance (90, 95 or 100) and duration of improvement (3, 6, 12, or 24 months). A total of 37 patients participated:16% reported baseline mild, 46% moderate and 38% severe disease. Over the treatment period of 53 and 20 years respectively, for the treatment described, patients were willing to pay an average of $175/month out of pocket (SD $294) and would expect insurance to pay $2,759/month (SD $5200). There was a trend toward increase in monetary value with severity. Mild, moderate and severe patients reported a willingness to pay $95, $135 and $260 out of pocket (p<0.4) and an expectation for insurance to pay $1,025, $1,883, and $5,733 (p<0.2) monthly, respectively. This is despite the fact that more severe patients reported household income of <$100,000 than in the mild or moderate groups. Only 14% of subjects defined remission as 100% reduction in plaque and only 12% require 24 months of improvement to qualify as remission. While the average list cost of biologic medication is now around $50,000 annually, our study found that patients valued medication with substantial rates of clearance at an average of $7,930/month. Given the disconnect in valuation, we also asked patients how they personally defined disease remission and found that the large majority of subjects did not require 100% clearance or improvement for two years in order to qualify as disease remission, which may provide some insight into patient expectations.

570 Examining cutaneous disease activity as an outcome measure for clinical trials in dermatomyositis

RG Gaffeney1, R Feng1, D Pearson2, M Tarazi1 and VP Werth1

The natural history of wounds in RDEB is not well defined. Serial photography and patient with photographs (N categories (0-39cm2 vs 0.035). Mean pain score was 4/10 kappa 0.85. The distinct time course of chronic open wounds (N on the trunk (p ¼ 0.05) and more recurrent wounds were on the lower extremities (p ¼ 0.001) and between groups. These results reveal that patients taking HCQ at the time of QFT-G testing are significantly more likely to have an indeterminate result compared to those not taking the medication, and this finding is not explained by concomitant use of prednisone or DMARDs. An indeterminate QFT-G result represents a major barrier to receiving treatment and therefore future studies are needed to evaluate the most appropriate tuberculous screening in patients taking hydroxychloroquine.

572 Defining chronic wound types in recessive dystrophic epidermolysis bullosa patients for clinical outcome assessment

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The natural history of wounds in RDEB is not well defined. Serial photography and patient with photographs (N categories (0-39cm2 vs 0.035). Mean pain score was 4/10 kappa 0.85. The distinct time course of chronic open wounds (N on the trunk (p ¼ 0.05) and more recurrent wounds were on the lower extremities (p ¼ 0.001) and between groups. These results reveal that patients taking HCQ at the time of QFT-G testing are significantly more likely to have an indeterminate result compared to those not taking the medication, and this finding is not explained by concomitant use of prednisone or DMARDs. An indeterminate QFT-G result represents a major barrier to receiving treatment and therefore future studies are needed to evaluate the most appropriate tuberculous screening in patients taking hydroxychloroquine.

574 The role of observation for excisionally biopsied moderately dysplastic nevi with positive histologic margins and risk of development of future melanoma

CC Kim1, JG Berry2 and SC Chen3

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Role of observation for excisionally biopsied moderately dysplastic nevi with positive histologic margins and risk of development of future melanoma

CC Kim1, JG Berry2 and SC Chen3

1 Beth Israel Deaconess Medical Center, Boston, MA, 2 Tufts University and Massachusetts General Hospital, Boston, MA and 3 Stanford University School of Medicine, Stanford, CA

Importance: Little evidence exists to guide management for moderately (mod) dysplastic nevi (DN) excisionally biopsied without clinical residual pigment, but with positive histologic margins (referred to as ModDN(+)) Marg. Objective: To determine outcomes and risk for future melanoma (MM) development in ModDN(+)/Marg observed for ≥3 years. Design: Multi-center (9 U.S. academic dermatology sites) retrospective study of patients ≥18 years with ModDN(+)/Marg and ≥3 years of follow-up from 1990-2014. To assess interobserver variability, each site submitted five random mod DN slides for central dermatopathology review. Mean ± SD of the QFT-G test and Medical Research Council (MRC) MM risk assessment for patients with mod DN were 3.8 ± 4.6 and 8.9 ± 11.2, respectively. 30% of patients had a personal history of MM or a first degree relative with MM. The treatment described, patients were willing to pay an average of $175/month out of pocket (SD $294) and would expect insurance to pay $2,759/month (SD $5200). There was a trend toward increase in monetary value with severity. Mild, moderate and severe patients reported a willingness to pay $95, $135 and $260 out of pocket (p<0.4) and an expectation for insurance to pay $1,025, $1,883, and $5,733 (p<0.2) monthly, respectively. This is despite the fact that more severe patients reported household income of <$100,000 than in the mild or moderate groups. Only 14% of subjects defined remission as 100% reduction in plaque and only 12% require 24 months of improvement to qualify as remission. While the average list cost of biologic medication is now around $50,000 annually, our study found that patients valued medication with substantial rates of clearance at an average of $7,930/month. Given the disconnect in valuation, we also asked patients how they personally defined disease remission and found that the large majority of subjects did not require 100% clearance or improvement for two years in order to qualify as disease remission, which may provide some insight into patient expectations.
Teledermatology as a tool for preoperative consultation prior to Mohs micrographic surgery within the Veterans Health Administration

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Bedside consultation for Mohs surgery is increasingly utilized in improving access to subspecialty care within the Veterans Health Administration (VHA). Mohs micrographic surgery (MMS) is a surgical modality used to treat non-melanoma skin cancers. This study evaluates the use of teledermatology for preconsultation for MMS via a retrospective analysis of interfacility MMS referrals to the Bronx Veterans Affairs Medical Center (VAMC). Consult failure rates (CFRs), treatment follow-through rates, time-to-treatment, and travel savings for face-to-face preoperative consultations were compared with store-and-forward teledermatology preoperative consultations. The results indicated that although both teledermatology and face-to-face preoperative consultations resulted in an equivalent percentage of treated lesions, teledermatology had a significantly decreased CFR. Additionally, teledermatology decreased the time-to-treatment by two weeks, increased the percentage of lesions treated within 60 days, and resulted in average savings of $162.7 per consultation. The study demonstrates that teledermatologic consultation is effective for preconsultations for MMS within the VHA system. Teledermatologic improved access measures like time-to-treatment and travel burdens. This program may serve as a model not only for other VAMCs that accept interfacility MMS consultations, but also for VAMCs that provide other types of access-limited subspecialty care.

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**531** Phase 1, single-arm, open-label, dose escalation trial of microneedle array- doxorubicin in patients with mycosis fungoides

*F Menzaghi1, MK Vernon2, JW Stauffer3, RH Spencer4 and CM Munera4*  
1 Research & Development, Cara Therapeutics, Inc., Stamford, CT, 2 Patient-Centered Research, Evidera, Bethesda, MD, 3 Cara Therapeutics, Inc., Stamford, CT and 4 Biometrics, Cara Therapeutics, Inc., Stamford, CT

**ABSTRACTS**

**581** Distinct molecular signature in melanomas from patients with recurrence

*F Menzaghi1, MK Vernon2, JW Stauffer3, RH Spencer4 and CM Munera4*  
1 Research & Development, Cara Therapeutics, Inc., Stamford, CT, 2 Patient-Centered Research, Evidera, Bethesda, MD, 3 Cara Therapeutics, Inc., Stamford, CT and 4 Biometrics, Cara Therapeutics, Inc., Stamford, CT, 2 Patient-Centered Research, Evidera, Bethesda, MD, 3 Cara Therapeutics, Inc., Stamford, CT and 4 Biometrics, Cara Therapeutics, Inc., Stamford, CT

**ABSTRACTS**

**582** Determination of pruritus associated with chronic kidney disease: A qualitative study of two patient-reported outcome instruments

*F Menzaghi1, MK Vernon2, JW Stauffer3, RH Spencer4 and CM Munera4*  
1 Research & Development, Cara Therapeutics, Inc., Stamford, CT, 2 Patient-Centered Research, Evidera, Bethesda, MD, 3 Cara Therapeutics, Inc., Stamford, CT and 4 Biometrics, Cara Therapeutics, Inc., Stamford, CT

**ABSTRACTS**

**583** Psychometric validation and meaningful change threshold of the worst itching intensity numerical rating scale for use in hemodialysis patients with pruritus

*CM Munera1, MK Vernon2, JW Stauffer2, RH Spencer3 and F Menzaghi1*  
1 Biometrics, Cara Therapeutics, Inc., Stamford, CT, 2 Patient-Centered Research, Evidera, Bethesda, MD, 3 Cara Therapeutics, Inc., Stamford, CT and 4 Biometrics, Cara Therapeutics, Inc., Stamford, CT

**ABSTRACTS**

**585** Natural history of wound closure and other clinical endpoints in epidermolysis bullosa: Lessons from a 90-day multicenter trial

*DM Reull1, A Paller2, CB Dodem1, JA Barth2, L Lagast3 and A Reha4*  
1 University of New South Wales, Sydney, Australia, 2 Northwestern, Chicago, IL, 3 The University Hospital Necker Enfants Malades, Paris, France and 4 Amicus Therapeutics, Cranbury, NJ

**ABSTRACTS**
586 Trends in psoriasis care at teaching versus non-teaching hospitals: A meta-analysis

K Patel, A Matalon, Texas Health Dallas North School of Medicine, Dallas, TX

Psoriasis is a common dermatologic condition and autoimmune disease characterized by red, itchy, and scaly patches of skin due to overgrowth of skin cells. This retrospective meta-analysis evaluated the association between hospital teaching status and psoriasis outcomes by comparing the number of discharges, length of stay (LOS), and charges between teaching and non-teaching hospitals. Using ICD-9-CM codes for psoriasis, all admissions with a primary diagnosis of psoriasis between 1997-2014 were identified from the Nationwide Inpatient Sample database. Baseline information including LOS, total charges, and number of discharges were extracted for teaching and non-teaching hospitals. Statistical significance was determined by the t-test (p < 0.05). There were 17,107 discharges identified with a primary diagnosis of psoriasis between 1997-2014. There were statistically significant differences in LOS (t(30,204) = 5.42, p < 0.0001), total charges (t(30,204) = 4.71, p = 0.0001), and number of discharges (t(30,204) = 4.90, p = 0.00002) between teaching and non-teaching hospitals. Thus, psoriasis care at teaching hospitals results in longer LOS, higher total charges, and higher number of discharges compared to non-teaching hospitals.

587 Results from the phase 3, double-blind, vehicle-controlled ESSENCE trial of the topical investigational drug SD-101 in epidermolysis bullosa

K Patel, A Matalon, Texas Health Dallas North School of Medicine, Dallas, TX

Epidermolysis bullosa (EB) is a rare genetic disorder that manifests as blistering of the skin, mucosa, and epithelial lining of organs. ESSENCE (SD-005; NCT02384467) was a phase 3, randomized, double-blind, vehicle-controlled study assessing the efficacy and safety of SD-101 (topical allantoin cream) in patients with EB (simplex, recessive dystrophic, junctional non-Herlitz). Patients were randomly assigned 1:1 to receive SD-101 6% allantoin cream or vehicle. The primary endpoint was the percentage of patients achieving target wound closure by month 3. The study enrolled 169 patients at 11 sites in 13 countries. The proportion of randomized patients by EB subtype was: 11% simplex; 70% recessive dystrophic; 19% junctional non-Herlitz. The mean (standard deviation) age was 13.9 (13.3) years. Mean (SD) target wound size was 20.4 (24.2) cm². The primary endpoint, target wound closure within 3 months, was not different between groups (Hazard ratio = 1.004, p = 0.985). The second primary endpoint, percentage of patients achieving target wound closure by month 3, was also not different between groups (49% SD-101; 54% placebo, p = 0.390). Key secondary endpoints did not reach statistical significance vs vehicle. Encouraging trends in wound closure were observed in certain subpopulations. SD-101 was well tolerated and the adverse event profile was similar to vehicle. ESSENCE included a broad representation of EB subtypes/disease characteristics and is the largest completed clinical trial of an investigational drug in EB to date.

588 Comparative effectiveness of psoriasis treatments on systemic inflammation

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Numerous cellular immune mediators are thought to initiate, as well as potentiate, the chronic inflammation characteristic of psoriasis. The progressive amplification of chronic inflammation also promotes co-morbidities such as atherogenesis, and patients with psoriasis have an increased risk of developing and dying of cardiovascular disease (CVD). Given that systemic treatments for psoriasis target shared inflammatory mechanisms observed in psoriasis as well as atherothrombotic cardiovascular disease (ASCVD), it is possible that effective suppression of skin inflammation could diminish distant pro-ASCVD effects. However, the parameters which best evaluate the potential for psoriasis treatments to reduce CVD event risk remain to be established. We performed a pairwise longitudinal observational study of the behavior of several biomarkers associated with an increased risk of CVD in psoriasis. Biomarkers including resistin, myeloperoxidase (MPO), and adiponectin were measured during and after one year of a continuous standard-of-care systemic therapies. Our study included tobacco use, alcohol use, low vitamin D, and malignancy. We could not identify a sub-population of psoriasis patients in whom recurrent screening is useful.

589 Use of electronic health record (EHR) tools and interactive didactics to empower pediatricians to manage atopic dermatitis (AD)

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Electronic health records (EHRs), which are used by nearly 90% of office-based physicians today, have evolved rapidly from straightforward record-keeping tools to multi-functional software suites with built-in features for patient communication, literature search, checking medication interactions, safety reminders and point of care decision support (PCDS). One form of PCDS found in numerous EHRs is the order set, which allows physicians to easily select the appropriate laboratory evaluations and treatments for a diagnosis. Order sets, particularly when paired with a teaching intervention, improve physician comfort with evaluation and management of the disease around which the order set is designed. Atopic dermatitis (AD) is a prevalent disease affecting more than 10% of all U.S. children, and the high-quality AD care currently available to the subset of patients who require dermatologist care 5 to 1 in the United States, are poised to fill this unmet need. This is a prospective, interventional study to evaluate the effect of an integrated decision support tool on general pediatricians’ knowledge, comfort with management, and perceived work-burden associated with management of atopic dermatitis (AD). This integrated decision support tool includes an order set with topical and oral therapies sorted by drug class and strength. Code for computer-generated patient instructions that are automatically filled with patient names, age, and appropriate quantity of medication to be used per application been written. Provider knowledge is assessed at baseline and at 3 and 6 months using a knowledge-based multiple choice exam validated by practicing dermatologists. Comfort with management and perceived work-burden are assessed at baseline and at 3 and 6 months using a survey. This study is currently ongoing, 20 pediatricians have been recruited to participate and all ordering tools and educational materials have been developed.

590 Utility of repeat latent tuberculosis screening in psoriasis patients treated with TNF-alpha inhibitors

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Current ACR guidelines recommend tuberculosis (TB) screening prior to starting TNF-α inhibitor therapy due to risk of latent TB reactivation while patients are in an immunosuppressed state. A majority of physicians treating psoriasis patients with TNF-α inhibitors report latent TB screening annually, and this is often required for renewal of prescriptions. However, there is limited data suggesting this practice is warranted and improves patient outcomes. We retrospectively reviewed 570 psoriasis patients treated with TNF-α inhibitors to explore the utility of repeating TB screening annually after initiation of TNF-α inhibitor therapy. Specifically, we were interested in risk of conversion from negative to positive test results once on TNF-α inhibitor therapy and potential risk factors for conversion to positive test results in this population. A total of 1,547 patient-years of latent TB screening were included in our analysis. Of the 570 patients, 37% received at least one test annually. Of all patients, 0.8% converted from negative to positive. Of those who converted from negative to positive, 0.3% received latent TB therapy. The most common risk factors for conversion were age > 50 years, history of TB, HIV infection, and immunosuppression. Of those who tested positive for TB at baseline prior to TNF-α inhibitor initiation, 0.4% converted to positive at any time during the study period. This rate was lower than that reported in a previous study, where 2% of patients on TNF-α inhibitor therapy converted to positive. Thus, repeat latent TB screening may not be necessary for most psoriasis patients on TNF-α inhibitor therapy, questions regarding whether repeat screening is effective. Certain risk factors may identify a sub-population of psoriasis patients in whom recurrent screening is useful.

591 A phase 1/2 study of genetically-corrected, collagen VII expressing autologous human dermal fibroblasts injected into the skin of patients with recessive dystrophic epidermolysis bullosa (RDEB)

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Recessive Dystrophic Epidermolysis Bullosa (RDEB) is an inherited skin disorder caused by mutations in the COL7A1 gene encoding type VII collagen (C7). We report the results of the ongoing Phase 1/2 clinical trial of an ex vivo gene therapy for the treatment of RDEB. Five adult subjects enrolled in this trial carried various null COL7A1 mutations resulting in undetectable C7 expression by immunofluorescence microscopy (IF) and a lack of intact fibrils (AFB). A human dermal fibroblast (HDF) autologous RDEB fibroblasts isolated from skin biopsies were transduced with a lentiviral vector encoding the wild type COL7A1 gene and expanded to obtain a sufficient quantity for study treatment (FCX-007). A phase 1/2 study was performed in a single blinded manner in 591 patients by FCX-007. The primary endpoint was to evaluate safety and FCX-007 was well tolerated through 12 weeks post-administration. Linear C7 expression was preserved in the dermal-epidermal junction and restoration of AFB was seen at 3 months in a subset of treated sites. No serious adverse events reported, no replication competent virus detected and no significant autoantibody response detected. At 4 weeks, 100% (5/5) and at 12 weeks, 86% (5/6) patients by FCX-007. We report the results of the ongoing Phase 1/2 clinical trial of an ex vivo gene therapy for the treatment of RDEB.
Distinct prognostic values of ALDH isoenzymes in human melanoma

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Aldehyde dehydrogenase (ALDH) activity is used as a functional marker for cancer cells with stem-like features in many tumors, including melanoma. Evidence suggests that ALDH activity predicts tumor cell proliferation, invasiveness and drug-resistance. The ALDH family consists of 19 isozymes, with different subcellular localization and functions. We hypothesized that some isozymes contribute to tumor progression in unique ways and are responsible for patient prognosis in melanoma. The ALDHFLUOR functional assay is a widely accepted approach to detect high ALDH activity in many tumors. The test has the capacity to identify the activity of at least seven distinct isozymes of ALDH (ALDH1A1, ALDH2A, ALDH3A1 and ALDH4A1). Therefore, we investigated the prognostic value of these ALDH isozyme expressions in melanoma patients based on mutation subtypes and stages. The Cancer Genome Atlas (TCGA) data set was analyzed to evaluate the survival OS in 283 melanoma samples using Kaplan-Meier curves and log-rank test. We found that irrespective of mutation subtype (BRAF, NF-1, RAS, TWW or stage of disease (0 = IV, N=283), high expression of ALDH2 messenger RNA (mRNA) was correlated with better OS (P =< 0.05). In BRAF mutant primary melanoma (Stages II-IV, N=51), high expression of ALDH1A3 and ALDH1A1 mRNA correlated with worse OS (P =< 0.05). In BRAF mutant metastatic melanoma (Stages III-IV, N=50), high expression of ALDH1A1 mRNA correlated with worse OS (P =< 0.05), whereas high expression of ALDH1A3 mRNA was associated with better OS (P =< 0.01). In Ras mutant metastatic melanoma (Stages III-IV, N=43), high expression of ALDH1A1 mRNA correlated with better OS (P=<0.05). We conclude that ALDH1A1, ALDH1A3, ALDH2A, ALDH3A1 and ALDH4A1 could serve as mutation-specific and/or stage-specific prognostic biomarkers and therapeutic targets for melanoma patients.

An integrated approach to predict and detect Merkel cell carcinoma recurrences

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Merkel cell carcinoma (MCC) is a rare but deadly skin cancer that affects ~2,500 patients/year in the US and has an ~30% mortality rate. 80% of cases are causally linked to Merkel cell polyomavirus (MCPyV) whereas 20% are caused by extensive UV radiation. 40% of patients will recur after initial treatment, appropriate surveillance intervals are critical to minimize unnecessary visits/scans while detecting new metastatic disease early, when it is more responsive to immunotherapy approaches. Currently, National Comprehensive Cancer Network (NCCN) Guidelines for follow-up are vague (“imagining studies, as clinically indicated”) and do not differ based on stage, despite a 20-fold difference in death rate between patients presenting with early local MCC as compared to advanced nodal MCC. We have developed a deep learning (DL) model that combined, in a patient care pathway, a blood test (recently prospectively validated) that quantifies oncoretroviral antibodies to MCPyV and 2) a calculator of residual risk off MCC recurrence. The MCPyV antibody test assists in management of all newly diagnosed MCC patients; non-antibody producers have a 42% higher recurrence risk than antibody producers and must be followed by imaging studies. For antibody producers, routine scans are not needed because serial blood tests reflect tumor burden and detect recurrences early, before they are clinically evident. The risk calculator is based on data from a registry of 1,400 MCC patients and integrates stage-specific recurrence data and other risk factored risk factors (sex, immune suppression, time since diagnosis). We estimate that using these tools could safely reduce visit/scan frequency by 40% or more, reduce approximately 1.8% of unnecessary visits/scans and result in well-maintained hair reduction (80%) one year after last treatment. In conclusion, the developed ex vivo MCC model allows correlation of histopathological data ex vivo with clinical outcomes.

Low versus high fluence light-induced hair removal: How hair follicle ex vivo studies translate into clinical results

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Professionals and home-use devices for hair removal deliver excellent hair reduction. Yet, how distinct hair follicle (HF) compartments are affected by various fluence levels and how this relates to clinical efficacy remains unclear. The goal of this study was to investigate the histomorphological changes of HFs exposed to a range of fluences ex vivo and to correlate these findings to clinical outcomes. Human scalp skin was treated with a range of laser fluences and commercial IPL device ex vivo followed by histological analysis. Clinical studies on efficacy of hair removal were done using a commercially available IPL, home-use device. Low fluence (5J/cm² or below at pulse durations of 10 ms or less) induced the anagen-catagen transition characterized by morphological changes similar to what occurs in vivo. An increase in fluence caused the appearance of a few apoptotic cells in the dermal papilla and in the outer root sheath. A high fluence (6J/cm² or more) was observed in all HF compartments, and massive cell death in dermal papilla and stem cell-enriched region of the outer root sheath. A single pulse of a home-use IPL device resulted in anagen-cataogen-like transition with occasional detection of apoptosis in the dermal papilla and outer root sheath, suggesting that 1) high hair reduction can be expected in vivo and 2) longer-term treatment might result in HF miniaturization due to cumulative effect on dermal papilla and outer root sheath cells. Indeed, clinical studies with home-use high fluence devices showed superior efficacy of hair reduction on female legs, armpits and bikini zones, with full hair regrowth after 4 treatments. Long-term application resulted in well-maintained hair reduction (80%) one year after last treatment. In conclusion, the developed ex vivo HF model allows correlation of histopathological data ex vivo with clinical outcomes.

Quality of care in dermatology: Validation of novel provider-reported and patient-reported measures

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A robust measure of quality of care should capture the clinical outcomes of patients (the end game). This study compared a provider-reported measure and a patient-reported measure in clinics for three different dermatology providers to determine the best assessment for quality of care. The provider-reported measure consisted of 4 questions (i.e., Severity, Change, Harm, and Response) in an e-record flow sheet. Providers completed the Severity question (i.e., lower the score the better the outcome). Cronbach's alpha and correlative analyses were performed in STATA. The provider-reported measure had high reliability for all providers (N=1,331 visits, 0=.766) and for returning patients (N=837 visits, 0=.728). Reliability was consistent across the three providers (a=.701 vs. a=.738 vs. 6.698). Similarly, the patient-reported measure had high reliability (N=741 visits, 0=.797). A subset analysis was performed for visits with the identical 4 questions between provider-patient measures. Significant correlation (r=.544, p=.001) and strong reliability (r=.704) was observed between the two measures, despite the higher mean patient outcome score (7.5 vs. 5.6). Provider and patient input are important for the assessment of quality of care. Our results illustrate the use of novel measures to evaluate quality of care in dermatology.

PD-L1 expression is an independent predictor of favorable outcome in patients with localized esophageal adenocarcinoma

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The prognosis of adenocarcinoma of the esophagogastric junction (AEG) is bad. The programmed cell death protein-1 (PD-1), a co-inhibitory receptor primarily expressed by T-cells, represents a potential therapeutic target. PD-1, PD-1 ligand 1 (PD-L1) and PD-L2 expression is a prognostic factors in some cancers; however their expression in AEG is unknown. We analyzed PD-L1, PD-L2 and PD-1 expression by tumor-infiltrating lymphocytes (TILs) and cancer cells in tumor specimen of 23 patients. We investigated TILs (TILs-PD-L1+) in 69%. We found PD-L2 expression by cancer cells (cancer cell-PD-L1) in 43.5% of patients whereas we observed PD-L1 expression by TILs (TILs-PD-L1) in 69%. We found PD-L2 expression by cancer cells and TILs in only 3.5% and 1.8%. 77.4% of tumors contained PD-1+ cancer cells and 81% PD-1+ TILs. Patients with increased expression of PD-1 by cancer cells and TILs showed significantly reduced OS and DFS, as determined by univariate, but not multivariate analysis. We found that expression of PD-L1 by cancer cells is an independent predictor for improved DFS and OS in multivariate analysis. Taken together, our data demonstrate that expression of PD-L1 cancer cells and TILs is an independent predictor of favorable outcome in AEG, whereas PD-L1 expression is associated with worse outcome and advanced tumor stage.

Treatment of urticaria in the United States in 2014 and 2015

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Urticaria is a common medical condition in the United States and is typically treated in the outpatient setting. How urticaria is treated is not well characterized. We analyzed the National Ambulatory Medical Care Survey (NAMCS) data for the years 2014 and 2015 (the most recent NAMCS data available). The NAMCS provides a representative sample of ambulatory visits to U.S. office-based physicians. We used SAS software to weight the data and to reflect representative national estimates. Urticaria comprises 3.07% of all visits and 0.18% of all dermatology visits. Based on the NAMCS sample, urticaria was the 16th most common dermatology diagnosis in 2014, and the 23rd most common dermatology diagnosis in 2015. Treatments for urticaria were prescribed in decreasing (frequency) include 2nd generation H1 blockers, 1st generation H1 blockers, topical corticosteroids, systemic corticosteroids, and H2 blockers. Specifically, the most common medications are cetirizine, diphenhydramine, hydroxyzine, prednisone, loratadine, and mometadone cream/solution. In 2014, omalizumab was approved for the treatment of urticaria; only three patients in the NAMCS database were treated with omalizumab for urticaria in 2014 and 2015. With the availability of new medications, treatment paradigms will likely change in the immediate future.
Quantification of activity and damage are essential to outcomes in morphea, in which effective treatment abrogates activity to stabilize damage. The only validated clinical measure incorporating these features is the Localized Scleroderma Cutaneous Assessment Tool (LoSCAT). However, LoSCAT scores that correlate with mild, moderate, and severe disease, which put an individual score into context, have not been determined. We performed a prospective cohort study to ascertain the clinical significance of individual LoSCAT scores and identify important changes in scores that correlates with minimal clinical important difference (MCID). 120 participants from the Morphea in Adults and Children Cohort were included with 1 study visit July 2014–June 2015. The LoSCAT and physician’s subjective assessment of activity and improvement were completed at every visit. Optimal score ranges for LoSCAT components corresponding with severity groups were determined by receiver operating characteristic (ROC) analyses with cut-off of 90% sensitivity and 80% specificity. Optimal score change for LoSCAT components associated with MCID were chosen based on similar ROC analyses of scores of the LoSD1, LoSAI, LoSD2, LoSA2, and LoSVE components in these scores. Mild, moderate, and severe disease correspondence with LoSAI activity scores of 0–4, 5–12, and >12; and physician global assessment of activity (PGA-A) scores of 0–10, 11–30, and >30. Mild, moderate, and severe disease damage corresponded with LoSD1 (damage scores) scores of 0–10, 11–15, and >15; and PGA-Damage scores of 0–18, 19–30, and >30. Improved activity was indicated by LoSAI score decrease of at least 2 points or 27.5% and PGA-A decrease of at least 4 points or 30. Improved damage was indicated by LoSD1 score decrease of at least 2 points. Worsening activity was indicated by LoSAI score increase of at least 2 points or 27.5% and PGA-A increase of at least 4 points. Worsening damage was indicated by LoSD1 increase of at least 25%. In sum, LoSCAT component score ranges can be used to contextualize patient disease severity and MCID for future observational and interventional studies.

Effect of stimuli on sun protective habits: A randomized double-blind controlled study

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Acne vulgaris is one of the most common dermatologic disorders and may result in profound negative physical and psychosocial side effects including scarring, low self-esteem, depression and suicidal ideation. This intervention aimed to improve child and adolescent acne care by providing photo- and text-based education through interactive education of clinicians and implementation of an electronic medical record (EMR) tool. Pediatricians associated with a tertiary children’s hospital healthcare system in San Diego (n=116) underwent intensive, case-based education on acne assessment and treatment based on published acne treatment guidelines. An EMR tool was implemented that guided classification of acne severity (mild, moderate, and severe) and generated severity-based care plans consisting of prescriptions, non-prescription materials, and customized educational materials for the use of our clinic. Subjects were assessed in 4 month blocks prior to and after implementation of the intervention, and included number of acne-coded visits, patients referred to pediatric dermatology, and prescribing patterns amongst the participating pediatricians. Data was ascertained via EMR accessed data collection, and survey of participating physicians. Analysis of data pre- and post-intervention revealed an 18% increase in the number of acne-coded visits by pediatricians (OR=1.18, p<0.001), and a 13% decrease in the number of patients referred to pediatric dermatology (OR=0.85, p<0.001). Prescribing patterns of the pediatricians post-intervention reflected improved adherence to practice guidelines with a 13% increase in frequency of topical retinoid prescriptions (OR=1.22, p=0.003) and a 15% decrease in frequency of clindamycin prescriptions (OR=0.89, p<0.001). Notably, practitioners perceived a decreased work burden of treating acne.
Health insurance status and outcomes among adults my mycosis fungoides in the United States
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Few studies have analyzed skin perfusion in patients with graft-versus-host disease (GVHD). To determine whether dermoscopic imaging, using skin pigmentation and epidermal changes, can be reliably early onset of multiple basal cell carcinomas (BCCs) among other major and minor criteria. BCNs frequently present in childhood, and despite the robust literature on management of BCNs and BCNs in adults, there is little guidance on management of BCCs and BCNs in pediatric patients. Typical management of BCCs includes excision and cryotherapy, but these options may be less favorable for pediatric patients who have anticipated high burden, given associated morbidity including pain and scarring. We report a retrospective case series that highlights the impact of disease progression in 4 pediatric patients with BCNs. 2 females and 2 males were diagnosed with BCNs at ages 1 month to 10 years. Initial pathology-confirmed BCNs presented at ages 7 to 10 years (in 3 patients) and were treated by standard approaches. In ongoing follow-up, concerns regarding lesions with nonconcordant features were identified such as pinpoint telangiectasias that potentially represented early changes of BCC and skin-colored papules resembling nevus BCC without bleeding or characteristic dermoscopy findings. Such lesions could have atypical morphology. Furthermore, Graft-versus-host disease (GVHD) is a complex immunological disorder that occurs as a result of an immune response of the graft against the recipient, and it can affect multiple organs, including the skin. Abnormal perfusion responses were observed in patients with GVHD, suggesting that skin blood flow may be a useful tool for monitoring disease activity and for identifying early signs of disease. These findings highlight the potential of dermoscopic imaging to detect early signs of skin disease in children and to inform management decisions.
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Secukinumab for patients with moderate-to-severe hidradenitis suppurativa

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BACKGROUND: Despite high survival rates of non-melanoma skin cancer (NMSC), many patients report unmet supportive care needs, increased cancer-specific and general distress, and higher levels of anxiety and depressive symptoms. Virtual reality (VR) has experienced tremendous growth in recent years with rapidly expanding applications. Proof of concept using VR to mitigate stressful healthcare experiences has already been demonstrated in various settings. OBJECTIVE: To assess the utility of a VR experience in the context of the Mohs surgical day as a means to ameliorate patient anxiety and pain. POPULATION: Patients undergoing Mohs micrographic surgery at a single tertiary care clinic. METHODS: After the first Mohs surgery stage was performed, patients completed a pre-virtual reality (VR) experience survey. Patients then participated in a 10-minute VR experience, and immediately after, completed a post-VR survey. Both surveys included a list of questions adapted from the Beck Anxiety Inventory (BAI). RESULTS: One hundred and two patients were enrolled in the study and 96 participants completed both the pre- and post-VR surveys. The average age was 62.5 years (SD 14.4). When comparing the post-VR survey with the pre-VR survey, there was a significant decrease in the number of patients who reported any level (mild, moderate, or severe) of the following symptoms: unable to relax (p = 0.002), fear of the worst happening (p = 0.0001), terrified or afraid (p = 0.0046), and nervous (p = 0.0003). Additionally, 77.5% of participants reported improved overall experience/satisfaction with the surgical day as a result of the virtual reality experience. CONCLUSION: Patients with NMSC undergoing Mohs micrographic surgery report symptoms of distress and anxiety during their surgical day. The current study suggests that virtual reality can alleviate anxiety and increase overall satisfaction for patients with NMSC during a Mohs surgical day.

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Clinical application of cultured stratified epithelial sheets for stable vitiligo of children

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BACKGROUND: Autologous cultured therapy has emerged as an effective treatment for stable vitiligo. But we need long time to follow up these children. OBJECTIVE: To investigate the safety and efficacy of autologous cultured epithelial sheets for the treatment of children stable vitiligo. Methods: Seven five children with stable vitiligo were included in this study. Keratinocytes and melanocytes from the patients were cultured according to modified Rheinward & Green’s technique under serum-free, feeder-free conditions. Patients were followed-up at 1, 3, 6, and 12 months post-transplantation. Results: Six one patients were segmental type of vitiligo. Scars at the donor sites were the most frequent adverse events associated with the procedure. Twenty eight (40%) patients achieved 100% repigmentation but someones graft sites colour were darker than normal skin around the lesion. Thirty one patients responded with repigmentation ranging from 75% to 100%. Total effective was 84.2%. The brightly refractile pigment cells rings can be seen by Confocal Laser Scanning Microscope (VivaScope 1500) and more numbers than that before surgery. Conclusion: Autologous epithelial sheet cultured is a safe and efficacious approach to cure stable vitiligo of children. But we need long time to follow up these children.
Proteomic profiling of zinc-induced skin cell proliferation: Activation of b-catenin pathway
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A 3D skin equivalent on a novel bi-layered nonwoven polymeric scaffold as a model to study keratinocyte differentiation and wound healing
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Aged skin requires appropriate skin care to counterbalance age-related pH increase and improve barrier function. This confirmatory, randomized study investigated efficacy of water-in-oil (w/o) emulsions (pH 4 or pH 5.8) on the volar forearms of elderly subjects (n=20) after 4 weeks of treatment. Further, after the treatment, the skin was challenged with an SDS in order to analyze the barrier protection properties of both formulations. This study evaluated skin pH, hydration and TEWL at baseline, after treatment and SDS challenge. Next, the stratum corneum lipid lamellar structure was analyzed and the lipid content was determined. After the 4-week treatment, pH 4 w/o emulsion resulted in a significantly lower skin pH (p<0.001) and improved the skin barrier compared to the pH 5.8 emulsion. Following SDS-induced barrier damage to the skin, the pH of all test areas increased, but the area treated with the pH 4 emulsion showed the lowest increase compared to baseline. In addition, even after the SDS challenge the skin area treated with the pH 4 emulsion still maintained significantly increased (p<0.001) length of intercellular lipid lamellae compared to the beginning of the study. Topical application of a w/o emulsion with pH 4 reacidifies the skin in elderly and has beneficial effects on skin moisturization, regeneration of lipid lamellae and lipid content and hence improves the epidermal barrier in aged skin.

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Galecitin-8 is upregulated in psoriasis and promotes IL-17A-induced keratinocyte proliferation by modulating mitosis
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TSG-6 is a hyaluronan-binding protein overexpressed in inflammatory conditions in reconstructed human epidermis
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Immune progenitor cells are enriched in psoriasis epidermis
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Genome-wide DNA methylation profiling identifies differential methylation in uninvolved psoriatic skin
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KMT2D epigenetically regulates p63 target enhancers to coordinate epithelial homeostasis
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Role of eutectic mixture of ceramides for reconstruction of stratum corneum intercellular lipid model
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Epidermal Structure and Barrier Function

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Galecitin-8 is upregulated in psoriasis and promotes IL-17A-induced keratinocyte proliferation by modulating mitosis. Galecitin-8 is a galactoside-binding lectin, which has been implicated in different stages of the cell cycle. In this study, we investigated the role of galecitin-8 in regulating keratinocyte proliferation in psoriasis. Our results showed that galecitin-8 expression was upregulated in psoriatic skin compared to normal skin, and that it promoted keratinocyte proliferation through the regulation of the cell cycle. This study highlights the potential role of galecitin-8 as a therapeutic target for psoriasis.

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TSG-6 is a hyaluronan-binding protein overexpressed in inflammatory conditions in reconstructed human epidermis. TSG-6, also known as HA-binding protein (HABP), is a hyaluronan-binding protein that plays a role in cell-cell interactions and matrix remodeling. In this study, we investigated the expression and function of TSG-6 in psoriasis. Our results showed that TSG-6 was upregulated in psoriatic skin compared to normal skin, and that it promoted keratinocyte proliferation through the regulation of the cell cycle. This study highlights the potential role of TSG-6 as a therapeutic target for psoriasis.

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Immune progenitor cells are enriched in psoriasis epidermis. Immune progenitor cells are a subset of immune cells that have the ability to differentiate into various immune cell types. In this study, we investigated the enrichment of immune progenitor cells in psoriasis epidermis. Our results showed that immune progenitor cells were enriched in psoriasis epidermis compared to normal skin, and that they played a role in the immune response to psoriasis.

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Genome-wide DNA methylation profiling identifies differential methylation in uninvolved psoriatic skin. DNA methylation is a epigenetic modification that plays a role in gene regulation. In this study, we investigated the DNA methylation landscape in uninvolved psoriatic skin. Our results showed that there were differential methylation patterns in uninvolved psoriatic skin compared to normal skin, and that these patterns were associated with the immune response to psoriasis.

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KMT2D epigenetically regulates p63 target enhancers to coordinate epithelial homeostasis. KMT2D, also known as TRIM32, is an epigenetic regulator that plays a role in gene expression. In this study, we investigated the role of KMT2D in regulating p63 target enhancers in the context of epithelial homeostasis. Our results showed that KMT2D epigenetically regulated p63 target enhancers to coordinate epithelial homeostasis.

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Role of eutectic mixture of ceramides for reconstruction of stratum corneum intercellular lipid model. Ceramides are essential components of the skin barrier, and their dysfunction is associated with skin disorders such as psoriasis. In this study, we investigated the role of eutectic mixture of ceramides in the reconstruction of the stratum corneum intercellular lipid model. Our results showed that eutectic mixture of ceramides promoted the reconstruction of the stratum corneum intercellular lipid model, and that it was a potential therapeutic target for skin disorders.

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Growth differentiation factor 11 (GDF11) modulation in skin rejuvenation
S Ipponjima1, Y Umino2, M Nagayama1 and M Denda2

Live imaging of granular cells during cornification in the epidermal equivalent model
S Iriyama, S Nishikawa, E Takai, H Yamamishi, N Kunizawa, T Hirao, J Hosoi and S Amano

Fluctuation of Caspase 14 caused by temperature and humidity unbalances the NMF production pathway and the process of keratinocyte enucleation
S Iriyama, S Nishikawa, E Takai, H Yamamishi, N Kunizawa, T Hirao, J Hosoi and S Amano

Laminin-S11 is a key component of epidermal basement membrane to maintain epidermal homeostasis
S Inyama, S Nishikawa, E Takai, H Yamamishi, N Kunizawa, T Hirao, J Hosoi and S Amano

Effects of polyols on lipid in an epidermal-equivalent model
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Live imaging of granular cells during cornification in the epidermal equivalent model
S Iriyama, S Nishikawa, E Takai, H Yamamishi, N Kunizawa, T Hirao, J Hosoi and S Amano

The third brain: When skins well-being struggles against stressful lifestyles
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Three-dimensional ultrastructural analysis of spatial relationship between granular cells and Langerhans cells in human epidermis

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Our previous ultrastructural studies of CLSM images of normal epidermis indicate that granular cells (GCs) in the basilar region of the stratum corneum (SC) are surrounded by Langerhans cells (LCs). We focused on the localization of GCs in the granular layer to unveil the fusion process of GCs. We generated mice expressing enhanced green fluorescent protein (EGFP) in the upper 10% of the SC, and visualized the localization and intracellular movement of GCs in situ. To investigate the fusion process of GCs and LCs, we generated mice expressing the fusion protein from the uppermost stratum granulosum (SG1 cells). Confocal microscopic analysis revealed that SG1 has two distinct pH layers, lower acidic (pH 5.33, SD 0.02) and upper neutral SC layers, rather than gradual change over SC layers. SC pH imaging in various parts of the body confirmed that the lower acidic SC layer consistently exists irrespective of the thickness and compactness of SC including paws. Topical application of pH-sensitive fluorophores generated high-resolution images, and they were lost when treated with lower acidic SC by histological analysis. These results suggested that the functions of cells were closely related with their localization in the epidermis.

ABSTRACTS | Epidermal Structure and Barrier Function

Effect of daily light stress on skin aging- Development of 3D skin reconstructed models

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As used in this study, the term daily light stress refers to how a person perceives their environment and the various physical, mental, or emotional interactions that person may encounter on a day to day basis, and how the consequences of these events accumulate to potentially become harmful and impact body homeostasis and health (e.g., chronic stress). Due to the variety of events that can be considered as stressful, added to the filter of one's own perception, measuring stress is not easy.

Intrinsic homeostatic mechanism of stratum corneum regulated by pH

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Stratum corneum (SC) provides protective barrier function and is composed of 15-20 layers of dead keratinocytes or corneocytes with a clarified homeostatic mechanism. pH has been considered to be one of the key regulators for SC homeostasis, while precise in vivo pH distribution in SC layers and its biological significance remain to be clarified. The purpose of this study is to assess the significance of pH regulation in SC by in vivo imaging. We fused two fluorescent proteins, pH-sensitive Venus and insensitive mCherry, and obtained real time pH value by concentration-independent ratiometric imaging. We generated mice expressing the fusion protein from the uppermost stratum granulosum (SG1 cells): Coroцит microscopic analysis revealed that SC has two distinct pH layers, lower acidic (mean pH 5.33, SD 0.02) and upper neutral SC layers, rather than gradual change over SC layers. SC pH imaging in various parts of the body confirmed that the lower acidic SC layer consistently exists irrespective of the thickness and compactness of SC including paws. Topical application of pH-sensitive fluorophores generated high resolution images, and they were lost when treated with lower acidic SC by histological analysis. These results suggested that the functions of cells were closely related with their localization in the epidermis.

In vitro.

Investigating small RNA extraction process through the study of different plant extracts

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As in humans, plants respond to environmental stresses by modifying their gene expression through the activity of smallRNAs that can rapidly regulate their gene expressions, and enable them to adapt to stress conditions. We have developed a novel extraction process maximizing the extraction potential of plant smallRNAs, in order to capture the benefits and the epigenetic potential of plants. A Baobab extract enriched in small RNAs was evaluated on aged fibroblasts. We evaluated the expression level of two key enzymes of the microRNA maturation, and observed them to have been maintained in senescent treated cells. In addition, the expression level of miR-100, whose decline has been associated with cellular aging, was observed to have increased in senescent fibroblasts treated with the extract. The development of plant extracts rich in small RNAs will help foster the generation of new plant extracts which can exhibit more efficient activity on specific markers in vitro, as was observed here on particular markers of cellular aging.

Reversible differentiation in HaCaT cells

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To elucidate events implicated in the control of epithelial growth and differentiation, normal human keratinocyte, cultured in appropriate media, provide an ideal experimental system. But their use can be limited by the complexities involved in their recovery from donors, cultivation and limited number of passages. To exclude these problems, the spontaneously immortalized human keratinocyte cell line HaCaT is often used as a model for the study of keratinocyte functions and differentiation. In this study, we compared the keratinocyte-differentiation markers expressed in HaCaT cells which were cultured in low Ca (0.05mM, LC-) high Ca (1.25mM, HC-) Dulbecco’s Modified Eagle Medium (DMEM) and reversed low Ca (1.25mM to 0.05mM, RL-) DMEM. The morphological changes were observed in LC-, HC- and RL-HaCaT cells. Next, RTPCR was performed to reveal the differentiation stage in these cells. The expression of involucrin, a keratinocyte differentiation marker, increased in HC-HaCaT and then was restored to the LC-HaCaT level in RL-HaCaT. However, the expression of ZO-1, a tight junction protein, was unchanged. These results suggest that reversible differentiation was induced depending on Ca concentration in HaCaT cells. Taken together, this reversible differentiation was induced depending on Ca concentration while maintaining proliferating property in HaCaT cells.
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Isoforme-specific functions of dermocrine in skin barrier maintenance and percutaneous immune response

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Dermocrine (DMKN) family members consist of four splice variants (a, b, y and c) in human and three variants (a, b and y) in mice. DMKN a, b and y isoforms are expressed specifically in the epidermis. DMKN b/γ-deficient mice (b/γ KO) recently generated by us and another group exhibited transitory hyperkeratosis and wrinkle formation with upregulated DMKN α expression, implicating a compensatory mechanism. Therefore, we established mice deficient for all three isoforms (a/β/γ KO). By day 10, b/γ KO showed more severe skin scales and wrinkle formation with constriction/autolemination of a tail, compared to b/γ KO. They also showed a growth retardation and dead under a low humidity condition. Increased TWL was evident in ~day 3 b/γ KO and ~day 14 α/γ/γ KO, but returned to normal thereafter. b/γ KO revealed thickening of granular layers with aberrant keratohyaline granules and cornified envelope fragility, all of these were more intense in α/γ/γ KO. Further investigation into the pathophysiology is in progress.

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Metabolic reprogramming maintains skin integrity in the absence of glucose transport and identifies a therapeutic vulnerability in psoriasis hyperplasia

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Glut1 facilitates glucose transporter expression in keratinocytes. Here, through the deletion of Glut1 in the epidermis, we demonstrate that Glut1 is indeed critical for the rapid proliferation of keratinocytes in vitro. Keratinocyte deficiency for Glut1 show energetic stress, poor proliferation, and increased levels of oxidative stress. Remarkably, Glut1 is dispensable for skin development, but essential for percutaneous immune responses. Glut1 deficient keratinocytes show alterations in sphingolipid, hexose, amino acid, and nucleotide metabolism in the absence of Glut1. Glut1 deficiency impaired keratinocyte proliferation and migration in response to UVB and excisional wounds. Moreover, both genetic and chemical Glut1 inhibition further reduced the inflammation associated with mouse and human organoid models of psoriasis. Thus, Glut1 is selectively essential for physiological hyperproliferation and its topical inhibition may be a novel treatment strategy for psoriasis hyperplasia and inflammation.

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Effects of short-term moisturizer application in different ethnic skin types - non-invasive assessment with optical coherence tomography and reflectance confocal microscopy

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The purpose of the present study was to evaluate the effectivity of three-dimensional light microscopy in reducing the required time and inter-rater discrepancies, especially in the case of percutaneous biopsy that is not familiar with the quantification methods. Three-dimensional microscopy provides more extended depth of penetration compared with conventional light microscopy and is known to be useful in clinical evaluation of thick biological specimens. Skin nerve biopsy together with the quantification of intraneuronal fiber density (IFDs) in multiple sections has been widely accepted for evaluating peripheral neuropathies. The skin samples were collected from the patient with post-herpetic neuralgia. Two investigators, a physician and a non-physician assessed the intraepidermal nerve fiber densities and required different gluing techniques including direct visualization of tissue slides and analysis with two- and three-dimensional images. Three-dimensional microscopy could produce images that enabled reliable evaluation of IFDs; the accuracy of analysis was statistically comparable between the physician and non-physician (p<0.05). Three-dimensional microscopy also enabled the non-physician to proceed meaningfully faster evaluation compared with the direct visualization method (p<0.01). Three-dimensional microscopy could be one of the useful methods to improve accuracy and convenience of the analysis of IFD especially for unaccustomed physicians or non-physicians.
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Lyonsomes support the degradation, signaling, and mitochondrial metabolism necessary for human epidermal differentiation

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Keratinocyte structural remodeling during epidermal differentiation, including a broad transformation of the proteome coupled with a reduction in total cellular biomass. This suggests that intracellular digestion of proteins and organelles is necessary for keratinocyte differentiation. Here, we use both genetic and pharmacologic approaches to demonstrate that lysosomal function and autophagy are both required for keratinocyte differentiation in organotypic human skin. Lysosomal activity was required for mTOR signaling and mitochondrial oxidative metabolism. In turn, mitochondrial reactive oxygen species (mtROS) and E2F1, a critical regulator of keratinocyte differentiation. Finally, treatment with exogenous ROS rescued the differentiation defect in lysosome-inhibited keratinocytes. These findings highlight a reciprocal relationship between lysosomes and mitochondria, in which lysosomes support mitochondrial metabolism and the associated production of mtROS. The mtROS released to the cytoplasm in suprabasal keratinocytes triggers autophagy and lysosome-mediated degradation necessary for epidermal differentiation. As defective lysosome dependent autophagy is associated with common skin diseases including psoriasis and atopic dermatitis, a better understanding of the role of lysosomes in epidermal homeostasis may guide future therapeutic strategies.

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Anti-pollution and anti-oxidative effects of two formulations containing Taraxacum officinale root extract on human skin explant

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Skin is exposed to external stress including UV or environmental pollutants such as particulate matter (PM) generated by human activities. PM contains harmful compounds (heavy metals or polycyclic aromatic hydrocarbons) that enter the body (BaP), which modulates xenobiotic metabolizing enzymes (XME). When adsorbed on the skin, some molecules penetrate regulate XME activity (i.e. Cytochrome CYP1B1) and generate deleterious effects (oxidative stress, inflammation). UVA exposition leads to oxidative stress and photoaging characterized by wrinkles and loss of skin tone. To evaluate anti-pollution properties of two formulations (SP30 and SPFS0+) containing Taraxacum officinale root extract, we analyzed, by RT-PCR, their effects on CYP1B1 over-expression induced by pollutants on human skin explants. To study protection against oxidative stress induced by UVA, we quantified Reactive Oxygen Species with a fluorescent probe (DichloroFluoRescerin Diacetate). To validate our in vivo model, penetration of the pollutants and their impact on metabolism were assessed by using BaP as a representative tracer. Radiolabeled compound was used to quantify the metabolites by high-performance liquid chromatography. Our results showed that both formulations reduced CYP1B1 overexpression induced by pollutants and decreased ROS production induced by UVA. In conclusion, we demonstrated the anti-pollution and the anti-oxidant properties of the two formulations. These formulations could protect skin against environmental stress by acting as a shield.

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Alternative splicing factor Sprr1 controls homeostasis of skin by regulating barrier formation and function

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The epidermis of the skin provides an essential barrier protecting the human body against infection and act as a barrier to the passage of water and other molecules between epithelial cells and integrity of TJ is a biophysical parameter of skin diseases such as atopic dermatitis or psoriasis. The epithelial-specific splicing factors Sprr1 are required for early mammalian development, including establishment of skin layer epithelial barrier functions. Ablation of epithelial cell-type specific splicing factors, Sprr1, in epithelial cells induces global changes in alternative splicing of numerous gene transcripts that are enriched for biological processes and pathways relevant to epithelial cell development, function, and TJ formation. Sprr ablation leads to a disruption of TJ integrity in vivo and in vitro, indicated by increased transepidermal water loss (TEWL) and leakage of inflammatory cells in the upper dermis. To overcome neonatal lethality, we generated inducible-Ahed KO mice by crossing K5.CreERT2 mice carrying a transgene with tamoxifen inducible Cre recombinase under the K5 promoter. The KO mice showed atrophic epidermis and cell death in hair follicles by topical treatment of high dose 4-OH Tamoxifen (Tam) at day 10. In contrast, the exposure to low dose 4-OH Tam for 3 weeks led to generation of eczematous lesion. Collectively, the results suggest crucial roles of Ahed in the development and maintenance of the epidermis.

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The barrier abnormality of early-onset pediatric atopic dermatitis results from abnormalities in tight junctions and epidermal lipids, but not differentiation proteins

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Pediatric atopic dermatitis (AD) is a common skin disease including psoriasis and atopic dermatitis, a better understanding of the relationship between lysosomes and mitochondria, in which lysosomes support mitochondrial metabolism and the associated production of mtROS. The mtROS released to the cytoplasm in suprabasal keratinocytes triggers autophagy and lysosome-mediated degradation necessary for epidermal differentiation. As defective lysosome dependent autophagy is associated with common skin diseases including psoriasis and atopic dermatitis, a better understanding of the role of lysosomes in epidermal homeostasis may guide future therapeutic strategies.

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Nicotinamide metabolism controls the proliferation/differentiation balance in human primary keratinocytes

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Nicotinamide (NAM) is a biophysical parameter of skin diseases such as atopic dermatitis or psoriasis. The epithelial-specific splicing regulators Sprr1 are required for early mammalian development, including establishment of skin layer epithelial barrier functions. Ablation of epithelial cell-type specific splicing factors, Sprr1, in epithelial cells induces global changes in alternative splicing of numerous gene transcripts that are enriched for biological processes and pathways relevant to epithelial cell development, function, and TJ formation. Sprr ablation leads to a disruption of TJ integrity in vivo and in vitro, indicated by increased transepidermal water loss (TEWL) and leakage of inflammatory cells in the upper dermis. To overcome neonatal lethality, we generated inducible-Ahed KO mice by crossing K5.CreERT2 mice carrying a transgene with tamoxifen inducible Cre recombinase under the K5 promoter. The KO mice showed atrophic epidermis and cell death in hair follicles by topical treatment of high dose 4-OH Tamoxifen (Tam) at day 10. In contrast, the exposure to low dose 4-OH Tam for 3 weeks led to generation of eczematous lesion. Collectively, the results suggest crucial roles of Ahed in the development and maintenance of the epidermis.

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Ahed plays crucial roles in homeostatic maintenance of epidermis

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Pediatric atopic dermatitis (AD) is a common skin disease including psoriasis and atopic dermatitis, a better understanding of the relationship between lysosomes and mitochondria, in which lysosomes support mitochondrial metabolism and the associated production of mtROS. The mtROS released to the cytoplasm in suprabasal keratinocytes triggers autophagy and lysosome-mediated degradation necessary for epidermal differentiation. As defective lysosome dependent autophagy is associated with common skin diseases including psoriasis and atopic dermatitis, a better understanding of the role of lysosomes in epidermal homeostasis may guide future therapeutic strategies.
Organic osmolytes improve cell volume regulation of aged human keratinocytes

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Elevated circulating levels of three pro-inflammatory cytokines (IL-1β, IL-6 and TNFα) are linked to the development of multiple, age-associated chronic disorders in aged humans (arthropathies, diabetes, cardiovascular disease). We report that reduced levels of circulating cytokines are not yet available, our recent studies in aged mice demonstrate that 1) age-associated epidermal dysfunction stimulates cutaneous pro-inflammatory cytokine production, accounting elevations in circulating cytokine levels, independent of liver or T-cell contributions; and 2) approaches that improve epidermal function reduce not only cutaneous, but also circulating cytokine levels. We determined here whether improvements in epidermal function reduce circulating levels of pro-inflammatory cytokines in 31 humans, treated twice-daily with a topical formulation previously shown to improve epidermal function in humans. Controls included 30 untreated, aged humans. Eleven additional volunteers served as young controls. Both epidermal functions and plasma levels of IL-1β, IL-6 and TNFα were measured at baseline and after 12 treatment. Aged humans displayed significantly higher basal levels of all 3 cytokines in aging-associated abnormalities in cutaneous function alone can mitigate age-associated systemic inflammation, perhaps attenuating the subsequent development of inflammation-associated chronic disorders.
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Activation of AMPK-SIRT1 and PPAR Adiponectin is known to exert biological effects through binding to its receptors, leading to...

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Characterisation of a novel murine model of atopic dermatitis L Smith1, S Catul1, L Casals-Dias1, C Valens1, M Campion1, S Coughlin1, G Fissner1 and M Stratigos1 1 Department of Dermatology, University College Dublin, Ireland, 2 Cardiovascular Research Institute, UC San Francisco, San Francisco, CA and 3 Systems Biology Ireland, University College Dublin, Dublin, Ireland

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In vivo confocal Raman spectroscopy and chemometric analysis on human stratum corneum: insights on composition, organization, aging and photoaging S Perticaroli, D Yeomans, K Ellis, K Wierzbowski, T Cambron and P Ray The Procter and Gamble Company, Mason, OH

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Identifying novel biomarkers of aging P Hall1, M Ozols1, CEM Griffiths2, REB Watson2 and AK Langton1 1 The University of Manchester, Manchester, England, United Kingdom and 2 University of Manchester, Manchester, England, United Kingdom

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The sphingolipid synthesis of keratinocyte is increased by adiponectin mediated by the activation of nuclear hormone receptor pathways S Hong1, H Sen2, K Shin1, K Park1, C Kim1 and S Sen1 1 Department of Dermatology, College of Medicine, Dankook University, Cheonan, Republic of Korea, 2 1Department of Dermatology, College of Medicine, Dankook University, Cheonan, Republic of Korea, 3 Food Science and Nutrition, Convergence Proc of Material Science, Chungnam National University, Daemyung, Republic of Korea and 4 Department of Dermatology, Chung-Ang University Hospital, Seoul, Republic of Korea

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Understanding the role of ABCA12 in the pathogenesis of harlequin ichthyosis E Enijnhout1, P Drenth1, NA Cley1, B Fell1, D Mine2, D Kelle1, A Enright1 and EA O'Toole1 1 TQML, London, England, United Kingdom, 2 GSK, Stevenage, England, United Kingdom and 3 ELL, Cambridge, England, United Kingdom

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In vivo confocal Raman spectroscopy and chemometric analysis on human stratum corneum: insights on composition, organization, aging and photoaging

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The sphingolipid synthesis of keratinocyte is increased by adiponectin mediated by the activation of nuclear hormone receptor pathways

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Understanding the role of ABCA12 in the pathogenesis of harlequin ichthyosis

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Effect of pregnancy on skin hydration and barrier function

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Introduction: Pregnancy is a particular physiological state inducing major changes to the woman skin. Previous work showed that areas like abdomen, subjected to a high mechanical stress, are greatly impacted, among which the hydration and the barrier function. The goal of our study is to evaluate the impact of pregnancy on these two properties on the abdomen using a non invasive biochemical approach. Methods: A clinical study has been conducted on 15 non-pregnant women and 26 pregnant women at the 6th month of pregnancy and 4 months after delivery. Skin surface samples have been collected from the abdomen using swab technique.

Natural Moisturizing Factors (NMF) quantity and ceramide content have been quantified by LC/UV and LC/MS respectively. Results: Concerning the hydration state, the results show that the amount of NMF is significantly decreased in areas that are not influenced by the abdomen. In pregnant women after delivery, NMF quantity tended to be lower than in non pregnant women, although not significantly. Our results also show that the amount of ceramides was significantly lower in pregnant women than in non pregnant women, and remained significantly lower after delivery. Conclusion: The abdomen skin is critically affected by the pregnancy state. Skin hydration and barrier function tended to be affected not only during pregnancy but also 4 months after delivery.

A facial treatment cleansing device enhanced delivery of topical skin care products


New cosmetic products are introduced for sale almost every day demonstrating improvement in many skin attributes. To satisfy and retain consumers, personal care companies continually introduce new products. Additional we are utilizing or developing new or modified delivery systems or devices that may enhance the efficacy, improve ease of use or consumer appeal of current products. For example, skin exfoliation can provide a boosting effect to topical skin care products that are subsequently applied. Skin exfoliation may be done by chemical exfoliators like glycolic acid or by physical exfoliators like sugar scrubs. Recently, we developed a skin treatment cleansing device that can eliminate the need for both chemical and physical skin exfoliation products. In a large scale aging study, 30 inbred strains were necropsied at 12, 20, and greater than 20 months of age. Ten strains were identified with nail lesions; the highest frequency was noted in NON/ShiLtJ mice. To determine if these lesions occurred in younger mice, NON/ShiLtJ mice aged 241 days of age were obtained. Lesions identified fell into two main categories: acute to chronic penetration of the third phalangeal bone through the hyponychium with associated inflammation and bone remodeling or metaplasia of the nail matrix and nail bed associated with severe orthokeratotic hyperkeratosis replacing the nail plate. Penetration of the distal phalanges through the hyponychium appeared to be the initiating feature. The acute to subacute inflammatory response was associated with osteolysis of the distal phalanges. The highest frequency of nail lesions was in the NON/ShiLtJ and NZW/LacI strains with a strong female predilection. Younger NON/ShiLtJ mice revealed that these lesions were intermittent or affected only one digit. The only other nail unit abnormality identified was sporadic subungual intraosseous epimyloid inclusion cysts that closely resembled similar lesions reported in humans, dogs, and horses.

Nail lesions in 30 old inbred mouse strains

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In a large scale aging study, 30 inbred strains were necropsied at 12, 20, and greater than 20 months of age. Ten strains were identified with nail lesions; the highest frequency was noted in NON/ShiLtJ mice. To determine if these lesions occurred in younger mice, NON/ShiLtJ mice aged 241 days of age were obtained. Lesions identified fell into two main categories: acute to chronic penetration of the third phalangeal bone through the hyponychium with associated inflammation and bone remodeling or metaplasia of the nail matrix and nail bed associated with severe orthokeratotic hyperkeratosis replacing the nail plate. Penetration of the distal phalanges through the hyponychium appeared to be the initiating feature. The acute to subacute inflammatory response was associated with osteolysis of the distal phalanges. The highest frequency of nail lesions was in the NON/ShiLtJ and NZW/LacI strains with a strong female predilection. Younger NON/ShiLtJ mice revealed that these lesions were intermittent or affected only one digit. The only other nail unit abnormality identified was sporadic subungual intraosseous epimyloid inclusion cysts that closely resembled similar lesions reported in humans, dogs, and horses.

Sebaceous gland abnormalities in fatty acyl CoA reductase 2 (Far2) null mice

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In a large scale screen for skin, hair, and nail abnormalities in null mice generated by The Jackson Laboratory’s KOMP center, homozygous mutant Far2tm2b(KOMP)Wtsi mice were found to develop focal areas of alopecia due to a subdiale sebaceous gland abnormality. As sebocytes mature in normal mice they become paler with lower amounts of a clear lipid containing vacuole. By contrast, the Far2/Far2 null mice had sebocytes that became brightly eosinophilic but did not rupture as they entered the sebaceous gland duct. Scattered throughout the dermis and often at the dermal hypodermal fat junction, were dystrophic hair follicles or ruptured follicles with a foreign body granulomatous reaction surrounding a free hair shaft (trichogranuloma). The Moshomin gland in the eyelid is one of several specialized sebaceous glands in the mouse. These glands had moderately to markedly dilated ducts associated with similar changes in the sebocytes as seen in the truncal skin. Skin surface lipid analysis of Far2 null mice revealed a lower level of wax esters, cholesterol ester, ceramides, and diacylglycerols compared to wildtype control mice. Similar changes have been reported in CORGEA A mice (Pigmentation, growth, and fertility) and other models with an increase in ceramides, and diacylglycerols compared to wildtype control mice. Similar changes have been reported in CORGEA A mice (Pigmentation, growth, and fertility) and other models with an increase in ceramides, and diacylglycerols compared to wildtype control mice. Similar changes have been reported in CORGEA A mice (Pigmentation, growth, and fertility) and other models with an increase in ceramides, and diacylglycerols compared to wildtype control mice. Similar changes have been reported in CORGEA A mice (Pigmentation, growth, and fertility) and other models with an increase in ceramides, and diacylglycerols compared to wildtype control mice.
668 Mitochondrial depolarization is a key component of CRF-induced apoptosis in human keratinocytes. The role of the mitochondrial apoptotic pathway in CRF-induced apoptosis was investigated. We treated keratinocytes with the CRF-2 receptor agonist clonidine, which has been shown to induce apoptosis in human keratinocytes. We observed a rapid depolarization of the mitochondrial transmembrane potential, followed by an increase in mitochondrial superoxide production. These changes were accompanied by the activation of caspase 3 and 7, indicating the initiation of the apoptotic cascade. The mitochondrial apoptotic pathway appears to be a central component of CRF-induced apoptosis in keratinocytes.

669 The mitophagy receptor NIX induces mitochondrial fragmentation during epidermal differentiation in an Lc3-independent manner. The mitophagy receptor NIX is a key regulator of mitochondrial homeostasis, but its role during epidermal differentiation remains unclear. We investigated the role of NIX in mitochondrial dynamics and barrier repair during epidermal differentiation. We observed that NIX is upregulated during epidermal differentiation and that it induces mitochondrial fragmentation. Interestingly, NIX-induced mitochondrial fragmentation is associated with increased barrier repair, indicating a potential role for NIX in maintaining skin barrier function.

670 Topically applied buffers of different pH and composition influence skin pH, barrier repair, epidermal differentiation, and inflammation. Topically applied buffers can influence skin pH, barrier repair, and epidermal differentiation. We investigated the effects of different pH and composition buffers on these parameters. We found that buffers with pH values of 4.0 or 5.6 induced lower skin hydration and SC integrity compared to a buffer with pH 7.0. The composition of the buffer also influenced its effects. For example, buffers containing ammonium glycolate (pH 4.0) were more effective than those containing citrate (pH 5.6) in inducing lower skin hydration and SC integrity.

671 Cross-talk between covalent DNA modifications and chromatin architecture. DNA dioxygenase Tet2 mediates the effects of architectural chromatin protein CTCF on epidermal barrier maintenance, inflammation and tumorigenesis. We investigated the role of Tet2 in regulating chromatin architecture and its effects on epidermal barrier maintenance and tumorigenesis. We found that Tet2-mediated DNA dioxygenation is important for maintaining a barrier-permissive chromatin architecture in the epidermis. This architecture is associated with reduced expression of genes involved in inflammation and tumorigenesis.

672 Dual role of the anaphase promoting complex/cyclosome in regulating stemness and differentiation in human primary keratinocytes. The anaphase promoting complex/cyclosome (APC/C) is a key regulator of cell cycle progression and differentiation. We investigated the role of the APC/C in regulating stemness and differentiation in human keratinocytes. We found that the APC/C is important for maintaining stem cell properties and suppressing differentiation. However, we also observed that the APC/C is involved in differentiation when it is activated under specific conditions. These findings highlight the complex role of the APC/C in regulating stemness and differentiation.

673 11ß-hydroxysteroid dehydrogenase type 1 activation induced by UVB might be a possible mechanism of skin barrier dysfunction due to UV exposure. Ultraviolet light (UV) radiation to the skin causes dose- and wavelength-dependent reactions, including skin barrier dysfunction. Long-term exposure to even low dose UV induces the alteration of skin barrier, presenting prominent ceramide decrease in the stratum corneum (SC) intercellular lipids. 11ß-hydroxysteroid dehydrogenase type 1 (11ß-HSD1), which converts inactive corticosterone to active cortisol in peripheral tissues and inhibits keratinocytes and fibroblasts proliferation, is known to be induced by UVB. We performed in vitro, in vivo, and ex vivo studies to elucidate whether skin barrier dysfunction induced by UV could be resulted from 11ß-HSD1 activation after UV irradiation. We observed that 11ß-HSD1 expression was induced by UVB irradiation, and that this expression increased with increasing doses of UVB. Furthermore, we found that UVB irradiation increased 11ß-HSD1 expression as well as inflammatory cytokines in vivo in SC. These results suggest that 11ß-HSD1 activation might contribute to skin barrier dysfunction induced by UVB.

689 APOBEC regulates NOTCH1 expression and keratinocyte differentiation. T. Dharmic, Y. Nakano, K. Waku, M. Otsuka, M. Muramatsu and K. Kabashima 1 Department of Dermatology, Kyoto University, Kyoto, Japan, 2 Institute of Medical and Dental Sciences, Kyoto University, Kyoto, Japan, 3 Kanazawa University, Kanazawa, Ishikawa, Japan, 4 Department of Dermatology, Kyoto University, Kyoto City, Japan APOBEC (adenosine deaminase, cytidine deaminating) is a deaminase that induces C to U deamination of RNAs and DNAs. In humans, seven APOBECs are known while rodent skin has just a single APOBEC. APOBECs are proposed to be involved in the host protective response against several viruses, such as HIV, through deamination of viral ssDNA. We have reported that APOBEC3A and 3B expression is increased in the epidermis of psoriasis. On the other hand, recent evidence suggests that APOBEC3A and 3B is involved in the mutagenesis of several cancers including skin cancer. In the present study, we investigated whether APOBECs mediated Notch1 treatment using keratinocytes and skin. APOBEC3A and 3B expression was increased in the epidermis of psoriasis. On the other hand, recent evidence suggests that APOBEC3A and 3B is involved in the mutagenesis of several cancers including skin cancer. In the present study, we investigated whether APOBECs mediated Notch1 treatment using keratinocytes and skin.
TRPV1 positive peripheral sensory nerves are required for prompt skin barrier repair

Department of Dermatology, Kyoto University, Japan

Peripheral sensory nerves appear to play an important role in cutaneous homeostasis. Patients with peripheral neuropathy suffer from various skin manifestations including frequent infection and impaired wound healing. However, role of sensory nerves in mediating repair process after epidermal barrier disruption are not fully understood. Here, we showed that transient receptor potential cation channel subfamily V member 1 (TRPV1) agonist-induced neuropathy mice exhibited delayed barrier repair after tape stripping. Gene expression analysis revealed that keratinocyte differentiation and barrier-related lipid processing were downregulated after barrier disruption in the neuropathy mice. Upon screening of neuropeptides in dorsal root ganglia, some neuropeptides, which were galanin, neuropeptide S, pituitary adenylate cyclase activating polypeptide (PACAP), somatostatin and substance P, were downregulated in the neuropathy mice. We showed that PACAP significantly promoted primary mouse keratinocyte differentiation. These findings suggest that PACAP is involved in epidermal repair under barrier disruption via promoting keratinocyte differentiation. Further understanding the precise roles on the mechanism of neuropeptides will provide opportunities to develop therapeutic agents which can manipulate barrier repair in patients with neuropathy.

Minimum role of filaggrin-gene mutations in the skin barrier function of atopic dermatitis

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Filaggrin-gene mutations were detected in 8 patients out of the 54 patients. Amount of filaggrin protein was evaluated using ELISA from scales in the lesions. Severity of the lesion was evaluated on sequencing with gDNA obtained from peripheral blood. Mutations found in Japanese were detected by genotyping with Taqman probes or direct sequencing. Amount of filaggrin protein was significantly lower in patients with filaggrin-gene mutations. These results suggest that filaggrin-gene mutations are not only producing sebum, but that they are also key regulators of the skin homeostasis. Therefore, it is necessary to study on complex in vitro model the influence of environmental factors on sebaceous glands, to better understand what happens in greasy and prone to acne skin. We developed different in vitro co-culture models combining the sebocytes and the most current bacteria observed near the sebaceous gland, Propionibacterium acnes, and UVA. Under P. acnes influence, we observed in sebocytes monolayers a decrease of released triglycerides (tenfold decrease) and a strong increase of free (acid lipids level fourfold increase). In the same co-culture model exposed to UVA radiations, we observed an increase of lipid per-oxidation (fourfold increase). To be closer to the real organization of the sebaceous gland, we developed a new model of sebocytes in clusters using human serum (HS). The efficiency of the model was confirmed by the presence of specific markers of the sebaceous gland (mucin 1, keratin 7). With an innovative 3D imaging technique, we also reached for the first time to count a huge number of lipid droplets (3000 droplets). This new model allowed us to better understand the influence of environmental factors on sebaceous gland. We observed in presence of HS an increase of the number (> 1 m3) and of the mean volume of lipid droplets (x2.8), confirming the functionality of this new model. The development of such in vitro complex sebaceous gland models including its environment could permit to evaluate more efficiently molecules intended for the treatment of skin prone to acne.

Site-specific microarray evaluation of spontaneous dermatitis in flaky tail mice

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Flaky tail mice (FTM) with gene mutations to filaggrin and TMEM79 are known to spontaneously develop atopic dermatitis (AD)-like dermatitis. Interestingly, we have already reported that the spontaneous development of dermatitis exhibits site-specificity, i.e., the face and neck develop spontaneous dermatitis, while the dorsal flank shows normal skin in old FTM. In fact, TEWL and SC pH increased, SC hydration decreased, and epidermis thickened in the neck, but not in the dorsal flank. The detailed pathogenic mechanisms of such site-specific emergence of dermatitis in AD remain unclear. To clarify the pathogenesis of site-specificity, we analyzed skin of the neck and dorsal flank in old FTM using a microarray. Many genes for keratin and keratin-associated protein were up-regulated in the neck compared with the dorsal flank. Several genes thought to be associated with the pathogenesis of AD, such as IL-4, 5, and 13, TSLP, TARC, loricrin, involucrin, transglutaminase 1, TMEM79, defensin, cathelicidin antimicrobial peptide, claudin 1, and EGFR, showed no significant change between the neck and dorsal flank. In other AD-associated genes, filaggrin was up-regulated in the neck, and some members of the kallikrein and serine peptidase inhibitor family were up-regulated. KEGG pathway analysis with DAVID ver. 6.8 revealed the most significant difference in the pathway of S. aureus infection between both sites (P < 0.001). In addition, multiple genes downstream of the proinflammatory receptor-activated receptor (PPAR) signaling pathway were down-regulated (P < 0.003), in line with our previous studies showing therapeutic effects of some PPAR activators in a hapten-induced murine AD model. Although further research is needed to fully understand the mechanisms associated with site-specific emergence of dermatitis in AD, including ones associated with S. aureus infection or PPARs.

The beneficial effect of Montecatini thermal water upon various enzymes including NADH dehydrogenase in modulation of epidermal keratinization

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Background: Today, the curative use of thermal water at the medical centre of Montecatini Terme is operated with insurance coverage, and further research is needed to fully understand the components of enriched mineral and its works by medical team. As a result, remarkable clinical studies have been conducted and it is proven that Montecatini thermal water is also useful on human skin problems such as psoriasis and atopic dermatitis. Meanwhile, further research on biological mechanism of epidermal keratinization is required more than ever. In this research, the influence to enzymatic mechanism on the epidermal keratinization by Montecatini thermal water, which is related to improvement of the action of this enzyme activity. Conclusion: This result gives a new perspective on psoriasis treatment mechanism by Montecatini thermal water, which is related to improvement of the multi-functional state of the stratum corneum, and activation of NADH dehydrogenase and Bleomycin Hydrolase.
Aryl hydrocarbon receptor activation upregulates a battery of antimicrobial genes in the pathogenesis of atopic dermatitis caused by air pollution

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The Aryl Hydrocarbon Receptor (AHR) is a multifaceted transcription factor involved in xenobiotic metabolism, immune cell development, and epidermal differentiation. AHR is activated by aryl hydrocarbons, of which TCDD is well-known. Coal tar (CT) is an activator of AHR and is used in the clinic as a treatment for atopic dermatitis (AD). To better understand AHR activation and downstream effects, we studied ligand-mediated DNA binding by AHR, its target gene expression, and overall differentially expressed genes in treated keratinocytes. TCDD and CT treated human primary keratinocytes were subjected to chromatin immunoprecipitation followed by deep-sequencing (ChiP-seq) and RNA-sequencing to study genomewide effects of AHR activation by both AHR ligands. TCDD and CT, as AHR ligands, show early (after 2 hours) upregulation of genes involved in detoxification pathways. Also, we observed genome wide integrations extending terminal differentiation pathways (e.g. IFI, FLG), antimicrobial proteins (e.g. PRI, SLPI, ST8SIA8), and tight junction proteins (CLDN4, OCLN), approximately 24 hours after AHR activation by CT and TCDD. The genomics binding data of the AHR indicates that the canonical cellular responses after CT and TCDD exposure steer primarily towards activation of xenobiotic metabolism pathways. The transcriptional analysis shows additionally differentially regulated genes in the aforementioned metabolism pathways, and the activation of the antimicrobial defense mechanisms of the epidermis. The latter may influence the interaction between host and microbes and could have important implications for the skin microbiome composition in coal tar treated AD patients.

Aryl hydrocarbon receptor-dependent expression of aldo-keto reductase 1C3 in the skin

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CT and TC induced transcriptional changes were translated to the protein level. SDS-PAGE/western blot analyses confirmed that the observed transcriptional upregulation of AKR1C3 correlates with an enhanced expression of the corresponding enzyme in AD keratinocytes. Thus, necroptosis and RIP3 in particular represent potential targets for treatment of AD. In vitro, we found that inhibition of RIP3 by its specific inhibitor RIP3 inhibitor (RIP3i) significantly reduced the survival of AD keratinocytes.

Aryl hydrocarbon receptor-dependent expression of aldo-keto reductase 1C3

M. Jargosch1, F. Löffler2, P. Pätzold3, L. Krause1, N. Gazzara-Stark1, T. Biedermann1, S. Eyerich1, K. Wotawa1

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In vivo assessment of cleaner mildness by confocal raman microscopy

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Skin cleansers contain surfactants, which may compromise the integrity of skin barrier to the penetration of external aggressors, resulting in skin irritation. Assessing cleaner mildness on skin is typically done by clinical evaluation and measurement of transepidermal water loss (TEWL) following exaggerated patch test or exaggerated wash test protocols. These methods are partly subjective and often with variable results. Previously, we demonstrated that the effects of a relatively harsh surfactant (0.1% sodium lauryl sulfate) on skin barrier function can be objectively assessed by measuring the concentration profile of a marker with a known safety profile (carnitine) that penetrates into the skin, using in vivo confocal Raman microspectroscopy (CRM). In the present work, we demonstrate that this method can be used to discriminate between different cleanser formulations according to their effect on skin barrier to external penetration.

The results of the confine penetration method are compared with the more commonly used exaggerated patch and exaggerated arm wash tests. The obtained results reflect the penetration depth of the cleanser penetration method agrees with the results of the exaggerated tests, confirming the validity of the method. In conclusion, the confine penetration test objectively evaluates the effects of topical cleansers on skin barrier to external penetration and can be used to assess cleanser mildness.

Characterisation of the response of claudin 1 to ultraviolet radiation: A bioinformatics and experimental study

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Human skin forms the main barrier between the internal system and the external environment. Claudin-1 in particular is crucial in two ways. First, it protects the internal system from external insults such as ultraviolet radiation and toxins. Second, it prevents excessive water loss and maintains water and electrolytes homeostasis. Tight junctions of the epidermis contribute to this barrier. Tight junctions are complexes of proteins that form a dynamic barrier and communicate with each other. Claudin-1 has an integral role in tight junctions. The aim of this study was to characterise the potential response of claudin-1 to UVR using bioinformatics to identify potential MPP cleavage sites or RIC sensitive sites. We also aimed to argue claudin-1, and MPP expression in keratinocytes irradiated by solar simulated UVR. Bioinformatics analysis showed that Claudin-1 possesses an appreciable number of UV absorbing amino acids. However, the most striking observation is that proteases MMP-9 (induced by UVR) showed the greatest number of predicted cleavage points on claudin-1 (24% of total claudin-1). MMP-9 and MMP-2 can potentiate tonotubular keratin formation.

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Keratin-14 dependent disulfide bonding contributes to epidermal homeostasis and barrier function through 14-3-3 and Hippo signaling in mouse skin

ABSTRACTS | Epidermal Structure and Barrier Function

3D epidermal models through physiological processes oscillate in a daily fashion. CR relies on a clock localized in the brain and on peripheral clocks found in tissues such as heart, liver or muscle. Their disruption has a dramatic impact on human physiology. The skin contains also a clock revealed by the expression of circadian rhythm proteins known as Clock genes (CLOCK, Clock, Per2 and Cry1). CR mechanism involves interdependent feedback loops of transcription and translation of these Clock genes. The dimer BMal/Clock drives transcription of Per and Cry genes whereas a negative feedback loop is initiated by the nuclear translocation of the dimer Per2/Cry1, inhibiting their own transcription. Newest and up to date to experiments to models used to study skin CR are done on cellular synchronized models and not on full skin. To address the consequences of CR alteration on skin physiology, we propose a skin explant model synchronized by desaminothreonine (DEX). The models relevance was confirmed by the trans-epidermal water loss. The observation of expression pattern of the ROR gene published data. Indeed, we observed the classical rhythmicity and anti-phasic expression between BMal and Per2 but also a rhythmicity for Cry1. We confirmed the non-rhythmic pattern of Clock marker. We confirmed the functionality of this model with the marker aquaporin-3, known to oscillate according to a CR. We used this model to study the impact of blue light (BL) on skin circadian markers. We found that BL disrupts skin CR, by abolishing the rhythmicity of BMal, Per2 and Cry1. We observed in BL condition a discrepancy in the cytoplasmic and nuclear localization of Cry1, suggesting that the negative feedback loop is imbalanced confirming the abolishment of rhythmicity observed. We demonstrate that a plant extract is able to protect the skin circadian rhythm against its disruption by blue light.

Synchronized skin explant model to study circadian rhythm alterations

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Circadian rhythm (CR) is an evolutionary system adjusting organism physiology to diurnal variations. Physiological processes oscillate in a daily fashion. CR rely on a clock localized in the brain and on peripheral clocks found in tissues such as heart, liver or muscle. Their disruption has a dramatic impact on human physiology. The skin contains also a clock revealed by the expression of circadian rhythm proteins known as Clock genes (CLOCK, Clock, Per2 and Cry1). CR mechanism involves interdependent feedback loops of transcription and translation of these Clock genes. The dimer BMal/Clock drives transcription of Per and Cry genes whereas a negative feedback loop is initiated by the nuclear translocation of the dimer Per2/Cry1, inhibiting their own transcription. Newest and up to date to experiments to models used to study skin CR are done on cellular synchronized models and not on full skin. To address the consequences of CR alteration on skin physiology, we propose a skin explant model synchronized by desaminothreonine (DEX). The models relevance was confirmed by the trans-epidermal water loss. The observation of expression pattern of the ROR gene published data. Indeed, we observed the classical rhythmicity and anti-phasic expression between BMal and Per2 but also a rhythmicity for Cry1. We confirmed the non-rhythmic pattern of Clock marker. We confirmed the functionality of this model with the marker aquaporin-3, known to oscillate according to a CR. We used this model to study the impact of blue light (BL) on skin circadian markers. We found that BL disrupts skin CR, by abolishing the rhythmicity of BMal, Per2 and Cry1. We observed in BL condition a discrepancy in the cytoplasmic and nuclear localization of Cry1, suggesting that the negative feedback loop is imbalanced confirming the abolishment of rhythmicity observed. We demonstrate that a plant extract is able to protect the skin circadian rhythm against its disruption by blue light.

Protease-activated receptor 2 activation reduces Claudin-1 expression in primary human keratinocytes

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Claudin-1 is an integral membrane protein and a component of tight junction (TJ) complex. Strong evidence from murine studies has highlighted a critical role played by claudin-1 in epidermis barrier function and more recently in inflammation. We and others have shown reduced expression of claudin-1 in Atopic Dermatitis (AD) skin. Mechanisms that regulate claudin-1 expression in AD skin are yet to be fully elucidated. Protease-Activated Receptor (PAR) 2 is a transmembrane G-protein-coupled receptor that can be activated by endogenous and environmental proteases (e.g. dust mites, allergens). Increased proteases activity has been shown in atopic skin. Activation of PAR2 has been associated to inflammation, altered epithelial barrier function and pruritus. In this study we aim to investigate the effect of PAR2 activation on claudin-1 expression and barrier integrity. We hypothesized that PAR2 activation would lead to an impaired TJ barrier function and pruritus. To this end, we undertook ex-vivo experiments on the human skin explant model.

Epidermal tissue differentiation requires the uncharacterized protein RED1

C Temmeau, DT Sessions, A Mah, L Lee, JY Shin and CS Lee, Dermatology, Stanford University, Stanford, CA

The human genome encodes >4,000 uncharacterized open reading frames (ORFs) with undiscovered biological functions. To identify potentially novel mediators of epidermal differentiation, we have termed Regulator of Epidermal Differentiation (RED1). An orthogonal screen also identified RED1 as highly down-regulated in primary human squamous cell carcinomas compared to patient-matched normal skin. As malignant neoplasms frequently demonstrate loss of differentiation, we hypothesized that RED1 is required for epidermal differentiation. To test this possibility, we undertook gain- and loss-of-function experiments in organotypic human epidermal tissue. Enhanced expression of RED1 accelerated differentiation in regenerated human skin while CRISP/Cas9-mediated depletion attenuated this process. In each condition, we used skin explant experiments to assess the global impact of altering RED1 expression using ChIP-seq to identify gene groups that control skin differentiation, development, and function. Proximity-dependent biotin ligation assays in organotypic human epidermal tissue identified validated RED1 target genes, including RUNX1 and PER4. One novel gene, RED1, which is known to be essential to skin differentiation, as well as genes not previously implicated in skin homeostasis, such as callycin binding protein (CACYBP). Gene set enrichment analysis suggests RED1 and its interacting proteins co-control distinct subsets of genes that influence epidermal differentiation and drive neoplastic transformation. These findings indicate that RED1 impacts on epidermal neoplasia. RED1 therefore represents a new regulator of epidermal differentiation with potential roles in skin carcinogenesis.
Then we screened various plant extracts and found that red clover extract activated HIF-1α transcriptional activity activated in a hypoxic environment decreased with age. Moreover, we found that ramapycin, HIF-1α inhibitor, decreased MCSP protein synthesis. In the pruritic dermatitis models, sustained Ca2+ increases were observed in nerves in the upper epidermal region independently of scratching, and depletion of neuronal nitric oxide synthase blocked Ca2+ increases. The pruritus dermatitis models suggest that epidermal barrier dysfunction is a key element which influences skin inflammation and itch. However, the epidermal nerve dynamics and activity have not been directly characterized. Here, by intravital imaging, we demonstrated that epidermal nerves were constantly extended and retracted, and occasionally underwent local dissolution in the normal mouse skin. Epidermal nerves were found only underneath the keratinocyte tight junctions in the normal mouse and human skin. In contrast, the pruritic models showed that epidermal nerve dysfunction is related to human atopic dermatitis samples, nerves appeared unprotected in the upper epidermal region with impaired tight junctions. In the pruritic dermatitis models, sustained Ca2+ increases were observed in nerves in the upper epidermal region independently of scratching, and depletion of epidermis-innervating neurons abolished pruritus. These data suggest that epidermal nerves dynamically maintain their positioning below the tight junctions, and that their activation is linked to the pruritus development caused by epidermal barrier dysfunction.

Capturing the role of epithelial-immune interactions to maintain tissue homeostasis
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The skin is an essential barrier that protects organisms from the external environment. Despite recent advances in understanding of skin diseases, the nature of epithelial and immune cell interactions in the skin and the role of immune cells in the maintenance of skin homeostasis remain largely unexplored. Pertinent knowledge of these interactions is critical for the treatment of skin diseases, including autoinflammatory and infectious skin diseases. We investigated the role of immune cell subsets within the skin in maintaining tissue homeostasis.

Imaging of the epithelial nerve dynamics and activity in the normal and pruritic dermatitis conditions
S. Takahashi1, T. Aihara1, H. Kawakami2, A. Kudo3, M. Amagai4 and T. Okada1 1 Grad School of Med Life Sci, Yokohama City Univ / RIKEN-IMS, Yokohama, Kanagawa, Japan, 2 RIKEN-IMS / Dept of Dermatol, Keio Univ School of Med, Yokohama, Kanagawa, Japan, 3 Dept of Dermatol, Keio Univ School of Med, Sinyoku, Tokyo, Japan, 4 Dept of Dermatol, Keio Univ School of Med / RIKEN-IMS, Sinyoku, Tokyo, Japan and 5 RIKEN-IMS / Grad School of Med Life Sci, Yokohama City, Kanagawa, Japan
Epidermal nerves detect stimuli that evoke pain and presumably itch. However, the epidermal nerve dynamics and activity have not been directly characterized. Here, by intravital imaging, we demonstrated that epidermal nerves were constantly extended and retracted, and occasionally underwent local dissolution in the normal mouse skin. Epidermal nerves were found only underneath the keratinocyte tight junctions in the normal mouse and human skin. In contrast, the pruritic models showed that epidermal nerve dysfunction is related to human atopic dermatitis samples, nerves appeared unprotected in the upper epidermal region with impaired tight junctions. In the pruritic dermatitis models, sustained Ca2+ increases were observed in nerves in the upper epidermal region independently of scratching, and depletion of epidermis-innervating neurons abolished pruritus. These data suggest that epidermal nerves dynamically maintain their positioning below the tight junctions, and that their activation is linked to the pruritus development caused by epidermal barrier dysfunction.
LRRC8A is essential for volume-regulated anion channel activity during hypotonic stress response in keratinocytes

ABSTRACT | Epidermal Structure and Barrier Function

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LRRC8A is essential for volume-regulated anion channel activity during hypotonic stress response in keratinocytes

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Mammalian cells regulate their cell volume not only to maintain their physiological function but also to counteract otherwise fatal osmotic stress. Although protected by the stratum corneum, underlying keratinocytes become a direct target to osmotic stress when the epidermal barrier function is disturbed. Upon exposure to hypotonic stress cells swell, which is counteracted by regulatory volume decrease (RVD). Volume-regulated anion channels (VRAC) are key players during RVD since they allow efflux of Cl\(^{-}\) into the extracellular space followed by osmotically driven water with the result that the initial cell volume is restored. Just recently, the essential VRAC component LRRC8A has been identified in different mammalian cell types. In our study, we investigated for the first time the expression and function of LRRC8A in keratinocytes in response to hypotonic stress. We revealed that LRRC8A is expressed in human epidermis as well as in primary keratinocytes NHK and HaCaT cells. We could show that hypotonic stimulation increased intracellular Ca\(^{2+}\) concentration. Importantly, VRAC activity and volume regulation did not require Ca\(^{2+}\) abnormalities, although VRAC activity was increased by extracellular Ca\(^{2+}\). In summary, our study demonstrates that LRRC8A is an important player during hypotonic stress response also in human keratinocytes. Since disorders related to impaired epidermal barrier function can be aggravated by osmotic stress our findings provide a starting point to evaluate LRRC8A as a novel target to milder detrimental osmotic effects.

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Expression and localization of the epidermal perturbing enzyme: Plasminogen

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The vegetable oils are commonly used as emollients in cosmetic formulations due to its moisturizing effect of cosmetic creams containing acefylline to correct hydration, and enhanced hexagonal or orthorhombic lateral-packing organization. Those modifications of lipid packing facilitate the moisturizing effect of acefylline. In this study, we investigated the effect of cosmetic creams containing acefylline on lipid neo-synthesis and on lamellar lipid organization. The vegetable oils are commonly used as emollients in cosmetic formulations due to its moisturizing effect. Those modifications of lipid packing facilitate the moisturizing effect of acefylline. In this study, we investigated the effect of cosmetic creams containing acefylline on lipid neo-synthesis and on lamellar lipid organization.

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Biological functionality and chemical properties of Brazilian vegetable oils by transcriptomic and metabolomic analysis

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The vegetable oils are commonly used as emollients in cosmetic formulations due to its moisturizing effect. Those modifications of lipid packing facilitate the moisturizing effect of acefylline. In this study, we investigated the effect of cosmetic creams containing acefylline on lipid neo-synthesis and on lamellar lipid organization. The vegetable oils are commonly used as emollients in cosmetic formulations due to its moisturizing effect. Those modifications of lipid packing facilitate the moisturizing effect of acefylline. In this study, we investigated the effect of cosmetic creams containing acefylline on lipid neo-synthesis and on lamellar lipid organization. The vegetable oils are commonly used as emollients in cosmetic formulations due to its moisturizing effect. Those modifications of lipid packing facilitate the moisturizing effect of acefylline. In this study, we investigated the effect of cosmetic creams containing acefylline on lipid neo-synthesis and on lamellar lipid organization. The vegetable oils are commonly used as emollients in cosmetic formulations due to its moisturizing effect.
26) was determined at 2.0 Å resolution. The physicochemical properties of the stratum corneum (SC) assures skin barrier integrity and can be affected by surfactants-containing cleansers by modifying skin pH and components. The aim of this study was to assess the effect of cleansers on the surface of human skin by confocal laser scanning microscopy (CLSM) in the fluorescence mode, by monitoring the labelling of acidic orange dye through the SC. First, we evaluated the application of SDS solutions (0.1-0.5-1-5%). We then compared 0.5% SDS solution with a body shower cleanser (product B) and SDS or test products diluted with acidic clorhydrate dye were applied on the surface of skin biopsies. After washes, CLSM imaging was performed with the Venuscope®2500 CLSM imaging software. For quantification of dye penetration, the stack projections were analyzed using ImageJ and internal Octopus softwares. The experiments were repeated on n=1 donors. Untreated skin biopsies exhibited a low level of dye penetration and this was expected for a normal, unmodified skin barrier. In contrast, we observed a significant, dose-dependent effect, increase of dye penetration through SC exposed to SDS (x1, x2, x4, x8, and x16), revealing alterations of the skin barrier. Comparison of test products showed that 0.5% SDS and product A induced a significant increase of dye penetration (x13.3 and x6, respectively) while no significant modification was found for product B, demonstrating that emollient shower oil containing Rhealba® oat Plantlet extract preserved SC and skin barrier. Our data showed that fluorescence dye penetration monitored with Venuscope®2500 CLSM imaging is a promising method to evaluate the effect of cosmetic products on skin barrier.

We previously determined the x-ray crystal structure of the wild-type keratin 1/10 helix 2B heterodimer at 3 Å resolution. The resolution of the structure was limited due to crystal packing effects from keratin 1/10 residue Cys401. Cys401Glu formed a disulfide linkage with Cys401 from another K1/10 heterodimer, creating an “X-shaped” structure. The disulfide-linked heterodimers formed a loose crystal packing arrangement, negatively impacting the achievable x-ray resolution. We hypothesized that mutation of Cys401Glu to alanine, which eliminates the disulfide linkage, would improve crystal packing and increase the structure resolution thereby providing a more accurate side chain electron density map. Indeed, when a Cys401Ala mutant was paired with its native keratin 1 (K1) heterodimer partner its x-ray crystal structure was determined to 2.0 Å resolution. The 2.0 Å resolution structure did not contain a disulfide linkage. Superposition of the wild-type K1/10 2B heterodimer structure onto the K1/10(Cys401Ala)2B 2B mutant structure has a root-mean-square-deviation (RMSD) of 1.62 Å; the variability in the atomic positions reflects the dynamic motion expected in this filamentous coiled-coil complexes. The electrostatic, hydrophobic, and contour features of the molecular surface are similar to the lower resolution wild-type structure. We postulated that elimination of the disulfide linkage in the K1/10(Cys401Ala)2B 2B mutant heterodimer structure could allow for the 2B heterodimers to bind/pack in the A22 molecular configuration for keratin intermediate filament assembly. Analysis of the crystal packing revealed an anti-paralleler tetrameric complexes of 2B heterodimers, however, their register is not consistent with models of the A22 molecular configuration. This must be considered in parallel biological and biochemical studies.

Cleansers provide the benefit of removing dirt and oil from skin, however, interaction between cleansers and stratum corneum may cause irritation. We previously demonstrated in epidermal equivalents, that decreased trans-epithelial electrical resistance (TEER) and increased cytokine release correlated with skin barrier damage and irritation potential induced by cleansers. The objective of this study was to correlate the barrier defects and cytokine production induced by in vitro and in vivo barrier and irritation impacts observed in clinical testing. Commercial cleansers with varying degrees of irritation potential were evaluated for their effects on skin barrier and irritation in exaggerated arm wash clinical studies and in human epidermal skin equivalent tissues in vitro. The correlation of in vitro TEER and interleukin 1α (IL-1α) changes with in vivo trans epidermal water loss (TEWL), dryness and erythema changes induced by the cleansers, were examined. A commercial harsh cleanser showing more dryness, erythema and higher TEWL in vivo, showed increased IL-1α release in vitro and decreased TEER in vitro. The findings suggest that the absence of a gentle daily cleanser foaming formula, were mild cleansers, producing less erythema and lower TEWL in vivo, and reduced inflammation and less barrier disruption in vitro. Additional cleansers were tested in both models, resulting in good correlation between TEER and IL-1α in vitro, and TEWL and visual grading in vivo. These results confirmed the predictive outcome and relationship between this in vitro model and in vivo clinical study results. Thus, this in vitro model can be used to determine skin barrier barrier, decreasing the speed and capacity of our evaluation process, and minimizing the need for clinical testing.

Development of a do-it-yourself skin equivalent system for use as a research tool
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Use of 3D skin tissue models can be advantageous over monolayer culture for evaluating the potential toxicity of materials that come in contact with skin. Despite the existence of commercially available tissue models, these models may not always be suitable for a specific research focus. Developing the technology to produce skin equivalents without prior expertise can also be a timely and costly undertaking. Here we demonstrate the construction of a three-dimensional human skin equivalent provided as a do-it-yourself kit for use in research laboratories. All components necessary to build the skin equivalent are provided in the 3D skin kit (MatTek, Ashland, MA), including keratinocytes, pre-cultured tissue culture inserts on which to produce the tissue and pre-formulated media. Our results demonstrate a novice user can successfully produce a 3D skin equivalent using the the 3D skin kit that is comparable to a commercially available human skin equivalent (Epiderm, LabCorp) and using normal neonatal keratinocytes. Skin equivalents produced with the 3D skin kit exhibited similar cytokine release profiles compared to the Epiderm kit at 12 hours. The availability of an easily user flexibly make MatTek 3D skin kit a powerful research tool for investigating multiple aspects of skin biology.
Effects of ammonia vapor exposure on viability in a human skin tissue model

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In this study, EpiderMFT, an in vitro 3D model of human skin, was used to evaluate the effects of ammonia vapor exposure in vitro. Prior to the experiment, the tissue was cultivated in a pressure-controlled, humidified incubator at a constant temperature of 37 °C. Following the exposure period of 6 h, the viability of the tissue was assessed using a colorimetric assay. The results showed a significant decrease in viability with increasing exposure concentrations, indicating that ammonia vapor can be harmful to the tissue. This study provides valuable insights into the effects of ammonia vapor exposure on human skin tissue in vitro, which is important for understanding the potential risks associated with ammonia exposure in industrial settings and other environments.

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Impact of essential fatty acids on the skin barrier function of tissue engineered skin substitutes

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Skin substitutes produced in vitro have a reduced permeability compared to normal human skin. This suboptimal barrier function may be caused by an alteration of the lipid metabolism such as the essential fatty acid (EFA) metabolism, which is a role of great interest in the organization of the stratum corneum. In fact, the synthesis of the ceramide containing a linoleate could be reduced by the essential fatty acid deficient growth media. In this study, we investigated the impact of linoleic acid (LA) and alpha-linoleic acid (ALA) in the formation of the skin barrier. To this end, healthy skin substitutes have been produced according to the self-assembly method of tissue engineering. Supplementation of the culture conditions with 10 μM of LA or 10 μM of ALA has been performed along with the appropriate controls. After a reconstruction of 63 days, the skin substitutes were analyzed using gas chromatography and percutaneous absorption techniques. The supplementation with both EFAs has no effect on skin morphology according to the macroscopic aspect and the histologic analysis. Both EFA supllementations have no effect on the epidermal thickness. Moreover, no inflammatory response is observed when these media are exposed or 24 hours post-exposure for cell viability using the MIT assay and for morphological changes indicative of toxicity. Data indicate that concentrations between 10-1000 ppm ammonia for up to 20 min did not have an immediate or delayed (24 h) effect on dermal tissue viability. Signs of toxicity, especially 24 h post-exposure were noted at 10,000 ppm ammonia and increased in severity with both increasing exposure time and concentration. To our knowledge, this is the first evaluation of antimicrobial factors and innate immunity in the skin substitutes, which together, we demonstrate the utility of the EpiderMFT model as a relevant substitute of human skin for evaluation of cytotoxicity in response to environmental insults.

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The effect of glucocorticoid receptor expression on the viability of tissue engineered skin substitutes

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In this study, we investigated the effect of glucocorticoid receptor (GCR) expression on the viability of tissue engineered skin substitutes. The tissue was cultured in vitro at the air-liquid interface and was exposed to either clean air or 1000 ppm ammonia for 24 hours. The viability of the tissue was assessed using a colorimetric assay. The results showed a significant decrease in viability with increasing exposure concentrations, indicating that ammonia vapor can be harmful to the tissue. This study provides valuable insights into the effects of ammonia vapor exposure on human skin tissue in vitro, which is important for understanding the potential risks associated with ammonia exposure in industrial settings and other environments.
Combination of retinaldehyde and hydroxyapatite mimics the effects of all-trans retinoic acid in reconstructed human epidermis

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Trans
Combination of retinaldehyde and hydroxylapatite mimics the effects of all-signatures of positive selection (by CMS scores) for EDC coding SNPs, we identified recent function is calibrated across the world. Using a population genetics approach to ascertain school of medicine, saint louis, mo and 2 Washington university school of medicine, St. Louis, MO, the expression of many genes encoded within the Epidermal Differentiation Complex locus is critical to skin barrier function, but we know very little about how skin barrier function is calibrated across the world. Using a population genetics approach to ascertain signatures of positive selection (by CMS scores) for EDC coding SNPs, we identified recent positive selection for an involucrin (IVL) haplotype specific to the HapMap CEU population. A direct and positive correlation (rho=0.95, r=0.89) was identified between the frequency of the IVL haplotype tagging SNP rs7545520 and northern latitude suggesting a selective advantage for the haplotype in northern regions. Linkage analysis and Genotype-Tissue Expression (GTEX) Project data revealed that the IVL CEU haplotype alone explains 11% of variation in expression that are recent and independent variants that explain reduced expression. Together, our genomic findings reveal population-specific calibrations of IVL expression that are recent and independent and further highlight the genetic innovation for skin barrier function as an adaptive trait.

Recent and independent emergences of population-specific enhancer eQTLs that modulate involucrin gene expression for human skin barrier calibration

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The expression of many genes encoded within the Epidermal Differentiation Complex locus is critical to skin barrier function, but we know very little about how skin barrier function is calibrated across the world. Using a population genetics approach to ascertain signatures of positive selection (by CMS scores) for EDC coding SNPs, we identified recent positive selection for an involucrin (IVL) haplotype specific to the HapMap CEU population. A direct and positive correlation (rho=0.95, r=0.89) was identified between the frequency of the IVL haplotype tagging SNP rs7545520 and northern latitude suggesting a selective advantage for the haplotype in northern regions. Linkage analysis and Genotype-Tissue Expression (GTEX) Project data revealed that the IVL CEU haplotype alone explains 11% of variation in expression that are recent and independent variants that explain reduced expression. Together, our genomic findings reveal population-specific calibrations of IVL expression that are recent and independent and further highlight the genetic innovation for skin barrier function as an adaptive trait.

Novel CYP26B1-selective inhibitor increases measures of epidermal barrier function in healthy, drier,’s, and ichthyotic reconstructed human epidermis

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The skin barrier, primarily by the stratum granulosum and stratum corneum of the epidermis, functions as our primary protection from dangerous pathogens, allergens, UV radiation, and mechanical injury, and plays a vital role in fluid and thermal regulation. This barrier is disrupted in several skin disorders ranging from atopic dermatitis, to rare genetic disorders, such as Darrier’s disease, ichthyosis. Current treatment options that are not adequately address patient needs, presenting significant efficacy or tolerability concerns. CYP26B1 is an isofrom of cytochrome P450 family 26 enzymes which are responsible for the elimination of all-trans retinoic acid (atRA) and retinoids. We have recently identified a novel trans-epidermal electrical resistance (TEER) characterizing variable expression of barrier and tight junction proteins and mRNAs, and observing morphological changes. In contrast, we observed barrier disrupting effects when RHEs were treated with all-trans retinoic acid or non-selective CYP26 inhibitors. These preliminary results may lead to a novel therapeutic strategy for rescuing skin barrier function deficiencies, as well as elucidate the mechanisms underlying skin barrier function.

The calcium sensing receptor regulates the calcium response to outside-in stimuli in live epidermis

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Our previous work has shown that keratinocyte differentiation, cell-to-cell adhesion, migration, epidermal barrier homeostasis, and wound healing processes. Barrier perturbation causes calcium mobilization from intracellular stores, resulting in a subsequent, transient elevation of cytosolic calcium levels in all epidermal layers. A key component of keratinocytes calcium signaling toolkit is the CaSR which transduces calcium signals by activating the store operated calcium entry and E-cadherin signaling pathways. We used genetic ablation to study the role of CaSR in signal transduction in live epidermis. K1cre/CaSr+/− and K14-cre mice were crossed with ROSA-GCaMP3 mice to create 1CaSR+/−/GCaMP3+ and 2CaSR+/−/GCaMP3+ mice expressing the cytosolic calcium sensor GCamp3. This system allowed us to use 2-photon excitation microscopy to visualize fast calcium dynamics in full thickness live epidermis. Dorsal skin biopsies were placed on an agar plate secured to a heated microscope stage. In both groups a correlation analysis was performed. In both groups the diffusion test revealed GAM of 0.3 pmol/cm2 sec. Rinsing the skin surface revealed an amount of 13.1 nmol/cm2 for GAM, while for AM 3.6 pmol/cm2 was calculated. There was a significant positive correlation between GAM and the TAM/AM rinsed from the skin surface (r=0.524, p=0.045). Tape stripping of the skin revealed an amount of TAM of 0.1 mmol/cm2, while for AM a concentration of 4 mmol/cm2 was calculated. There was no significant correlation (r=0.081, p=0.671). While the results from rinsing the skin sample might represent mainly the upper extracellular skin, the higher values due to tape stripping appear likely to represent a deeper, much more intracellular measurement. The significant correlation between GAM and TAM/AM rinsed from the skin surface indicates that the GAM trap in the diffusion test primarily assesses the extracellular compartments of the skin.

Metabolism analysis reveals an essential role for glucose in epidermal differentiation

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Advances in high throughput metabolomics enable discovery of new essential roles for metabolites in epidermal differentiation. A metabolome-wide analysis was performed during keratinocyte differentiating using 5 types of mass spectrometry that detect >14,000 analytes in all major metabolite classes. Unexpectedly, glucose was a top increased metabolite, which suggested a major role for glucose in epidermal differentiation. Functional studies in epidermal tissue showed that intracellular glucose elevation was required for induction of 224 of 386 (58%) terminal differentiation genes. Metabolites in glucose catabolic pathways were unchanged in differentiation, suggesting that accumulation of glucose itself was required. Consistent with this, decreasing cellular glucose levels, both by restricting available glucose as well as by increasing intracellular glucose catalyzing enzymes, HK2 and G6PD, blocked differentiation. Additionally, glucose restriction was rescued by non-metabolite insulin, suggesting a growth factor-driven increase in the expression of glucose. Pharmacologic inhibition studies revealed that 3 glucose transporters, GLUT1, GLUT3 and SGLT1, were essential for both glucose accumulation and epidermal differentiation. Glucose affinity chromatography followed by mass spectrometry identified the IRF6 transcription factor as a glucose binding protein in differentiating keratinocytes. IRF6 was essential for normal epidermal differentiation and was verified to bind glucose directly at high affinity. An IRF6 mutant found in both ectodermal dysplasia and cancer displayed diminished glucose binding. These data support a model in which epidermal differentiation is dependent on high affinity glucose transporters that enable accumulation of glucose, which in turns binds and enables IRF6-driven differentiation gene induction.
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Fundamental supply of cutaneous microcirculation in the Chinese Han population: Measurements by a full-field laser perfusion imager

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BACKGROUND: Cutaneous microvascular perfusion is believed to link with many diseases, and should be assessed. There are several papers on basal cutaneous microcirculation perfusion in different races, while the data in Chinese is vacant. OBJECTIVE: The aim was to establish the database of absolute fundamental supply of cutaneous microcirculation in the Chinese Han population. METHODS: With a full-field laser perfusion imaging (FFLP), the skin blood flow can be quantified. Cutaneous perfusion values were determined in 17 selected skin areas in 406 healthy participants aged between 20 and 80 years (mean 35.0±5.113). Essential parameters such as weight, height were also measured, and BMI were calculated and analyzed. The perfusion values were reported in Arbitrary Perfusion Units (APU). RESULTS: The highest cutaneous perfusion value fell on eyelid (931.20±242.59 in male and 967.83±225.49 in female), and prehilum had the lowest value (89.09±30.28 in male and 85.08±33.59 in female). The values were higher in men than women on the back of fingers, hand, cheek, neck, earlobe and lower in the forehead, nose, fingertips, changes, neck, earlobe. CONCLUSION: Cutaneous perfusion varies with skin regions. There is a tendency to measure higher perfusion values in men than in women. And the values are irrelevant with age or BMI.

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Noninvasive detection and analysis of ceramide in skin and blood in Chinese healthy population

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METHODS: The forearm cortical skin stratum corneum samples were obtained from 11 healthy subjects by a non-invasive method that cyanoacrylate adhesive method after signing the informed consent form; 4 blood samples were obtained by intravenous blood collection after written informed consent. Identification of ceramide in skin and blood samples is used by Ultimate/M1000 high performance liquid chromatography coupled with Q-Exactive TM quadrupole - electrostatic field orbital trap. Data was analyzed using full-flow lipid analysis software (Lipid Search) after collected. Results: Experiments were performed on 11 skin samples and 4 blood samples for ceramide characterization. Peaks can be seen in all skin samples with response intensities between 10^5 and 10^6, with peaks in some blood sample. Positive ions and negative ions were collected respectively for the samples. Except for the target ceramides reported in the literature, more than 300 other lipids were detected under positive ion conditions and more than 30 were detected under negative ion conditions, the results show that the ceramides in the skin samples are mainly long-chain ceramides and the blood samples are mainly short-chain ceramides. Conclusion: In this experiment, the qualitative analysis of ceramide in the samples can be carried out quickly and easily under the premise of maximizing the noninvasive and the differences in the distribution of ceramides in different tissues of the human body are demonstrated, indicating the importance of ceramide for skin barrier and the application prospect in the cosmetics industry.

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Truncated neurokinin 1 receptor (NK1R) is the predominant form of NK1R in human keratinocytes

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Neurokinin 1 receptor (NK1R) plays a key role in human pruritus, and antagonists of NK1R such as aprepitant and serlopitant are being developed as anti-pruritic agents. Recent data from our laboratory indicate that aprepitant may produce elements of its anti-pruritic activity via NK1Rs in keratinocytes. NK1R exists in two molecular forms: a full-length form that has 407 amino acids and a truncated spliced variant which ends at amino acid 311. Tissue distribution studies of the two forms of NK1R indicate that both are widely distributed throughout the body, including throughout the skin. However, the distribution of the two forms of NK1R in human keratinocytes has not been studied. In this study, we examined the expression of the full-length and truncated forms of NK1R in HaCaT human keratinocytes using quantitative PCR (qPCR). qPCR was performed using the TaqMan gene expression system. The TaqMan assay probe for full-length NK1R was available for a shelf item but the TaqMan probe for the truncated form was custom synthesized. Using qPCR, we find that the expression of truncated NK1R (Ct = 31) is much higher (>10-fold) than the full-length NK1R (Ct = 37), indicating that NK1R in human keratinocytes is predominantly of the truncated form. To determine whether a predominance of the truncated NK1R is seen in primary human keratinocytes, we repeated our analyses using primary keratinocytes from a 23-year-old female. In these cells, similar to HaCaT cells, truncated NK1R was the predominant form. The Ct value for full-length NK1R was 34 whereas the Ct value for truncated NK1R was 26, giving a >200-fold higher expression of the truncated variant. These data represent the first report in the literature that truncated NK1R is the predominant form of NK1R in human keratinocytes. Future studies will determine whether the distribution ratio of the two forms of NK1R in keratinocytes is associated with various dermatologic disease states.

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The polarity protein atypical kinase C controls desmosome organization and adhesive strength

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Desmosomes are adhesive complexes that anchor keratin filaments to the plasma membrane and critical for epidermal cohesion and differentiation. Interference with desmosomal components lead to a variety of autoimmune, inherited, and infectious skin diseases. How desmosomal protein composition and dynamics is controlled to enable upward migration of differentiating keratinocytes while maintaining epidermal integrity is largely unknown. Using an unbiased (phospho)-proteomics approach we now identify the polarity protein atypical kinase C (aPKC) as a central regulator of desmosomes. Loss of mammalian aPKC alters desmosomal protein expression and revealed a novel aPKC-dependent phosphorylation site on the phemphigus antigen Desmoglein 3 (Dsg3). Immunofluorescence analysis showed that aPKC+/− keratinocytes fail to enrich desmosomal components in linear junctional rings. Surprisingly, aPKC−/− keratinocyte sheets are more resistant to mechanical stress, and preliminary results suggest that aPKC-dependent phosphorylation of Dsg3 might control adhesive strength. We are now asking whether local activation of aPKC regulates layer dependent desmosome complexes formation and hence integrate adhesive strength and dynamics with epidermal stratification. In conclusion, our results identify aPKC as an important regulator of desmosomal adhesive strength.

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Ceramide synthase 4 controls epidermal lipid composition and barrier function

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Ceramide synthases (CerS) are key regulators of ceramides, which are central components of the epidermal lipid barrier. How epidermal lipid homeostasis is controlled and which ceramide synthases are essential for epidermal homeostasis is largely unknown. Previously, we showed that CerS4 controls hair follicle stem cell maintenance. By inactivating CerS4 in vivo, we now identify CerS4 as a key regulator of epidermal barrier maintenance but not formation. Large scale lipidomics and proteomics showed that loss of CerS4 interferes with epidermal lipid homeostasis resulting in an increase in the amount of key epidermal surface lipids, like cholesterol and glycosyl ceramides. Moreover, loss of CerS4 also increased filaggrin expression as well as processing, which together, lead to acanthosis and hyperkeratosis. Thus, whereas CerS3 controls epidermal barrier formation, CerS4 is central for skin barrier maintenance. In agreement, proteomics revealed a switch from CerS3 to predominantly CerS4 and 5 in adult epidermis. Together, our data show that an imbalance in ceramide synthases alters epidermal lipid homeostasis and drives structural, functional and pathological relevant skin barrier alternations, thus providing novel insight into lipid associated skin disorders.
Identification and molecular characterization of a CDC20 mutation in a novel mosaic variegated aneuploidy syndrome with premature aging phenotypes


The spindle assembly checkpoint (SAC) ensures proper chromosome segregation during mitosis. When deficient, it causes mosaic variegated aneuploidy (MVA), a chromosome anomaly characterized by mosaic random multiple aneuploidies. Defects in SAC can cause premature aging, which has been suspected in mouse studies, but remains elusive in humans. Here, we identified a de novo 46-8-year-old proband with MVA and premature aging phenotypes. The proband showed developmental delay without mental retardation in childhood. Premature aging phenotypes appeared from her 20s to 40s, including poikiloderma, subcutaneous fat loss, total hair loss, cataracts, renal failure, and total anemia with bone marrow hypoplasia. G-band analysis showed MVA in ~15% of her peripheral blood leukocytes and did not have microcephaly or the childhood cancers that typically appear in known MVA syndromes.

Whole-exome sequencing identified a missense mutation of the SAC gene CDC20 in the proband, but not in her parents, while no disease-causing mutations were found in the genes of premature aging or MVA syndromes. An immunoprecipitation assay revealed that the mutation significantly reduced the binding affinity of CDC20 to the N-terminus of BUBR1, an important regulator of SAC. The HCT116 cells in which the proband mutation was introduced via CRISPR/Cas9 showed SAC deficiency similar to the peripheral blood leukocytes of the proband. These results indicate that the mutation is pathogenic. Our findings indicate that CDC20 is a novel causative gene of MVA syndrome and suggest that SAC defects could cause premature aging in humans.

Expanding the phenotypic spectrum of junctional epidermolysis bullosa with respiratory and renal involvement (JEB-RR) with a novel TRPS1 mutation

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Junctional Epidermolysis Bullosa with Respiratory and Renal Involvement (JEB-RR) is a rare subtype of Epidermolysis Bullosa accompanied by severe interstitial lung disease and nephrotic syndrome. It is caused by bi-allelic mutations in the integrin alpha 3 (ITGA3) gene, which is a constituent of the basal membrane of skin, lungs, and kidneys. Eight total patients with JEB-RR have been described in the literature, all with lung and kidney disease with variable levels of skin involvement and early demise. Here we report a 17 year old male, who presented from childhood with prethial blistering, urethral strictures, localized hypotrichosis, and first toe dystrophy. He was hospitalized in our medical center due to severe bilateral interstitial pneumonia. Previous biopsies revealed subepidermal blistering, but Collagen 4 staining failed to detect the precise level of separation. Due to the unique clinical presentation, we performed whole exome sequencing, which revealed a homozygous c.821G>A in the TRPS1 gene, a mutation that is consistent with the clinical presentation. Clinical and histological findings confirmed the diagnosis of JEB-RR and the patient showed significant improvement with ITGA3 gene therapy.

Intravenous allogeneic mesenchymal stromal cell therapy in adults with recessive dystrophic epidermolysis bullosa is safe, improves quality of life and reduces itch

T Department of Dermatology, University of Leuven, Leuven, Belgium, 2 Division of Dermatology, King’s College London, London, England, United Kingdom, 3 St. John’s Institute of Dermatology, King’s College London, London, England, United Kingdom, 4 King’s College London, 5 The J. McGrath Institute for Genetic Disease, Gene Regulation, and Gene Therapy | ABSTRACTS

Recessive dystrophic epidermolysis bullosa (RDEB) is an inherited mechnonullous disorder associated with blisters, chronic wounds, scars, contractures, pain, and itch. The molecular basis of RDEB is a lack of basement membrane type VII collagen (COL7). Currently, no effective therapy exists. We conducted a phase I/II open label clinical trial giving two infusions of bone marrow-derived allogeneic mesenchymal stromal cell (MSCs), 2-4 x 10^6 cells/kg, 2 weeks in adults with RDEB with or without conditioning or no type. No serious adverse events were reported up to 12 months post-MSCs. Regarding efficacy, one individual showed a slight transient increase in C7 levels (days 28-60 only). However, clinical benefit of RDEB improved in 4 subjects with a decrease in disease activity at day 28 and day 60 post-MSCs compared to baseline for the BEBSS, EBDASI activity and the QOLB scores. Leuven Itch Score subscales of frequency, severity and consequences of itch showed a significant reduction at days 28 and 60 post MSCs. In serum, HMGBl levels reduced after MSCs at day 28 and 60 compared to baseline. RNA-seq data from both blood and skin revealed upregulated MAPK, Death receptor signaling and JAK stats pathways after cell therapy. This clinical trial demonstrates that MSC infusion is safe in adults with RDEB and can lead to clinical benefits that persist for at least 2 months. Our data also highlight HMGBl as a potential biomarker for cell therapy intervention and identify key signaling pathway changes that occur in response to MSC infusion.

Efficient reframed gene therapy for recessive dystrophic epidermolysis bullosa using CRISPR/Cas9

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Efficient reframed gene therapy for recessive dystrophic epidermolysis bullosa using CRISPR/Cas9 showed SAC deficiency similar to the peripheral blood leukocytes of the proband. These results indicate that the mutation is pathogenic. Our findings indicate that CDC20 is a novel causative gene of MVA syndrome and suggest that SAC defects could cause premature aging in humans.

Intravenous allogeneic mesenchymal stromal cell therapy in adults with recessive dystrophic epidermolysis bullosa is safe, improves quality of life and reduces itch

T Department of Dermatology, University of Leuven, Leuven, Belgium, 2 Division of Dermatology, King’s College London, London, England, United Kingdom, 3 St. John’s Institute of Dermatology, King’s College London, London, England, United Kingdom, 4 King’s College London, 5 The J. McGrath Institute for Genetic Disease, Gene Regulation, and Gene Therapy | ABSTRACTS

Recessive dystrophic epidermolysis bullosa (RDEB) is an inherited mechnonullous disorder associated with blisters, chronic wounds, scars, contractures, pain, and itch. The molecular basis of RDEB is a lack of basement membrane type VII collagen (COL7). Currently, no effective therapy exists. We conducted a phase I/II open label clinical trial giving two infusions of bone marrow-derived allogeneic mesenchymal stromal cell (MSCs), 2-4 x 10^6 cells/kg, 2 weeks in adults with RDEB with or without conditioning or no type. No serious adverse events were reported up to 12 months post-MSCs. Regarding efficacy, one individual showed a slight transient increase in C7 levels (days 28-60 only). However, clinical benefit of RDEB improved in 4 subjects with a decrease in disease activity at day 28 and day 60 post-MSCs compared to baseline for the BEBSS, EBDASI activity and the QOLB scores. Leuven Itch Score subscales of frequency, severity and consequences of itch showed a significant reduction at days 28 and 60 post MSCs. In serum, HMGBl levels reduced after MSCs at day 28 and 60 compared to baseline. RNA-seq data from both blood and skin revealed upregulated MAPK, Death receptor signaling and JAK stats pathways after cell therapy. This clinical trial demonstrates that MSC infusion is safe in adults with RDEB and can lead to clinical benefits that persist for at least 2 months. Our data also highlight HMGBl as a potential biomarker for cell therapy intervention and identify key signaling pathway changes that occur in response to MSC infusion.
Suprabasal acantholytic blisters in oral mucosa caused by homozygous nonsense mutation in desmoglein 3 gene

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Desmoglein 3 (Dsg3), encoded by DSG3 gene, is the major component of desmosomes which function to maintain integrity of epidermis. Dsg3 deficient mice develop ichthyosis and mucosa and hypotrichosis, caused by loss of cell-cell adhesion. Autoantibodies against human Dsg3, called as mucosal pemphigus vulgaris, leads to suprabasal acantholysis histologically and blister formation limited to the mucosa clinically. A patient with Dsg3 deficiency, however, has never been reported. A 1 year-old female baby presented with recurrent erosions in the oral and laryngeal mucosa after birth. Her conjunctival and genital mucosa is spared and her hair was normally growing. Histological examination of skin biopsy showed suprabasal acantholytic blisters, but direct and indirect immunofluorescences were negative. Whole genome sequencing from her DNA identified a novel homozygous nonsense mutation in desmoglein 3. Using direct sequencing and restriction fragment length polymorphism assays, we found that her parents harbor the heterozygous mutations in DSG3. Immunofluorescence in immunoblotting showed high levels of Dsg3 in keratinocytes from her skin as well as oral mucosa. Electron microscopy of her skin biopsy shows mature desmosomes in the basal layer. In conclusion, we describe a new hereditary disease featuring recurrent mucosal erosions caused by homozygous mutation in DSG3.

Reduced microbial diversity is a feature of recessive dystrophic epidermolysis bullosa wounds

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Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a rare heritable skin disease characterized among other things by non-healing wounds. Interestingly, while some wounds heal rapidly, others seem to be more reluctant to heal, becoming chronic and increasing the risk of infections, scarring and even skin cancer. Recent studies have shown the great importance of patient immune status and microbiome composition in EB and other skin diseases. However, their contribution in EB wound healing has not yet been explored. Moreover, murine studies suggest a direct link between microbial infection and both scar formation and wound induction. In this study, we performed deep transcriptome profiling of RDEB wounds and normal skin by shotgun metagenomic sequencing. We sampled 18 RDEB patients from 2 different continents (Chile and Austria), including their respective age/gender matched and household controls. Results showed a remarkably similar pattern of microbial diversity between patients and controls in intact forehead skin, with Propionibacterium as the predominant genus. Greater differences were observed when compared non-wounded with wounded skin in RDEB patients, differences that are maintained even across continents. Comparing the number of different genus identified revealed an overall trend for reduced diversity in RDEB wounds in comparison with RDEB unwounded skin. Similar to other chronic wound settings, Corynebacterium and Staphylococcus were the most abundant genera colonising RDEB wounds in both patient cohorts. To our knowledge this is the first example of culture-independent microbial profiling in any sub-type of EB and demonstrates a reduction in microbial diversity comparing RDEB patients with controls and comparing RDEB wounded with non-wounded skin.

HUNTING for genes that affect inflammatory skin disease in 4,071 cases and 40,430 controls

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We performed a genome-wide association study (GWAS) of 2,801 psoriasis cases, 1,270 atopic dermatitis cases and 40,430 controls from the Nord-Trøndelag Health Study (HUNT). Through genotyping and dense imputation mapping, we tested almost 15 million genetic variants across the genome. The first phase association analysis showed allele frequencies and effect sizes comparable to other European populations, which confirms high-quality phenotype and genotypes generated from HUNT. We will expand our analyses to refined phenotypes after linkage to individual-level information from regional- and national health registries, including diagnosis from primary and tertiary care, in addition to relevant medical information from the Norwegian Prescription Database. To enable investigations of synergies between DNA, RNA and proteins for psoriasis, we have collected skin biopsies from 50 psoriasis cases and 30 controls. In addition, we have developed a functional genomic assay for candidate genes from in silico gene candidates for downstream functional investigation and potential novel drug targets.

Japans initiative on rare and undiagnosed diseases patients: To bring their diagnostic odyssey to an end, and beyond

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We have taken measures for specially defined rare diseases, called Nan-Byo (difficult illness) in Japanese since 1972. While this governmental support has benefited Nan-Byo patients, those suffering from unidentified conditions do not fall into the scheme and cannot receive subsidies nor chances for consultation. To identify such rare and often undiagnosable diseases, we must integrate systematic diagnosis by medical experts through global data matching, thereby solving the NoF problem. Thus, AMED launched the Initiative on Rare and Undiagnosed Diseases (IRUD) to construct a holistic medical network utilizing the existing next-generation sequencing cores, and further expanded the consortium in the Japans universal healthcare system. To deal with the 34,000+ patients estimated by the survey for potential IRUD target, the outpatient-based diagnostic network has grown to include 34 Clinical Centers and 140+ hospitals to register 7,640 trio-samples, 35.2% of which was successfully diagnosed. This IRUD Registry, storing patients clinical-genetic data generated in a globally standardized format, has already matched 11 novel diseases and been working on 200 NoF cases, including various cutaneous disorders. For the remaining 60% of still-undiagnosed patients, the functional genomics approach (model organisms, patient specific heptal networks) is expected to model organism research network. Furthermore, AMED is aiming to advance the IRUD towards not only an improved diagnosis but also novel treatments by utilizing patient’s international collaboration. We believe in its significant contribution to the ongoing global endeavors, involving players in basic research, applied research, and societal implementation.
Filaggrin gene (FLG) promoter polymorphisms are associated with atopic dermatitis but not ichthyosis vulgaris in Japan
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Atopic dermatitis (AD) is an inflammatory and immunological skin disease. Mutations in the gene encoding filaggrin (FLG) were shown to be predisposing factors for AD in various populations. However, the rate of FLG mutations in AD is low in some populations. Studies have shown that patients without FLG mutations also have reduced filaggrin expression suggesting that other factors controlling filaggrin expression, including promoter polymorphism, might be important. The involvement of FLG promoter polymorphism in AD has not been determined. In this study, we performed genetic analysis of FLG promoter region in AD patients (n=124) and used array-based sequencing of FLG for a more comprehensive evaluation of the AD dataset. We assembled 43 laboratory variables into a database. We performed Kaplan Meier survival and hierarchical clustering. Seven of the 25 TTD patients with mutations in XPD died (median survival 15 yr) compared to only 1 death in the 15 TTD patients with mutations in TTDN1, 1 in GTF2E2, and 6 unknown. We gathered clinical information from NIH visits combining multiple cohorts, we can produce a reliable model and ensure accurate interpretations are made about the roles coheritabilities play in psoriasis.

Mucin type VII collagen distorts outcome in human skin graft mouse models
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Integrative copy number variation and filaggrin variation are associated with atopic dermatitis in African Americans
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Filaggrin (FLG) loss of function (LOF) mutations have been associated with increased prevalence and persistence of atopic dermatitis (AD). AD is more common in African-Americans (AA) than whites. In previous studies, FLG LOF mutations were rarely found in AA. Thus, we used array-based sequencing of FLG for a more comprehensive evaluation of FLG variation and to assess integrative copy number variation (CNV) within the 3’ portion of FLG exon 3 and 4. Overall, we identified seven novel CNVs. The MVF for any CNV was 0.006 but not IV (P=0.472) compared with normal controls. Allele frequency was not different. To determine transcriptional differences between the genotypes, we performed FLG promoter activity assay in keratinocytes and found that the G allele has reduced promoter activity (P=0.05). Together, our study suggested that FLG promoter polymorphisms are significantly associated with AD and may explain differences in FLG expression in AD.

Segmental odonto-maxillary dysplasia is caused by mosaic variants in the gene encoding beta-actin
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Segmental odontomaxillary dysplasia (SOD) is a rare sporadic disorder of unknown aetiology affecting the face, presenting at birth or in early childhood. Affected individuals have progressive hypodontia, reduced or decreased pigmentation, erythema, asymmetry, dental anomalies and commissural lip tissues. Here we present detailed phenotypic, histological and radiological data from a cohort of eight patients, and investigate the genetic basis of the disorder. We hypothesised that this condition could be due to a post-zygotic mutation in utero. Sequencing of DNA extracted directly from paired samples of affected skin and blood demonstrated variants in the gene encoding beta-actin (ACTB) that mosaic in affected tissue but undetectable in blood. Beta-actin variants have previously been discovered as the cause of a proportion of Beckers naevi. We propose that SOD should be included under the spectrum of Beckers naevus syndrome, and highlight it as a diagnosis frequently missed in paediatric dermatology.

Genetic Disease, Gene Regulation, and Gene Therapy | ABSTRACTS
Gentamicin induces premature termination codon readthrough and restores laminin 332 in junctional epidermolysis bullosa harboring nonsense mutations

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Herlitz junctional epidermolysis bullosa (H-JEB) is an incurable, devastating, often fatal, inherited skin disease. H-JEB is caused by loss-of-function mutations in LAMC1, LAMB3 and LAMC2, leading to complete loss of laminin 332, the major component of anchoring filaments which mediate epidermo-dermal adherence. LAMB3 mutations occur in 80% of H-JEB patients. About 95% of these mutations are nonsense and lead to premature termination codons (PTC). In this study, we evaluated the ability of gentamicin to induce nonsense mutation readthrough in 293T cells and H-JEB laminin β1-null keratinocytes transfected with expression vectors encoding 8 different LAMB3 nonsense mutations. We found that gentamicin induced readthrough and expression of full-length laminin β1 in all 8 nonsense mutations tested. We next used lentiviral vectors to generate stably transfected H-JEB cells harboring the R635X and C290X hotspot nonsense mutations. Treatment of these cells with various concentrations of gentamicin resulted in synthesis and secretion of full-length laminin harboring the R635X and C290X hotspot nonsense mutations. Treatment of these cells with gentamicin demonstrated that gentamicin can suppress PTC mutations and restore laminin 332 expression and function in H-JEB cells with nonsense mutations. We conclude that gentamicin may offer a readily available, novel therapy for H-JEB and other inherited skin diseases caused by PTC mutations.

Use of hierarchical clustering and principal component analysis for deep phenotyping of patients with mutations in XPD (ERCC2): trichothiodystrophy (TTD), xeroderma pigmentosum (XP) and PTX/TDT

M. Levon, P. Hanonz, D. Tamura, E. Helfer, S. Khar, M. Schobeh-Knudsen, J. N. Okwara and K. H. Kramer, 1 Wayne State University, 2 LCBG, NCI, Bethesda, MD, 3 LCBG, NCI, Bethesda, MD, 4 H1, NIH, Baltimore, MD, 5 Trichothiodystrophy (TTD) and xeroderma pigmentosum (XP) are rare autosomal recessive diseases with mutations in the XPD (ERCC2) gene, a DNA repair/transcription helicase. TTD and XP are characterized by multisystem clinical abnormalities and decreased survival yet have different phenotypic features. XPD/TTD overlap patients have features of both diseases. We followed a cohort of 68 individuals with defects in the XPD gene (24 TTD, 11 XP and 34 TTX/TDT patients) from 1971 to 2017. We gathered clinical information from multidisciplinary NIH vitiligo. NIH electronic health record systems and outside medical records of each patient, we assembled 258 clinical and laboratory variables into a database. We used Kaplan Meier survival curves, hierarchical clustering and principal component analysis to determine the primary features that distinguish each disease. We found that median survival of the TTD patients (15 yr, 9 deaths) was significantly lower (p < 0.0001) than that of the XP (46 yr, 8 deaths) and XPD/TTD (60 yr, 2 deaths) patients. Among the TTD patients, poor prognostic features (Hazard Ratio > 10) include low birth weight, neonatal colloidion membrane, macular, cryptorchidism, short stature, low weight, ataxia, peripheral neuropathy and not toilet trained by 2 yr. Deep phenotyping and principal component analysis can help identify important phenotypic differences that may improve clinical diagnosis, predict disease progression, and guide treatment for our patients and other patients with rare disorders.

Mosaic RAS/MAKP variants cause sporadic vascular malformations which respond to targeted therapy

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Vascular malformations (VMs) are comprised of abnormal connections of arteries, veins, lymphatics, and capillaries. VMs can occur in any anatomic location and may be sporadic or inherited. VMs can be simple fibrofatty or highly complex chimeric malformations with arteriovenous fistulas, with both structural and functional components. VMs arise from developmental dysregulation, whether genetic or nongenetic, and are composed of abnormal structures and behave in an abnormal fashion. VMs are highly heterogenous and have been divided into capillary, venous, arteriovenous and lymphatic. VMs are managed on an individual basis considering lesion location, composition, symptoms and patient preference. We report a kindred with mosaic RAS/MAKP null mutations, who presented with sporadic intracranial and extracranial VMs in which known genetic causes had been excluded. Each patient in this kindred had at least one VM, which lead to disfigurement, overgrowth, stroke, pain and/or life threatening bleeding. Our hypothesis was that mosaic RAS/MAKP null mutations lead to dysregulated cAMP signaling and dermal connective tissues and may be a useful molecular target for alleviating the skin aging and skin cancer.

Simultaneous gene-editing and reprogramming of epidermolysis bullosa simplex fibroblasts

V. Linclau, J. D. Tamura, K. T. Van Den Broeck, K. C. Graf, K. C. Butterfield, D. R. Roop, G. Bilosousova and I. Kuypet, 1 Gates Center for Regenerative Medicine, University of Colorado, Aurora, CO

Epidermolysis bullosa simplex (EBS) is an autosomal dominant skin fragility disorder caused by mutations in the keratin (K) 5 or K14. Although current therapy for EBS is limited to wound care, advances in reprogramming somatic cells into induced pluripotent stem cells (iPSCs) offer the possibility of developing new approaches for EBS treatment. The iPSC-based therapeutic approach for EBS relies on the generation of patient-specific iPSCs, which then undergo genetic editing and differentiation into skin cells suitable for transplantation. One of the main hurdles in advancing the iPSC-based therapy into the clinic is the complexity of a multi-step manufacturing process to produce genetically corrected iPSC-derived epithelial sheets. To reduce the manufacturing complexity of an iPSC-based therapy for EBS, we have combined our previously presented high-efficiency RNA-based reprogramming approach with a specific CRISPR/Cas9-mediated knock-out of the mutant allele of the K14 gene into a one-step procedure. We established a fibroblast line from an EBS patient carrying a hot-spot exon 25 mutation C273T at codon 125 in K14 and successfully reprogrammed it to multiple isogenic EBS iPSCs lines with a specific knock-out of the mutant K14 allele (KO EBS iPSCs) within 4 weeks of initiating simultaneous gene editing and reprogramming. Simultaneous reprogramming and gene correction not only reduces the complexity of the iPSC-based therapy but also improves safety of the approach by avoiding lengthy cell culture periods, drug selection, and multiple sub-cloning steps. We are currently differentiating these KO EBS iPSCs into keratinocytes to determine if these gene edited iPSC-derived keratinocytes can be used for EBS therapy by employing a mouse xenograft model.

Simultaneous gene-editing and reprogramming of epidermolysis bullosa simplex fibroblasts

J. Cogan, 1 Y. Hou, 1 M. Hirsch, 1 M. Hao, 1 V. Alexeev, 2 and D. Woodley, 1

Epidermolysis bullosa simplex (EBS) is an autosomal dominant skin fragility disorder caused by mutations in the keratin (K) 5 or K14. Although current therapy for EBS is limited to wound care, advances in reprogramming somatic cells into induced pluripotent stem cells (iPSCs) offer the possibility of developing new approaches for EBS treatment. The iPSC-based therapeutic approach for EBS relies on the generation of patient-specific iPSCs, which then undergo genetic editing and differentiation into skin cells suitable for transplantation. One of the main hurdles in advancing the iPSC-based therapy into the clinic is the complexity of a multi-step manufacturing process to produce genetically corrected iPSC-derived epithelial sheets. To reduce the manufacturing complexity of an iPSC-based therapy for EBS, we have combined our previously presented high-efficiency RNA-based reprogramming approach with a specific CRISPR/Cas9-mediated knock-out of the mutant allele of the K14 gene into a one-step procedure. We established a fibroblast line from an EBS patient carrying a hot-spot exon 25 mutation C273T at codon 125 in K14 and successfully reprogrammed it to multiple isogenic EBS iPSCs lines with a specific knock-out of the mutant K14 allele (KO EBS iPSCs) within 4 weeks of initiating simultaneous gene editing and reprogramming. Simultaneous reprogramming and gene correction not only reduces the complexity of the iPSC-based therapy but also improves safety of the approach by avoiding lengthy cell culture periods, drug selection, and multiple sub-cloning steps. We are currently differentiating these KO EBS iPSCs into keratinocytes to determine if these gene edited iPSC-derived keratinocytes can be used for EBS therapy by employing a mouse xenograft model.
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**MiRNA signature in atopic dermatitis**

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**ABSTRACT**

The skin is a major site for the surveillance and response to external insults such as allergens and pathogens. While some miRNAs are expressed in the skin, the role of miRNA expression in atopic dermatitis (AD) is unknown. Here, we present miRNA expression profiles in PBMCs and plasma of both children with AD and healthy controls. Supervised analysis of miRNA expression, validated by qRT-PCR, identified miR-451a and miR-142-5p as AD signatures. These results provide the first demonstration, to our knowledge, of direct in vivo gene expression by KB103, producing full length trimeric C7 by IDIF and immunoblot, and efficient delivery in the dermis of the EDS patients, while as expected, there was no noticeable impairment in patients. It has been shown to play a critical role in oncogenesis and in erythroid lineage differentiation, however, its function is skin biology is unknown. Altered expression of miR-223 and miR-451a was confirmed in a larger number of samples using miRNA qRT-PCR. Then, we analyzed plasma samples from AD and healthy babies to identify differentially expressed miRNAs circulating in blood. Among four differentially expressed miRNAs, circulating miR-451a was found again to be most significantly downregulated in patients. In summary, we investigated miRNA profile in PBMCs and plasma of AD children at first year of life and identified for the first time that miR-451a is associated with atopic dermatitis in children.

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**Successful in vivo COL7A1 gene delivery and correction of recessive dystrophic epidermolysis bullosa (RDEB) skin using an off the shelf HSV-1 vector (KB103)**

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**ABSTRACT**

RDEB is a devastating genetic blistering disorder caused by null mutations of COL7A1, the gene coding for collagen VII (C7). As an alternative to currently studied ex vivo gene replacement or bone marrow replacement therapies for RDEB, which require highly specialized facilities and considerable expense, we developed an off the shelf therapy consisting of a modified replication-deficient HSV-1 vector (KB103) directing COL7A1 expression for direct in vivo administration to RDEB skin either by intradermal injection or by topical administration. Primary RDEB patient keratinocytes and fibroblasts showed efficient transduction (by KB103), producing full length trimeric C7 by IDIF and immunoblot, and efficient C7 deposition at the dermal-epidermal junction in organotypic culture. Linear basement membrane zone expression of full length human C7 was noted by IFD in skin of C7 deficient RDEB mice following either intradermal injection to intact skin, or topical application to wounded skin. IDIF and PCR studies showed increased C7 expression in vivo following reaplication without increased inflammation or other adverse events. Clinical grade KB103 is currently under GMP manufacture and will undergo release testing and characterization for optimal formulation. The feasibility and safety in animal studies needed to support phase-I clinical trial have been completed with no KB103 related adverse event reported. In total these results provide the first demonstration, to our knowledge, of direct in vivo gene replacement therapy to the skin and implicate KB103 as a potentially convenient and widely applicable method for in vivo COL7A1 gene therapy for RDEB patients.

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**Topically-delivered gene suppressing noncontract targets IL-17RA for psoriasis**

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**ABSTRACT**

Activated interleukin (IL)-23/Th17 signaling is critical for psoriasis pathogenesis, and systemic IL-17 inhibitors are highly effective therapy. However, most patients have mild to moderate disease and no targeted topical therapeutic option. We developed a topically-delivered and highly efficient dermal targeting bacterial ART which regulates noncontract, comprised of antisense DNA or siRNA in a dense spherical configuration. These spherical nucleic acids or SNAs penetrate the skin barrier to knock down cutaneous gene targets. We generated anti-human and anti-mouse liposomal DNA SNAs targeting the IL-17RA receptor (ILRA) and tested their efficacy in reducing IL-17RA and improving markers of psoriasis in human 3D and imiquimod-induced mouse models of psoriasis. SNAs reduced human keratinocyte IL-17RA expression by 83% versus scrambled SNA, and TNF, and NF-kB, phosphorylated markers of IL-17RA SNA and IFNB4, TNF, and PI3 kinase pathway by 52%; p<0.001). Immobilizing and ELISA (NC16A, BP230 and Col7) were performed on patients’ biopsies and serum samples. We found that 25/37 patients had at least one serologic test positive (21) or doubtful (4) with 12 patients demonstrating ELISA positivity to more than one protein. However, only 3/37 patients 2 with RDEB and 1 with EB and IGA against BP230/COL7 met minimal diagnostic criteria for pemphigoid of positive DIF or IF on salt split skin (SSS). Furthermore, only one of these three had both positive and his revertant patch never blistered, suggesting that these antibodies were non-pathogenic. Wide presence of positive serological tests for antibodies to the BMZ in EB might be an effect of chronic wounds, similar to physical aging as studied in the elderly population. The clinical relevance of autoantibodies in EB is disputable, especially those detected by ELISA or immunoblot, that do not bind back to human control skin by means of IF on SSS.

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**Ex-vivo nonlinear microscopy imaging of Ehlers-Danlos syndrome-affected skin**

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**ABSTRACT**

Ehlers-Danlos syndrome (EDS) is the name for a heterogeneous group of rare genetic connective tissue disorders with an overall incidence of 1 in 5000. Nonlinear microscopy techniques can be utilized for non-invasive in vivo label-free imaging of the skin. Among these techniques, two-photon absorption fluorescence (TPF) can visualize endogenous fluorophores, such as elastin, while the morphology of collagen fibers can be assessed by second-harmonic generation (SHG) microscopy. In our present work, we performed TPE and SHG microscopy imaging on ex vivo skin samples of classical EDS and vascular EDS patients and healthy controls. We detected irregular, loosely dispersed collagen fibers in a non-parallel arrangement in the dermis of the EDS patients, while as expected, there was no noticeable impairment in the elastic content. In vivo nonlinear microscopic imaging could be utilized for the non-invasive diagnostic testing of EDS and the assessment of the skin status of EDS patients in the future.
763 Genome-wide association study and comprehensive HLA analysis of pemphigus
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Pemphigus is an autoimmune blistering disease, and pemphigus vulgaris (PV) and foliaceus (PF) are the two clinical subtypes. The development of HLA molecules has been suggested, but specific HLA risk variants as well as novel risk variants outside the MHC region remain to be discovered. We performed a two-stage genome-wide association study of pemphigus in the Chinese Han population in 166 patients and 844 healthy controls with a follow-up analysis in an additional 1105 patients and 996 healthy controls. Further, a comprehensive association analysis of the MHC region was performed by HLA imputation and further validation by next-generation sequencing and genotyping. Novel associations with PV were discovered on 12q24.33, located within RAN and STX11 (P = 1.3 × 10^-6) and with PF on 1p10 (P = 6.9 × 10^-7), respectively. The association patterns of RAN-R114* alleles are different between two subtypes. Discovery of these risk loci has advanced our understanding of the genetic basis of pemphigus susceptibility, and provided opportunities for risk prediction and preventive treatment for pemphigus, in particular for PV.

764 miR-146a and miR-146b regulate members of the IL-1β family in human primary keratinocytes
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Pemphigus and atopic dermatitis (AD) are chronic inflammatory skin diseases, which pathogenesis is not fully understood. miR-146a and miR-146b (miR-146ab) have been shown to inhibit inflammatory responses of keratinocytes in pemphigus and AD. The interleukin (IL)-1 cytokine family has both pro- and anti-inflammatory members. We analyzed the expression of IL-1 family genes in the skin of pemphigus and AD patients and studied their regulation by miRNAs. miR-146ab targeted members of the IL-1β family in human primary keratinocytes. We showed that TP63-mediated expression of members of the IL-1 family changes in the skin of pemphigus and AD patients as compared to healthy controls and that these cytokines are also differentially expressed between the two diseases. To study which cytokines might affect the expression of these genes in the skin during inflammation, we stimulated keratinocytes with a set of pro-inflammatory cytokines known to affect skin responses in pemphigus and AD. We identified a robust upregulation of IL-1α, IL-1β, IL-18, IL-36α, IL-33, IL-37 and IL-38 in response to IFNγ and TNF-α. IL-36α, IL-36γ and IL-38α were upregulated by IFNγ, TNF-α and IL-17A. The transfection of miR-146a mimics reduced the levels of IL-1α, IL-1β, IL-33, IL-36α, IL-36γ, IL-36α and IL-38. Our data demonstrate that IL-1 family genes are differentially expressed between the two diseases and indicate that miR-146ab control the pathways that activate the expression of IL-1 family genes in keratinocytes.

765 Anti-inflammatory properties of a unique mixture of botanical extracts in in vitro models of psoriatic inflammation
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Psoriasis is a chronic inflammatory disease of the skin affecting 2-3% of the U.S. population. First-line therapies such as corticosteroids effectively reduce disease flares but have significant side effects. The purpose of this study was to evaluate a natural extract (CF) as a potential topical therapy for psoriasis. CF is a unique mixture of hydro-ethanolic extracts derived from 25 plant species known to contain anti-inflammatory compounds. To simulate psoriatic inflammation, primary human keratinocytes were treated with a combination of IL-17A and TNF-α, which are known to contribute to psoriasis pathogenesis. Co-administration of CF resulted in dose-dependent suppression of IL-6 release into the growth medium. CF suppressed IL-17ATNF-α-induced release of IL-8 by 70%, exceeding the suppression seen with clinically relevant concentrations of the potent topical corticosteroid, clobetasol propionate (5%). We performed microarray analysis and identified 20,000 differentially expressed probes in CF- and vehicle-treated keratinocytes in the presence of CF vs. vehicle. Notably, this gene expression signature was significantly associated with the inhibition of several psoriasis-relevant inflammatory and stress response pathways and was consistent with the anti-inflammatory gene expression signature of CF even under unstimulated conditions. Finally, we show in a human skin explant model of psoriasis that CF could suppress IL-17A production by 54% (3 donors, p<0.001). Together, these data suggest CF is a potent, non-steroidal anti-inflammatory capable of repressing both innate and adaptive immune responses associated with psoriasis.

766 The phenotypic spectrum of vascular anomalies associated with postzygotic mosaic variants in G-proteins
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Background: Vascular anomalies (VA) are a heterogeneous group of congenital malformations affecting the vascular system. They are usually classified according to the vessel affected, or clinical presentation. They result from non-random somatic mutations occurring in the germline. The phenotypic spectrum of vascular anomalies associated with postzygotic mosaic variants in G-proteins is not fully understood. The aim of this study was to investigate OSMR mutation spectrum of sporadic and familiar PLCA patients in the mainland of China, and to analyse any genotype-phenotype correlation. Methods: This study was carried out on 64 sporadic PLCA, 51 familial PLCA and 10 unaffected controls collected from 24 unrelated Chinese families. Genomic DNA was extracted from peripheral blood samples. Mutation screening was performed by sanger sequencing of 18 OSMR exons. Results: One patient was identified with a postzygotic mosaic mutation in OSMR in the heterozygous state. The silent mutation was novel and the patient showed significantly lower median age of onset (23.5 years) than the sporadic patients (32 years). Sequence analyses demonstrated OSMR missense mutation rate in familial PLCA Patients (65.7%) was significantly higher than that of sporadic patients (34.5%). Conclusion: The present data indicated OSMR mutation was not only a cause of familial PLCA, but also played an important role in the pathogenesis of sporadic PLCA, although we did not know the exact molecular mechanism. Meanwhile, the homozygous OSMR mutation appeared more frequently in female patients and in young patients with PLCA.

767 Mechanisms contributing to the skin phenotype of ectodermal dysplasia caused by TP63 mutations
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Mutations in the transcription factor TP63 underlie ankyloblepharon-ectodermal defects-leaf lipodystrophy syndrome (AEC), a form of ectodermal dysplasia. The molecular mechanisms underlying skin fragility and epidermal differentiation defects in these patients are not fully understood. Animal models of AEC did not fully recapitulate the AEC patient skin phenotype. We developed a human skin cell-based system that expresses mutant TP63 at physiological levels in a cellular background that is genetically susceptible to AEC. Skin fibroblasts from two AEC patients were reprogrammed into induced pluripotent stem cells (iPSC). Next, we corrected the TP63 mutations in these iPSC using Crispr/Cas9, thereby generating congenic cell lines that were identical except for the presence or absence of the TP63 mutation. The iPSC were differentiated into keratinocytes (iPSC-K) and subjected to a comparative transcriptome analysis. We identified defects in several desmosomal components in AEC iPSC-K. Exposure to calcium revealed abnormal desmosomal assembly and reduced cell adhesion in AEC iPSC-K. We also identified differentiation defects in AEC iPSC-K. These findings are consistent with immunofluorescence staining experiments demonstrating reduced and aberrant expression of desmosomal components and differentiation markers in AEC patient skin. In addition, our results provide evidence that two desmosome-linked signaling pathways (gama2 and p38) are disrupted in AEC. We found that TP63 mutations in the expression of OSMR mRNA and protein in affected skin and healthy controls. Conclusion: The present data indicated OSMR mutation was not only a cause of familial PLCA, but also played an important role in the pathogenesis of sporadic PLCA, although we did not know the exact molecular mechanism.
Filaggrin 2 deficiency causes generalized peeling of the skin
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Peeling skin syndromes form a large and heterogeneous group of inherited disorders characterized by superficial epidermal detachment, often associated with inflammatory features. We studied a consanguineous family featuring non-inflammatory peeling of the skin exacerbated by exposure to heat and mechanical stress. Whole exome sequencing revealed a homozygous nonsense mutation in FLG2, encoding filagrin 2, which co-segregated with the disease phenotype in the family. The mutation resulted in decreased FLG2 RNA and filagrin 2 protein in the patient’s epidermis. Filagrin 2 deficiency was shown to prevent epidermal differentiation in skin equivalents. Moreover, Filagrin 2 was found to co-localize with corneodesmosin which plays a crucial role in maintaining cell-cell adhesion in the uppermost epidermal layer. Absence of filagrin 2 in the human skin was associated with markedly decreased corneodesmosin expression. Accordingly, we showed that FLG2 down-regulation leads to reduced corneodesmosin expression and disrupts keratinocyte cell-cell adhesion in the dispaese dissociation assay. This effect was aggravated by temperature elevation, correlating the clinical phenotype. Taken together, the present data suggest that filagrin 2 is essential for normal differentiation and cell-cell adhesion in the cornified cell layers.

Histological characteristics and genetic study of bullous pemphigoid in Chinese han population
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Bullous pemphigoid (BP) is a potentially fatal subepidermal blistering autoimmune disease, which characteristically affects elderly patients who present with large tense bullae on the entire skin and frequently the extremities. Comparing to other bullous disorders, the genetic data of BP is sparse and inconsistent. Various association results were reported for BP in different studies, such as HLA-DQB1*06:01 (DRB1*11:01) and DRB1*04:03 with Caucasian BP, DQB1*03:02/DRB1*11:01/DRB1*04:03 with Japanese BP. Aiming to discover the HLA profile of Chinese BP patients and to analyze the histological characteristics of BP, we conducted a genetic association analysis study in a sample of 575 pemphigoid patients (collected from 2006-2017) and 976 healthy controls of Chinese descent. We firstly analyzed the basic profile of Chinese BP patients and to analyze the histological characteristics of BP, we conducted a genetic association analysis study in a sample of 575 pemphigoid patients (collected from 2006-2017) and 976 healthy controls of Chinese descent. We firstly analyzed the basic profile of Chinese BP patients and to analyze the histological characteristics of BP, we conducted a genetic association analysis study in a sample of 575 pemphigoid patients (collected from 2006-2017) and 976 healthy controls of Chinese descent. We firstly analyzed the basic profile of Chinese BP patients and to analyze the histological characteristics of BP, we conducted a genetic association analysis study in a sample of 575 pemphigoid patients (collected from 2006-2017) and 976 healthy controls of Chinese descent. We firstly analyzed the basic profile of Chinese BP patients and to analyze the histological characteristics of BP, we conducted a genetic association analysis study in a sample of 575 pemphigoid patients (collected from 2006-2017) and 976 healthy controls of Chinese descent. We firstly analyzed the basic profile of Chinese BP patients and to analyze the histological characteristics of BP, we conducted a genetic association analysis study in a sample of 575 pemphigoid patients (collected from 2006-2017) and 976 healthy controls of Chinese descent. We firstly analyzed the basic profile of Chinese BP patients and to analyze the histological characteristics of BP, we conducted a genetic association analysis study in a sample of 575 pemphigoid patients (collected from 2006-2017) and 976 healthy controls of Chinese descent.

Development of a zebrafish model for CARD14-associated human disorders
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CARD14 is prominently expressed in the epidermis and plays a central role in the activation of the NF-κappa B signaling pathway. Gain-of-function mutations in CARD14 have recently been shown to cause two papulo-squamous diseases: familial poikiloderma and phrygian rubber pilaris (PRP). To study CARD14 role in the pathogenesis of those diseases, we overexpressed human CARD14 in zebrafish, a system increasingly used to model human skin diseases. CARD14 overexpression induced marked morphological changes in the fish developing epidermis, including a granular surface, spiky pigmentation and foci of linear thickening. In situ hybridization demonstrated keratinocytes of uneven size and shape. Scanning electron microscopy revealed abnormal keratinocyte proliferation and surface microvilli. Developmentally, overexpression of CARD14 had a profound effect on anterior-posterior axis symmetry, noticed mainly at the caudal pole, leading to anomalies ranging from curved tails to complete absence of caudal development associated with asymmetric eye development. Quantitative RTPCR showed strikingly increased expression of NF-κappa B and some of its downstream targets, in CARD14-overexpressing embryos, compared to controls. Taken together, our data indicate that CARD14-overexpressing zebrafish represents a legitimate model for the study of CARD14-associated inflammatory dermatoses.
A new phenotype combining hidradenitis suppurativa with Dowling-Degos disease caused by a founder mutation in PSENEN

M. Pflumm1, A. Malmhagen-Schwarz1, N. Malchini1, R. Bochner2, A. Peled3, J. Mohammad4, T. Hillenmeyer1, A. Ga1, A. Hainer4 and E. Sprecher1 1 Tel Aviv Medical Center, Tel Aviv, Israel, 2 Tel Aviv Medical Center, Tel Aviv, Israel, 3 Tel Aviv Medical Center, Tel Aviv, Israel, 4 Tel Aviv Medical Center, Tel Aviv, Israel. Hidradenitis suppurativa (HS) is a chronic inflammatory disease of the hair follicle unit which can be caused by mutations in four genes (including PSENEN) encoding the $\gamma$-secretase complex. Both HS and DDD typically involve the flexural areas of the body. During the past 10 years, we encountered 4 patients who presented with clinical features consistent with both DDD and HS and aimed at identifying the genetic cause of coexisting DDD and HS. We initially excluded KRT14, POGLUT1 and POFGUT1 mutations in all 4 patients. We then screened PSENEN for pathogenic mutations using Sanger sequencing since this gene was recently reported to be associated with the double phenotype displayed by our patients. We identified in all 4 affected individuals an hitherto unreported heterozygous 168T>G, p.V65X mutation in PSENEN. In summary, our study confirms this novel mutation for the mutation in all 4 patients. Using qPCR and RNA extracted from patient keratinocytes, we observed a significant decrease in the expression of PSENEN as well as POFGUT1 which had previously been implicated in DDD. DDD as well as HS-associated genes have been shown to encode important regulators of Notch signaling. Accordingly, using a reporter assay, we demonstrated decreased Notch activity in patient's keratinocytes. The present data confirm the genetic basis of the combined DDD-HS phenotype and suggest that Notch signaling may play a central role in the pathogenesis of this rare condition.

SAM syndrome is characterized by extensive phenotypic heterogeneity

S. Tabiei1, M. Mohammad2, E. Cohen-Barak2, A. Ga1, E. Mamlouk1, O. Sarig1, S. Shahel3 and E. Sprecher1 1 Tel Aviv Medical Center, Tel Aviv, Israel, 2 Haemek Medical Center, HaZafon, Israel, 3 Haemek Medical Center, HaZafon, Israel, 4 Tel Aviv Medical Center, Tel Aviv, Israel. Desmogleins are trans-membranal proteins traditionally considered to play a critical role in the maintenance of cell-cell adhesion in the epidermis. Severe skin dermatitis, multiple alleles and metabolic wasting (SAM) syndrome is a rare and usually inherited condition caused by bi-allelic mutations in DSG1 encoding desmoglein 1. The disease was initially reported to manifest with life-threatening erythroderma, failure to thrive, atopic manifestations, recurrent infections, hypotrichosis and palmoplantar keratoderma. Very little is currently known about the phenotypic spectrum of this rare condition. Here we studied three new cases of SAM syndrome. Consistent with previous data, using whole exome and direct sequencing, SAM syndrome was found in all cases to result from homozygous mutations in DSG1. The characterized result in DSG1 and subsequent examination of transcript levels in control patients were found to display a variable range of cutaneous, extra-cutaneous and immunological abnormalities when compared to previous descriptions of the syndrome. Additionally, there was no correlation between the extent of skin involvement and the existence of allergic manifestations. The present data emphasizes the fact that SAM syndrome is characterized by extensive phenotypic heterogeneity, suggesting the existence of potent modifier traits.

The genetic landscape of the healthy human skin

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A unique mixture of botanical extracts attenuates keratinocyte inflammation associated with atopic dermatitis

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SMARCAD1 haploinsufficiency underlies Huriez Syndrome and associated skin cancer susceptibility

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We identified heterozygous splice site mutations in the skin-specific isoform of SMARCAD1 in patients with mosaic Huriez syndrome, autosomal dominant Huriez syndrome is characterized by scleroatrophy of the fingers and toes, palmoplantar keratoderma, and susceptibility to cutaneous squamous cell carcinoma. We identified heterozygous splice site mutations in the skin-specific isoform of SMARCAD1 to human skin cancer susceptibility. Functional analysis revealed complete loss of expression of the mutated allele in patient skin thus linking SMARCAD1 deficiency to human skin cancer susceptibility. Functional analysis revealed reduced proliferation, senescence and constitutive increase in DNA double-strand breaks in SMARCAD1 deficiency to human skin cancer susceptibility. Functional analysis revealed complete loss of expression of the mutated allele in patient skin thus linking SMARCAD1 deficiency to human skin cancer susceptibility.

The p63 - iRHOM2 signalling axis in the keratinocyte stress response

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We have identified inherited gain-of-function mutations in RHOM2 encoding (iRHOM2) associated with a palmar keratoderma and squamous oesophageal cancer syndrome (termed TOC). iRHOM2 is a key mediator of ADAM17 (TACE) enzymatic activity involved in shedding of ADAM17 substrates. Towards understanding the upstream regulator of iRHOM2, the sequence was analysed by bioinformatics software. A p63 binding site was found and binding was confirmed by utilising a ChIP-seq dataset in both human and mouse. We found that iRHOM2 is a novel target of p63 and both p63 and iRHOM2 can differentially regulate cellular stress associated signalling pathways. In normal keratinocytes, p63 positively regulates iRHOM2, while iRHOM2 antagonizes p63 expression. We identified two copy-number variations in patients with reduced keratinocyte hyperproliferation. In contrast, in hyperproliferative keratinocytes including palmar keratoderma, there is an auto-regulatory feedback loop occurring between p63 and iRHOM2. We demonstrate that this p63: iRHOM2 signaling axis regulates cell survival and oxidative stress via modulation of survivin and cytochrome c, respectively. Furthermore, the antioxidant compound Sulforaphane can downregulate p63: iRHOM2 expression, leading to reduced inflammation, cell survival and ROS production. Together these findings elucidate a novel p63 associated pathway that identifies iRHOM2 as a potential therapeutic target to treat hyperproliferative skin disease and neoplasia.
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Generation and characterization of a mutant rat with targeted ablation in
Samd9, the gene responsible for normophosphatemic familial tumoral
calcinosis
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In humans, loss-of-function mutations in the Sterile a Motif Domain Protein 9 (SAMD9) gene
are associated with normophosphatemic familial tumoral calcinosis (NFTC), a rare heritable
disorder characterized by progressive deposition of calcified masses in cutaneous and subcutaneous tissues upon trauma. The mechanisms of SAMD9 in the ectopic mineralization
process of skin remain unknown. Due to the lack of the corresponding gene in mouse, we
generated a Samd9 mutant rat using transcription activator-like effector nucleases (TALEN)
which target the 5 coding region of the rat Samd9 gene. The Samd9 mutant rat carrying a
c.67delC mutation is predicted to cause out-of-frame translation and a premature stop codon.
RT-PCR and immunofluorescent labeling for SAMD9 in the mutant rats was entirely negative,
confirming that the rats are Samd9 null. Similar to human NFTC patients with SAMD9 mutations, blood biochemistry did not reveal differences in serum calcium, phosphorus and
parathyroid hormone levels between wild type and Samd9 null rats. Histopathology of a
number of organs in 40-day old rats revealed significantly increased renal mineralization in
the Samd9 null as compared to wild type rats. In addition, early growth response 1 (EGR1), a
transcription factor with a known role in the regulation of inflammation and tissue mineralization, was found to be upregulated in the Samd9 null rats by immunofluorescent labeling.
The increased EGR1 expression in the absence of SAMD9 suggests that EGR1 can serves as a
molecular target for the treatment of NFTC in which skin inflammation precedes ectopic
mineralization.

Genetic modifiers for ectopic mineralization: The paradigm of PXE
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Pseudoxanthoma elasticum (PXE), a prototype of heritable ectopic mineralization disorders, is
caused by inactivating mutations in the ABCC6 gene encoding a putative efflux transporter
ABCC6. The phenotypic spectrum of PXE is highly variable with inter- and intra-familial
heterogeneity. There is mounting evidence suggesting the presence of predisposing genetic
factors, yet to be identified, that modify the PXE phenotype. Using inbred mouse strains with
the same mutant allele in the Abcc6 gene as novel models of PXE, yet with high degrees of
variability of mineralization phenotypes, we sought to identify genetic modifiers beyond the
PXE-causing gene Abcc6. We crossed the KK/HIJ strain displaying the most severe mineralization phenotype with either unaffected C57BL/6J mice that are wild type for the Abcc6
allele (KK x B6 cross) or with mildly affected DBA/2J mice carrying the same Abcc6 allelic
mutation with KK/HlJ (KK x D2 cross). The F1 progeny were backcrossed to the KK/HlJ
parental strain and N2 mice were generated. Genomic DNA was genotyped using the GigaMUGA SNP Array. The severity of tissue mineralization was based on a semi-quantitative
measurement of lesion size and severity by histopathology. Quantitative trait locus (QTL)
analysis revealed several chromosomal regions that regulate the phenotype severity in a
number of organs: Cardiac mineralization (Chr7, KK x B6 cross, LOD ¼ 10.5, p ¼ 0.0001;
Chr3, KK x D2 cross, LOD ¼ 3.37, p ¼ 0.038), lung mineralization (Chr7, KK x B6 cross, LOD
¼ 3.64, p ¼ 0.006), renal mineralization (Chr7, KK x B6 cross, LOD ¼ 7.37, p ¼ 0.0001;
Chr5, KK x B6 cross, LOD ¼ 4.12, p ¼ 0.006; Chr5, KK x D2 cross, LOD ¼ 5.67, p ¼ 0.0001),
and muzzle skin mineralization (Chr7, KK x B6 cross, LOD ¼ 15.67, p ¼ 0.0001). These QTLs
contain potential modifier genes that need to be refined by additional analysis.

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Identification of amino acid residues in ABCC6 important for substrate
interaction
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Pseudoxanthoma elasticum (PXE) is an autosomal recessive disease characterized by progressive ectopic calcification of the elastic fibres in the dermis. Inactivating mutations in the
gene encoding the hepatic efflux transporter ABCC6 underlie PXE. We have recently found
that ABCC6 is involved in the release of nucleoside triphosphates (NTPs), predominantly ATP,
from hepatocytes. Outside the hepatocytes, but still within the vasculature of the liver, the
ectonucleotidase ENPP1 converts released ATP into AMP and inorganic pyrophosphate (PPi),
a crucial inhibitor of soft tissue calcification. Lack of ABCC6-mediated hepatic ATP release
therefore results in severely reduced plasma PPi levels and explains the ectopic calcification
phenotype seen in PXE patients. This firmly links ABCC6 to the release of ATP from (liver)
cells. Being involved in ATP efflux is an unusual function for an ABC transporter. Most ABC
transporters use the energy released by intracellular ATP hydrolysis to transport specific
substrates across membranes, often against steep concentration gradients. ABCC6s closest
relative is ABCC1, an efflux pump with broad substrate selectivity. Recently, the structural
basis of substrate recognition by ABCC1 was elucidated. Based on the available molecular
structure of ABCC1 we have generated a molecular model of ABCC6 to identify amino acid
residues potentially important for substrate interaction. We subsequently evaluated in
HEK293 cells if mutating these amino acids affects cellular ATP release. The optimized
cellular real-time ATP efflux assay that we developed also provides an excellent tool to
determine the pathogenicity of ABCC6 mutations of unknown clinical significance.

Tsc2 haploinsufficiency accelerates tumor progression in a mouse model of
TSC skin tumors
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Individuals who are genetically mosaic tend to exhibit a less severe disease than those with a
germline mutation. In tumor syndromes such as tuberous sclerosis complex (TSC), this
reduced severity has been attributed to the lower likelihood of sustaining a 2nd-hit mutation in
tumor cells when the individual is comprised of wild-type and mutant cells (mosaic) rather
than homogeneous mutant, haploinsufficient cells (germline). Here we investigated whether
the microenvironment surrounding the tumor cells contributes to disease severity. We
compared tumor formation in our previously characterized Prrx1-cre-driven conditional Tsc2
knockout mice on a homozygous Tsc2 floxed (Tsc2cKOfl/fl) genomic background to Tsc2
conditional knockout mice on a haploinsufficient background (Tsc2cKOfl/-). Both developed a
thickened dermis, highly vascular tumors in the limbs and skin, and kidney cystadenomas. 8
week old Tsc2cKOfl/- mice showed a greater number of ventral skin vascular tumors measured
from histological sections than Tsc2cKOfl/fl mice (tumor area 13.3%  8.9% for Tsc2cKOfl/- vs
3.2%  2.3% for Tsc2cKOfl/fl, p¼0.03) and increased kidney cystadenomas (tumor area 34%
 20% for Tsc2cKOfl/- vs 8.3%  8.6% for Tsc2cKOfl/fl p¼0.01). Median survival was 20
weeks in Tsc2cKOfl/- and 28 weeks in Tsc2cKOfl/fl mice (p¼2.36E-07, Log-Rank test). Since
we had previously shown that the chemokine CCL2 plays a role in TSC tumorigenesis, we
used ELISA to measure serum levels in 8 week old mice. Serum levels of Ccl2 were 91  31
pg/mL for Tsc2cKOfl/- and 56  12 pg/mL for Tsc2cKOfl/fl mice (p¼0.043). Tsc2 haploinsufficiency through changes in the microenvironment may have a role in the greater
disease severity of individuals with germline mutations compared to mosaic individuals.

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Hairless mouse Hr mutation induces skin commensal bacterial changes and
skin mastocytosis
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We observed that Hairless (Hr-/-) mice skin has an aberrant expression of chymase and
trypsin-like activity that correlated with the accumulation of mast cells (MCs) in their dermis
(skin mastocytosis). The accumulation of MCs in Hr-/- mice corresponded to an increased
release of MC mediators when the mice were stimulated with 48/80, a Mrgx MC specific
degranulation agent, indicating that MCs were functional and mature.To understand the
underlying pathogenesis of Hr-/- mastocytosis, we investigated both intrinsic and extrinsic
factors that could lead to the excessive number of MCs present in the hairless skin including:
vitamin D receptor (VDR) pathway, commensal composition and stem cell factor (SCF)
expression in the skin of Hr-/- mice compared to their littermates. Given that Hr gene has
been reported to be disruptive in the VDR pathway, 1,25-Dihydroxyvitamin D (VD) i.p. injections were performed onto Hr-/- and their littermates; however, no changes were noticed in
MC number after VD i.p. injections. KIT and its ligand, SCF are essential elements for the mast
cell development. KIT mutations (that lead to constitutive activation) are commonly detected
in patients with mastocytosis. Moreover, previous studies from our lab demonstrated that MC
maturation and migrationcan be determined by skin commensal gram-positive bacteria
through the TLR2-SCF pathway. Interestingly, when we analyzed the skin microbiome
composition on WT and Hr-/- mice, we found that Hr-/- mouse skin contained a lower
amount of bacteria, but that bacteria composition presented with a higher ratio of grampositive bacteria than in WT mice. Compatible with these results, compared to wild type
littermate mice, hairless mice had higher lipoteichoic (LTA) in the epidermis (quantified by
immunofluorescence), ligand of Toll-like receptor 2 (TLR2), and also higher expression of SCF
along skin epidermis. Our study reveals that the hairless gene changes the mouse skin
structure leading to a different skin commensal bacteria population, higher SCF expression in
the skin and also mastocytosis.

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Genomic tools identify overlapping Mendelian disorders and provide rationale
for treatment of a patient with concurrent acrodermatitis enteropathica and
epidermolysis bullosa
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Pasteur Institute of Iran, Tehran, Iran
Whole exome sequencing (WES) has recently entered into clinical setting. Here we report a
patient affected by two overlapping inherited diseases, acrodermatitis enteropathica (AE) and
epidermolysis bullosa (EB). Genomic tools identified two homozygous variants in the patient
and provided insight for treatment of her complex phenotype. A 17-year-old female
demonstrated widespread erythematous and desquamative plaques, generalized alopecia and
blisters, growth failure, impaired taste and smell, regression of sexual development, and nail
dystrophy. Several skin biopsies at different times suggested EB diagnosis. The laboratory
findings included decreased plasma zinc level but normal alkaline phosphatase and albumin.
She was treated by oral elemental zinc which was appropriate for patients affected by EB, but
the patient was unresponsive to it. WES and whole genome homozygosity mapping identified
the presence of two homozygous mutations within the runs of homozygosity in COL7A1 and
SLC39A4.The presence of COL7A1 mutation explained the existence of tissue separation on
pathological examinations. SLC39A4 mutation suggested that the lower zinc level is not a
secondary finding due to malabsorption which is frequent among EB patients, but instead, it is
compatible with diagnosis of AE. These results indicate the existence of two Mendelian
disorders in the patient. This primary zinc deficiency was treated with high-dose to overcome
the inherited defect in intestinal zinc absorption. After one-week the skin plaques began to
disappear and hair growth was initiated, development of secondary sexual characteristics and
remarkable weight gain were observed.


Genetic Disease, Gene Regulation, and Gene Therapy | ABSTRACTS

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Genome-wide single nucleotide polymorphism-based autozygosity mapping facilitates identification of mutations in consanguineous families with epidermolysis bullosa

A Juiotto4, A Kundaje2 and P Khavari5

The routine diagnosis of heritable skin and connective tissue disorders is complicated by the fact that in this group of disorders, clinical manifestations may result from genetic or phenotypic heterogeneity and the existence of new genes and/or novel disease subtypes. Autozygosity mapping (AM) has been proven to be a useful adjunct in the molecular diagnosis of homozygous autosomal recessive (AR) diseases. We investigated the utility of AM for the molecular diagnosis of hereditary AR disorders, using epidermolysis bullosa (EB) as a paradigm. We applied this technique to a cohort of 46 distinct EB families using genome-wide single nucleotide polymorphism (SNP) array-based AM to guide targeted Sanger sequencing of EB candidate genes. Initially, 39 of the 46 cases (84.7%) were diagnosed with homozygous mutations using this method. Individually, 26 cases, including the seven initially unresolved cases, were analyzed with an EB-targeted next-generation sequencing (NGS) panel. This approach identified mutations in five additional cases, initially undiagnosed due to presence of compound heterozygosity, deep intronic mutations, or runs of homozygosity below the set threshold of 2 Mb, for a total yield of 44 out of 46 cases (95.7%) diagnosed genetically. The yield of 84.7% using AM-guided sequencing was remarkably similar to that of the independent use of our previously reported NGS targeted panel, in which potential causative variants were identified in 76 of 91 (83.3%) families with EB. Thus, AM is an expeditious and cost efficient approach to identify mutated genes consanguineous families.

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Postzygotic dominant-negative mutations of RH0A cause a mosaic neuroectodermal syndrome

DERRY2, A Sotiri1, S Khosravianmikh1, Y Dufoeur1, P Kuentz2, V Carmignac1, D Bessis1, V Doboly1, S Polubothu6, F Faravelli1, V Kimler1, L Faivre1, M Ross3 and J Riviere2

In vitro genome editing in iPSCs via CRISPR/Cas9 for recessive dystrophic epidermolysis bullosa mutations

J Jackow1, Z Guo1, H Abaci1, Y Doucet1, J Shin1, C Hansen1, J Salas2 and A Christiano1

Ballelic COL7A1 editing in iPSCs via CRISPR/Cas9 for recessive dystrophic epidermolysis bullosa mutations

J Jackow1, Z Guo1, H Abaci1, Y Doucet1, J Shin1, C Hansen1, J Salas2 and A Christiano1
Exome, genome, and cDNA sequencing reveal KDSD mutations cause two forms of ichthyosis and identify retinoids as pathogenesis-directed therapy.

Punctate palmoplantar keratoderma type 1 (PPPK1, OMIM# 148600) is a rare autosomal disease and is classified into two types based on the causative genes.

The microRNAs (miRNAs) function as global negative regulators of gene expression and have been associated with a multitude of skin biological processes such as aging. To understand genome-wide changes in miRNA expression in human skin from Asian individuals during aging, we analysed by miRNA array full-thickness skin samples categorized into young, intermediate and elderly groups. Based on filtering criteria of a fold change ≥5 and an adjusted p-value ≤0.05, the expression of 21 miRNAs was found to be modulated during skin aging. More specifically, the expression of 8 miRNAs were altered between young and elderly groups, 3 miRNAs were modulated between intermediate and elderly groups. Strikingly, no miRNA appeared to be altered between young and intermediate groups. Using advanced bioinformatics, we identified predicted target genes and cellular pathways related to identified miRNAs. The miRNA-targeted genes were found to be associated with several biological processes related to methylation, cell cycle, apoptotic process, mitochondrion and regulation of stress fibre assembly. We also found a subset of the potential dysregulated-miRNA target genes belonged to the insulin signalling pathway. We confirmed with qPCR that miRNA expression of IGF-1 and its receptor were downregulated in the elderly group. Determining a miRNA signature in the skin of Chinese individuals for aging is a first step towards a better understanding of the interplay between miRNAs and protein-coding genes during intrinsic aging. Moreover, miRNAs differentially expressed in the skin of Chinese individuals may be the foundation to understand specific ethnic-related physiological characteristics.
Intradermal injection of bone marrow-MSC corrects recessive dystrophic epidermolysis bullosa in a xenograft model

C Ganier, M Titeux, S Gaucher, J Peltzer, J Lataillade, A Ishida-Yamamoto

Psoriasis is a common inflammatory skin disease, with considerable genetic contribution. Genome-wide association studies have successfully identified a number of genomic regions that contribute to the risk of psoriasis. However, it is challenging to pinpoint the functional causes and then further decipher the genetic mechanisms underlying each region. In order to prioritize potential functional causal variants within psoriasis susceptibility regions, we integrated the genetic association findings and functional genomic data publicly available, i.e., histone modifications in relevant immune cells. We characterized a pervasive enrichment pattern of psoriasis variants in five core histone marks across immune cell types. We discovered that genetic alleles within psoriasis association regions might influence gene expression levels through significantly affected the binding affinities of 17 transcription factors. We established a catalog of 654 potential functional causal variants for psoriasis and suggested that they significantly overlapped with causal variants for autoimmune diseases. We identified a number of potential causal variants that overlapped with the peaks of five histone marks in primary CD4+ T cells. Its alternative allele affected the binding affinity of transcription factor Ikaros. This study highlights the complex genetic architecture and complicated mechanisms for psoriasis. The findings will inform the functional experiment design for psoriasis.

Nervous system development and function are dependent on the proper regulation and patterning of gene expression. This process is controlled by transcription factors that bind to enhancer elements in the genome and activate transcription. One such transcription factor is the nuclear hormone receptor Pbx1, which plays a critical role in the development of the nervous system. In this study, we examined the role of Pbx1 in regulating gene expression during nervous system development. We used a combination of genomic and transcriptomic approaches to identify Pbx1 target genes and to analyze their expression patterns in the developing nervous system. We found that Pbx1 regulates the expression of several genes that are important for nervous system development, including genes involved in axon guidance, synaptic plasticity, and cannabinoid receptor signaling. These findings suggest that Pbx1 plays a key role in regulating gene expression during nervous system development and may have implications for understanding the development of nervous system disorders.
Cardiomyopathy in epidermolysis bullosa simplex patients with mutations in KLHL24 gene

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Epidermolysis bullosa simplex (EBS) is a genetic skin disorder characterized by the formation of blisters at sites of friction and trauma. Mutations in genes encoding for basement membrane proteins cause the disease. One such protein is collagen type VII (COL7A1), which is involved in the attachment of keratinocytes to the extracellular matrix. A recent study investigated the genetic cause in a female newborn with EBS with pyloric atresia. An amniocentesis detected a deletion of COL7A1 due to mutations in genes encoding for basement membrane proteins. Here we investigate the genetic cause in a female newborn with JEB with pyloric atresia. An amniocentesis detected a low-level fetal mosaic trisomy of chr 2. Immunofluorescence mapping showed a junctional split and absence of immunoreactivity for integrin β6. Sequence analysis of the ITGA6 gene (chr 2) revealed a homozygous frame-shift insertion leading to a premature stop codon. This mutation was found in a heterozygous state in the mother and absent in the father. Segregation analysis with chr 2-specific short tandem repeat markers showed exclusive maternal inheritance of chr 2 demonstrating evidence for uniparental disomy due to trisomic rescue. A mutation was found in a heterozygous state in the mother and absent in the father. Segregation analysis with chr 2-specific short tandem repeat markers showed exclusive maternal inheritance of chr 2 demonstrating evidence for uniparental disomy due to trisomic rescue. A homozygous frame-shift insertion leading to a premature stop codon was confirmed which provide justification for inclusion of COL7A1 officially as the 20th gene.

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KLHL24 gene

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817 Integrated methylobe and transcriptome analysis to dissect key biological pathways for psoriasis in Chinese Han population

T Yang, Q Xue, J Song, J Zhang, G Zhang, Y Yang, J Wang. Dermatology and Department of Dermatology, the First Affiliated Hospital, Anhui Medical University, Hefei, Anhui, China

Psoriasis (Ps) is a chronic recurring hyperproliferative and inflammatory skin disease caused by the dysregulation of keratinocytes and immune cells. Risk factors include genetic and environmental factors. Recent studies have shown that DNA methylation (DNAm) could modulate gene expression in the pathogenesis of Ps. However, the relationship between whole-genome DNAm and gene expression in Ps has not been studied yet. In this study, we used our previous methylobe and transcriptome dataset from the same 60 samples and reanalyzed differentially methylated sites (DMRs) and differentially expressed genes (DEGs) by comparing the involved Ps (PP) compared to nonPs (PN) and the cardiovascular system. PXE is caused by inactivating mutations in the ABCC6 gene encoding a putative efflux transporter ABCC6. It was recently discovered that absence of ABCC6-mediated ATP release from the liver and consequently reduced PPi levels in plasma underlie Ps. In this study, we examined whether inhibition of tissue-nonspecific alkaline phosphatase (TNAP), the enzyme that degrades PPi to PP, would increase plasma PP levels and counteract ectopic mineralization. First, Abcc6-/− mice, despite unchanged plasma PPi levels. Plasma levels of pyridoxal 5’-phosphate, a byproduct of PPi metabolism, were significantly reduced in PP compared to NN and 2,194 genes in PP compared to PN. A total 656 genes with reverse correlation between expression and methylation overlapped in PP compared to NN and PP compared to PN. Further enriched GO categories included circulatory system development and inflammation responses to various stimuli and cytokine activity. KEGG analysis revealed superior Rap1 signaling pathway and cytokine-cytokine receptor interaction. Our results provided the most comprehensive correlation analysis of transcriptome and methylobe in Ps. Further KEGG analysis suggested Rap1 signaling pathway may play a role in the development of psoriatic skin, which has not been reported previously. This integrated analysis provides additional insights into the pathogenic mechanisms involved in Ps.

818 Multigene next-generation sequencing panel identifies a spectrum of mutations in consanguineous families affected by ichthyoses

L Youssefian1, H Vahidnezhad2, A Sanidid3, S Zeinali1, S Sotoudeh1, H Mahmoudi1, L Youssefian1, H Vahidnezhad2, A Sanidid3, S Zeinali1, S Sotoudeh1, H Mahmoudi1

Ichthyoses are a heterogeneous group of congenital epidermal skin diseases characterized by scaling, hyperkeratosis and dyskeratosis. In this study, we characterized the ichthyoses spectrum in consanguineous families in Iran. We performed multigene next-generation sequencing (NGS) panel that encompasses 46 genes related to ichthyoses. Pathogenic mutations were identified in 151 cases (84% detection rate). The most frequent mutations were identified in TGM1, while other genes with a notably high rate of disease-causing mutations included PNPLA2, CERS, and CYP4F22. The pathogenic variants were not found in 28 of the 179 probands (15.6%), suggesting the presence of additional candidate genes. In our cohort we found mutations in 113 of 125 families in autosomal recessive congenital ichthyoses associated genes. Twenty-five families were found to have mutations in 7 genes associated with different syndromes in which the ichthyosis is one of the clinical manifestations: Chanarin-Dorfman, Sjogren-Larsson, neonatal ichthyosis and sclerosing cholangitis (NISCH), chondrodysplasia punctata, X-linked dominant, keratitis-ichthyosis-deafness (KID), Netherton, and ichthyosis with hypotrichosis. These results depict a marked difference between the molecular basis of ichthyoses in poorly-defined countries in comparison with well-characterized Western populations.

820 Customized gene-targeted next generation sequencing panel identifies a spectrum of mutations in consanguineous families affected by ichthyoses

L Youssefian1, H Vahidnezhad2, A Sanidid3, S Zeinali1, S Sotoudeh1, H Mahmoudi1

Ichthyoses are a heterogeneous group of congenital epidermal skin diseases characterized by scaling, hyperkeratosis and dyskeratosis. In this study, we characterized the ichthyoses spectrum in consanguineous families in Iran. We performed multigene next-generation sequencing (NGS) panel that encompasses 46 genes related to ichthyoses. Pathogenic mutations were identified in 151 cases (84% detection rate). The most frequent mutations were identified in TGM1, while other genes with a notably high rate of disease-causing mutations included PNPLA2, CERS, and CYP4F22. The pathogenic variants were not found in 28 of the 179 probands (15.6%), suggesting the presence of additional candidate genes. In our cohort we found mutations in 113 of 125 families in autosomal recessive congenital ichthyoses associated genes. Twenty-five families were found to have mutations in 7 genes associated with different syndromes in which the ichthyosis is one of the clinical manifestations: Chanarin-Dorfman, Sjogren-Larsson, neonatal ichthyosis and sclerosing cholangitis (NISCH), chondrodysplasia punctata, X-linked dominant, keratitis-ichthyosis-deafness (KID), Netherton, and ichthyosis with hypotrichosis. These results depict a marked difference between the molecular basis of ichthyoses in poorly-defined countries in comparison with well-characterized Western populations.

821 Network analysis of pro-inflammatory genes shared by psoriasis and attherosclerosis identified via RNA-seq

D Seth, JB Golden1, B Richardson3, M Cartwright1, TS McCormick1, KD Cooper1, and LA Elenich1

Attherosclerosis and psoriasis are both chronic disorders of the skin and atherosclerosis is a major complication of psoriasis. Using RNA-seq, we identified 1,460 unique genes that differed in expression and methylation levels in PP compared to NN and 2,194 genes in PP compared to PN. A total 656 genes with reverse correlation between expression and methylation overlapped in PP compared to NN and PP compared to PN. Further enriched GO categories included circulatory system development and inflammation responses to various stimuli and cytokine activity. KEGG analysis revealed superior Rap1 signaling pathway and cytokine-cytokine receptor interaction. Our results provided the most comprehensive correlation analysis of transcriptome and methylobe in Ps. Further KEGG analysis suggested Rap1 signaling pathway may play a role in the development of psoriatic skin, which has not been reported previously. This integrated analysis provides additional insights into the pathogenic mechanisms involved in Ps.

822 The proteome profiling of hair anchorage from hair plucks across the human hair cycle

LA Elenich1, P Dwivedi1, T Chaudhary1, J Winget3, B Fisher3 and M Davis4

Hair anchorage plays a crucial role in the hair growth cycle (anagen, telogen and exogen) and is critical for hair growth. Using hair plucks from healthy, untreated subjects, we studied the proteome profiling of hair anchorage from hair plucks across the human hair cycle. Our results highlight the dynamic changes in the hair anchorage proteome during the telogen to anagen transition, not only is there a loss of proteins under the existing database, with implications for genetic counseling, prenatal genetic testing and diagnosis of antenatal abnormalities. In conclusion, additional DEGs were identified that expand the existing database, with implications for genetic counseling, prenatal genetic testing and diagnosis of antenatal abnormalities. In conclusion, additional DEGs were identified that expand the existing database, with implications for genetic counseling, prenatal genetic testing and diagnosis of antenatal abnormalities. In conclusion, additional DEGs were identified that expand the existing database, with implications for genetic counseling, prenatal genetic testing and diagnosis of antenatal abnormalities. In conclusion, additional DEGs were identified that expand the existing database, with implications for genetic counseling, prenatal genetic testing and diagnosis of antenatal abnormalities.
ABSTRACTS | Genetic Disease, Gene Regulation, and Gene Therapy

823 Enhancer connectome functionally interrogates GWAS-identified SNPs associated with inflammatory skin conditions

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The vast majority of polymorphisms for human dermatological diseases fall in noncoding DNA regions, leading to difficulty interpreting their functional significance. Recent work utilizing chromosomal conformation capture (3C) technology in combination with chromatin immunoprecipitation (ChIP) has provided a systematic means of linking noncoding variants within active enhancer loci to putative gene targets. Here, we apply H3K27ac H3C1p high-resolution contact maps, generated from primary human T cell subsets (CD4+ Naïve, Th17, and Treg), to a set of 21 dermatologic conditions. These conditions include 528 disease-associated intergenic single nucleotide polymorphisms (SNPs) with a minimum significance p ≤ 5.0x10−8 from over 100 genome-wide association studies (GWAS), identified 1,490 H3C1p enhancer targets. SNPs from inflammatory conditions not only showed a significant enrichment bias within the human leukocyte antigen locus, but also targeted several key factors within the JAK-STAT signaling pathway. A focused profiling of systemic lupus erythematosus-specific HIC1p enhancer interactions with interferon regulatory factor 8 (IRF8) and various members of the Ikaros family of zinc-finger proteins, known for their role in the development of T cell differentiation and function, demonstrates the ability of H3K27ac H3C1p to assign functional relevance to GWAS-identified variants, providing novel insight into the genomic regulatory mechanisms underlying dermatologic diseases.

825 Functional prediction of missense variants using in silico bioinformatics tools

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Next-generation sequencing (NGS) technologies can identify on average 20,000 missense variants per exome, out of which very few can be held responsible for causing the underlying disease. Because of this sheer amount of variants, it is not feasible for the researchers to empirically examine their individual or collective phenotypic or pathological effects. Therefore, bioinformatics tools have been introduced to predict the effect of genetic variants. Some tools are based on sequence conservation approach like SIFT, MutationAssessor and LammMmA, whereas tools like PolyPhen2 and CADD integrate different information resources and use a machine learning based approach to predict the variants. As far as consensus approaches are concerned, Condel combines the results from other tools. However, none of the existing tools can actually predict the biological consequences of the missense variants in the protein. A large number of mutations was extracted from public database ClinVar, namely 715 gain-of-function (GOF) and 4125 loss-of-function (LOF) variants and was annotated using the Ensembl tool VEP. All of these variants have supporting evidence of their functional significance in terms of literature tests, clinical reports and research projects. Our results show two peaks of mean prediction scores across GOF and LOF variants (p < 0.05) indicating a non-random difference for each in silico tool available through VEP apart from MutationAssessor. This difference aligning with the prediction score distribution across all the variants helped us to choose cut-off scores for identifying GOF and LOF variants. These cut-off prediction scores resulted in correct prediction of 71% GOF and 75% LOF variants. Therefore, the cut-off scores of different in silico tools can be useful to predict the functional significance of a missense variant. However, they should be used more as an indicator as they need to be interpreted with further evidence on pathogenicity.

826 Functional genomic analysis of the IL2RA susceptibility region in alopecia areata

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Alopecia areata (AA) is an autoimmune disease that causes hair loss by T-cell autoimmunre action against the hair follicles. We previously published a GWAS and meta-analysis to search for common alleles contributing to AA risk and identified 14 genomic regions harboring potential susceptibility genes. In this study, we conducted targeted resequencing on several GWAS regions in 122 cases and focused on the functionality relevant IL2RA locus. Interleukin-2 (IL2) is involved in T cell proliferation and survival making it essential for maintaining immune tolerance. Various IL2RA haplotypes have been shown to regulate IL2 Receptor expression on CD4+ T cells inducing Treg cells. Several polymorphisms surrounding the IL2RA locus have been reported to be associated with type 1 diabetes (T1D), rheumatoid arthritis, vitiligo, and AA. Haplotype-dependent gene expression can confer either risk or protection from autoimmunity. For example, rs417359 (haplotype07) contains an R risk allele in AA and T1D. We identified novel variants in the IL2RA region that show rare enrichment in AA and were replicated in our whole exome sequencing, thus prioritizing these variants as candidates for functional studies. We discovered 458 variants, 65 of these were classified as rare enriched defined as a variant present in less than 1% of population in population databases and in 3 or more patients in our cohort. These rare enriched variants fall mainly within introns, intergenically or downstream of IL2RA. Using an algorithm (FUN-LDA) that predicts functional effects of certain non-coding genetic variants in tissue types, we localized the majority of these 65 rare enriched variants in the context of cell types and found they mostly fall in the immune cell cluster. Overall, this analytical pipeline helps discover and place rare enriched disease variants in the context of specific cell types for future functional studies.

827 Role of the autophagy protein, Syntaxin 17 (STX17), in melanogenesis and alopecia areata

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Sudden whitening of the hair is a clinical observation in Alopecia Areata (AA) that is known as 'canities subita', a phenomenon in which scalp hair appears to turn completely white due to the rapid and preferential attack of pigmented hair follicles (HF). This observation led to the hypothesis that HF melanocyte-specific antigens play a key role in AA disease onset. Recently, essential autophagy proteins have been found to have pleiotropic roles in the regulation of melanin production and melanosome formation in the melanogenesis pathway. Interestingly, we have observed a protein expression analysis of melanoma tissue types, we localized the majority of these 65 rare enriched variants in the context of cell types and found they mostly fall in the immune cell cluster. Overall, this analytical pipeline helps discover and place rare enriched disease variants in the context of specific cell types for future functional studies.

828 Reversal of a core, keratinocyte-autonomous inflammatory program linking diverse cutaneous rashes

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In humans, gain-of-function (GOF) mutations in RHBDF2 cause the skin disease tylosis. We generated a mouse model of the human skin disease tylosis that show GOF mutations in RHBDF2 cause tylosis by enhancing the amount of AREG secretion. Additionally, we demonstrate that AREG is an essential shiedade of AREG, and mouse models of tylosis lacking AREG specifically in the skin exhibit a full hair coat with no signs of hyperplasia or hyperkeratosis. Furthermore, we show that genetic disruption of AREG ameliorates skin pathology in mice carrying the tylosis type 1 disease mutation. Collectively, our data suggest that RBHDF2 plays a critical role in regulating EGFR signaling and its downstream events, including development of tylosis, by facilitating enhanced secretion of AREG via ADAM17. Thus, targeting AREG could have therapeutic benefit in the treatment of tylosis.

829 Functional deletion of amphiregulin restores the normal skin phenotype in a mouse model of the human skin disease tylosis

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In humans, gain-of-function (GOF) mutations in RHBDF2 cause the skin disease tylosis. We generated a mouse model of the human skin disease tylosis and show that GOF mutations in RHBDF2 cause tylosis by enhancing the amount of AREG secretion. Additionally, we demonstrate that ADAM17 is an essential shiedade of AREG, and mouse models of tylosis lacking ADAM17 specifically in the skin exhibit a full hair coat with no signs of hyperplasia or hyperkeratosis. Furthermore, we show that genetic disruption of AREG ameliorates skin pathology in mice carrying the tylosis type 1 disease mutation. Collectively, our data suggest that RBHDF2 plays a critical role in regulating EGFR signaling and its downstream events, including development of tylosis, by facilitating enhanced secretion of AREG via ADAM17. Thus, targeting AREG could have therapeutic benefit in the treatment of tylosis.
**829**
The EDC enhancer 923 is required for Ivl, Smcp, and Lce6a gene expression and chromatin accessibility.

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The mechanisms by which gene expression is activated at the chromatin level are poorly understood. The presence of distinct nuclear envelope rearrangements (IERCs), focus locus genic signals, and chromatin compaction upon keratinocyte differentiation, provides a unique opportunity to address this question. Previously we identified a role for the epidermal-specific 923 enhancer in dynamic chromatin remodeling. Here we sought to investigate whether 923 is necessary for EDC activation using CRISPR/Cas9-mediated deletion. 923+/- KO mice (both 923+/- and 923+/-) appeared phenotypically normal under homeostatic conditions. However, transcriptional profiling of 923de/- embryonic skin captured during epidermal development (E14.5–NR) revealed decreased expression of genes involved in the cornified envelope (E14.5–NR), and spermidine mitochondrial-associated cytochrome rich protein (Smcp). The requirement for the 923 enhancer was further validated in 923de/- newborn keratinocytes. ATAC-seq in 923de/- and wild-type littermate newborn keratinocytes further identified more closed chromatin (higher ATAC-seq peaks) observed at the 5' and 3' ends of the EDC delB, indicating a requirement for the 923 enhancer for chromatin organization at the EDC boundaries. Male fertility defects associated with decreased 923 enhancer target gene Smcp expression in 923de/- male testes were observed with reduced progeny and slower sperm motility. Our genomic study reporting in vivo functional effect for the deletion of an epidermal-specific enhancer, identifies a requirement for 923 in proximal Ivl, Lce6a, and Smcp target gene expression, higher level chromatin accessibility in the EDC, and downstream male fertility function for its Smcp target gene, thus addressing a knowledge gap for enhancer function in chromatin level gene expression.

**831**
Disseminated superficial actinic porokeratosis: A novel G311R MVK mutation associated with decreased enzyme activity in a French family

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MVK gene mutations are responsible of hyper-IgD syndrome and heterozygotes are associated with decreased enzyme activity in a French family.

**833**
A genome-wide association study identifies novel loci associated with severe acne and implicates hair follicle development in acne molecular pathogenesis

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Acne is a common chronic inflammatory disease affecting the pilosebaceous unit in the skin. The pathogenic mechanisms, and how to treat it, are poorly understood.

**834**
CRISPR/Cas9 based COL7A1 genomic editing in recessive dystrophic epidermolysis bullosa (RDEB) via non-viral polymer delivery system

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RDEB is an orphan, intractable, often lethal and usually progressive disorder caused by mutations in exon 80 of the COL7A1 gene which results in absent or dysfunctional Collagen VII production, leading to skin blistering and premature aging (9 months+) Fabry rats developed an unkempt appearance, alopecia, and xeroderma. At necropsy, increased effort was required to section the aged Fabry rat skin. These changes prompted us to histologically examine the skin of 3 Fabry and 3 WT 80-week-old males. The dermal connective tissue layer was thickened and denser in Fabry rat skin, and a prominent band of histiocytes was observed. CDDO staining confirmed the abundance of macrophages in Fabry, but not WT, dermis. Lipidema was apparent in Fabry rat skin, a finding consistent with Fabry disease. Fabry rats weighed less than controls at the age of 8 months. Fabry animals were also noted, suggesting occlusion and thus providing a xeroderma explanation. Fabry rats frequently developed lesions characterized as circular to oval alopecic plaques with erosions and hemorrhagic crusting. The lesions had a predilection for the dorsal head and back and were more commonly seen in Fabry female rats than males. On the microscopic level, the lesions were observed to demonstrate focal ulceration, epidermal spongiosis with microvesiculation, and overlying serum crust. In the dermis, there was a marked cellular infiltrate consisting of histiocytes, lymphocytes, and abundant eosinophils. Importantly, no vascular pathology was observed in the lesions. Although angiorkeratomas were not observed in Fabry rats, the skin findings indicate that inflammation and lipodema may be prominent mediators of Fabry disease phenotypes in humans.

**880**
Fabry rat skin findings demonstrate potential roles of inflammation and lipidopa in Fabry disease

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Fabry disease is an X-linked lysosomal disease caused by α-galactosidase A deficiency. Patients experience proteinuria, cardiomyopathy, angina, and stroke. Dermatological manifestations include sweating abnormalities and angiorkeratomas.

To gain a better understanding of disease mechanisms, we generated a Fabry rat model using CRISPR/Cas9 technology and confirmed the absence of α-galactosidase A activity in tissues.

Wild type, 923+/-, and Fabry rats were indistinguishable at young ages (weaning-6 months), but aging (9 months+) Fabry rats developed an unkempt appearance, alopecia, and xeroderma. At necropsy, increased effort was required to section the aged Fabry rat skin. These changes prompted us to histologically examine the skin of 3 Fabry and 3 WT 80-week-old males. The dermal connective tissue layer was thickened and denser in Fabry rat skin, and a prominent band of histiocytes was observed. CDDO staining confirmed the abundance of macrophages in Fabry, but not WT, dermis. Lipidema was apparent in Fabry rat skin, a finding consistent with Fabry disease. Fabry rats weighed less than controls at the age of 8 months. Fabry animals were also noted, suggesting occlusion and thus providing a xeroderma explanation. Fabry rats frequently developed lesions characterized as circular to oval alopecic plaques with erosions and hemorrhagic crusting. The lesions had a predilection for the dorsal head and back and were more commonly seen in Fabry female rats than males. On the microscopic level, the lesions were observed to demonstrate focal ulceration, epidermal spongiosis with microvesiculation, and overlying serum crust. In the dermis, there was a marked cellular infiltrate consisting of histiocytes, lymphocytes, and abundant eosinophils. Importantly, no vascular pathology was observed in the lesions. Although angiorkeratomas were not observed in Fabry rats, the skin findings indicate that inflammation and lipodema may be prominent mediators of Fabry disease phenotypes in humans.

**883**
Genetic Disease, Gene Regulation, and Gene Therapy | ABSTRACTS
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HLA-DQB1 amino acid position 87 and DQB1*0301 are associated with Chinese Han SLE

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Objectives: To further identify susceptibility loci associated with Chinese Han SLE methods. We extracted SNPs of 1047 cases and 1205 controls subjects and SNP data of 612 cases and 2193 controls individuals in previously SLE GWAS. Then we carried out imputation and stepwise conditional analysis of classical HLA alleles, amino acids and SNPs across the MHC region in SLE GWAS using newly constructed Han-MHC reference panel. Results: We mapped the most significant independent association to HLA-DQB1 at amino acid position (P=8.7×10^-7) and an independent association at HLA-DQB1*0301 (Conditional P=1.4×10^-7).

836

Non-viral gene therapy for Recessive Dystrophic Epidermolysis Bullosa (RDEB) using a highly branched poly-α-amino ester polymer (HPAE-E) (P=10^-11; HLA-A amino acid position 152, P=5.37×10^-42; HLA-DRB1*03:01 and HLA-DQB1*02:01, P=3.37×10^-6). Except HLA-DRB1*03:01, KIT mRNA expression levels were decreased in patients with SLE (P<1.0×10^-5) compared with those in healthy controls and that of GPR78 was increased (P=4.44×10^-11). The eQTL study demonstrated that KIT mRNA expression levels were significantly associated with rs2855772, r31316627 and r10018951 which were significantly associated with SLE in the Han Chinese population (r2=1.5×10^-4). Our study suggests that multiple noncoding variants are associated with SLE in the Han Chinese population.

837

Genome-wide association study identifies three novel susceptibility loci for systemic lupus erythematosus in Han Chinese

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Objectives In order to determine the presence of novel susceptibility genes for systemic lupus erythematosus (SLE), we used imputed data from our previously published genome-wide association studies (GWAS) in Han Chinese population to generate candidate association signals and performed a replication study. Methods GWAS data from 1,047 SLE cases and 1,205 controls were pre-phased using SHAPEIT and imputed using IMPUTE 2 with 1000 Genomes Project reference data (phase 1 integrated set, March 2012, build 37). Eighty-two single nucleotide polymorphisms (SNPs) underwent further replication studies in 11,755 samples. A meta-analysis of these studies was performed. Results Real-Time PCR (RT-PCR) and expression quantitative trait loci (eQTL) were used to determine gene expression differences and regulatory effect of SNPs, respectively. Results We identified three novel genome-wide significance loci (KIT, GPR78 and TRAPPC11) encompassing three noncoding variants (rs2855772, r31316627 and r10018951) which were significantly associated with SLE in the Han Chinese population (P<1.5×10^-4). KIT mRNA expression levels were decreased in patients with SLE (P<1.0×10^-5) compared with those in healthy controls and that of GPR78 was increased (P=4.44×10^-11). The eQTL study demonstrated that KIT mRNA expression levels were significantly associated with rs2855772, r31316627 and r10018951 which were significantly associated with SLE in the Han Chinese population (r2=1.5×10^-4). Our study suggests that multiple noncoding variants are associated with SLE in the Han Chinese population.

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Fine-mapping analysis of the HLA region for vitiligo in Chinese Han population

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Vitiligo is an autoimmune disease characterized by patchy depigmentation of skin and hair. The clinical manifestations are resulted from progressive loss of melanocytes for which the mechanism is still unclear. Most recently, evidence has been accumulated to support the autoimmune and genetic hypotheses. In recent decades, association evidence has been obtained within the HLA region for the risk of vitiligo. However, the genetic association structure is unclear largely due to extensive linkage disequilibrium in this region. To elucidate the genetic architecture of the HLA region for vitiligo in Han Chinese population, our group carried out a dense sequencing study for the HLA region in 10,689 healthy control samples, and constructed the a Han-MHC reference panel. In this study, we performed a fine-mapping analysis of the HLA region in 2,818 Han Chinese subjects through a HLA imputation method with the newly built Han-MHC reference panel. We identified three new HLA alleles HLA-DQA1*02*02, HLA-DQ7*01:02 and HLA-DPB1*17:01 and confirmed four known alleles for the risk of vitiligo. We further revealed via conditional analysis that HLA-DQB1 amino acid position 33 (OR=1.79, P=1.87×10^-11) and HLA-DQB1 amino acid position 66 (OR=1.79, P=1.87×10^-11) was the strongest risk factor for vitiligo, which explained 8.60% of the phenotypic variance could be explained by all reported variants in the Han Chinese population. These findings show the genetic architecture of the HLA region for vitiligo in Han Chinese population and expand our understanding on the roles of HLA coding variants in the pathogenesis of vitiligo.

839

Four HLA amino-acid variant and two HLA allele confers risk for leprosy in the Han-population

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Leprosy risk is strongly associated with variation within the major histocompatibility complex (MHC). The HLA-DRA1, but its genetic architecture has yet to be fully elucidated. Here, we conducted a large-scale fine-mapping study of leprosy risk by imputing class I and II human leukocyte antigen (HLA) genes and corresponding amino acid variation sites from the GWAS data reported before (706 leprosy cases and 1225 controls were included) with Han-population-specific reference panel. We identified six independent risk variants, including four HLA amino-acid variants (HLA-DRA1 amino acid position 71, P=1.28×10^-42; HLA-DQB1 amino acid position 35, P=1.75×10^-16; HLA-C amino acid position 116, P=5.12×10^-13; HLA-A amino acid position 152, P=1.16×10^-13) and two HLA allele (HLA-DRA1*04:03*02, Pcond=6.7×10^-10 and HLA-DPA1*03:01, P=9.1×10^-10). Except HLA-DQA1 amino acid position 33, other five sites were first reported associated with leprosy. The results of this study provided new insights into the genetic architecture of leprosy risk in the Han population and illustrate the value of HLA imputation for fine mapping causal variants in the MHC.

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Novel mutations in Chinese Han patients with tuberous sclerosis complex: Case series and review of the literature

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Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disease characterized by hamartomas in multiple organ systems. This study was performed in one familial and two sporadic cases with TSC. Two novel mutations (c.1884_1887delAAAG and c.5266A>G) were identified by direct DNA sequencing. Of the four mutations, c.1884_1887delAAAG and c.1960G>C were found in a family and identified in the same allele by TA cloning sequencing. However, c.1960G>C was reported to be non-pathogenic. Furthermore, correlations between genotypes and phenotypes of Chinese Han patients since 2014 were performed by paired chi-square tests in our literature reviews which has not been reported. The results showed patients with TSC2 mutations had a higher frequency of mental retardation and a lower frequency of seizures and skin lesions. Genetically, they had a higher frequency of familial inheritance.
Our study highlighted that immune-mediated processes were involved in the pathophysiology demonstrated a high tendency for PV susceptibility genes to be associated with autoimmunity.

To identify additional genetic susceptibility loci for pemphigus vulgaris (PV), we performed the first genome-wide association study in 2,420 PV cases and 1,031 controls, and we validated in independent samples of 252 cases and 1,852 controls. We identified rs11218708 (P = 3.3 × 10^-12; OR = 1.54) at 11q24.1 to be significantly associated with PV. Further fine-mapping analysis of PV risk in the MHC region demonstrated three independent variants predisposed to PV, HLA-DRB1*14:04 (P = 2.47 × 10^-6; OR = 2.47), and rs7454108 at the TAP2 gene (P = 2.78 × 10^-12; OR = 3.25), and rs1051336 at the HLA-DRA gene (P = 3.06 × 10^-6; OR = 3.1), by stepwise analysis. A systematic evaluation by gene- and pathway-based analyses demonstrated a high tendency for PV susceptibility genes to be associated with autoimmunity.

Our study highlighted that immune-mediated processes were involved in the pathophysiology of PV and illustrated the value of imputation to identify variants in the MHC region.

Here, we investigated whether the HMGB1 treatment could serve as a promising therapy for diseases of the cutaneous, and possibly non-cutaneous tissues with severe intractable damages, such as DEB. Consistent with this anti-cell death gene profile, the number of bone marrow Pdgfrα positive cells significantly decreased without the HMGB1 treatment. Thus, these results indicated that the activation of tissue regeneration, possibly by systemic delivery of HMGB1 promotes tissue regeneration by activating PDGFRα cells in a mouse model of epidermolysis bullosa.
Identification of a novel pathway linking TGF-β, fibroblast and integrin α5β1 that promotes invasion in basal cell carcinoma

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We previously reported that pericytes were major source of integrin-ligand MEGF-E8, and MEGF-E8 enhanced angiogenesis. Several studies have demonstrated that MEGF-E8 negatively regulates fibrosis in lung and respiratory tract. The aim was to elucidate the role of MEGF-E8 in skin fibrosis in systemic sclerosis (SSc). We demonstrated that the expression of MEGF-E8 around blood vessels in the dermis and serum MEGF-E8 levels in SSc patients were significantly reduced. It has been recognized that latent-TGF-β binds to integrin αvβ3, leading to the activation of TGF-β. We found that MEGF-E8 bound to integrin αvβ3 and inhibited TGF-β-induced collagen type I, Smad and CTGF in SSc fibroblasts, suggesting that MEGF-E8 bound to integrin αvβ3 may inhibit the activation of TGF-β signaling in SSc fibroblasts. In a mouse model of bleomycin-induced fibrosis, MEGF-E8 KO mice exhibited enhanced pulmonary and skin fibrosis compared to WT mice. Furthermore, bleomycin-induced dermal thickness was significantly inhibited by injection of MEGF-E8. In studies using tight-skin mice, the deficient expression of MEGF-E8 significantly enhanced both pulmonary and skin fibrosis. These results suggest that vascularopathy-induced reduction or dysfunction of pericytes and endothelial cells may be associated with the decreased expression of MEGF-E8 in SSc skin, and that this decrease might attenuate the inhibitory effect on the activation of TGF-β signaling in SSc fibroblasts by MEGF-E8, resulting in the exacerbation of skin fibrosis. The inhibitory regulation of fibrosis by MEGF-E8 may be involved in the pathogenesis of SSc and integrin-modulating therapy could be promising for fibrosis in SSc.

Inhibitory regulation of MEGF-E8 on fibrosis in systemic sclerosis via modulating TGF-β signaling

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Pemphigus foliaceus IgG autoantibodies block heterophilic trans-interaction of desmoglein 1 and desmocollin 1 without intracellular signaling
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It is controversial if the desmoglein 1 (Dsg1) and desmocollin 1 (Dsc1) isomer is involved in the desmoglein isomer expression is due to these inhibition of Dsg interaction or intracellular signaling events that cause desmosome destabilization or both. A recent study showed that heterophilic binding of Dsg and Dsc is important for adhesive unit of desmosomes. Here we analyzed that PF autoantibodies can block the formation of Dsg of Dsc of Dsg1 and Dsc1 were produced by a mammalian expression system and then coated on microscope. Beads coated with Dsg1 alone or Dsc1 alone showed minimal aggregation while a mixture of Dsg1 beads and Dsc1 beads showed strong aggregates after 30 min interaction, confirming that the heterophilic binding of Dsg1 and Dsc1 is dominant. The aggregation required a mature form of Dsg1 and Dsc1, consistent with adhesion being a property only of mature, not proprotein.

To determine if PF Abs can directly block Dsg/Dsc adhesion, we tested anti-Dsg1 mAbs isolated from a PF AB panel by transfer to Dsg1 or Dsc1-coated beads. In this assay, human Dsg1 or Dsc1-SCF beads were putative trans-adhesive interface of Dsg1 could block the aggregation of Dsg1 and Dsc1 beads. On the other hand, non-pathogenic Dsg1 IgG or SCF mAbs did not inhibit aggregation. These findings indicated that this in vitro aggregation head assay can discriminate pathogenic vs. non-pathogenic PF Abs. Furthermore, sera from 8 PF patients with active disease all inhibited the aggregation of Dsg1 and Dsc1 beads. When paired sera obtained from 3 PF patients in active phase and in remission were compared, the former inhibited aggregation much better than the latter, as determined by aggregate size. These findings reveal direct evidence that PF autoantibodies cause steric hindrance of the heterophilic trans-interaction of Dsg and Dsc that provide adhesion to desmosomes. Furthermore, this assay will be a valuable and simple tool to assess pathogenic strength of PF autoantibodies.

Growth Factors, Cell Adhesion and Matrix Biology | ABSTRACTS

Three hyaluronic acid synthases differently regulate epidermal and dermal hyaluronan production in murine contact hypersensitivity model
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Hyaluronan is a characteristic pathological feature of allergic contact dermatitis (ACD), but its pathogenesis has not been fully elucidated. We previously demonstrated that spongiosis increases hyaluronan synthase (HAS) mRNA expression and accumulates hyaluronan (HA) in the intercellular space associated with decreased E-cadherin expression and that inflammatory cytokines, e.g., IFN-γ, IL-4 or IL-13, augment HAS mRNA expression with increased HA production and decrease E-cadherin expression by normal human epidermal keratinocytes in vitro and also induce spongiosis of organ cultured epidermises. To further clarify the HA metabolism of the epidermis in ACD, we used murine ACD model. We first examined HAS1, HAS2, and HAS3 mRNA expression of the epidermis and dermis in the elicitation phase of ACD in wild-type (WT) mice with absolute qPCR. In the ammonium thiocyante-sparated epidermis, HAS3 mRNA expression was significantly upregulated and HAS2 mRNA expression was almost undetectable but HAS1 mRNA expression was not detectable. On the other hand, in the dermis, HAS1 mRNA expression was upregulated and HAS2 mRNA expression was detectable but not altered, while HAS3 mRNA expression was not detectable. Consistent with these data, histochemical staining using hyaluronic-acid-binding protein (HABP) revealed that HA deposition was observed both in the epidermis and dermis. Next, to explore the role of HAS 1 in ACD, we used HAS1 KO mice (HAS1 KO). Unexpectedly, however, the epidermal HAS1 KO caused increased HA deposition in the elicitation phase, suggesting the role of HAS other than HAS1.

The other hand, ear swelling was significantly reduced in HAS1 KO mice compared with that in WT mice at 6, 12 and 24 h but not at 48 and 72 hrs after hapten challenge. These results suggest that HAS1, HAS2, and HAS3 differentially regulate HA production in ACD and possibly modulate inflammatory response of ACD.

The natural phychochemical dehydroabietic acid induces regeneration of collagen fibrils in UV-irradiated human dermal fibroblasts
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Acne is a multifactorial skin disease with abnormal keratinization, sebaceous gland hyperplasia, inflammation, and folliculitis. Manifestation of comedones and inflammatory acne is not fully elucidated. Since keratinocytes lack androgen receptors, we hypothesized that fibroblasts interact with androgenic hormones to modulate keratinocyte proliferation and differentiation. We cultured dermal fibroblasts with testosterone (T), dihydrotestosterone (DHT), alpha-estrogen (Ea) and beta-estrogen (Eb), and examined growth factors expression. We observed two to four-fold increase in keratinocyte proliferation and migration caused by O3 exposure was completely inhibited by disrupting EGFR crosstalk and activation of downstream signaling pathways are required for the observed acceleration in wound healing in a diet-induced obese diabetic mouse model, topical application of O3 oil visibly promoted healing with a 2.3-fold reduction in the epidermal gap and 1.7-fold increase in granulation tissue compared with vehicle-treated mice at 8 days after initiation (when wounds in nondiabetic mice had closed). Immunohistochemistry staining showed a 2.5-fold increase in HG staining in O3 oil treated mice and a 2.3 fold increase in keratinocyte (KC) migration (p<0.05) at the wound edge at 4.8 days post wounding. Brief exposure of monolayer cultured normal KCs to O3 (20 μM, 2 h) daily increased cell proliferation in high glucose (HG; p<0.01) within 24h and normal glucose (NG; p<0.01 NG vs. HG+O3) by 2.7h in WST assays. Exposure to O3 also enhanced cell migration (p<0.01, HG vs. HG+O3) by 2.4h and p<0.05 NG vs. NG+O3 by 60h in mouse KCs and p<0.1 HG vs. HG+O3 by 6h and p<0.05 NG vs. NG+O3 by 18h in human KCs in scratch assays. Under NG condition, brief O3 exposure increased EGF-stimulated p-ERK by 1.7-fold, p-AKT at Thr 386 and p-P38 by 2.1-fold and p-ATK at Ser 473 by 2.9-fold, and increased IGF-1 stimulated p-ERK by 1.5-fold. Interestingly, stimulation with EGF or IGF-1 reversed HG-induced inhibition on PI3K/Akt and ERK activation in the presence of O3, suggesting that brief O3 exposure ameliorate skin cell function under HG condition. The increase in KC proliferation and migration caused by O3 exposure was completely inhibited by disrupting EGFR and IGF1R simultaneously. These findings suggest that topical exposure to O3 requires activation through induction of growth factors from dermal fibroblasts. Interaction of growth factor findings suggested that androgens could modulate keratinocyte proliferation and differentiation. Tyrosine kinase inhibitors suppressed proliferation of keratinocyte co-cultured with fibroblasts stimulated by T and DHT. Immunostaining of acne lesions showed higher expression of these growth factors in dermus compared to non-lesional skin. Our findings suggests could modulate keratinocyte proliferation and migration through induction of growth factors from dermal fibroblasts. Interaction of growth factor pathways between fibroblasts and keratinocytes would have roles in the sex hormone-dependent acne pathogenesis.

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Targeting hyaluronan in the skin alters reactive adipsigenesis in the colon
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Dermal white adipose tissue is activated upon skin infection or injury and this process of reactive local adipsigenesis is an essential part of innate immune defense of the skin against bacteria. In the current study, we showed that reactive adipsigenesis also takes place in the colon in response to injury stimulated by dextran sodium sulfate (DSS). In the epithelial setting of the intestine, reactive adipsigenesis is important to resist bacterial translocation but also promotes inflammation and colitis. Since adipsigenesis is dependent on hyaluronan (HA) in the surrounding extracellular matrix (ECM), we investigated mice over-expressing human-Hyaluronidase 1 (Hyal1) during embryogenesis (Eha/Hyal1) or only in skin (K14/Hyal1 mice). Eha/Hyal1 mice showed less adipsigenesis of skin and colon, less inflammation in both organs, and increased bacterial invasion into both sites. K14/Hyal1 mice had normal HA in their colon and less HA in skin as expected after K14 targeted Hyal1 expression. Unexpectedly however, K14/Hyal1 mice with DSS colitis had increased adipsigenesis in the submucosal layer of the colon when compared to controls with DSS. Skin targeting of Hyal1 led to less adipsigenesis in colon (p = 0.04). Our results demonstrated that adipsigenesis is important for the control of colitis. The role of Hyal1 in the skin and colon is currently under investigation.

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Fibroblast heterogeneity in human adult dermis: IGF-1/TGF-ß1 signal modification to rejuvenate reticular fibroblasts
T Quan1, Z Qin 1, T Purohit 1, Y Shao 1, J Baldassare 2, JJ Voorhees 1 and LipoTrue Inc., New Brunswick, NJ

Currently, the roles of IGF and TGF-ß1 in human skin fibroblasts (GFs) are not fully understood. Using a ground-breaking discovery of human-derived, non-GMO GFs that can produce the protein and warrants rapid and reproducible production of non-GMO GFs for wound healing and skin regeneration, stimulating differentiation, growth and migration of keratinocytes and fibroblasts and synthesis of extracellular matrix. We present the improvement of skin conditions after application of GFs obtained through the unique process of transient expression in N. benthamiana. The adult plant is transfected with a vector that contains the synthetic transcript identical to the human gene, and then high purity proteins are expressed by the plant. This system avoids the use of animal or bacterial cells to produce the protein and warrants rapid and reproducible production of non-GMO GFs for their use in cosmetics and medicine. Plant-derived GFs are identical to the human ones and were showed in our studies to restore and improve skin condition. Plant-Inalignus-Growth Factor 1 (plant-IGF1) increased keratinocytes growth (58%, p < 0.01) and wound regeneration. Furthermore, we obtained a ground-breaking result showing the activation of chymotrypsin-like detoxifying activity of the proteasome in primary human dermal fibroblasts (54%, p = 0.001) decreasing UVB-induced protein carboxylation (66%, p = 0.001) as a consequence. Plant-IGF1 also increased cytokinin 14 expression (68%, p = 0.001) and augmented epidermal thickness (33%, p < 0.001) in human skin explants. Finally, it improved skin conditions in vivo, decreasing the melanin content and reducing the number, depth and volume of wrinkles in the undereye area. Plant-Epidermal Growth Factor (plant-EGF) was demonstrated to regenerate by 100% the 1mm wounds in vitro and increase the levels of hyaluronic acid secreted by primary human keratocytes (41%, p < 0.01). It also increased the content of young (123%) and mature (26%) collagen, as well as elastin (45%, p < 0.05) in human skin explants. Plant-EGF improved skin appearance in vivo reducing the ulcer (up to 90% in depth up to 32%) and number of face wrinkles (11%), reducing the skin roughness (12%) and increasing skin elasticity (13%, p < 0.01).

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Desmoglein 3 acts as a mechanosensor in keratinocytes
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The desmosomal cadherin desmoglein 3 (Dsg3), plays a crucial role in cell-cell cohesion and tissue integrity that is highlighted in the autoimmune blistering disease pemphigus vulgaris where Dsg3 serves as a major autoantigen (PVA). Increasing evidence suggests that Dsg3 acts as a surface regulator in cell-mechano-transduction. However, little is known about its direct response to mechanical forces. The aim of this study was to investigate the role of cyclic strain and substrate stiffness on Dsg3 expression, compared with E-cadherin a well-characterized mechanosensor. Both oral and skin keratinocytes were tested using the Flexcell system and were subjected to mechanical stretching for various time periods. Although no apparent change was found at the mRNA level in response to strain for up to 24 hours, increased protein expression was detectable for Dsg3 and associated junctional proteins (to a lesser extent for E-cadherin). A focused, temporal gene expression analysis showed the activation of chymotrypsin-like detoxifying activity of the proteasome in primary human dermal fibroblasts (54%, p < 0.001) decreasing UVB-induced protein carboxylation (66%, p < 0.001) as a consequence. Plant-IGF1 also increased cytokinin 14 expression (68%, p < 0.001) and augmented epidermal thickness (33%, p < 0.001) in human skin explants. Finally, it improved skin conditions in vivo, decreasing the melanin content and reducing the number, depth and volume of wrinkles in the undereye area. Plant-Epidermal Growth Factor (plant-EGF) was demonstrated to regenerate by 100% the 1mm wounds in vivo and increase the levels of hyaluronic acid secreted by primary human keratocytes (41%, p < 0.01). It also increased the content of young (123%) and mature (26%) collagen, as well as elastin (45%, p < 0.05) in human skin explants. Plant-EGF improved skin appearance in vivo reducing the ulcer (up to 90% in depth up to 32%) and number of face wrinkles (11%), reducing the skin roughness (12%) and increasing skin elasticity (13%, p < 0.01).

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Use of plant derived-growth factors to improve skin condition
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The use of plant derived growth factors (GFs) for skin regeneration, stimulating differentiation, growth and migration of keratinocytes and fibroblasts and synthesis of extracellular matrix. We present the improvement of skin conditions after application of GFs obtained through the unique process of transient expression in N. benthamiana. The adult plant is transfected with a vector containing the synthetic transcript identical to the human gene, and then high purity proteins are expressed by the plant. This system avoids the use of animal or bacterial cells to produce the protein and warrants rapid and reproducible production of non-GMO GFs for their use in cosmetics and medicine. Plant-derived GFs are identical to the human ones and were showed in our studies to restore and improve skin condition. Plant-Inalignus-Growth Factor 1 (plant-IGF1) increased keratinocytes growth (58%, p < 0.01) and wound regeneration. Furthermore, we obtained a ground-breaking result showing the activation of chymotrypsin-like detoxifying activity of the proteasome in primary human dermal fibroblasts (54%, p < 0.001) decreasing UVB-induced protein carboxylation (66%, p < 0.001) as a consequence. Plant-IGF1 also increased cytokinin 14 expression (68%, p < 0.001) and augmented epidermal thickness (33%, p < 0.001) in human skin explants. Finally, it improved skin conditions in vivo, decreasing the melanin content and reducing the number, depth and volume of wrinkles in the undereye area. Plant-Epidermal Growth Factor (plant-EGF) was demonstrated to regenerate by 100% the 1mm wounds in vivo and increase the levels of hyaluronic acid secreted by primary human keratocytes (41%, p < 0.01). It also increased the content of young (123%) and mature (26%) collagen, as well as elastin (45%, p < 0.05) in human skin explants. Plant-EGF improved skin appearance in vivo reducing the ulcer (up to 90% in depth up to 32%) and number of face wrinkles (11%), reducing the skin roughness (12%) and increasing skin elasticity (13%, p < 0.01).

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EGRF proteomics reveals novel Epha2-dependent trafficking and signaling pathways in epidermal keratinocytes
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Intercrine-signaling systems must be precisely controlled to maintain epidermal homeostasis. Receptor trafficking (RTKs) are integral in regulating these cell-cell communication networks. In particular, Epha2 and EGF transduce distinct signals to dictate keratocyte fate. Our work shows that loss of Epha2 impairs keratinocyte differentiation and barrier formation. Underlying this defect are changes in EGF signaling, localization, and degradation. We hypothesize that EGF2 promotes epidermal differentiation through upstream, negative regulation of EGF signaling. To probe how EGF2 dampens EGF, we performed loss-identification proteomics (BioID) to identify disparages in EGF-interacting proteins in keratinocytes with or without EGF2. As a result of these studies, we are the first to define a BioID proteome for EGF in 3D epidermal cultures having identified 121 putative interactors. Of these, 87 are novel compared to reported EGF associations. This protein subset was significantly upregulated upon EGF2 and did not express a greater change in protein abundance upon EGF2 treatment, which was characterized by a decrease in EGF receptor (EGFR) levels. Among this group, we identified 113P3E, which was the most significantly upregulated. AP2, an EGFR mutant that constitutively activates EGFR, increased apical localization of 113P3E and expression of LipoTrue Inc., New Brunswick, NJ

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CCN2/Connective tissue growth factor regulates G1 to S phase cell cycle progression in human skin fibroblasts
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CCN2 is a matricellular protein of the CCN family of extracellular matrix-associated proteins. CCN2 exerts a variety of functions, in a tissue and cell type-specific manner. In connective tissue, CCN2 promotes production of extracellular matrix, and contributes to the pathogenesis of fibrotic diseases. We report here the unexpected observation that CCN2 functions as a critical regulator of cell cycle progression in primary cultured human skin fibroblasts. Depletion of CCN2 in fibroblasts, by siRNA-mediated knockdown, resulted in near-complete inhibition of proliferation. Different siRNAs, which targeted either coding or non-coding CCN2 transcript sequences, yielded similar levels of CCN2 knockdown and inhibition of proliferation. Different siRNAs, which targeted either coding or non-coding CCN2 transcript sequences, yielded similar levels of CCN2 knockdown and inhibition of proliferation. Different siRNAs, which targeted either coding or non-coding CCN2 transcript sequences, yielded similar levels of CCN2 knockdown and inhibition of proliferation.
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**Absence of collagen VII binding to thrombospondin 1 promotes activation of TGF-beta in recombinant dystrophic epidermolysis bullosa**

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**Mutations in the gene encoding collagen VII (C7) cause the devastating blistering disease recessive dystrophic epidermolysis bullosa (RDEB). This disease is characterized by severe skin fragility and non-healing wounds aggravated by scarring and fibrosis. The altered skin architecture in RDEB also allows for development of fatal squamous cell carcinoma.**

Previously demonstrated that the matricellular protein thrombospondin 1 (TSP1) is increased in RDEB fibroblasts and that C7 expression modulates TSP1. Because TGF-beta is increased in RDEB and correlates with disease severity, and TSP1 is shown to activate TGF-beta, we investigated the role of TSP1 in TGF-beta activation in RDEB patient cells. Knock-down of TSP1 in RDEB fibroblasts caused decreased expression of SMAD2 and SMAD3, a reduction in the number and branching of newly formed blood vessels in the endocytic mutant. Together, this data suggests that VE-cadherin endocytosis and SMAD2/3 activation are both involved in TSP1 dependent TGF-beta activation in RDEB cells.

**Moreover, our data identifies TSP1 as a possible target for reducing fibrosis in the tumor-promoting dermal microenvironment in RDEB patients.**

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**VE-cadherin internalization coordinates endothelial cell functions during vascular development**

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**Formation of the hierarchically branched vascular system is driven by the angiogenic sprouting of endothelial cells from pre-existing vessels during early development. In order to form new blood vessels, endothelial cells must polarize and migrate collectively, while at the same time maintain vessel integrity and barrier function. This is accomplished in large part through the plasticity of cell-cell junctions and cell adhesion molecules, although the mechanisms underlying this plasticity remain poorly understood. Vascular endothelial cadherin (VE-cadherin) is the principal cell-cell adhesion molecule of the endothelial adherens junction, and is critical for vascular development, maintenance of vascular permeability in adults, and angiogenesis during tissue repair. Our study identified IRPA (intronic promoter transcripts; produced via intron retention and alternative polyadenylation (IRPA). As these transcripts reduce TGF-beta signaling, their overexpression in various human cell types was shown to enhance VE-cadherin endocytosis, a key mechanism of TGF-beta signaling. These results suggest a novel role for IRPA in the regulation of vascular development and function.**

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**A splicing switch provides dual layer control of laminin alpha 3 isoforms for squamous cell carcinoma**

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**The laminin 3 gene (LAMA3) generates two major isoforms through alternative promoters; LAMA3A (3A) and LAMA3B (3B). The ratio between these is implicated in squamous cell carcinoma (SCC), with high 3A relative to 3B correlating with progression. However, when we investigated the ratio of laminin (LM) 3A and 3B, it is found that each of the isoforms is distinct in function to support adhesion and migration. Previously, we reported reduced splice variant 3B promoter-driven transcripts, produced via insertion and alternative polyadenylation (APA). As these transcripts reduce 3B abundance, they could be involved in LAMA3A regulation. In this context, we showed that knock-down of LM3A increased the expression of LM3B, a mechanism involving alternative splicing of the LM3A promoter. In addition, we demonstrated that IGF-1 may be a new regulator of the LAMA3A-LM3B ratio at the transcript level, and in turn LM3A expression at the protein level. These findings have implications for wound repair and SCC.**

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**An exemplary claim substantiation for the firming / anti-sagging activity of a synthetic tripeptide**

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**Insulin-like growth factor 1 increases expression of decorin and biglycan as well as their molecular sizes in primary cultured human dermal fibroblasts. Their size increases are due to lengthened GAG chains, because treatment with chondroitinase ABC shifted decorin and biglycan to the original core protein size. In addition, protein levels of chondroitin sulfate (C7B) 1 and 3 were increased. We also observed higher mRNA levels of decorin, CHSY1, and CHSY3 but not that of biglycan. Increased transcriptional level of decorin is blocked by U0126, an MEK/ERK inhibitor and treatment with CREB siRNA, suggesting that IGF-1 induces decorin expression through activating MAPK/Erk/CREB pathway. IGF-1 also reduced ADAMES, which degrades biglycan, and its knockdown with siRNA resulted in augmented biglycan protein level, suggesting that increase of biglycan level by IGF-1 may be resulted from reduction of ADAMTS5. Taken together, our results suggest that IGF-1 may be a new regulator of decorin and biglycan in the skin.**

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**Growth Factors, Cell Adhesion and Matrix Biology | ABSTRACTS**

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**Secretion of TGFβ1 by fibroblasts and macrophages is executed by secretory autophagy**

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**TGFβ1 is a pleiotropic cytokine with cell type-specific effects modulating growth, survival and differentiation. It plays key roles in tissue fibrosis, inflammation and tumorgenesis. TGFβ1 is regulated as small latent complex (SLC), consisting of a LAP (latent-associated peptide) prodomain and the mature growth factor. SLC is tethered to latent TGFβ1 binding protein (LTBP) to form the large latent complex (LLC). Only this complex is efficiently released to the extracellular space where it is sequestered by anchorage to the extracellular matrix (ECM). Fibroblasts and key cells depositing ECM and their function crucially depends on autocrine TGFβ1 signaling. While signaling from different TGFβ receptors and extracellular activation of TGFβ1 are well understood, information on its intracellular trafficking and secretion is sparse. We have previously shown that TGFβ1 is secreted as a 3.5 kDa prepro-peptide by several cell types and that it is cleaved after secretion. In the present study, we reveal the involvement of secretory autophagy for TGFβ1 release from human and murine fibroblasts and macrophages. LAP-TGFβ1 co-localized with GRP94, involved in selective cargo for trafficking in specialized Golgi-derived vesicles. These structures were also positive for the autophagosomal marker LC3B, and EM analysis detected LAP-TGFβ1 in autophagic vacuoles.**

**Immunoprecipitation revealed that GRP94 specifically directs not only TGFβ1 but also other mammalian Atg-like proteins via an LC3-interacting motif (LIR). Depleting GRP94 or mutating the LIR motif severely impaired TGFβ1 secretion. Of note, inhibition of autophagy effectively blocked TGFβ1 secretion in human and murine fibroblasts and macrophages, underscoring the crucial importance of autophagosome formation for TGFβ1 secretion.**

**The regulated secretion of the potent cytokine TGFβ1 through this unconventional autophagy-dependent mechanism adds another level of control to TGFβ1 bioavailability and may offer novel targets for designing therapeutic agents that modulate TGFβ1 activity.**
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Hypoxia induced Cyt61 productions involved in the pathogenesis of infantile hemangio
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Our previous studies have shown that secreted extracellular matrix-associated protein Caveolin-2 is a common feature in the affected organs. Here, we performed a hypoxia-induced Cyt61 productions involved in the pathogenesis of infantile hemangioma (IHE). The mechanisms are still unclear, yet abnormalities of hypoxia, as well as involuting IHE(P<0.001). Moreover, hypoxia inducer CytC treatment dramatically induced Cyt61 production in HEMCs isolated from IHE patients in a time-dependent manner, reaching a peak at 12 hours. In hemangioma-derived stem cells (HEMCs) from IHE patients, PDTC and S检察官(1:25) obviously abolished Cyt61-stimulated VEGF-A synthesis, indicating that Cyt61 upregulation in HEMCs may contribute to their migration on NF-kB activation. Taken together, our results reveal a novel role of Cyt61 in promoting blood vessels proliferation under hypoxia in IHE via upregulation of VEGF-A production by HEMCs. Targeting of Cyt61 may represent a novel strategy in IHE treatment.

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E-cadherin binds desmoglein to facilitate desmosome assembly
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Desmosomes are intercellular adhesive junctions composed of desmocollin (Dsc) and desmoglein (Dsg) proteins. While previous studies demonstrated that classical cadherins are required for desmosome assembly, the mechanistic role of classical cadherins in desmosome formation is unknown. Here, we use single molecule force measurements, super resolution imaging, and structure/function analysis to resolve the roles of E-cadherin (Ecad) in desmosome assembly. AFM measurements reveal that Ecad forms short-lived interactions with Dsg2 but does not bind to Dsg1. Force measurements quantitate that Ecad and Dsg2 bind in an antiparallel manner, with a dissociation rate constant of 175 s⁻¹ at 25°C. In contrast, Dsg2 forms long-lived bonds with Dsg2, but interacts only weakly with Dsc2. Structure/function analysis shows that Ecad mutations that interfere with Ecad adhesion interactions or with Dsg2 interactions prevent timely desmosome assembly. Furthermore, super resolution imaging of desmosomes in human keratinocytes shows that while Ecad is localized to nascent desmosomes, it is excluded as desmosomes mature. Together, the data suggest a model in which initial Ecad/Ecad interactions promote the recruitment of desmosomal proteins via binding to Ecad to sites of nascent cell-cell contacts thus spatially coordinating and facilitating desmosome assembly.

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Comparison of the role of the EGF receptor ligand amphiregulin in normal versus malignant epithelial cells
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Amphiregulin (AREG) is the predominant EGF receptor (EGFR) ligand in normal human skin keratinocytes (KC) and loss of AREG function leads to growth arrest in G2/M/cellcycle, accompanied by PCP dysfunction. We previously reported that AREG silencing in two head and neck squamous cell carcinoma (HNSCC) cell lines (UMSCC22A and -22B, derived from the same patient, leads to a strong reduction in cell proliferation very similar to that observed in skin KCs (cell numbers reduced by ~80%). To further characterize the role of AREG in epithelial biology of normal and malignant oral epithelial cells, we assessed AREG function in a KC cell line derived from normal oral mucosa (OKF4) as well as in a panel of five HNSCC cell lines (UMSCC22A, -22B, -199, -497 and -812). Our results indicated that AREG expression in OKF4 was very similar to human skin KCs, with AREG mRNA being expressed at much higher levels than any other EGR ligand (7-fold more than TGFα and ~15 fold more than AREG and HBEGF). In contrast, EGFR ligand expression in the HNSCC cell lines was much more heterogeneous, with EGFR, HBEGF, and/or TGFα being expressed at levels similar to or higher than human skin KCs. Tetracycline (Tet)-inducible shRNA-mediated AREG silencing in stably transfected TetR-modified HNSCC lines efficiently reduced AREG transcript levels in all 5 lines (81.6 - 94.1% after 48 h of Tet treatment). Tet-induced AREG silencing led to a strong reduction in OKF4 cell growth (74.3% decrease in cell number relative to no Tet controls) but only to modest reductions of cell counts in the HNSCC cell lines (0.8 - 51.2%, average 31.3%). There was no correlation between AREG expression levels and silencing-mediated growth inhibition in the HNSCC lines. Together, our data show that while AREG plays an important role in the autocrine proliferation of normal oral KCs that is very similar to skin-derived KCs, HNSCC cells are much more heterogeneous with respect to AREG-driven autocrine EGFR signaling, likely due to aberrant downstream signaling.

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Epithelial integrin α3 impacts the composition of the cellular microenvironment
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Integrin α3β1 is widely expressed in epithelia at the cell-matrix and cell-cell interfaces. Loss of function mutations in ITGA3 cause intestinal lung disease, nephropathy syndrome and epidermolysis bullosa (IJBNE). The mechanisms are still unclear, yet abnormalities of the basement membranes are a common feature in the affected organs. Here, we performed a global unbiased analysis to detect changes in the abundance of soluble and deposited extracellular proteins secreted by integrin α3 deficient keratinocytes (A3) compared to normal human keratinocytes (Co). Cells were SILAC labeled, and the soluble and insoluble extracellular compartments were collected separately. Proteomic analysis was performed using experimental settings and bioinformatics data processing pipeline described before. In the identified proteins (EEMC) 167 proteins were identified as differentially regulated in A3 versus Co. Of the 217 proteins detected in the soluble ECM, 26% were regulated in A3. Gene expression studies revealed that regulation of EEMC proteins was mainly mediated by integrin α3. To demonstrate that the observed regulations are the direct consequence of the absence of integrin α3, we induced the rescue of α3 in the A3 cells by stable transduction with retroviral particles containing the full-length human ITGA3 (A3+α3). Using these cells in 2D and 3D-organotypic co-culture (OTC)models, we demonstrated that integrin α3 expression in keratinocytes switches the laminin-rich ECM to a fibronecin-rich ECM and significantly impacts the abundance of several EEMC proteins including nephrilin and chondroitin sulfate proteoglycan. Furthermore, these proteins were similarly regulated in the skin, kidney and lung samples of patients with IJBNE, supporting the relevance of our findings in vivo.

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Planar cell polarity-disrupting mutations alter Celsr1-mediated cell adhesion and dynamics
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Planar cell polarity (PCP), the collective and organized polarization of cells along a tissue plane, is essential to early embryonic development and tissue organization in complex, multicellular organisms. A striking example of PCP is the ordered alignment of body hairs in mammalian skin. Deficiencies in PCP components leads to neural tube defects, cardiomyopathies, as well as, abnormal hair patterning in the skin. Atypical cadherin Celsr is the principal component of an asymmetric junctional complex that regulates PCP. How Celsr mediates adhesion interactions to coordinate asymmetric PCP protein localization and function remains poorly understood. Using the mammalian epidermis as a model system, along with junctional recruitment and cell aggregation assays, we confirmed that Celsr1 mediates calcium-dependent, homophilic cell adhesion. Interestingly, disease-associated PCP variants Celsr1C198S and Celsr1D197S, which harbor mutations in the extracellular cadherin domain, still mediated cell aggregation. These mutants lack the ability to enrich at epithelial junctions, suggesting that intercellular adhesion alone cannot explain Celsr1 functions. Surprisingly, Celsr1C198S aggregates segregated from Celsr1D197S clusters when mixed, demonstrating this point mutation generates a novel Celsr1 variant with altered homophilic binding properties. Further, Celsr1C198S displayed broader cell surface distribution and reduced junction localization relative to WT even when in adhesive aggregates. Stable association of Celsr1 at the membrane was also substantially reduced in Celsr1⁺/⁻ mice lacking the membrane. These observations indicate that a fibronectin-rich ECM and significantly impacts the abundance of several EEMC proteins including nephrilin and chondroitin sulfate proteoglycan. Furthermore, these proteins were similarly regulated in the skin, kidney and lung samples of patients with IJBNE, supporting the relevance of our findings in vivo.
Elevated expression of osteopontin splice variants in nonmelanoma skin cancer compared to normal skin and adult keratinocytes

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Osteopontin (OPN) is a matricellular protein, whose expression is increased in cancer, and which plays a critical role in regulating cell adhesion and proliferation. This study investigated the expression patterns of OPN splice variants in normal skin, nonmelanoma skin cancer, and adult keratinocytes. RT-qPCR analysis revealed significantly higher OPN splice variant expression in nonmelanoma skin cancer compared to normal skin. Additionally, adult keratinocytes expressed several OPN splice variants that are different from those expressed in normal skin. The results suggest that OPN splice variants may have different regulatory roles in normal skin, skin cancer, and adult keratinocytes.

Platelet expression of GFs in PRP samples prepared from patient volunteers by quantitative RT-qPCR

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Platelet-rich plasma (PRP) is a concentrate of autologous platelets enriched in growth factors (GFs). This study investigated the expression of platelet-derived GFs in PRP samples prepared from patient volunteers. The study found that detectable transcript levels were noticed for IGF and HGF. Variability in platelet expression of GFs could determine patient response to PRP therapy.

Human adult keratinocytes express OPN-a, OPN-b, and OPN-c splice variants

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Human adult keratinocytes express several OPN splice variants, including OPN-a, OPN-b, and OPN-c. This study investigated the expression of OPN splice variants in human adult keratinocytes (HaCaT). The results showed that HaCaT keratinocytes express OPN-a, OPN-b, and OPN-c splice variants. The study suggests that the expression of OPN splice variants may have different regulatory roles in normal skin, skin cancer, and adult keratinocytes.

Integration of mechanotransduction and matrix remodeling

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Mechanotransduction is a critical process that regulates cell function and development. This study investigated the integration of mechanotransduction and matrix remodeling in keratinocytes. The results showed that mechanotransduction and matrix remodeling are linked through the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs).

Nonmelanoma skin cancer is marked by increased expression of OPN splice variants

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Nonmelanoma skin cancer is marked by increased expression of OPN splice variants. This study investigated the expression of OPN splice variants in nonmelanoma skin cancer compared to normal skin. The results showed that nonmelanoma skin cancer expresses several OPN splice variants, including OPN-a, OPN-b, and OPN-c. The study suggests that the expression of OPN splice variants may have different regulatory roles in normal skin, skin cancer, and adult keratinocytes.

IL-9-mediated differentiation and migration of hair follicles

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IL-9 is a cytokine that regulates the differentiation and migration of hair follicles. This study investigated the role of IL-9 in the differentiation and migration of hair follicles. The results showed that IL-9-mediated differentiation and migration of hair follicles are dependent on the activation of the IL-9R signaling pathway.
Divergent trends in acne vulgaris interest and research a decade of lost face

value in the United States

A Park1 and J Okhovat2

Journal of Investigative Dermatology (2018), Volume 138

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Comparison of Sensitite YeastOne® colorimetric antifungal panel with routine CSLI tests against dematiae fungi

Y Lim S1, Y Cai2, T Zhang2, X Li3, G Li2 and C Zhang2

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Detection of scabies by anti-house dust mite antibody immunoblot test

W Eng1 and R Norman2

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Rapid differentiation of dermal fibroblastic stem cells into adipocytes (reactive adipogenesis) enhances skin immunity.

Enhancing this response could be clinically beneficial, and we hypothesized that retinoids might play a role in the regulation of this process. Upon induction of adipogenesis under differentiation conditions, 3T3-L1 preadipocytes showed a rapid and transient increase in cathepsin D (Campi mRNA expression (31-fold over control, p value < 0.0001). Addition of retinoid acid (RA) or retinol further enhanced this response (~120 fold vs. control, p < 0.0001). The presence of RA also enabled sustained elevated levels of Campi expression over time. This transcriptional response also led to higher CRAMP protein levels as detected by immunoblotting of cell supernatants. The increased protein expression translated to increased anti-microbial activity as detected by the assay of capacity of culture supernatant to kill S. aureus. In these antimicrobial assays, culture supernatant from 3T3-L1 adipocytes treated with RA showed higher activity than control supernatants. RA also significantly reduced the bacterial persistence, indicating that retinoids could play a role in enhancing the skin immunity.

Retinoids improve innate immune defense by enhancing reactive dermal adipogenesis

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Discovery of a receptor-dependent step in cathelicidin activation of inflammation identifies a novel therapeutic target for psoriasis and rosacea

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The cathelicidin antimicrobial peptide LL37 is a critical element in host defense but inappropriate expression breaks tolerance to self-nucelic acids and promotes inflammatory skin diseases such as psoriasis and rosacea. To understand the mechanism responsible for LL37-dependent inflammation we performed RNA-Seq on human keratinocytes (NHEK) exposed to LL37 and synthetic U1 RNA (U1), a self non-coding RNA released upon tissue damage. Compared to LL37 or U1 RNA alone, the transcriptional response of NHEK to the combination of both LL37 and U1 was unique and included a notable Type 1 interferon signature (167 genes were uniquely increased by 2-fold or more). Screening of a peptide library derived from LL37 showed that the ability of LL37 to penetrate membranes was not necessary for breaking tolerance. A combination of LL37 and U1 activated both binding of U1 to scavenger receptors on NHEK and macrophages. Use of siRNA against scavenger receptors disrupted this binding and inhibited the inflammatory cytokine response to the combination of LL37 and U1 (P < 0.01 in qPCR, P < 0.001 in PLA). This suggested a 3-way binding interaction with scavenger receptors, LL37 and U1 was required. Inhibitors of endocytosis further established that the ability of LL37 to stimulate expression of IL-6 and interferon-β1 was dependent on clathrin-mediated endocytosis. With this information, a library screen showed binding could be blocked by a competitive peptide and resulted in inhibition of the cytokine and interferon response to LL37 and U1. Analysis of psoriatic lesional skin showed that the binding of LL37 to scavenger receptors occurs in human skin. These results indicate that the inflammatory activity of LL37 is mediated by a cell surface, receptor-dependent interaction that is potentially therapeutically targetable.

β-Defensin 103 characterizes a distinct molecular phenotype of human acral melanoma, by its correlated expression with IL-17A and IFNγ-mediated immune genes, as well as MC1R-mediated pigmentary signatures

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β-Defensin 103 (β-Def103) is highly expressed in stratum corneum and regulated by IL-17A and IFNγ. We recently identified an as MC1R receptor agonist, which increases levels of pigmentation in mammals, and regulates melanoma cell migration. Our human melanoma enrichment of melanoma signatures was not different between acral vs. non-acral melanoma genes, as well as MC1R-mediated pigmentation signatures (MITF (p < 0.49)) (p < 0.05). Those correlations were not significant in normal skin (p = 0.83), IL-17F (p = 0.86), IL-20 (p = 0.62), TYR (p = 0.59), DEFB103B (p = 0.83), the expression of IL-17A pathway genes (IL-17A (p = 0.77), IL-17F (p = 0.50), IL-23 (p = 0.49), IL-19 (p = 0.86), IL-20 (p = 0.97), IFNγ pathway genes (IFNα (p = 0.49), and MCIR-mediated signatures (MTF (p = 0.62), TYR (p = 0.49). Those correlations were not significant in normal skin (p > 0.05). Thus, we propose that DEF103B is a novel access of keratinocytes to melanocytes via MCIR, which could be a potential mechanism for abnormally dark pigmentation and invasive tumor progression of acral melanoma.

MicroRNA-17-92 cluster promotes the proliferation and the chemokine production of keratinocytes: Implication for the pathogenesis of psoriasis

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MicroRNA-17-92 (miR-17-92) is a miRNA cluster that regulates cell growth, metabolism, and inflammation. Its expression is temporally regulated in inflamed young skins. In contrast, a delayed and weaker inflammatory response with a seemingly defective production of specialized pro-resolving mediators was noticed in older skins. Using principal component analysis, we have also shown that in young skin arachidonic acid pathway was highly mobilized with subsequent biosynthesis of both pro- and anti-inflammatory mediators (PG2, TXB2) and pro-resolving mediators (LXα4, LXb4). In old skins, the EP2/DHA pathways were rather mobilized without biosynthesis of final pro-resolving mediators. Hence, the metabololipidomic profiling of old skins uncovered an endogenous resolution program of inflammation that was associated with dysregulated and/or absence of pro-resolving mediators. Taken together, the present results seem to indicate a role for lipids to reestablish cutaneous inflammation during aging and to rescue failed resolution in aged skin.

β-Defensin 103 characterizes a distinct molecular phenotype of human acral melanoma, by its correlated expression with IL-17A and IFNγ-mediated immune genes, as well as MC1R-mediated pigmentary signatures

1 Gattefosse, Saint-Priest, Rhone-Alpes, France, 2 Ambiotis, Toulouse, Languedoc-Roussillon, France

The skin immune system is regulated by bioactive lipids that initiate and amplify inflammation but control its efficient ending also called resolution. When dysregulated, bioactive lipid mediators contribute to skin pathologies by unresolved inflammation leading sometimes to chronic inflammation or fibrosis. Recently, age-associated alterations in inflammation and resolution programs were reported in aged mice allowing us to hypothesize that inflammation and its resolution could be impaired in aged human skins. Using PMA-treated skin explants from young (24±4 y) old and 58±3 y donors, we have performed a metabololipidomic study using LCMS-MS. We have shown that prostaglandinE2 (PG2), a pro-inflammatory mediator as well as the lipoxins LxA4, LxB4, pro-resolving biomarkers were produced and temporally regulated in inflamed young skins. In contrast, a delayed and weaker inflammatory response with a seemingly defective production of specialized pro-resolving mediators was noticed in old skins. Using principal component analysis, we have also shown that in young skin arachidonic acid pathway was highly mobilized with subsequent biosynthesis of both pro- and anti-inflammatory mediators (PG2, TXB2) and pro-resolving mediators (LXα4, LXb4). In old skins, the EP2/DHA pathways were rather mobilized without biosynthesis of final pro-resolving mediators. Hence, the metabololipidomic profiling of old skins uncovered an endogenous resolution program of inflammation that was associated with dysregulated and/or absence of pro-resolving mediators. Taken together, the present results seem to indicate a role for lipids to reestablish cutaneous inflammation during aging and to rescue failed resolution in aged skin.

MicroRNA-17-92 cluster promotes the proliferation and the chemokine production of keratinocytes: Implication for the pathogenesis of psoriasis

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1 Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi’an, Shaanxi, China and 2 Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi’an, Shaanxi, China

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Prebiotic stimuli alter gene expression in skin

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Skin microbiome has an important role as host guardian, contributing to several physiological processes including skin barrier maintenance and protection against pathogenic microorganisms. It is postulated that skin microbiome communicates with skin cells regulating protein turnover and microbial adhesion to keratinocytes. Prebiotics are widely used for improving the health of digestive tract that reflects itself on skin surface. Most ingredients used in topical use formulations might have an unknown prebiotic effect that contributes to its performance on skin. Little is known about the mechanisms triggered by specific ingredients that have prebiotic effect in skin gene expression. In this study, we tested different prebiotic technologies in a cellular model and showed that they are able to modulate significantly gene expression related to skin maintenance, differently modifying skin milieu depending on the technology used. Moreover, these ingredients interfere in microorganism adhesion to keratinocytes and prebiotics and the microbiome are important to maintain skin health and that these technologies can be addressed to preserve skin integrity.
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Skin microbiome has an important role as host guardian, contributing to several physiological functions including skin tanning, epidermal differentiation, and barrier function. Skin microbiome plays a crucial role in maintaining skin health. In recent years, there has been a growing interest in understanding the role of the skin microbiome in various skin disorders, including acne, atopic dermatitis, and psoriasis. These disorders are characterized by dysbiosis, where there is a imbalance in the microorganisms that colonize the skin. Understanding the role of the skin microbiome in these disorders is crucial for developing new therapeutic strategies. In this study, we investigated the role of the skin microbiome in psoriasis, a chronic inflammatory skin disorder that affects millions of people worldwide. We found that the skin microbiome of patients with psoriasis is different from that of healthy controls. Specifically, we observed changes in the abundance of certain microbial taxa, which may contribute to the pathogenesis of psoriasis. We also found that the skin microbiome of patients with psoriasis is associated with increased expression of pro-inflammatory cytokines, suggesting a role for the skin microbiome in the development of psoriasis.

**Skin pathology**

Psoriasis is a chronic inflammatory skin disorder that is characterized by the development of raised, red, itchy plaques on the skin. These plaques are covered by a scaly, silver-white substance called scale. The disease is characterized by increased proliferation of keratinocytes, the cells that make up the skin. In psoriasis, the turnover of these cells is accelerated, leading to the formation of plaques.

**Microbiome analysis**

We used 16S rRNA gene sequencing to analyze the skin microbiome of patients with psoriasis and healthy controls. We observed changes in the abundance of certain microbial taxa in the skin of patients with psoriasis. Specifically, we found an increase in the abundance of Firmicutes and a decrease in the abundance of Bacteroidetes in the skin of patients with psoriasis. We also observed changes in the abundance of specific bacterial species, such as Staphylococcus and Propionibacterium, which are known to be associated with skin disorders.

**Conclusion**

Our findings suggest that the skin microbiome plays a role in the development of psoriasis. Understanding the role of the skin microbiome in psoriasis is crucial for developing new therapeutic strategies. In the future, we aim to investigate the potential of targeting the skin microbiome as a therapeutic strategy for psoriasis.

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**Aberrant expression of the antimicrobial protein REGA1 in psoriasis**

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**Background**

Psoriasis is a chronic inflammatory skin disorder that affects millions of people worldwide. It is characterized by the development of raised, red, itchy plaques on the skin. The disease is characterized by increased proliferation of keratinocytes, the cells that make up the skin. In psoriasis, the turnover of these cells is accelerated, leading to the formation of plaques.

**Objective**

The aim of this study was to investigate the expression of the antimicrobial protein REGA1 in psoriasis.

**Materials and Methods**

We used immunohistochemistry to analyze the expression of REGA1 in skin biopsies from patients with psoriasis and healthy controls. We also performed qPCR to analyze the expression of REGA1 in skin cell lines.

**Results**

We found that the expression of REGA1 was decreased in skin biopsies from patients with psoriasis. We also found that the expression of REGA1 was decreased in skin cell lines from patients with psoriasis.

**Conclusion**

Our findings suggest that the expression of the antimicrobial protein REGA1 is decreased in psoriasis. This may indicate a role for the skin microbiome in the development of psoriasis. In the future, we aim to investigate the potential of targeting the skin microbiome as a therapeutic strategy for psoriasis.

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** Skin remodeling after probiotic stimuli **

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**Background**

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host. The skin microbiome plays a crucial role in maintaining skin health. In recent years, there has been a growing interest in understanding the role of the skin microbiome in various skin disorders, including acne, atopic dermatitis, and psoriasis. These disorders are characterized by dysbiosis, where there is a imbalance in the microorganisms that colonize the skin. Understanding the role of the skin microbiome in these disorders is crucial for developing new therapeutic strategies. In this study, we investigated the role of the skin microbiome in psoriasis, a chronic inflammatory skin disorder that affects millions of people worldwide. We found that the skin microbiome of patients with psoriasis is different from that of healthy controls. Specifically, we observed changes in the abundance of certain microbial taxa, which may contribute to the pathogenesis of psoriasis. We also found that the skin microbiome of patients with psoriasis is associated with increased expression of pro-inflammatory cytokines, suggesting a role for the skin microbiome in the development of psoriasis.

**Methods**

We used 16S rRNA gene sequencing to analyze the skin microbiome of patients with psoriasis and healthy controls. We also performed qPCR to analyze the expression of REGA1 in skin cell lines.

**Results**

We found that the expression of REGA1 was decreased in skin biopsies from patients with psoriasis. We also found that the expression of REGA1 was decreased in skin cell lines from patients with psoriasis.

**Conclusion**

Our findings suggest that the expression of the antimicrobial protein REGA1 is decreased in psoriasis. This may indicate a role for the skin microbiome in the development of psoriasis. In the future, we aim to investigate the potential of targeting the skin microbiome as a therapeutic strategy for psoriasis.

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**Background**

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We used 16S rRNA gene sequencing to analyze the skin microbiome of patients with psoriasis and healthy controls. We also performed qPCR to analyze the expression of REGA1 in skin cell lines. **Results**

We found that the expression of REGA1 was decreased in skin biopsies from patients with psoriasis. We also found that the expression of REGA1 was decreased in skin cell lines from patients with psoriasis. **Conclusion**

Our findings suggest that the expression of the antimicrobial protein REGA1 is decreased in psoriasis. This may indicate a role for the skin microbiome in the development of psoriasis. In the future, we aim to investigate the potential of targeting the skin microbiome as a therapeutic strategy for psoriasis.
Identification of allergens for wheat-dependent exercise-induced anaphylaxis developed by sensitization to grass pollen

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We identified several cases of wheat-dependent exercise-induced anaphylaxis (WDEIA) of which typical symptoms are eyelid edema. Although we have identified -5 gladin as a major wheat allergen for typical type of WDEIA, these cases are not sensitized with -5 gladin, but strongly sensitized to grass pollens. These cases possibly developed as pollen-food allergy syndrome (PFAS-WDEIA) due to cross reaction between wheat proteins and grass pollen proteins. In this study, we aimed to identify cross-reacting wheat allergens to grass pollen. We used sera obtained from 5 patients who were diagnosed as PFAS-WDEIA according to the clinical symptoms and skin prick test or/and serum levels of specific IgE to wheat and grass pollens. All of the 5 patients had serum IgE specific to grass pollen (class 4-6) and 4 patients had IgE specific to crude wheat proteins (class 2-4) but not IgE specific to -5 gladin with ImmunoCAP (Pharmacia). For specificity of the assay, we used sera obtained from the patients as IgE-antibodies (anti-IgE antibody) and found no cross reactivity. allergens for wheat-dependent exercise-induced anaphylaxis were identified in the sera of patients with PFAS-WDEIA. These results suggest that wheat and grass pollen may share common allergens.

Age-associated changes in the human skin immune environment

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Aging is accompanied by significant alterations in the function of the immune system associated with increased susceptibility to bacterial and viral infections, and increased risk of cancers. Skin with its complex network of immune cells and factors acts as a first line of physical and immunological defense against environmental insults. However, age-related changes in skin immune system is largely unknown. To establish a comprehensive skin immune atlas, we screened over 200 human skin samples obtained as part of our human tissue biobank project to identify cohorts of sun-protected skin from the same anatomical site of young and elderly women (12 samples in each group). First, we studied the age-related changes in Langerhans cells (LCs), which are skin resident dendritic cells (DCs) and play a sentinel role as the front line immune barrier against foreign allergens and pathogens. We found that the number of epidermal LCs is significantly reduced with age. In stark contrast, we observed a significant increase in dermal DCs together with increase in CD4+ and CD8+ T cells in the skin of elderly woman. In the following study, we aimed to study the age-related changes in the skin immune environment, which will have clear implications for skin health, inflammatory diseases and cancer.

Ablation of basophils reduces ILC2-dependent atopic dermatitis-like inflammation in mice overexpressing interleukin-33 in the skin

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We utilized IL33Tg mice, which express IL-33 protein in the skin, to study the role of basophils in the development of atopic dermatitis-like inflammation. We depleted basophils using anti- IgE antibody and observed a significant increase in dermal DCs together with increase in CD4+ and CD8+ T cells in the skin of IL33Tg mice. The dermatitis that developed in DT-treated IL33 Bas-TRECK Tg mice was milder, and the concentration of Th2 cytokines in the skin was lower than in control mice. These results suggest that basophils exacerbate IL-33-induced, ILC2-mediated AD-like dermatitis.

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908 Various pro-inflammatory stimuli, including particulatematter pollution, induce hyperpigmentation in 3Dreconstructed skin models in vitro

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Induction of melanin biosynthesis is a tightly regulated process that can be modulated via internal and external stimuli. Externally, the skin often is exposed to environmental stimuli and stresses such as solar UV and infrared light, as well as to environmental pollution, all of which are able to modulate skin pigmentation. Post-inflammatory hyperpigmentation (PH) is a common, acquired pigmentation disorder caused by inflammatory dermatochromes (including acne-related hyperpigmentation), infections, reaction to drugs, dermatologic procedures, and external injury (burns, frictions, trauma). PH may occur in all skin phototypes but is most common among individuals with Fitzpatrick skin phototypes III to V. In this study, we investigated in vitro the consequences of the application of various stimuli on cultured keratinocytes and melanocytes, and on reconstructed human epidermis. We investigated the potential of P. acnes application to induce hyperpigmentation in a reconstructed pigmented epidermis model. We prepared a dermal cell monolayer bilayered on Matrigel to resemble certain types of environmental pollution (e.g., particulate matter, ultrafine particles) and UV light on 3D models. In these in vitro testing approaches on skin reconstructed models represent a promising tool for monitoring in vitro the potential of various stimuli to induce hyperpigmentation, and for the evaluation of biofunctional ingredients or other chemical substances that may demonstrate protective activity.
The cytosolic dsRNA-sensing pathway mediated by MDA5/MAVS/IRF3 is critical for the induction of type I and III IFNs after viral infection of skin keratinocytes.

Histopathologic patterns in maculopapular drug eruptions and relation with systemic involvement

Drug eruptions are generally present as a maculopapular rash with a benign course and rapid healing. Systemic features such as fever, lymph node enlargement, or involvement of the skin, liver, and viscera are present in some patients with maculopapular eruption (MPE). Although some recent studies exist for DRESS syndrome, clinical and histopathologic findings of MPE with systemic features have not been studied well. Patients with MPE on dermatology inpatient clinic, from January 2012 to January 2017, evaluated in this retrospective descriptive study. Patients were separated into three groups according to the evidence of systemic involvement and severity, MPE with mild systemic symptoms (MMP), MPE with severe systemic symptoms (sMPE) and MPE with severe systemic symptoms (sMPE). Histopathologic patterns and total number of histopathologic pattern in same lesion were compared between each groups. Total number of patients were 41. There were 18 patients in MPE group, 14 patients in sMPE group and 9 patients in sMPE group. Superficial perivascular lymphocytic infiltration was the main histopathologic change in all patients. Interphase changes and deep dermal infiltration found to be related with systemic involvement (p<0.001). Eczematous pattern, deep dermal fibrosis and interphase dermatitis, deep dermal infiltration and gene expression studies in mice, we demonstrate that the late phase of MPE with systemic involvement of immunohematologic significance, in mice and men, with systemic involvement of immunohematologic significance, in mice and men, with systemic involvement of immunohematologic significance, in mice and men, with systemic involvement of immunohematologic significance, in mice and men, with systemic involvement of immunohematologic significance, in mice and men, with systemic involvement of immunohematologic significance.
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TRPM2-dependent NLRP3 inflammasome activation exacerbates the oxidative stress-driven immune response in patients with vitiligo. S Liu1, L Zhang2, C Zhang3, Y Wang2, Y Gao1 and C Li1 1 Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi’an, Shaanxi, China, 2 Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi’an, Shaanxi, China, 3 Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi’an, Shaanxi, China

The NLRP3 inflammasome plays a key role in sensing pathogens and danger signals in the innate immune system and is thought to be involved in oxidative stress-driven undesirable immune response of vitiligo, a common skin disease characterized by melanocyte destruction by CD8+ T cells. However, the mechanism by which the NLRP3 inflammasome activates and induces vitiligo is not clear. In this study, we revealed that NLRP3 and IL-1β expression increased in the epidermis and lesional patients with vitiligo, and IL-1β was upregulated by TRPM2 channels, could up-regulate chemotaxis-related proteins, including CXCL10 and CXCL2, in CD8+ T cells. However, the mechanism by which the NLRP3 inflammasome activates and thereby contributes to dysregulated autoimmune response in patients with vitiligo.

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New role of exogenous HMGB1 for human keratinocyte under acute inflammatory event. M Kusakabe1, Y Imai1, K Y asuda2, K Nakanishi2, T Y oshimoto3 and K Y amanishi1 1 Dermatology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan, 2 Immunology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan, 3 Immunology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan

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Characterization of eicosanoid species altered in psoriatic skin. M Beamer1, W Swindell2, LC Tsoi3, Y Liang4, M Sarkar5, X Xing1, M McDonald1, JJ Nos- thes1, V Scott1 and JE Gadzukson1 1 Dermatology, U of M, Ann Arbor, MI, 2 Univ. of Michigan, Athens, OH, 3 Univ. of Michigan, Ann Arbor, MI, 4 Dermatology Discovery AbbVie, North Chicago, IL and 5 Department of Dermatology, University of Michigan, Ann Arbor, MI

Eicosanoids are a class of signaling molecules that are made by the enzymatic or non-enzymatic oxidation of arachidonic acid or other polyunsaturated fatty acids. Eicosanoids influence a diverse physiology as well as pathologic processes including inflammatory reactions. While some work has been done on individual eicosanoid species in psoriasis, no unbiased global profiling of eicosanoid species has been published to date in psoriasis. Using a targeted approach, we measured the concentrations of 161 eicosanoids in 10 lesional and 10 non-lesional and 10 control skin using liquid chromatography in conjunction with mass spectrometry. Eicosanoid levels were normalized against total protein concentration. Using a false-discovery-rate (FDR) rate of p<0.01, 9-HETE (9.5-fold, FDR<0.01), 5-HETE (3.3-fold, FDR<0.01), 10 HDoHE (2-fold, FDR<0.01) in psoriatic skin compared to either non-lesional or control skin. Resolvin D1, which is a product of 11-lipoxygenase (11-LOX) metabolism,mediated by 11-LOX, was increased in lesional skin. Furthermore, the 12-lipoxygenase (12-LOX) metabolites of lipoxins and leukotrienes were increased in lesional skin. Strikingly, the 15-LOX metabolites of resolvins and protectins were markedly decreased in lesional skin. These results provide new paradigms for the mechanisms that trigger skin inflammation and they depict key data to develop future therapeutic options.
Skin resident innate lymphoid cells play an integral role in homeostatic regulation of sebaceous glands via TNF/Lymphoid receptor signaling to maintain skin homeostasis. T Kobayashi, D Kim, B Vossin, T Doebel, A Truong, J Lee, J Jo, F Kennedy, H Kong and K Nagao NIAH/NIH, Bethesda, MD

Epithelial and immune cells in skin have co-evolved to establish sophisticated barrier systems against microorganisms. Innate immunity is essential for host defense, however, the role of innate immune cells on the regulation of epithelial homeostasis remains underappreciated. Here, we uncovered spatial and functional diversity of skin-resident innate lymphoid cells (ILCs), via defining different transcriptional and regolome landscapes. A unique subset of ILCs, which were preferentially localized in close proximity to sebaceous glands, required CRD for localization and relied on cooperative effects of IL-7 and TSLP for skin-residency. Strikingly, the absence of IL-21 in Rag2 Il2rg-/- mice led to sebaceous hyperplasia. 16S rRNA microbiome analysis revealed that, while lymphocytes were essential to regulate Gram-positive cocci (Staphylococcus), a SA pathway was induced in keratinocytes. Interestingly, both PSM and NCa pathways were characterized in sebaceous hyperplasia, which was accompanied by excess production of sebum. Sebaceous hyperplasia was also observed in the selective absence of epithelial and dermal ILCs in Il7 Tdlp/- mice. Human sebocytes cultured with various cytokines that were differentially expressed by epithelial ILCs revealed that TNF/Lymphoid receptor ligands mediate innate lymphocyte responses with significant downregulation of the Notch ligand Jag2. Indeed, siRNA knockdown of Jag2 inhibited sebocyte proliferation in vitro. Furthermore, ablation of Tnf/Lymphoid receptor signaling in vivo (Tof Il7 Tdlp/- mice) led to sebaceous hyperplasia. 16S rRNA microbiome analysis revealed that, while lymphocytes were essential to regulate Gram-positive cocci (Staphylococcus), a SA pathway was induced in keratinocytes.

17 Candidalysin, a virulence factor of Candida albicans, activates human keratinocytes through MAPK and NF-kB pathways
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Pсориазis is a chronic autoimmune and inflammatory skin condition mediated by keratinocytes, T cells and cytokines. Recent evidence implicates inflammatory cytokines such as IL-17, IL-22 and IL-23 in the pathogenesis of pсориазis. Interestingly, these cytokines are involved in the host response against Candida species, which are known to exacerbate the psoriatic process. It is thought that superantigens and toxins of Candida can worsen pсориазis symptoms, however, the fungal virulence factors responsible for the psoriatic exacerbation are not well understood. Recently, candidalysin, a cytotoxic peptide-toxin secreted by C. albicans hyphae was found to be an essential factor required for C. albicans infections. Given that candidalysin activates epithelial immune responses and damages host epithelial membranes, we hypothesized that candidalysin might activate keratinocytes, therefore playing a key role in the inflammatory process in skin pсориазis. In this study, we examined the effects of candidalysin on human keratinocytes and clarified the underlying molecular mechanism. Treatment of normal human keratinocytes with increasing doses of candidalysin resulted in dose-dependent production of various cytokines and chemokines, including IL-12, IL-6, IL-8, GM-CSF and MIP-3α, which have been implicated in pсориазis pathogenesis. Candidalysin also caused cell damage, as confirmed by activation of the MAPK phosphatase MP1 and c-Fos pathways that are associated with cell damage. Furthermore, candidalysin induced phosphorylation of ERK and MAPKs of ERK, p38 but not JNK. Indeed, we confirmed that MAPK and NF-kB signaling pathways were required for the candidalysin-induced keratinocyte activation, as evidenced by the inhibitory effects of ERK, p38- and NF-kB-specific inhibitors. Together, these findings suggest that candidalysin might act as a trigger for the exacerbation and perseverance of skin pсориазis through activation of keratinocytes.

Quorum sensing between bacterial species on skin protects against barrier disruption and inflammation promoted by Staphylococcus aureus
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Staphylococcus aureus (SA) is associated with increased severity of atopic dermatitis (AD) and promotes skin barrier disruption and Th2/Th17 inflammation in murine models. To investigate the mechanisms responsible for this phenotype, we tested the response of human keratinocytes and murine skin models to SA with targeted gene deletions in specific toxins. A SA phenol-soluble modulin (PSM) was responsible for induction of transacylase activity and IL6 in keratinocytes. Interestingly both PSM and SA secreted proteases were crucial for inducing barrier damage in mice (increased transepidermal water loss (TEWL) and transacylase activity). Indeed, a PSM mutant, and a SA mutant under control of the agr quorum-sensing system and thus we hypothesized that this might be a target for other members of the skin microbiome to influence SA. Using a clinically isolated S. hominis (SH) strain and a beta-lactam resistant strain, we demonstrated that a secreted factor (<3Da) from SH, identified as a novel autoinducing peptide (AIP), could prevent transcription of SA toxins by inhibiting agr activation in SA (baseline SA agr activity:1794.39±150.27RFU) vs 188.32±22.6 (P<0.05). Inhibition of SA agr growth but prevented the response of keratinocytes to SA (baseline SA transepacylase activity:7.94±1.64(D04315m/h) vs 17.74±4.68(D04315m/h) with SH). Inhibition of agr activity also potentially prevented skin inflammation and barrier damage in mice (baseline TEWL with SH vs SA: 8.3 ± 2.2 vs 25.1 ± 2.7, P<0.05). The SH-defined multiple species and strains of skin microbes that can turn off SA agr activity including SH, S. epidermidis, and S. warneri. Overall these findings show the benefits of a diverse skin microbiome and provide support for a new therapeutic rationale of applying specific communal microbes to AD patients.

Urinary squamous cell carcinoma antigen in psoriasis patients: The first pilot study
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This is the first pilot study aimed to explore the level of urinary squamous cell carcinoma antigen (SCCA) in psoriasis patients and its relationship with disease severity. Fifteen psoriasis patients were treated with adalimumab. At different visits before and after treatment, quantitative body surface area (qBSA) was obtained from standardized digital body images of the patients, and pсориазis area severity index (PASI) was also monitored. Urine squamous cell carcinoma antigen (SCCA) were detected by using Microparticle Enzyme Immuno-Assay. The urine SCCA were also tested in 20 healthy volunteers as normal control. We found that the SCCA level in psoriasis group was significantly higher than in the normal control group (31.2±22.9ng/ml vs. 11.7±2.5±1 ng/ml, P<0.05). The SCCA level in psoriasis group was significantly higher than in the normal control group (31.2±22.9ng/ml vs. 11.7±2.5±1 ng/ml, P<0.05). A slightly higher but non-significant urine SCCA level was observed in the female psoriasis patients (vs. male patients, P > 0.05). However, after treatment, the serum SCCA levels were not significantly decreased (P > 0.05). And the urine SCCA level was not correlated to PASI or qBSA. In conclusion, urine SCCA is elevated in psoriasis patients, but not correlated with disease severity or treatment response.
922 HIV-1 trans-infection of T-cells via dendritic cells requires endosomal sorting and polarized trafficking to the virological synapse
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Dendritic cells (DC) are the ‘sentinels' of the immune system and play an essential role in the presentation of antigenic material to T-cells. Human Immunodeficiency Virus 1 (HIV-1) is taken into DC mediating degradation and transported to a virological synapse (VS) to promote T-cell infection. DC studies have led to the identification of several restriction factors that aid viral transmembrane, however the virus is trafficked through the cell to the VS within DC is still unclear. A high-throughput siRNA method was developed to monocyte derived dendritic cells (MoDCs) to identify membrane trafficking proteins involved in the transfer of HIV from DC to T-cells. Characterization of siRNA hit showed that a functional endosomal to polarized transport is required for efficient HIV transfer to T-cells and trapping virus in early endosomal-derived compartments significantly reduces viral trans-infection. Novel proteins identified by the siRNA screen share common roles in cargo sorting and transport from endosomal compartments and polarized transport to the plasma membrane. In addition, depletion of these target proteins resulted in an accumulation of virus in intracellular compartments and reduced VS formation between DC and T-cells. Identification of cellular candidates involved in viral transfer from DC to T-cells is critical for the development of potential drug therapies to combat HIV-1 infection in vivo.

924 Functional divergence of skin Human Polyomaviruses (HPyVs)
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Human Polyomaviruses (HPyVs) are prevalent in many different types of tissues including skin. As its name implies, some members of this family have oncogenic potential. Merkel Cell Polyomavirus (MCPyV) plays a causative role in about 80% of Merkel Cell Carcinomas. However, other closely related HPyVs do not appear to be oncogenic. The functional diversities that underlie differences in transforming potential remain unclear. The small T antigen of MCPyV has been reported to have transforming properties on mammalian cells. Here, we studied the functional properties of small T antigen from HPyVs and 7, two other prevalent skin HPyVs, which appear to have minimal oncogenic potential, Lentiviral expression of the small T antigens from HPyVs and 7 induced cellular senescence in primary dermal fibroblasts. HPyV6 and 7 small T transduced primary fibroblasts showed a flat and enlarged cell morphology, significantly slower cell growth rate, and high senescence associated beta galactosidase staining upon differentiation. We determined that the MCPyV small T increased the expression of several inflammatory cytokines, known as the senescence associated secretory phenotype (SASP), to an even greater extent than HPyV6 and 7 small T. Together, our data shows that the small T antigens of HPyV6 and 7 are functionally distinct from MCPyV and may help to explain the different diseases caused by the viruses. Future mechanistic study will be performed in order to address the mechanism by which this functional divergence could occur.

925 Neonatal priming shapes preferential capacity for immune tolerance to skin commensal vs. pathogenic bacteria
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Life in a microbial world requires both tolerance to commensal microbes and protective responses to infectious pathogens. However, mechanisms enabling the host to establish a privileged relationship with commensals vs. pathogens remain largely unknown. Using tetraplets for a model bacterial antigen, we can track the antigen’s fate. T cell response to a commensal, S. epidermidis (SE-2w), and a prototypical pathogen, S. aureus USA300 (SA-2w). As previously shown, SE-2w colonization of neonatal skin establishes immune tolerance to this commensal and enriches for 2w-specific (2w) regulatory T cells (Treg). In contrast, we find that neonatal SA-2w colonization does not protect against skin inflammation nor enrich 2w Tregs upon subsequent SA-2w challenge. Consistent with a model in which different T cell priming upon initial microbial exposure sets the immune phenotype that subsequently dictates tolerance or immunity, neonatal SA-2w but not SA-2w colonization was alone sufficient for enrichment of 2w Tregs. Moreover, the distinct responses to SE and SA were maintained following concurrent skin colonization, suggesting that the host can simultaneously distinguish friend from foe and that this occurs at the level of naïve T cell priming by dendritic cells (DCs). Using fluorescently-marked SE and SA strains to track DC uptake and genetic models of DC depletion, we are currently investigating the relevant populations involved in this response. RNA sequencing of C57B6 T cells from the skin of SE vs. SA-colonized neonates as well as functional studies in IL-1R-/- mice support IL-1β as a key upstream regulator shaping these distinct responses. Notably, neonatal colonization with an Δagr SA-2w mutant enables enrichment of 2w-specific Tregs. In ongoing work, we are investigating the individual contribution of age-regulated SA molecules in skewing away from host tolerance.

926 Innate antiviral immunity is activated upon skin injury via IL-27/STAT1 axis
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Activation of antiviral immunity is critical to control infection following skin injury. While the induction of antibacterial host defense molecules is well characterized, the role of antiviral immunity and regulation of antiviral proteins (AVPs) in the skin is largely unknown. Here, we show that AVPs are potently induced following acute traumatic skin injury in human keratinocytes and primary murine murine wound models, we discovered that excisional punch biopsy-induced wounding triggers production of multiple AVPs, including OAS1, OAS2, and OAS1 (p=0.05). Since we previously reported that IL-27 plays a functional role in skin wound repair, we hypothesized that IL-27 might stimulate AVP expression in the skin. Indeed, IL-27 induced OAS2 both in vitro in human keratinocytes and in vivo upon s.c injection into mouse skin (p<0.01) in a dose- and time-dependent manner. Addition of IL-27 neutralizing AB to wounds or genetic KO of the IL-27 receptor significantly decreased OAS1 and OAS2 expression. To examine this bias further, we investigated the role of IL-27 in vivo using a model of delayed skin injury. We conclude that the IMQ mouse model of PSO is not suitable to answer any questions related to AHR-CYP1A1 axis and CYP1A1-dependent CYP1A activity has a direct impact on AVP expression. In fact, the same results could be well explained by a potential metabolism of IMQ by AHR-dependent cytomegalovirus P450 (CYP) 1A enzymes. By combining CYP activity (EROD) assay and HPLC-MS techniques, we here demonstrate that IMQ is a substrate for CYP enzymes in human UC. A modulation of AHR-dependent CYP1A activity has a direct impact on AVP levels. Moreover, IMQ competes with gymnema, a selective CYP1A1 substrate, indicating that in KC IMQ is primarily metabolized by CYP1A1. In line with this, further experiments were conducted with murine hepatic microsomes revealed a rapid metabolism of IMQ, which was completely abrogated by CYP1A inhibition. Taken together, our data provide evidence that a stimulation or inhibition of the AHR-CYP1A1 axis directly influences the pharmacokinetics of IMQ. For the IMQ mouse model of PSO, we conclude that the CYP1A1-mediated metabolism of IMQ in KC determines the bioavailability of the inflammatory drug for effector T cells, which are the critical drivers of the inflammation. We conclude that the IMQ mouse model of PSO is not suitable to answer any questions related to AHRs role in the pathogenesis of PSO.

927 Peripheral nerves are involved in the development of Staphylococcus aureus-induced skin inflammation possibly via recruiting basophils
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Staphylococcus aureus (S. aureus) is known to be correlated to disease severity of atopic dermatitis (AD) and to be associated with dysbiosis. We firstly attempted to generate a dermatitis induced by heat killed S. aureus (HKSA), and found that it was accompanied with extensive basophil infiltration. To evaluate the role of basophils in this model we used conditional basophil knockout mice. The ear swelling and the infiltrating cells in basophil-depleted mice were significantly attenuated compared with those in wild-type mice. In addition, basophils infiltrated into the dermal cell existed in the close vicinity of peripheral nerves, which is consistent with the lesion skin of human AD. Next, we hypothesized that peripheral nerves may contribute to the basophil infiltration in S. aureus-induced skin inflammation. For this reason, mice were systemically desensitized by retinoic acid to ablate TRPV1+ nociceptors. Interestingly, the ear swelling and the number of infiltrating basophils in denerve mice were attenuated compared with those in wild-type mice. We found that the lesional T cells priming upon initial microbial exposure sets the immune phenotype that subsequently dictates tolerance or immunity, neonatal SA-2w but not SA-2w colonization was alone sufficient for enrichment of 2w Tregs. Moreover, the distinct responses to SE and SA were maintained following concurrent skin colonization, suggesting that the host can simultaneously distinguish friend from foe and that this occurs at the level of naïve T cell priming by dendritic cells (DCs). Using fluorescently-marked SE and SA strains to track DC uptake and genetic models of DC depletion, we are currently investigating the relevant populations involved in this response. RNA sequencing of C57B6 T cells from the skin of SE vs. SA-colonized neonates as well as functional studies in IL-1R-/- mice support IL-1β as a key upstream regulator shaping these distinct responses. Notably, neonatal colonization with a Δagr SA-2w mutant enables enrichment of 2w-specific Tregs. In ongoing work, we are investigating the individual contribution of age-regulated SA molecules in skewing away from host tolerance.
The loss of Langerhans cells in the pellagra skin lesion is preceded by the downregulation of CCR2 chemokine signaling

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Pellagra, acrodermatitis enteropathica and biotin deficiency are caused by the deficiency of specific nutrients. These patients commonly present with similar skin dermatitis and show shared histological alterations, including vacuolarization and necrosis of keratinocytes in epidermis. Recent studies have shown that number of epidermal Langerhans cells decreased at these skin lesions. However, the precise reasons, why Langerhans cells decrease or disappear, have not yet been elucidated. In this study, we generated several nutrient deficient model mice including pellagra. After BALB/c mice were fed with niacin-deficient diet or control diet for 4 to 12 weeks (n=3-6), we performed microarray analysis using RNA extracted from mice skin. We found Langerhans cells determined by CD207 were substantially decreased or eventually disappeared in pellagra model mice skin even without any pathological change. The microarray data also shows the decreased expression of CCR2 and CCL2, which is a known to be essential for the recruitment of blood-borne precursor cells of Langerhans cells. Our observations suggest that the lack of niacin disturbs the crucial expression of CCR2 and CCL2 in keratinocytes of Langerhans cells which are subsequently decreased by the loss of recruitment of precursor cells to the lesional skin after prolonged inflammation.

High-fat diet exacerbates neutrophilic folliculitis by facilitating sequential chemokine expressions by keratinocytes and neutrophils

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Neutrophilic folliculitis is a common skin condition caused by various factors such as bacterial infections, physical or chemical irritation. It has been reported that obese individuals develop severe neutrophilic folliculitis more often than normal weight or lean individuals. However, whether and how obesity affects the development of neutrophilic folliculitis has not been well investigated. Using a high-fat diet (HF)-induced obese model and a pooled 12-hour diet-induced macrophage polarization model, we found that HF-fed obese mice exhibited severe focal accumulation of neutrophils that formed numerous intra-epidermal neutrophilic abscesses in the skin. Expression profiles of cytokines and chemokines associated with neutrophilic folliculitis signaling from CXCR2, a chemokine receptor for chemokine (CX-C motif) ligand 1 (CXCL1) and CXCL2, was critical for the neutrophil accumulation in the skin, because neutrophil infiltration was completely abolished in mice lacking CXCR2. Flow cytometric analysis revealed that CXCL1 was produced mainly in the hair follicle keratinocytes within three hours after application of PMA, while CXCL2 was produced in the epidermal neutrophils around three to six hours after application of PMA, both of which were significantly up-regulated in HF-fed mice than in normal diet-fed mice. Free fatty acids, including palmitate acids and stearic acids that are abundantly contained in HFD, significantly upregulated CXCL1 and CXCL2 expression from keratinocytes and neutrophils, respectively. Taken together, our observations raise the possibility that HFD is involved in the exacerbation of neutrophilic folliculitis in obesity by sequentially upregulating CXCR2 in gands in keratinocytes and neutrophils.
Lymphangiogenic factor represents a promising new approach for the treatment of chronic inflammatory skin diseases. Our results are consistent with those of a previous study, which demonstrated that lymphangiogenic factor drives CD8+ T cell trafficking through lymphatic vessels, leading to the depletion of CD8+ T cells and alleviation of disease symptoms. This finding suggests that lymphangiogenic factor may be a novel therapeutic target for the treatment of chronic inflammatory skin diseases.

In summary, our study highlights the potential of lymphangiogenic factor as a therapeutic agent for the treatment of chronic inflammatory skin diseases. Further research is needed to fully understand the mechanisms underlying its therapeutic effects and to develop effective clinical strategies for its use in patients with such conditions.
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ILC2s and Type 2 immunity influence haircell follicle stem cell proliferation and skin homeostasis

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Type 2 immunity is a major driver of inflammatory allergic skin disease. However, the fundamental role of ILC2s in the context of skin disease is not well understood. Here we show that type 2 innate lymphoid cells (ILC2s) are the predominant skin tissue-resident immune cells that secrete type 2 cytokines in homeostasis. ILC2s in the skin have a distinct subset of activating signals when compared to tissue-resident ILC2s from other tissues. The activation of ILC2s and secretion of IL-13 is coupled to the stage of the hair follicle cycle. Deletion of ILC2s or the type 2 immunity signaling axis leads to increased proliferation of the hair follicle stem cells in a model of depilation followed by hair growth. Repeat depletion of mice deficient in type 2 immunity leads to stem cell senescence as evidenced by elevated p16(CDKN2a). This work identifies a previously uncharacterized function for type 2 innate immunity in homeostasis, and highlights the crosstalk between ILC2s and type 2 immune responses with hair follicle stem cells.

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Tmem79 deficient mice sequentially develop dermatitis associated with S. aureus non-dominant and dominant dysbioses

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S. aureus-dominant dysbiosis has been reported to be associated with atopic dermatitis (AD). In this study, we investigated the association of dermatitis with dysbiosis caused by the deficiency of Tmem79, the responsible gene for AD-like dermatitis observed in flaky tail (ft) mice. We generated complete knockout mice of Tmem79 (-/-) to eliminate the influence of truncated Tmem79 protein expression in ft mice, and analyzed the time-dependent changes of dermatitis and skin microbiota. Skin severity score and scratching behavior analysis under SPF condition revealed that Tmem79(-/-) mice spontaneously developed dermatitis at 34 weeks old (first phase), followed by temporal improvement and subsequently more severe dermatitis after 12 weeks old (second phase). Skin microbiota composition analysis by 16S rRNA sequencing and quantification analysis of the total amount of 16S rRNA PCR amplicon which would reflect the total number of microbiota revealed no major compositional change with five-fold increment of the total amount of 16S rRNA PCR amplicon at the first phase of dermatitis, and S. aureus-dominant dysbiosis with gradual increment of the PCR amplicon amount at the second phase of dermatitis. Anti-inflammatory treatment with cephalaxin and enrofloxacin cocktail improved both phases of dermatitis. Although this treatment reversed dermatitis with S. aureus-dominant dysbiosis in the second phase, it recurred after suspension of the antibiotic treatment. Taken together, Tmem79(-/-) mice sequentially developed dermatitis associated with S. aureus non-dominant and dominant dysbioses. Although the elucidation of molecular function of Tmem79 is still remaining, Tmem79(-/-) mice would potentially provide an important clue for clarification of mechanism of dermatitis with both S. aureus non-dominant and dominant dysbioses.

**944**

A journey into psoriasis: From the adipose tissue to the skin

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Psoriasis is a chronic inflammatory skin disease characterized by complex pathogenic mechanisms, involving multiple cytokines, chemokines and other pro-inflammatory mediators. Psoriasis is associated with several comorbidities including obesity and type 2 diabetes. To date, there are no published reports assessing the effects of psoriasis-signature cytokines on adipose tissue-derived mediators and vice versa. In this study, we seek to determine: (i) the pathogenic role of psoriasis-signature cytokines in the development of adipocyte-related disorders, and (ii) the role of adipose tissue-derived mediators in triggering and/or amplifying skin inflammation. Thus, we treated whole human adipose tissue with IL-17 or TNF-α alone, and with the combination of both cytokines, investigating their effects on expression (RT-PCR) and production (ELISA) of adipokines and inflammatory factors (CC20, IL-23, IL-8 and IL-6). Subsequently, we performed ex vivo skin organ culture assays, incubating healthy skin biopsy with the collected supernatants of the above-mentioned adipose tissue experiments. Firstly, we observed that IL-17 and TNF-α induced an additive or synergistic effect on adipose tissue gene and protein expression. Additionally, the adipose tissue-derived mediators promote inflammation in normal skin organ cultures. These data could support the hypothesis that the adipose tissue acts as source of pro-inflammatory mediators playing a relevant role in the pathogenesis of psoriasis.

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Resistin-like molecule α provides vitamin A-dependent antimicrobial protection of the skin

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Vitamin A controls the immune system through its effects on epidermal and mucosal barrier integrity, skin infections, mucosal immunity and airway inflammation, but the mechanistic basis of these effects remains unclear. Here, we show that resistin-like molecule (RELm) α, a distinct adipokine, can be induced by retinoic acid in keratinocytes and confers resistance to bacterial infection. RELmα expression required dietary vitamin A and could be induced by isoretinoin, a therapeutic retinoid (vitamin A analog) that is frequently used to treat inflammatory skin disease. The expression of RELmα in human skin is regulated by vitamin A and occurs by indirect transcriptional mechanisms. RELmα expression directly protects skin from bacterial infection of the skin in a vitamin A-dependent manner. Human skin expressed Resistin, a closely-related member of the RELm family. Human Resistin also required retinoids for its expression and killed bacteria, indicating a conserved function for RELm proteins in skin innate immunity. Our results identify members of the RELm family that provide vitamin A-dependent antimicrobial protection of the skin. These findings provide insight into why skin immunity requires adequate dietary vitamin A and suggest why vitamin A analogs such as isoretinoin are effective treatments for human skin disease.
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**STAT3 deficiency in keratinocytes promotes serum IgE production in response to Staphylococcus aureus epicutaneous exposure**

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Autoimmune dominant hyper-IgE (HIES) syndrome is a primary immunodeficiency disorder characterized by elevated serum IgE levels, severe eczema and recurrent Staphylococcus aureus and Candida albicans skin infections. Dominant-negative mutations in STAT3 are found in majority of the HIES patients. However, the role of impaired STAT3 signaling in contributing to the increased IgE levels in HIES is not well understood. Herein, we used a mouse model of atopic dermatitis-like skin inflammation induced by S. aureus epicutaneous exposure in cre/delta-cKO mice with cell-specific deletion of STAT3 in keratinocytes (K5-CreERT2; STAT3fl/fl) and CD4+ T cells (CD4-creERT2×STAT3fl/fl) mice. We found that S. aureus epicutaneous exposure induced B cell expansion and markedly elevated serum IgE levels along with concomitant increases in serum IgG and IgA levels. Unexpectedly, mice with STAT3 deletion in keratinocytes but not in CD4+ T cells had significantly higher serum IgE levels compared with wildtype (WT) mice. CD4+ T cells were required for the IgE production, since CD4- T cell depletion resulted in significantly reduced levels of serum IgE. Interestingly, we found S. aureus epicutaneous exposure induced significantly higher mRNA levels of IL-17A, IL-17F, IL-12p40, and IL-23p19 in the skin of mice with STAT3 deletion in keratinocytes compared with WT mice. Finally, S. aureus epicutaneous exposure of mice infected in both IL-17A and IL-17F failed to increase serum IgE levels. Together, these results indicate that S. aureus epicutaneous exposure in the setting of impaired STAT3-signaling in keratinocytes enhanced IL-17A/F responses that resulted in elevated serum IgE production. This provides insights into a novel role of S. aureus epicutaneous exposure and STAT3-signaling in keratinocyte regulation of IgE production and provides a mechanistic explanation for the increased serum IgE levels in HIES.

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**Targeting pathogenic interactions between Rac1 and NCK1 in psoriasis**

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Rac1 and NCK1 have been proposed to be key regulators of actin dynamics and cell motility. To identify novel targets, we recently identified a screen for potential drug entities that could be repurposed against psoriasis, enriching for inhibitors of the Rac1-NCK1 interaction, which leads to disfiguring lesions and systemic inflammation. Immunosuppression is used to target the inflammatory component, however, these drugs are often insufficient to provide adequate disease control. To identify novel targets, we conducted a CRISPR screen in mouse keratinocytes, and identified benzamil as a novel, cost-effective, immune-sparing therapy for psoriasis, targeting the epithelium in psoriatic skin.

**948**

**Microbiome of hidradenitis suppurativa**

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Hidradenitis suppurativa (HS) is a chronic, painful inflammatory skin disease. It affects the pilosebaceous unit and leaves patients with cysts, nodules, and draining sinus tracts. The etiology of HS has not yet been fully elucidated. The occasional efficacy of antibiotics in HS suggests a role for bacteria in the pathogenesis of the disease, although this remains controversial as many HS lesions do not show signs of primary infection. We conducted a case-control study to evaluate the bacterial microbiome of HS. We collected samples of the surface and follicular microbe of 11 individuals with HS and 10 healthy controls. Samples were collected from HS lesions, HS peri-lesional skin, and healthy control skin in the groin and axilla. DNA was sequenced using the V3-V4 hypervariable region of the 16S ribosomal RNA gene and data analysis was performed using the QIIME (Quantitative Insights Into microbial Ecology) pipeline. OTU (Operational Taxonomic Units) were constructed and annotated using the Greengenes database. We compared the Shannon Diversity Index and β-diversity of HS to control and following injury-induced skin inflammation. However, after co-housing ft/ft mice with wildtype mice, which reduces or intermixes the skin microflora, resolved the inflammation, indicating a pathogenic role for both IL-1β and the skin microbiome. Based upon 16S rDNA sequencing of skin swabs, the microbial diversity was stable in ft/ft mice prior to and following injury-induced skin inflammation. However, after co-housing ft/ft mice with wildtype mice, there was a significant shift in the skin microbial diversity, which corresponded with the resolution of skin inflammation and IL-1β dysregulation. Deeper taxonomic analysis revealed that the co-housing of ft/ft mice altered the abundance of specific bacterial species that likely contributed to the resultant skin inflammation. Taken together, skin microbiome alterations were sufficient to induce aberrant IL-1β responses that mediated chronic skin inflammation, providing novel targets for therapeutic intervention in AD.

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**Severity of pediatric atopic dermatitis correlates to hemolytic potential of S. aureus isolates from atopic lesions**

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Atopic dermatitis (AD) is one of the most prevalent diseases of childhood and impacts quality of life for patients and their families. AD is characterized by pruritus and eczematous skin changes and is known to result from various environmental and genetic factors. During AD flares, microbial diversity declines as the population of Staphylococcus aureus increases. The contribution of S. aureus to the pathogenesis of AD is well established but, the mechanism of this contribution is poorly understood. The hemolytic exotoxin, α-hemolysin, is known to damage the skin barrier and promote pruritus through stimulation of mast cell degranulation and histamine release. However, exotoxins are produced differentially in diverse S. aureus strains. We aimed to determine if differential hemolytic exotoxin expression in S. aureus isolates from AD patients correlates to disease severity. We collected skin swabs from the three most prevalent co-habiting S. aureus isolates from each participant along with venous blood. Clinical severity was graded based on a global assessment of disease severity in our initial pilot study on 5 patients and SCORAD for 14 Assessment included severity of lesions, body surface area involvement, and patient reported itch. Samples were grown on selective media and multiple colonies picked based on differences in colony morphology. Hemolytic activity of >700 isolates was determined based on zone of lysis (mm) on rabbit blood agar. We found a significant difference in hemolysis between healthy controls (0.52 ± 0.09 mm) and moderate/severe AD patients (1.79 ± 0.5 mm) with concomitant increases in serum IgG and IgA. Unexpectedly, mice with STAT3 deletion in keratinocytes but not in CD4+ T cells had significantly higher serum IgE levels as well as venous blood levels (p<0.0001), and moderate and severe (p<0.0001) AD patients. The data indicate that disease severity is linked to the exotoxin producing potential of S. aureus, with moderate and severe AD flares being colonized by high toxin producers. Better understanding of the role of S. aureus in AD pathogenesis is key to future drug development initiatives.

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**Serum leucine-rich-2 glycoprotein is a biomarker for the effectiveness of biologic therapies in psoriasis**

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LGR5, a member of the alpha-2 glycoprotein (LRG), a glycoprotein-containing leucine-rich repeat, is produced by hepatocytes, endothelial cells, neutrophils and macrophages during systemic and local inflammation. We recently found that the serum levels of LRG were elevated in psoriasis patients and well correlated with Psoriasis Area and Severity Index (PASI) with a correlation coefficient of -0.8 in a large cohort of patients. Moreover, treatment of the inflamed skin with either topical antibiotics (Neosporin) or co-housing with wildtype mice, which reduces or intermixes the skin microbiota, resolved the inflammation, indicating a pathogenic role for both LRG and the skin microbiome. Based upon 16S rDNA sequencing of skin swabs, the microbial diversity was stable in ft/ft mice prior to and following injury-induced skin inflammation. However, after co-housing ft/ft mice with wildtype mice, there was a significant shift in the skin microbial diversity, which corresponded with the resolution of skin inflammation and IL-1β dysregulation. Deeper taxonomic analysis revealed that the co-housing of ft/ft mice altered the abundance of specific bacterial species that likely contributed to the resultant skin inflammation. Taken together, skin microbiome alterations were sufficient to induce aberrant IL-1β responses that mediated chronic skin inflammation, providing novel targets for therapeutic intervention in AD.
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Hyposis inducible factor-1z regulates the inflammation by downregulating Langerhans cell functions in a murine irritant dermatitis model
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Hyposis inducible factor (HIF)-1z regulates oxygen homeostasis through the control of erythropoietin and the expressions of multiple genes involved in a variety of biological processes, including cell proliferation, adhesion, and migration. HIF-1 is widely expressed in various types of immune cells, such as dendritic cells (DCs), which are the important sentinels of skin immune responses. DCs typically become activated in inflamed tissues, where oxygen tensions are usually low and migrate to draining lymph nodes to induce acquired immune responses. Since the functions of HIF-1 in DCs are still controversial, we sought to evaluate in vivo function of HIF-1 in skin DCs. We newly generated langerin positive cell specific HIF-1-deficient mice (langerin-HIF-1z/-) and applied a cotton oil induced irritant contact dermatitis (ICD) model. Intriguingly, langerin-HIF-1z/- mice showed exacerbated inflammation in response to ICD stimulation compared to wild type mice. In addition, the expression levels of concomitant molecules on Langerhans cells (LCs) are significantly up-regulated in langerin-HIF-1z/- mice, which suggests that langerin-HIF-1z/- DCs may not be papillitis. These results suggest that HIF-1 may regulate the inflammation of murine ICD by modulating the activation/maturity status of LCs. We then assessed the effect of HIF-1 on skin DCs in vitro by culturing single cell suspension from mice ear under normoxic or hypoxic conditions. Consistent with in vivo findings, we found that LC activation/maturation induced by lipopolysaccharide stimulation was impaired under hypoxic condition when compared to normoxic condition. Together, these results may provide a new therapy to modulate the skin immune response under hypoxic condition.

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Adaptive responses to skin microbe control the pathogenesis of experimental psoriasis
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Psoriasis is a chronic, inflammatory skin disease that affects 

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Maresin-1 inhibits IL-23 receptors via down-regulation of RORÎ³ expression and internalization in an imiquimod-induced psoriasis model mouse
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The anti-inflammatory effect of u4 polysaturated fatty acid has been confirmed in various inflammatory disease models. Maresin-1 (MaR1) is derived from u4 fatty acid, docosahexaenoic acid (DHA), and has strong anti-inflammatory effects on various inflammatory disease models. The aim of this study was to elucidate the effect of MaR1 on cutaneous inflammation, which had been unclear. In an imiquimod (IMQ)-induced psoriasis model mouse, the MaR1 reduced ear swelling response via suppression of IL-17A expression. Although MaR1 had no inhibitory effect on IL-23, MaR1 suppressed IL-17A production. MaR1 down-modulated IL-23 receptors (IL-23R)-mediated IL-23R expression through inhibition of retinoic acid related orphan receptor γT (RORγT) expression. Furthermore, we discovered that MaR1 also down-regulates the IL-23-induced expression of the pro-inflammatory cytokines IL-1β, IL-6, IL-12p70, IFN-γ, and TNF-α. At present, two distinct receptor internalization mechanisms are known. One pathway via clathrin-coated pits and the other via caveolae. To clarify which mechanism is related to MaR1-mediated IL-23R internalization, we examined the effects of nystatin, which disrupts internalization processes, including cell proliferation, adhesion, and migration. HIF-1 is widely expressed in various types of immune cells, such as dendritic cells (DCs), which are the important sentinels of skin immune responses. DCs typically become activated in inflamed tissues, where oxygen tensions are usually low and migrate to draining lymph nodes to induce acquired immune responses. Since the functions of HIF-1 in DCs are still controversial, we sought to evaluate in vivo function of HIF-1 in skin DCs. We newly generated langerin positive cell specific HIF-1-deficient mice (langerin-HIF-1z/-) and applied a cotton oil induced irritant contact dermatitis (ICD) model. Intriguingly, langerin-HIF-1z/- mice showed exacerbated inflammation in response to ICD stimulation compared to wild type mice. In addition, the expression levels of concomitant molecules on Langerhans cells (LCs) are significantly up-regulated in langerin-HIF-1z/- mice, which suggests that langerin-HIF-1z/- DCs may not be papillitis. These results suggest that HIF-1 may regulate the inflammation of murine ICD by modulating the activation/maturity status of LCs. We then assessed the effect of HIF-1 on skin DCs in vitro by culturing single cell suspension from mice ear under normoxic or hypoxic conditions. Consistent with in vivo findings, we found that LC activation/maturation induced by lipopolysaccharide stimulation was impaired under hypoxic condition when compared to normoxic condition. Together, these results may provide a new therapy to modulate the skin immune response under hypoxic condition.

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Stenotic changes of cerebral arteries and impaired brain glucose metabolism by long-lasting inflammatory cytokine release from dermis, but rescued by anti-IL-1 therapy
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We recently demonstrated that persistent release of IL-1b from skin inflammatory cells induces abdominal aortic remodeling such as vascular structure and wall fragility. Another critical and concerning organ with vascular system is brain. However, the risk for cerebral vascular involvement of long-lasting over production of IL-1b contributing to vasculature have not been elucidated so far. We addressed this question by using skin inflammatory mouse model, keratin-14-drivers transgenic mice (K14-Cre). Here, we closely monitored the mice until they were 6-months old. Here we demonstrated that sustained severe skin inflammation causes vascular sclerotic changes in brain. Functional change was also observed. Surprisingly, the pathology was ameliorated by simultaneous treatment with anti-IL-1 neutralization. Over-production of IL-1b in inflammatory skin in atopic dermatitis and psoriasis may be a risk factor for cerebral vascular disorders.

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Psoriatic keratinocyte-derived exosomes with enriched proteasome subunits promote neutrophil activation and psoriasis development
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Psoriasis is a chronic, inflammatory skin disease that affects approximately 2% of the global population. A key player in the pathology of psoriasis is the immune system. In various skin inflammatory disease models, Psoriatic keratinocyte-derived exosomes, or theirs specific cargoes, as therapeutic candidates for psoriasis.

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TNFα- and IL-17A-mediated induction of IL-36 cytokines in human keratinocytes
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Four different IL-36 cytokines are known: The agonistic IL-36α, IL-36β, IL-36γ and the antagonistic IL-36Ra. Expression of IL-36 cytokines is elevated in psoriatic skin and found to be regulated by TNFα and IL-17A. The regulatory mechanisms of IL-36 expression are unknown. This study explores these underlying molecular mechanisms. IL-36α, -γ and -Ra mRNA expression were increased in lesional compared with nonlesional psoriatic skin and with controls. In contrast, IL-36β was only found overexpressed in lesional compared with nonlesional skin. Human keratinocytes stimulated with either TNFα, IL-17A or a combination were found to express IL-36γ mRNA and protein in a time-dependent manner, with synergistic effect of TNFα and IL-17A. The same synergistic and time-dependent TNFα/IL-17A-induced IL-36γ expression was seen in both nonlesional and lesional skin. Chemical inhibitors, we found that TNFα-mediated IL-36γ mRNA and protein expression were regulated through ERK1/2 and NF-κB. In contrast, p38 MAPK was involved in IL-17A-mediated IL-36γ expression. Using siRNA knockdown, IL-36γ, an important protein in psoriasis, was found to be involved in IL-36α-mediated IL-36γ mRNA expression and in TNFα-mediated IL-36β and IL-36α expression. Moreover, IL-17A-induced expression of IL-36γ, -γ and -Ra was mediated through ACT1. To further explore the role of IL-36γ in the regulation of IL-36, the IL-36γ-expressing keratinocyte cell line HaCaT was transfected with IL-36γ siRNA and we demonstrated that the IL-23-induced expression of IL-36β, -β and -Ra was significantly reduced in IL-36γ-deficient mice compared with wildtype mice. These findings demonstrate a signaling pathway that very likely regulate IL-36 expression in psoriasis.
The effect of Hsp90 inhibition on TNF-α and IL-17A-mediated signaling

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Although atopic dermatitis (AD) is one of the most common human skin diseases, there is a scarcity of suitable animal models for AD, none of which satisfactorily mimic at least most of the key characteristics of human AD. Here, we report the development of a humanized AD mouse model that does so. Healthy human skin from adult female volunteers was transplanted onto SCID/beige mice. Autologous PBMCs were obtained from the same volunteers and pre-treated in vitro with high level IL-4 in order to induce the Th2 polarization pathway associated with active AD. And with lipopolysaccharide for TLR stimulation. When these cells were intradermally injected into the xenotransplants, within 2 weeks, this methodology elicited characteristic abnormalities in previously healthy human skin that mimics an unparallelled range of key features of human AD in vivo. Compared to vehicle-treated mice, experimentally induced AD lesions in human skin grafts completely normalized within two weeks after topical treatment with glucocorticosteroids (dexamethasone 50μg/ml) or tacrolimus 1 μM (10 μM), i.e., the most effective AD therapies. Given the skin pathogenesis of neurogenic skin inflammation induced by increased stress in the triggering/aggravation of human AD, also exposed xenotransplanted mice to 24 h of pressure (isocnic) stress one day after AD lesions had first been experimentally induced as indicated above and then brought into remission with topical dexamethasone. That this promoted reappearance of the typical histopathology of AD lesions in human skin xenotransplants 14 days post-stress further underscores how strikingly this new animal model recapitulates human AD. This also is the first report of mite viability by motility and scattered light intensity (SLI) of each mite was measured in darkfield images. 28% of the group, and observed after 0, 2, 6, 24, 48 and 72 hours. Mite motility was quantified, and the technique, incubated in DMSO at 0%, 5% (non-toxic), 10%, 20% and 50% (toxic) (n = 10 groups), and observed after 0, 2, 6, 24, 48 and 72 hours. Mite motility was quantified, and the scattered light intensity (SLI) of each mite was measured in darkfield images. 28% of the observed mites showed no movement at one or more time points, but were later observed to move indicating their viable status. A significant decrease in the SLI combined with a loss of motility was observed after 2 hours in DMSO at 50% concentration (p < 0.001), whereas no change in SLI was detected when mites were incubated in culture medium only. Based on these results, a 25% or greater decrease in SLI was selected as a threshold to indicate loss of viability. When using only motility as a marker of viability, the proportion of viable mites after 72 hours in 0%, 5%, 10% and 20% DMSO were 60%, 50% (p < 0.05), 10% (p < 0.05) and 0% (p < 0.05) respectively whereas when both motility and viability were considered, the survival proportions at 72 hours were 100%, 90% (p < 0.05), 70% (p < 0.05) and 60% (p < 0.05) respectively. Evaluation of mite viability by motility combined with SLI allows for more accurate toxicity assays of proposed anti-Demodex agents in the treatment of rosacea.
**ABSTRACTS | Innate Immunity, Microbiology, Inflammation**

964 Challenges in skin microbiome in vitro modelling
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Skin microbiome has an important role as host guardian, contributing to several physiological functions. However, knowledge of maintenance function in patients with inflammatory skin diseases is limited. Understanding the role of skin microbiome is crucial for clinical practice and development of personalized treatments. In our study, we investigated the impact of microbiome alterations on cytokine production in an in vitro model of skin inflammation. Using a 3D skin model, we observed a near absence of cytokine release in the control condition, while the treatment with microbiome modulators resulted in increased cytokine production. These findings suggest that microbiome alterations may have a significant impact on cytokine production in skin inflammation, providing new insights into the role of skin microbiome in dermatological diseases.

965 Expression and role pruritic receptors on monocyte-derived Langerhans cells
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Langerhans cells (LCs) are the only professional antigen presenting cell of the human epidermis under steady state conditions. Their role in various types of skin inflammatory conditions has been most extensively researched in mouse models, yet there is a brevity of information regarding their exact role in human skin. Most research focused on LCs uses primary cells isolated from the epidermis, which are activated as a consequence of the procedure. The possible role of LCs in the most prevalent skin symptom, pruritus, has not been investigated to date. In our current study we utilized monocyte-derived LCs as a model of LCs that have not undergone maturation to investigate the expression of receptors that have been implicated in pruritic signaling in both neuronal and non-neuronal elements of the skin. Of the investigated receptors LCs express histamine receptors H1R, H1R4, toll-like receptor 3 (TLR3), Transient receptor potential vanilloid (TRPV) 1, TRPV1 and TRPV4. TLR3 agonist with agonists to TLR3, TRPV1, TRPV4 and an agonist of the nociceptors C-fiber, GSK-101679A, respectively in the upregulation of CD86 expression, as well as that of CXCL2, CCL20, CCR7, all of which are potent T-cell activators. Interestingly all three activators also increased the expression of TLR5, which could conceivably prime LCs to be more easily activated by subsequent inflammatory stimuli. Overall our results highlight the possible role of novel proinflammatory pathways in LCs, and hints at the possible role of these antigen presenting cells in the development of pruritus.

966 The human cutaneous microbiome composition changes after coal tar treatment of both healthy and atopic dermatitis skin
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Bacterial components of the skin microbiome are known to contribute to atopic dermatitis (AD) pathogenesis in humans. Most notably, Staphylococcus aureus colonization and infection has been shown to correlate to disease severity and response to treatment. Coal tar treatment is effective in AD patients, and we recently found CT to influence host defense mechanisms by induction of antimicrobial proteins in keratinocytes. We therefore postulated that skin microbiome changes after CT treatment may aid in its therapeutic effect. We used 16S rRNA marker gene sequencing to identify bacterial taxa that are present on the inner elbow of ten healthy individuals and eight AD patients, before, during, and after CT (vehicle) treatment. We observed a near absence of Staphylococcus on healthy individuals and increased abundance of Staphylococcus in lesional AD skin microbiomes. During CT treatment in AD, Staphylococcus decreased in comparison to vehicle control, albeit that Staphylococcus also decreased during vehicle control, but to a lesser extent. Our study indicates that CT treatment affects the skin microbiome by altering the microbial composition of AD skin, shifting it towards that observed in healthy individuals. This indicates a hitherto undiscovered aspect of the mode of action of CT treatment. We propose that CT provides a long-term therapeutic effect on AD skin by shaping the skin microbiome and the host-microbe interaction leading to a milieu that is less prone to inflammation.

967 Melanin concentrating hormone modulate glycolytic reprogramming for regulating NLRP3 inflammasome activation via Hsp90/Hif-1α in psoriasis
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A high rate of Glycolysis is crucial in driving energy metabolism and immune response, especially in skin disease. Psoriatic skin characteristic with hyper-proliferated keratinocytes and chronic inflammation. Here, we investigated that the energy consumption of psoriatic keratinocyte primarily supported by glycolysis process. Melanin concentrating hormone (MCH), a neurotransmitter to regulate energy hemostasis and inflammation, have been found highly expressed in psoriatic serum (p<0.0001) and positive correlation to PASI. From mass spectra data analysis, MCH up-regulates Heat shock protein 90 (Hsp90) by PI3K-AKT pathway. The over expression of Hsp90 protects hyper-proliferation of keratinocytes in triggering pyruvate kinase subtype(PK)M2 dependent glycolytic process. The enhanced PKM2 reveals with a dimer, or monomer configuration that as a proinflammatory role, to further activate NLRP3 inflammasome. Consistently, the in vivo experiments exhibited mouse primer keratinocyte undergoing a glycolysis process by stimulating both with Imiquimod, mixed inflammation factors and MCH. Inhibit MCH receptor 1 or promote PKM2 into a tetramer configuration ameliorate the IMQ-induced epidermis thickness and skin inflammation. Taken together, the present study suggests MCH as an initial factor contributes to psoriasis development.

968 IL-36γ is a strong inducer of IL-23A expression in human 3D psoriasis skin models consisting of lesional epidermal keratinocytes and dermal fibroblasts
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Recent evaluation of IL-36γ indicates a key role in psoriasis pathogenesis. In order to unravel the effects of IL-36γ on psoriasis immunopathology in more detail, we treated human organotypic 3D psoriatic skin models with IL-36γ. We found that IL-36γ induces cytokine production of IL-23A even in the absence of proinflammatory cytokines. Our results suggest that IL-36γ provides a new psychometric feature for the diagnosis and treatment of psoriasis.

969 ATP from human keratinocytes by mechanical stretching is one of the causes of Koebner phenomenon
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Koebner phenomenon has been described in numerous skin diseases such as psoriasis. In patients with psoriasis, new psoriatic skin lesion often appears at the site of cutaneous traumatic stimuli. The pathogenesis of the Koebner response in psoriasis and other disease is not well understood. Here we report that mechanical stretching of human keratinocytes leads to rapid release of ATP. ATP is commonly considered as a classical danger signal, which stimulates immune responses. We hypothesized that ATP from keratinocytes is one of the causes of Koebner phenomenon. We established a cell stretch system to investigate stretch-evoked ATP release from normal human epidermal keratinocytes. ATP was detected with a luciferase-based bioluminescence assay. The stretch chamber and an extension device were set on a photon imaging system, and ATP bioluminescence during stretch stimulation was detected and visualized with a VIM camera (Hamamatsu Photonics, Japan). In this system, we can measure ATP release on time and the sensitivity is higher than usual ATP measurement system by microplate reader. Actually, we detected ATP release from human keratinocytes by mechanical stretching. The amount of ATP was dependent on stretched length. ATP has recently been shown to stimulate cutaneous innate and adaptive Th17 inflammatory response. In our study, we also demonstrated that ATP from human keratinocytes by mechanical stretching promotes IL-1β expression of human keratinocytes or monocyte-derived Langerhans cells involved in the induction of Th17 immunity. Therefore, ATP from mechanical stretching of keratinocytes may be important for the pathogenesis of psoriasis.

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970 Synergistic effect of EGFR/MEK inhibitors and Propionibacterium acnes in the induction of IL-36α-mediated cutaneous adverse reactions

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Propionibacterium acnes (P. acnes) is a skin commensal bacterium that can cause skin inflammation under certain conditions. EGFR/MEK inhibitors are used to treat certain skin diseases, and P. acnes has been implicated in the pathogenesis of skin infections. The synergistic effect of EGFR/MEK inhibitors and P. acnes on cutaneous adverse reactions was investigated in this study.

972 Deep mining of the diversity of eukaryotic viruses colonizing human skin

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Skin is the largest organ of the human body, and it is colonized by a diverse community of bacteria, fungi, and viruses. Understanding the structure and function of the skin virome is crucial for the development of effective therapies for skin diseases. This study aimed to deep mine the diversity of eukaryotic viruses colonizing human skin.

973 IKK/NF-κB signaling in keratinocytes controls TNF-dependent necroptosis and apoptosis-mediated skin inflammation

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IKK/NF-κB signaling controls inflammation and cell death and is important to maintain tissue homeostasis. Keratinocyte-specific deletion of IKK (IKKα and IKKβ) leads to TNF-dependent skin disease, resulting in early postnatal death of the mice. We identified increased numbers of apoptotic and necrotic keratinocytes in these mice and reasoned that keratinocyte death could trigger inflammation. RIPK1 induces cell death via kinase activity-dependent functions. To address the role of kinase activity of Receptor Interacting Protein Kinase (RIPK)-dependent cell death in IKK2E-KO mice, we crossed them with Ripk1D138N/D138N knock-in mice expressing kinase inactive RIPK1 D138N. We found that IKK2α/β and IKKβ/α mice did not develop skin lesions, demonstrating that RIPK kinase activity is required for development of skin lesions in IKK2E-KO mice. We further dissected out the RIPK1 kinase activity-dependent roles of necroptosis and necrosis in IKK2E-KO mice by crossing them to the mice lacking RIPK3 or MLKL, which are essential mediators of necrosis. We found that the IKK2α/β or IKKβ/α mice were strongly protected from skin lesion development, showing the important role of RIPK3-MLKL-mediated necroptosis in triggering skin inflammation in IKK2E-KO mice. Since inhibition of necroptosis strongly ameliorated but could not fully prevent the skin lesions in IKK2E-KO mice, we generated IKK2E-KO/Ripk3-/ mice to assess whether FADD/Casp8-dependent apoptosis contributes to the remaining inflammation in IKK2E-KO mice.

974 Sphingosine 1-phosphate receptor 1 and 2 control proinflammatory cytokine response to S. aureus in normal human keratinocytes

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Sphingosine 1-phosphate (SIP) is a bioactive lipid mediator generated from skin barrier ceramides during S. aureus infection. SIP regulates a myriad of cell activities such as differentiation, proliferation, migration, and angiogenesis via SIP receptors (SIP1 and SIP2). S. aureus skin lesions occur in patients with normal skin barrier function, and we hypothesized that SIP receptors on keratinocytes could be a bystander for bacterial invasion. At first we investigated the expression of SIP1R in S. aureus infected skin and found we have a high epidermal SIP2R expression in human impetigo skin. Second, we used a specific genetically engineered mouse to mouse the role of the SIP2 gene in neutrophil recruitment. We tested the neutrophil recruitment in SIP2R-/- mice in response to MRSA sterile bacterial supernatant. SIP2R-/- mice showed a reduced dermal neutrophil infiltration at the site of infections. Next, we investigated the pathways that connect SIP1R, SIP2R and S. aureus skin lesions in the context of pro-inflammatory cytokines and receptors, such as IL-1β, IL-1b, TNFα, and IL-8. We found that SIP2R-/- mice knock down NHEK, JIE013, a SIP2R antagonist, also affected NHEK TNFα transcription, but not NHEK IL-8 transcription as measured by qPCR. However, JIE013 significantly decreased NHEK IL-8 secretion, as measured by ELISA. This data suggests that SIP2R plays a role in neutrophil recruitment to S. aureus skin lesions.
Perforin-2: A novel antimicrobial protein that kills intracellular bacteria in healthy skin, but not in chronic ulcers

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Bacterial pathogens are important to consider as wound healing in chronic ulcers, however the mechanisms by which this occurs remain largely unknown. We examined the role of a novel antimicrobial protein, Perforin-2 (P-2), a membrane-attack-complex-perforin domain containing protein important for immunity against intracellular bacteria. We previously shown that wound infection with Staphylococcus aureus in P-2 KO mice resulted in bacterial dissemination and death. Infection was absent in P-2 KO despite systemic infection. We analyzed P-2 expression in human skin and diabetic foot ulcer (DFU) at a single cell resolution using novel PrimeFlow assay. This approach identified P-2 transcript in both CD45+ and CD45- cells. In contrast to healthy skin, PrimeFlow analyses from DFU revealed P-2 suppression in CD45+ cells and basal keratinocytes, suggesting their inability to eliminate intracellular pathogens. We investigated P-2 role in restoration of barrier function utilizing human ex vivo wound model. P-2 was measured by infection of human wounds with S. aureus strains isolated from chronic ulcers. Infection resulted in suppression of P-2 in a strain specific manner causing delayed healing. Our data suggest that P-2 may have dual property in barrier restoration, acting as an innate immune effector and wound healing stimulator, while its suppression in DFU contributes to chronicity of infection. Thus, P-2 may be a new target for prevention of chronic wound infections.

Enhanced platelet-activating factor synthesis facilitates acute and delayed effects of intoxicated thermal burn injury

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Patients who suffer thermal burns while intoxicated with ethanol (approximately half of all severe injuries) experience more complications, longer hospital stays, and almost twice the mortality than non-intoxicated burn patients. Murine models of intoxicated thermal burn injury have revealed that the injury manifests with an acute pro-inflammatory response and delayed immunosuppression but the mechanisms driving these effects are still unclear. The lipid mediator Platelet-activating factor (PAF) promotes inflammation and immuno-suppression in response to skin injury but it has not been characterized in intoxicated thermal burn. To determine the role of the PAF pathway in this system, we hypothesized that pre-incubation of a keratinocyte cell line with ethanol augmented thermal burn-mediated PAF production, and lipid extracts exhibit PAF-agonistic activity. We measured PAF levels, and lipid extracts revealed the presence of PAF rather than its non-enzymatically generated oxidized forms, suggesting this effect involves enhanced enzymatic synthesis of PAF. Inhibitor and enzyme assays indicated involvement of alcohol dehydrogenase and phospholipase A2 in the ethanol enhanced PAF biosynthesis. Pretreatment of mice with systemic ethanol or human adipose tissue with topical ethanol resulted in an augmented skin production of PAF with antigen in response to thermal burn injury. Moreover, wild-type mice receiving systemic ethanol prior to thermal burn exhibited an acute increase in serum IL-6 and an attenuated delayed-type hypersensitivity (DTH) immune response, while PAFR deficient mice were unaffected. These results demonstrate that ethanol + burn triggers high levels of PAF which mediate the inflammatory and immunosuppressive effects of this injury. These studies have potential therapeutic implications for managing intoxicated burn injury.

Oral tolerance induction in murine model of food allergy caused by epicutaneous sensitization

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Recent studies suggest that the sensitization to food allergens commonly occur through the skin. We previously established a mouse model of epicutaneous sensitization using ovalbumin (OVA). BALB/c mice are sensitized by three-time application of OVA to tape-stripped skin (1-week sensitization at 2-week intervals) and oral challenge of OVA undertaken. Allergic symptoms such as fall of rectal temperature are observed after oral challenge (Yu R et al, Experimental Dermatology. 2017;26;778-784). In the present study, we examined whether oral administration of food is effective for the prevention and treatment of food allergy caused by epicutaneous sensitization using this murine model. Mice were orally given 20 % OVA water for 1 week before epicutaneous sensitization (before-sensitization group) or during the sensitization phase (during-sensitization group). Oral administration of OVA after oral challenge of OVA in both groups was suppressed compared with non-treated control. In addition, the degranulation of mast cells in jejunum and the infiltration of basophils into jejunum were also inhibited in both groups. Furthermore, the induction of Treg in mesenteric lymph nodes was up-regulated in both groups compared with non-treated control. Thus, oral immunotherapy is effective for the prevention and treatment of food allergy caused by epicutaneous sensitization.

The microbial flora of clinically infected cutaneous metastases

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Cutaneous metastases (CM) represent skin involvement from internal malignancies or in-transit metastases from high-risk skin cancers. Knowledge of the microbial flora of skin lesions in patients with cutaneous metastases is critical towards mitigating associated symptoms of discomfort, malodor, and pain, all of which may negatively impact quality of life and cutaneous health. We characterized the microbiota and antimicrobial management of cutaneous metastases. We conducted a retrospective chart review of patients with symptomatic CM seen by Dermatology Service at Memorial Sloan Kettering Cancer Center between August 2006 and June 2015. We identified 64 patients with cutaneous metastases and clinical evidence of skin infection. Culture swabs yielded 17 distinct bacterial and fungal species. We detected pathogenic and/or opportunistic bacteria in 50% of skin lesions. The most commonly isolated organisms were Staphylococcus aureus and Pseudomonas aeruginosa. Patients treated with oral antibiotics, alone or in combination with topical agents, had a statistically significant better improvement in infectious symptoms than those treated without oral antibiotics. Our study showed that the normal skin microbial flora is disrupted in patients with infected skin metastases. Oral antibiotics may provide benefit when used as first-line therapy of infected skin lesions in patients with symptomatic cutaneous metastases. Further studies may include obtaining bacterial, viral, and fungal cultures of all patients with symptomatic cutaneous metastases and analysis of these samples using next-generation sequencing.
Keratinocytes derived TGFβ is not required for immune homeostasis in mouse skin

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TGFβ1 is essential for the epidermal retention of memory T cells and Langerhans cells, but the contribution of TGFβ1 to the maintenance of skin immune cells is currently unclear. Since the majority of cells in epidermis are keratinocytes (KC), we hypothesized that TGFβ1 from KC might be important for skin homeostasis and the maintenance of skin immune cells. To examine this, we generated K1CreERT2TGFβ1/YPF mice which enable tamoxifen-inducible specific ablation of TGFβ1 in KC. Systemic application of tamoxifen was lethal likely due to esophageal inflammation. Topical application of 4-hydroxytamoxifen (4-OHT) once a day for 2 days was well tolerated. We confirmed the deletion of TGFβ1 in bulk, thymus and interfollicular keratinocytes based on YFP expression and TGFβ1 mRNA 23 and 84 days post 4-OHT treatment. Mice with KC specific deletion of TGFβ1 developed acanthosis without observable skin inflammation, implicating a TGFβ1 autocrine/paracrine suppression of KC proliferation. We also found that ablation of TGFβ1 in KC has no effect on the numbers of epidermal-resident Langerhans T (L) cells and human lymphoid. A modest decrease in ERCT was observed. In dermis, we did not find any alteration in numbers of DC subsets, 2β T cells or dermal γδ T cells. Thus, under homeostatic conditions deletion of TGFβ1 in KC enhances epidermal acanthosis but has limited effect on cutaneous residence of most immune cells.

Superoxide dismutase 3 controls Th2 cytokine-mediated allergic inflammation through inhibition of perisentin

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Superoxide dismutase 3 (SOD3), an antioxidant enzyme that plays an important role in regulation of inflammation. However, the role of SOD3 and the mechanism(s) by which SOD3 inhibits the inflammation in several autoimmune diseases remain to be elusive. In this study, we aimed to investigate the mechanisms by which SOD3 inhibited the T helper (Th) 2 cytokine-mediated inflammatory response. Our data showed that SOD3 suppressed interferon-gamma (IFN-γ), IL-4, IL-5 and IL-13-mediated pro-inflammatory cytokine production from Th2 cells upon challenge with major allergens. Our findings indicate that SOD3 is an important regulator of allergic inflammation by inhibiting Th2 cytokine production.

Intracellular TLR7/9 signalling in infected dendritic cells is responsible for the generation of protective immunity against Leishmania major

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Intracellular immune responses against intracellular pathogens such as Leishmania major are mediated by dendritic cells (DCs). However, the role of TLR7/9 signal transduction in DCs for Leishmania infection remains unclear. In this study, we investigated the role of TLR7/9 in DCs by using MyD88-/- and TRIF-/- macrophages and DCs. Our results demonstrated that TLR7/9 signalling in DCs is essential for the generation of protective immunity against Leishmania major. Therefore, TLR7/9 signalling in DCs plays a crucial role in the protection against Leishmania infection.

Pluripotent stem cell model of Nakajo-Nishimura syndrome untangles proinflammatory pathways mediated by oxidative stress

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Nakajo-Nishimura syndrome (NNS) is an immunoproteasome-associated autoinflammatory disorder caused by a mutation of the PSMB8 gene. Although dysfunction of the immunoproteasome causes various cellular stressors, it is not well-known how this leads to autoinflammation. To investigate the mechanisms of autoinflammation, we developed a pluripotent stem cell (PSC) model line with PSMB8 mutation. Activity of the immunoproteasome in PSMB8-mutant PSC-derived myeloid cell lines (MT-MLs) was reduced even without stimulation compared to non-mutant-MTs. Additionally, MT-MLs showed an overproduction of inflammatory cytokines and chemokines, with elevated expression of monocytes and neutrophils. Treatment with p38 MAPK inhibitor and antioxidative agents decreased the abnormal production of cytokines and chemokines. The current PSC model revealed a specific ROS-mediated inflammatory pathway, providing a platform for the discovery of alternative therapeutic options for NNS and related immunoproteasome disorders.

Cytosolic nucleic acid induced signaling events in keratinocytes

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In the last decade, novel findings on disturbed intracellular signaling of professional immune cells and keratinocytes in psoriasis have led to therapeutic targeting of signaling events. However, our knowledge is still not completed on the altered signaling in keratinocytes by known pathogenic factors, such as cytosolic nucleotide fragments. These cytosolic nucleotide fragments induce pro-inflammatory activation and pro-viral reactions in keratinocytes, but other inflammatory events are barely studied. Therefore, we aimed to analyze these in keratinocytes. The HPV-KER cell line was transfected with the synthetic DNA/RNA analogue poly(dA:dT)/poly(dC:dG), cytokine expression was measured by real-time RT-PCR. Signal transduction was studied by Western blot, luciferase reporter assay and specific inhibitors and activated protein kinase C (PKC) activity was assessed by Western blot. Our results showed that poly(dA:dT)/poly(dC:dG) induced the expression of IL-6 and TNF-α, which was associated with an increase in PKC activity. These results suggest that cytosolic nucleotide fragments can induce pro-inflammatory signaling events in keratinocytes, which may be a potential target for therapeutic intervention.

Genome wide mapping identifies regulation of MAPK kinase pathway as key genetic determinant of allergic contact dermatitis

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The identification of genetic determinants underlying allergic contact dermatitis (ACD) is hampered by the complexity of the disease. In the current study, we aimed to identify genetic variants that contribute to the risk of ACD using a genome-wide association study (GWAS) approach. We analyzed a panel of 460 individuals with ACD and 125 control individuals. The GWAS analysis identified a genetic variant located on chromosome 6 (rs1364116) that was associated with the risk of ACD. The variant was located in the promoter region of the MAPK kinase 1 (MAP2K1) gene, which encodes a critical component of the mitogen-activated protein kinase (MAPK) signaling pathway. These findings suggest that variants in the MAPK kinase 1 (MAP2K1) gene contribute to the risk of ACD.
Evaluation of pharmacological responses in InflammaSkin®: a fully human full-thickness ex vivo skin model reproducing key features of psoriatic lesions

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We have recently established a new fully human ex vivo skin model for psoriasis based on the activation and differentiation of skin resident T cells in NativeSkin® skin explants. We have demonstrated that intradermal injection of anti-CD3 and -CD28 antibodies promotes the activation of resident T cells and culture in a chemically-defined medium supplemented with IL-1β, IL-23 and TGF-β induces Th17/T17 T cell polarization. T cell activation and differentiation into a Th17/T17 phenotype were secreted cytokines and a deterioration of tissue integrity and viability, indicating anti-inflammatory effects. In conclusion, we have developed a new fully human ex vivo skin model that successfully reproduces key features of skin inflammation observed in psoriatic lesions and showed the use of this model to assess efficacy of topically applied test compounds. Further development of the model will aim at investigating different treatment administrations, such as subcutaneous injection in an InflammaSkin® model with adipose tissue.

Comparative study of the skin lesions of Nakajo-Nishimura syndrome and PSM89-related autoimmune syndrome with cutaneous adverse reactions by a proteasome inhibitor

M Mellett1, B Meier1, D Mohanan2, R Schairer3, P Cheng2, TK Satoh1, B Kiefer2, S Nobbe2, M Thome2, E Contassot3 and L French1 1 Dermatology, University of Zurich, Zurich, Switzerland, 2 Dermatology, University Hospital Zurich, Zurich, Switzerland, 3 Biochem-Research Domain-Containing Protein 14 (CARD14), have been associated with an increased susceptibility to chronic inflammatory skin diseases, psoriasis and pitting in nails. However, the physiological impact of CARD14 gain-of-function mutations as drivers of disease pathogenesis remains to be fully determined. Here, we report that heterozygous CARD14 gain-of-function mutation alone is sufficient to drive IL-23/IL-17-mediated psoriasiform skin inflammation in vivo. This study, we examined skin specimens of Japanese cases with different clinical presentations and their immunohistochemical features on accumulation of ubiquitin and phospho-related p62 were compared with those of the cutaneous erosions in NNS and the PSM89-related autoimmune syndrome cases. Similar results observed in NNS and CARD14-induced lesions suggest the presence of common mechanism, while the PSM89-related lesion showed the distinct results and a different mechanism is predictable.
Innate Immunity, Microbiology, Inflammation | ABSTRACTS

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In contrast to mycelial cells, inflammasome activation in keratinocytes is dependent on JNK and p38 mitogen-activated protein kinases

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In addition to their barrier functions, keratinocytes are immunologically active cells responding to environmental stressors. Indeed, they express pattern recognition receptors (PRRs) that can sense various stimuli including microbes, chemicals and radiation and release cytokines, chemokines and alarmins. Keratinocytes can secrete the pro-inflammatory cytokine IL-1β in response to ultraviolet B (UVB) but the regulation of this process remains incompletely understood. Inflammasomes are key intracellular signaling platforms containing PRRs known as NOD-like receptors that are required for the processing and release of biologically active IL-1β by caspase-1. Inflammasomes have been extensively studied in myeloid cells. Recently, it has been reported that JNK-N-terminal kinase-1 (JNK1) is involved in inflammasome activation in myeloid immune cells, an observation that we confirmed in vivo and ex vivo and conditioned 1% OA and RNA interference. In contrast, we discuss about inflammasome activation mechanisms in keratinocytes. Here, we demonstrate that not only JNK but also p38 MAPK are required for inflammasome activation and IL-1β secretion in primary human keratinocytes exposed to UVB or nigericin, a well-known inflammasome activator derived Streptococci bacteria. Using selective small molecule inhibitors, siRNA gene silencing and CRISPR/Cas9-based deletion, we identified the p38 alpha and p38 isoforms as critical regulators of inflammasome activation as revealed by ASC oligomerization and IL-1β secretion in primary keratinocytes exposed to UVB or nigericin. Altogether, we demonstrate that the activation and regulation of the inflammasome in keratinocytes is not identical to that of myeloid cells.

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MicroRNA-31 promotes psoriasis by activating macrophages through metabolic reprogramming

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A growing number of evidences highlight the crucial roles of metabolic reprogramming in skin diseases. In particular, the diabetic phenotype, while the underlying mechanisms remain largely unknown. Here we report macrophage intrinsic microRNA-31 (mir-31) is markedly induced in the skin of psoriatic patients and imiquimod-induced mouse model of psoriasis. The genetic deficiency of mir-31 in macrophages protects mice from imiquimod-induced psoriasiform lesions, decreases acanthosis and reduces the disease severity. Furthermore, Protein Phosphatase 6 (PP6) is identified as a direct target of mir-31 in macrophages and its dysregulation is responsible for psoriasis severity. We further demonstrate that PP6 interacts with phosphorylates fructose-2,6-bisphosphate isoforms 1 (PFKFB3), a major 6-phosphofructo-2-kinase of aerobic glycolysis. Decreased PPs leads to an impaired kinase function of PFKFB3, eliciting truncated glycolytic capacity and pro-inflammatory cascades in macrophages. Thus, we conclude that macrophage glycolytic metabolism is regulated by mir-31/PP6 axis to adapt to psoriasis proceedings.

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Atopic dermatitis model on RHE in presence of S. aureus and infiltrating immuno-competent cells

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Atopic dermatitis (AD) is a common skin disease linked to a dysregulation of the immune system and an impaired epidermal barrier. AD lesions show an increased susceptibility to infection by S. aureus, which triggers the production of thymic stromal lymphopoietin (TSLP) by keratinocytes. Based on Reconstituted Human Epidermis (RHE) with a double porous polycarbonate filter allowing THP-1 monocyte infiltration, a unique in vitro model has been system and an impaired epidermal barrier. AD lesions show an increased susceptibility to infection by S. aureus, which triggers the production of thymic stromal lymphopoietin (TSLP) by keratinocytes. Based on Reconstituted Human Epidermis (RHE) with a double porous polycarbonate filter allowing THP-1 monocyte infiltration, a unique in vitro model has been developed to better investigate the cutaneous immune response activated by S. aureus in AD. RHE with impaired barrier were colonized with S. aureus at 2x10^5 cells/mL. THP-1 infiltrated the RHE for 4 hours. Tissue response was assessed after 4 hours (atopic rash), 16 and 48 hours (delayed inflammation) by qRT-PCR of inflammatory genes (TNF-α, TLR-2, anti-inflammatory IL-10). For selected biomarkers protein expression was confirmed by immunoblotting. Thp-1 successfully infiltrated the colonized RHE and differentiated towards a dendritic phenotype. S. aureus colonization of the RHE co-cultured with Thp-1 also decreased filaggrin expression. Human β-defensin 2 increased by more than 100-fold in the AD model, reflecting keratinocyte reaction to S. aureus. The THP-1-RHE cell migration model seems to fully recapitulate the features of AD in vitro by taking into account the keratinocyte innate and inflammatory response and the immune-mediated response in presence of a S. aureus stable colonization.

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Skin commensal bacteria drive the wound healing response by initiating pDC recruitment and activation in injured skin

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Wound healing of the skin is a complex process that involves the coordinated induction of inflammation and re-epithelialisation. We have previously shown that this process is triggered by pDC infiltration of injured skin and their activation to produce type I IFNs, although the initiating mechanisms are still elusive. Here we used tape stripping of murine skin or skin blister induction of human volunteers to skin injury models to demonstrate that skin commensal bacteria are required to initiate this process. First, we show that the skin commensal bacteria activate neutrophils to express CXCL10 which drives recruitment of pDC into injured skin. Second, we demonstrate that the presence of the skin microbiota is essential for the ability of CXCL10 to activate pDC. In fact, CXCL10 was found to preferentially bind and kill bacteria, leading to the formation of complexes with bacterial DNA. The subsequent release of CXCL10-bacterial DNA complexes into the extracellular environment triggered the activation of pDC via TLR9. By inhibiting pDC infiltration and activation, the depletion of skin microbiota profoundly dampened the inflammatory response in injured skin and delayed the wound closure. Our data uncover a fundamental role of the skin microbiota in the initiation of inflammatory and healing responses in skin wounds and suggest that the host exploits granulocytosis to efficiently restore barrier integrity.

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EMT-like phenotype in normal keratinocytes driven by TLR3 activation

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Toll-Like-Receptor 3 (TLR3), a pattern recognition receptor that recognizes dsRNA, activation results in NFκB or interferon-regulatory factor 3 (IRF3) mediated gene transcription of inflammatory cytokines, chemokines and alarmins. Keratinocytes can secrete the pro-inflammatory cytokine IL-1β by TLR3 activation in keratinocytes is not identical to that of myeloid cells.

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Prebiotic colloidal oatmeal supports the growth of S. epidermidis and enhances the production of lactic acid

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We previously demonstrated that 1% colloidal oatmeal promoted the growth of Staphylococcus epidermidis (S. epidermidis), Staphylococcus aureus (S. aureus) and Propionibacterium bacteria acnes (P. acnes). More importantly, we showed that colloidal oatmeal increased the growth rate of S. epidermidis more than that of S. aureus. We now demonstrate in an in vitro competition assay that 1% colloidal oatmeal selectively promoted the growth of S. epidermidis when S. epidermidis and S. aureus were cultured together. These data support a prebiotic mechanism for colloidal oatmeal in balancing the skin microbiome by supporting the growth of a skin health-associated species in the presence of an opportunistic pathogen. Short chain fatty acid (SCFA) analysis revealed that the metabolite profile of S. epidermidis grown in the presence of 1% colloidal oatmeal under aerobic conditions was more similar to S. epidermidis grown under anaerobic conditions in the absence of colloidal oatmeal, indicating S. epidermidis utilizes fermentation pathways to metabolize colloidal oatmeal. As a result, S. epidermidis grew faster and generated more lactic acid, a fermentation byproduct. Lactic acid is one of skins natural moisturizing factors (NMF) that provide skin benefits such as moisturization and exfoliation. RNA sequencing of S. epidermidis grown in aerobic and anaerobic conditions under the presence of colloidal oatmeal showed that the metabolic phenotype is a result of multiple players, and not likely driven by a single downstream mediator of TLR3 signaling.
1000 Molecular mapping of necrobiotic lipodermoida for identification of disease mechanisms and novel therapeutic strategies
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Necrobiotic lipodermoida (NL) is a chronic, disfiguring granulomatous skin disorder primarily involving lower legs. Although associated with diabetes, the etiology and mechanisms underlying NL are unknown, and treatment remains challenging. We hypothesized that comprehensive characterization of the RNA transcriptome in NL may increase the understanding of the underlying processes and highlight possible therapeutic opportunities. A total of 118 NL and 5 normal skin samples were subjected to whole genome RNA sequencing. In addition, independent validation was performed using Nanostring panel (594 immune-specific genes), demonstrating high concordance in the set of genes identified as differentially expressed by both platforms, notably highly enriched for immune system genes. We thereby uncovered the immune response characteristics of NL, highlighting potential therapeutic targets.

1001 Mast cells and microvascular network expansion in asymptomatic atopic dermatitis
S170
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Atopic dermatitis (AD) is a chronic inflammatory skin disease affecting children and adults worldwide. Yet, few studies have investigated the initial phase leading to lesions. We recently reported that skin-resident mast cells (MC) initiated pathogenic remodeling in prelesional atopic dermatitis (AD), using a human AD-like preclinical model. Further computer-assisted morphometric analysis performed on digitized images of CD11 stained skin sections revealed a significant increase of microvasculature in the hypodermis after a single antigen ovalbumin exposure, compared to controls. Next, polar coordinates of MC and neovessels were plotted establishing that 80% of MC were located within 25 μm radius of a vessel. This close interaction established by polar mapping strongly suggested angiongenic functions of MC in pre-lesional AD. Moreover, MC directly mediated this newly discovered pathologic angiogenesis, as 1) MC were the sole source of Vascular Endothelial Growth Factor-A (VEGF) at this stage, 2) neovessels correlated with MC-restricted VEGF mass in skin sections, and 3) no angiogenesis was evidenced in the absence of MC in this AD model when using MC-deficient mice. Since neovascularization promotes remodeling through recruitment of inflammatory cells, we are proposing that local MC targeting may represent an attractive prophylactic strategy for early AD because of their pro-remodeling and angiogenic functions through the release of VEGF.

1002 The relevance of CMV reactivation in immunompaired patients suffering from chronic inflammatory skin diseases
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Natalie Garzorz-Stark1, Philipp Speth1, Manja Jargoch1, Tilo Biedermann2, Kilian Eyerich1, Stefanie Eyerich1. 1 Department of Dermatology, TU Munich, Germany. 2 ZAUM, TU Munich and Helmholtz Center, Germany. Reactivation of latent CMV infection is a serious complication in immunompaired patients. In particular, patients with chronic inflammatory skin diseases (CISD) under therapy-induced immunosuppression are at risk. In a retrospective study, 48 patients suffering from a CISD whose lesions worsened despite immunosuppressive treatment were included. FPEX: tissue was examined for the presence of CMV DNA by PCR and could be detected in 1/18 chronic ulcers/pseudogangrenous (5.6%). Next, we analyzed the serum prevalence of CMV and CMV DNA in lesional skin in patients with CISD in whom long-term immunosuppressive therapy had been initiated (n=29). 21/29 patients (72.4%) were seropositive for anti-CMV IgG compared to the seroprevalence in the general population (50% and 65%). Anti-CMV IgM was detected in 5/29 patients (17.2%). Thereof one patient (A) was diagnosed with pseudogangrenous lesions. Co-localization of CMV infected cells was detected by in situ hybridization. Despite being treated with high-dose steroids and specific therapy regimens (inflammas in A and rituximab in B), lesions worsened. In all three patients, treatment with ganciclovir was initiated leading to profound improvement of skin lesions and hence health status. In conclusion, seropositivity of CMV is elevated in patients suffering from CISD under immunosuppressive treatment. The awareness of the phenomenon of CMV reactivation and prompt antiviral treatment might improve health status also in immunocompromised patients with CISD.

1003 Malignant T cells inhibit anti-cancer immunity in cutaneous T cell lymphoma
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In cutaneous T cell lymphoma (CTCL), the malignant T cells are a source of suppressive Th2 cytokines, such as IL-4, and progressive impairment of cellular immunity is a hallmark of the disease. IL-4 is known for its capacity to sustain Th2 cell differentiation, when acting directly on T cells, but can also initiate an IL-12 dependent negative regulatory feedback loop and initiate protective Th1 immune response when present during the initial activation of dermicitic cells (DC). Interestingly, we found an association of increased IL-4 production and, at the same time decreased IL-12 levels, with advanced stage CTCL. Neutralization of IL-4 restored Th1 but not Th17 immune responses in CTCL, and DC activation was directly suppressed by IL-4. Using in vitro models, we found that malignant T cells inhibit the development of a protective non-malignant effector CD4+ T cell population.

1004 Evaluation of genetic variants in hidradenitis suppurativa patients by exome sequencing
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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease affecting up to 2% of adults. Recently, autosomal dominant genetic aberrations in the gamma-secretase pathway have been identified in HS families. Still, little is known about genetic variants in HS patients. Saliva samples of HS patients as well as their unaffected parents were collected and subject to whole exome sequencing (HiSeq4000). Data were analyzed for Mendelian transmission patterns and de novo mutations. In silico prediction tools such as CADD and FATHMM were used to predict the functional importance of potential disease-associated variants. Four families with one affected child and two healthy parents, as well as one family with two affected siblings and their healthy parents were analyzed. Five out of six HS patients were male (83%). On average, HS patients were 38 (26-46) years old and reported an average disease duration of 12 (3-35) years. Patients had an average body mass index of 28.8 (22.4-34.8). Of significance, 9/14 patients ever had severe acne. On average, 6 (4-8) of 10 blood lipid parameters were in the normal range, 0.7 (0-1.6) of 10 lipids were in a pre-diabetic state and 0.2 (0-0.7) of 10 lipids were in the diabetic range. HbA1c levels were in the normal range (6.3 ± 1.1). No significant difference was noted compared to a healthy control population. Out of all HS patients, male (83%), 23/30 patients (76.7%) were smokers. The most common co-morbidities were asthma (73.3%). IMQ induced elevated pro-inflammatory Th1 response in one HS patient. Malignant T cells inhibit anti-cancer immunity in cutaneous T cell lymphoma. The awareness of the phenomenon of CMV reactivation and prompt antiviral treatment might improve health status also in immunocompromised patients with CISD.

1005 The skin: A new player in the control of whole-body glucose homeostasis
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Psoriasis is an independent risk-factor for development of insulin resistance and Type 2 diabetes (T2D). Here we used the immunompaired (IMQ) mouse model of psoriasis to investigate the effects of skin inflammation on insulin sensitivity and beta-cell function. To induce skin inflammation in mice, 75 mg of 5% IMQ cream (Aldara, Meda Pharma) was applied topically to a shaved dorsal region for 4 consecutive days (n = 3–8). Control mice were treated with vehicle. On the fifth day, mice were either fasted for glucose tolerance testing, or sacrificed in the fed state with blood and tissues collected for analysis (pGCP, ELISA and IF). All data is expressed as mean ± SEM. Treatment of mice with IMQ induced thickening, erythema and increased inflammatory gene expression in the skin (IL1b: 26.49 ± 5.45; Il6: 25.0 ± 0.49; Ifn-γ: 20.9 ± 2.0; Il17: 1.42 ± 0.27) and reduced GLUT4 gene expression (0.58 ± 0.08) in subcutaneous adipose tissue, which was dose dependent. Increased inflammatory gene expression was also observed in liver and skeletal muscle of IMQ mice. Fed serum insulin levels were elevated in IMQ-mice (1.44 ± 0.23 ng/ml vs controls 0.47 ± 0.15 ng/ml). Additionally, IMQ-mice displayed improved glucose tolerance, increased insulin and c-peptide response to glucose, and increased beta cell proliferation (Ki67). Skin inflammation induces tissue inflammation and markers of insulin resistance. Potential compensation to insulin resistance was also seen in the ileals. These results may support a novel pathophysiological pathway mediating insulin resistance development and T2D progression.
1006
Type 2 immune cells selectively interact with skin sensory nerve fibers in atopic dermatitis
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Chronic itch is frequently cited as the most debilitating symptom of atopic dermatitis (AD). Although it has been recently implicated as a critical feature of AD, the mechanisms by which itch is triggered are not well understood. To explore this neuroimmune interface, we generated a dual fluorescent reporter mouse line in which sensory nerves express tiflomato and immune cells express IL-2-specific enhanced GFP (eGFP). Using intravital two-photon imaging of skin lesions in a mouse model of AD, we observed that a subset of IL-4-eGFP+ type 2 immune cells markedly reduced their instantaneous speed upon interacting with sensory nerve fibers but resumed rapid transit after leaving the fibers. Overall, IL-4-eGFP+ immune cells that associated with sensory nerve fibers had a lower mean speed over the course of imaging compared to IL-4-eGFP+ cells that did not make contact with sensory fibers. In contrast, type 1 and type 3 immune cells did not demonstrate similar neuroimmune interactions. Collectively, we have identified type 2 immune cells that selectively interact with sensory nerve fibers in the skin. These observations provide a novel platform to identify key mediators of neuroimmune interactions that underlie AD.

1007
Analysis of cutaneous microbiota between two age-group of Caucasian women
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No significant levels of chemokines and inflammatory mediators (eg. CCL2 (p < 0.01), n = 5) and CCL3 (p < 0.05), n = 3) after incubation for 24 hours with even a low number of live Demodex mites. The contrasting effects on inflammatory pathways described here provide insight into how these complex organisms may coexist with host cells under normal skin conditions (expression to keratinocytes in low numbers) without disrupting the biobalance, and yet have the capacity to induce significant cellular inflammatory reactivity when their population is markedly increased (keratinocytes) or when they are exposed to other cells (sebocytes and PBMCs) as may occur in the skin of patients with rosacea.

1008
Type 1 immunity induces a senescence-like growth arrest in malaria parasites
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Type 1 immunity induces a senescence-like growth arrest in the remaining plasmodium parasites. One day of treatment with INF-γ caused a stable growth-arrest over 14 days in surviving Plasmodium (P) falciparum liver stages. INF-γ induced small non-proliferating plasmodium forms in human, monkey and mouse hepatitis Atoxoa. Treatment killed the large majority of the remaining parasites confirming that these small forms are alive. In vitro data showed that mammalian p21 can interact with parasite cyclin-dependent kinases (CDKs) PFKc, PKA and PKC. Inhibition of these CDKs with Artemisinin severely impairs the growth of P.falciparum, without killing the parasites. Therefore, we asked whether INF-γ and plasmodium parasites induce p21 in mammalian hepatocytes and whether mammalian p21 can arrest P.falciparum in vivo. Liver-infection with plasmodium parasites leads to an interferon-response and induced p21 but not p16 protein levels inside primary hepatocytes. The p21 protein was further enhanced by treatment with INF-γ and tumor necrosis factor. Most importantly, p21-p210 had a major defect in controlling the onset and the extent of parahepatitis. Hence, type 1 immunity did not only kill malaria parasites but induced a p21-dependent senescence-like growth arrest in the remaining plasmodium stages. This INF-γ-induced senescence-like growth arrest significantly contributed to the containment of the disease.

1009
Demodex mites modulate skin inflammation: Potential role in rosacea
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Low number of Demodex mites reside in the upper pilosebaceous follicles of healthy human facial skin in contact with keratinocytes without inducing inflammation. In rosacea patients, the greatly increased follicular mite population and the resulting follicular distress/disruption exposes them to both sebocytes and PBMCs (Peripheral Blood Mononuclear Cells). The aim of this study is to evaluate how Demodex mite density modulates inflammatory keratinocytes, sebocytes and PBMCs. Mites isolated from human skin using the Modified Standardized Skin Biopsy (MSSSB) were incubated with human keratinocytes (HaCaT), rat preputial sebocytes (RPs) and human PBMCs for 24 hours at low (1 mite/cm2) and high (5 mites/cm2) densities. TL2 expression and inflammatory mediators release were assessed by flow cytometry, qRT-PCR, Luminesky assay and ELISAs. Keratinocytes showed a higher gene and protein expression of TL2 when they were incubated with mites. However, only a high mite density significantly increased the gene expression and release of inflammatory mediators (eg. KLK5 (p < 0.05) and CCL2 (p < 0.05), n = 5). RPs and PBMCs released significant levels of chemokines and inflammatory mediators (eg. CCL2 (p < 0.05), MIF (p < 0.05), CXCL1 (p < 0.001), TNFα (p < 0.01) and IL-6 (p < 0.01), n = 3) after incubation for 24 hours with even a low number of live Demodex mites. The contrasting effects on inflammatory pathways described here provide insight into how these complex organisms may coexist with host cells under normal skin conditions (expression to keratinocytes in low numbers) without disrupting the biobalance, and yet have the capacity to induce significant cellular inflammatory reactivity when their population is markedly increased (keratinocytes) or when they are exposed to other cells (sebocytes and PBMCs) as may occur in the skin of patients with rosacea.

1010
Numerical eczema is a distinct clinical entity with overlapping features of both, psoriasis and atopic eczema
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Numerical eczema (NE) is a chronic inflammatory skin-disease that is characterized by a numerical analysis of cutaneous microbiota between two age-group of Caucasian women. This study will support new approaches in order to rebalance skin microbiota and prevent age-related skin disorders.

1011
IL-17 is crucial for pсорiatic inflammation, but also initiates an inflammatory feedback loop via signaling into keratinocytes
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The IL-17 cytokines play a key role in the development of psoriasis, as they are the only cytokies that can colonize the skin, leading to a higher diversity. In conclusion, our study represents the first identification of skin microbiota shift during aging of Caucasian women. This study will support new approaches in order to rebalance skin microbiota and prevent age-related skin disorders.

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1012 ECRG4 mediates early neutrophil recruitment and wound healing in a murine model
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Precise regulation of inflammation is essential to wound healing. Our laboratory has identified Esophageal Cancer Related Gene 4 (ECRG4) as a novel chemokine. Pretreatment with this protein releases a peptide that recruits leukocytes in infection and tumor models. We hypothesized that the wound's protease-rich environment also releases this chemotactic peptide. Here, we utilized murine injury models to demonstrate that ECRG4 mediates early neutrophil recruitment and that its deficiency delays wound closure. Injury models included excisional wounds and subcutaneous implantation of polyvinyl alcohol (PVA) sponges, which model a sterile injury. Inflammatory cell infiltration in ECRG4 knockout (KO) mice and wildtype controls (WT) were analyzed by flow cytometry at 24 hours. Excisional wounds were also used to assess the histology of wound repair, Gr1+ cell infiltration and the kinetics of wound closure. We found that ECRG4 synthetic peptide increased recruitment of leukocytes in the sterile injury model, while ECRG4 KO mice demonstrated a 5-fold decrease in inflammatory cell infiltration. In the subcutaneous implantation injury model, ECRG4 KO mice also demonstrated a 4-fold decrease in neutrophil recruitment in excisional wounds, with a delay in wound closure. There was no significant change in macrophage recruitment. The phenotype of uninjured ECRG4 KO mice was assessed by hemogram and flow cytometry of spleen and bone marrow and demonstrated no underlying hematologic abnormalities. These findings demonstrate the importance of ECRG4 as a chemotactant for neutrophils in the early inflammatory response and suggests that ECRG4 has a role in inflammatory response to wounding and a concomitant delay in wound closure, while exogenous ECRG4 peptide increases early neutrophil recruitment. Further experiments will clarify ECRG4's mechanism of action on myeloid cells. This work identifies ECRG4 as a therapeutic target for modulating wound inflammation.

1014 The balanopreputial microbiome in male genital lichen sclerosus
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Patient outcomes from male genital lichen sclerosus (MGLSc) are limited by the paucity of information about the etiology. The balanopreputial microbiome of MGLSc patients was recently found to be distinct from that of control men. The aim of this study was to further characterize the balanopreputial microbiome of MGLSc patients. Patients with MGLSc and 20 healthy controls. In the balanopreputial sac, the proportional abundance of Fusobacterium spp. and Streptococcus spp was higher in MGLSc (p = 0.04). In vivo, the relative abundance of Fusobacterium spp. was higher in MGLSc (p = 0.04). In vivo, the proportional abundance of Fusobacterium spp. was higher in MGLSc (p = 0.03). The phenotype of uninjured ECRG4 KO mice was assessed by hemogram and flow cytometry of spleen and bone marrow and demonstrated no underlying hematologic abnormalities. These findings demonstrate the importance of ECRG4 as a chemotactant for neutrophils in the early inflammatory response and suggests that ECRG4 has a role in inflammatory response to wounding and a concomitant delay in wound closure, while exogenous ECRG4 peptide increases early neutrophil recruitment. Further experiments will clarify ECRG4's mechanism of action on myeloid cells. This work identifies ECRG4 as a therapeutic target for modulating wound inflammation.

1015 Effect of an emollient on the skin fungal microbiome of atopic dermatitis (AD) patients
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Atopic dermatitis (AD) is associated with changes in skin bacterial microbiome (BMI). Emollient treatment (ET) induces change in BMI in AD, but its effect on fungal microbiome (FM) and their inter-correlation was unknown. We used Ion-Torrent sequencing to characterize the FM of AD patients in response to ET. Skin swabs were collected from lesional (LS) and non-lesional skin (NS) of AD patients suffering from moderate AD, after informed consent and according to GCP guidelines. Genomic DNA (gDNA) was extracted and used for FM sequencing analyses as described earlier. Principal components analyses (PCA), diversity, correlation and correlations analyses were performed using parametric tests (p < 0.0001). PCA showed NS after ET exhibited widespread variability vs UT skin, while there was no noticeable difference in LS 6 genera (including Aspergillus) were present only in UT, while 13 were unique to EFT. Shannon index of NS was significantly increased post-treatment, compared to pre-treatment swabs (p < 0.05). LS showed no difference in diversity. Ascomycota was the most abundant phylum in most samples, followed by Basidiomycota. Accumulation of genus Chromobacterium was significantly reduced in post-treatment samples in both LS (p = 0.047) and NS (p = 0.033), while that of Rhizomucor was significantly increased in EMT compared to UT samples. In UT skin, Basidiomycota exhibited positive correlation with Firmicutes bacteria and negative correlation with Proteobacteria, while Ascomycota exhibited negative correlation with Firmicutes but positive correlation with Proteobacteria. These correlations were reversed in EMT samples. EMT may induce beneficial changes in FM, and augment interactions between microbes and microbiota balance on skin in AD. Clinical relevance of these results need to be investigated.

1016 Lysis by bacteriophages can modulate Cutibacterium (formerly Propionibacterium) acne-induced immune responses
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The Gram-positive skin commensal, Cutibacterium (formerly Propionibacterium) acne is thought to play a role in acne pathogenesis, in part by inducing a cutaneous inflammatory response. The emergence of antibiotic resistance in clinical C. acne isolates, along with adverse side effects associated with current treatments, have highlighted the need for improved acne therapeutics. We previously characterized bacteriophages that infect C. acne strains and found that these viruses, found on both acne and healthy skin, have limited genetic diversity and the ability to kill a broad range of clinical C. acne isolates, suggesting their potential utility for acne treatment. However, the effect of phage killing on human immune responses is unknown. We hypothesize that C. acne phages, by degrading the bacterial cell wall and inducing lysis, can influence the human immune response to C. acne, likely via modulation and/or destruction of bacterial-associated innate immune ligands. To test this, we first measured the effects of bacteriophage-mediated killing of acne lesions and normal skin, finding that phage killed bacteria induce lower levels of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6, as well as compared with live bacteria, and this activity is dependent on phage killing. Simultaneous treatment with phage and live bacteria similarly affects cytokine secretion, and also promotes reduced secretion of IL-10, IL-12, and IFN-γ. As phage-mediated killing of C. acne can attenuate inflammatory pathways induced by these bacteria, and consequently, may hold potential as a therapeutic modality for acne.
1018 Neutrophil extracellular traps and type 1 IFN contribute to autoimmunity in hidradenitis suppurativa  
Hidradenitis suppurativa (HS) is a neutrophilic inflammatory skin disorder with an unknown etiology primarily affecting intertriginous areas. Considering the predominant cellular infiltrate, we sought to understand the role of neutrophil extracellular traps (NETs) in HS. In perilesional blood samples from HS patients, neutrophils had enhanced NETosis and WB analysis revealed that these NETs possessed proteins recognized by autoantibodies (AAb) present in HS serum, namely antibodies against IL17B. Furthermore, serum from HS patients had significant titers of total IgG and contained AAbs against citrullinated proteins, including filaggrin. In mice, a model of dermal thickening and inflammation, we found that injection of mice with footpad HS fluid elicited decreased neutrophil infiltration and a significant increase in neutrophil extracellular traps (seen by electron microscopy).  
Moreover, NETs were confirmed in HS tissue via immunofluorescent detection of citrullinated histone 4 (cit-H4). With ELISA, HS tissue homogenates revealed a positive correlation of detected cit-H4/double-stranded DNA complexes with disease stage ($r^2=0.710; p=0.0034$). Finally, HS tissue displayed a significant upregulation of type I interferon (IFN) genes. Taken together, these results suggest unreported roles of autoimmunity and neutrophils in the pathogenesis of HS, identifying NETs as a source of AAbs and the type I IFN signature in HS tissue, which could impact alterations in therapeutic approaches.

1020 Assessment of the anti-inflammatory effects of cannabidiol and its fluorinated derivative in vitro and in vivo models of atopic dermatitis  
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Fluorination can significantly increase the efficacy of the active components in pharmaceticals. The aim of the study was to assess the potential anti-inflammatory effects of cannabidiol (CBD), the major non-psychotropic component of the plant Cannabis sativa, and its fluorinated derivative (HUF-101) in various experimental systems modeling atopic dermatitis (AD). For the in vitro AD model, keratinocytes were challenged with the combination of Staphylococcus aureus enterotoxin B and rhic stromal lymphoprotein and expression of certain marker molecules were assessed by RT-qPCR and ELISA. For the in vivo model, mice were sensitized with 2% oxazolone (OXA) before elicitation. Test compounds were applied topically (1 and 10 μM) after inducing skin inflammation and edema formation (in the ears) was measured at 24 hours post elicitation. In vivo expression of certain inflammatory cytokines (e.g. interleukin [IL]-1α, IL-1β, IL-6 and IL-8) were significantly down-regulated upon the administration of CBD and HUF-101. However, HUF-101 exhibited significantly higher potency in comparison to CBD. In the in vitro model, topical application of 1μM CBD significantly reduced the OXA-induced ear edema; however, 10 μM CBD exerted insignificant effect. In contrast, HUF-101 attenuated OXA-induced edema formation at both concentrations. Intriguingly, the anti-inflammatory potency of HUF-101 was significantly greater than that of CBD. Our study provides the first evidence that CBD and its fluorinated derivative exert significant anti-inflammatory actions in models of AD. Further pre-clinical clinical studies are needed to exploit the therapeutic potential of certain CBD derivatives in inflammatory skin conditions.

1021 Distinct Cutibacterium acnes strains isolated from lesional and non-lesional regions of acne promote differential immune responses  
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We recently reported the essential roles of mast cells (MC) and sphenoid metabolite sphenogline-phosphate (SIP) in pre-lesional skin remodeling observed in female mice, using a human atopic dermatitis (AD)-like preclinical model. In human adults, females have a greater propensity to develop AD than males. Accordingly, most AD mouse models only utilize females as males do not exhibit AD-like changes. We previously showed epidermal and dermal thickening with cellular infiltration that occurred in the hypodermis of female mice after a single exposure to antigen ovalbumin (OVA), compared to controls and prior to IgE elevation. Using male mice in a similar preclinical model, we observed hypodermal cell infiltration after OVA exposure although to a lesser extent than in female mice ($p<0.0001$), but no skin layer thickening or increased skin SIP levels, compared to female mice. Moreover, the number of activated mast cells was not increased in male mice, as opposed to females. The current work supports our previously reported results establishing MC as major effectors of remodeling in pre-lesional AD. In sum, we identified that the absence of local mast cell activation and elevated SIP levels observed in male mice may explain gender differences at the onset of AD.

1022 Identification of mycobacteria species in skin tissue using amplification and melt curve analysis of the hsp65-gene  
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Species identification of mycobacteria by molecular methods in formalin fixed embedded skin tissue has been suboptimal. Conventional microbiological analysis (AKA Procedural Probe) is performed at the time of diagnosis but requires weeks, and traditional species-level identification is challenging. Patients that received culture for mycobacteria and histopathology of skin biopsy were studied (2011-2017). There was performed on automated BACTEC-MGIT broth incubation system. RT-qPCR melt curve assay of the hsp65-gene was performed on both follow-up growth. Consensus primers amplified the hsp65-gene, and fluorescence resonance energy transfer probes were used. Conventional identification from solid media was also done. We identified mycobacterial growth in 27 of 944 (2.86%) unique patients. The number of tissue specimens per patient ranged from 1-21. The mean number of samples from positive patients was 2.13 (95% CI 1.13-3.12). Mean age of patients with positive culture was 62.3 ± 18.5 years and no gender differences were observed. The cutaneous locations where the M. abscessus were found were fingers 18.5%, feet 10.5%, hands 8.5%, neck 7.5%, and back 4.5%. Histopatology revealed supplicative granuloma 48.1%, non-suppurative granuloma 18.5%, and mixed inflammation 66.6%. Paucibacillary disease was seen in 85.1%. The rapidly growing species identified were M. chelonae 29.6%, M. abscessus 14.8%, M. fortuitum 11.1%, and M. gordii 3.7%. The slowly growing species were M. avium-intracellulare complex 14.8%, M. marinum 14.8%, M. tuberculosis complex 7.4%, and M. gordonae 11.1%. Diagnostic yield of M. chelonae was 14.8% in 2021-2017, following positive culture. For comparison, growth and species level identification can require weeks using conventional methods. Thus, the use of this assay provides rapid and accurate species identification allowing for more timely initiation of therapy.
**1024**

**Dissecting the molecular interdependence of skin inflammation and obesity**

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Ponipiao, a chronic inflammatory skin disease, is often compromised by comorbidities with obesity being the most prevalent. Epidemiological studies clearly associate obesity with ponipiao and the aim of the present study was to investigate how obesity alters skin immune responses. To this end, an obesity model was established by feeding of male C57Bl6 mice with high-fat diet (HFD), 60% fat. To investigate the effect of obesity on Th1/Th1-mediated skin inflammation, we used a model of 2.4,6-trinitrochlorobenzene (TNCB) contact hyper-sensitivity. We observed a challenging sensitized response in a significant increase of ear swelling of obese mice with an elevation of IFNγ- and IL-17-positive cells in draining lymph nodes (DLN) and an increase of IL-17, IL-21, IL-6, TNF in the serum. Th2 cytokin IL-4 was undetectable increased (reduced) due to IL-22 (enhanced) MECP2 due to HFD. Interestingly, the ear swelling of mice against fluorescent isothiocyanate (FITC) was diminished in obese mice. This suggests a selective enhancement of Th1/Th1 and a decrease of Th2 immune responses by obesity. Investigating underlying mechanisms, we found increased expression of IL-12, IFNg, TNF, IL-6, and IL-22 in obese mice. IL-22 is the adipose-specific cytokine in obese mice. TNF, IL-6, IL-17, and macrophages were also significantly upregulated in TNCB-CHS skin of obese mice. On the contrary, Foxp3 and TGF-beta were downregulated. Further experiments showed that dermal gamma/delta T cells are the major source of IL-17 elevated due to obesity. Unexpectedly, the depletion of gamma/delta T cells resulted in significant increase of ear swelling in obese mice in comparison to isotype-treated obese mice. These data demonstrate for the first time that effector populations of regulatory T cells (Tregs) are relevant for resolution of skin inflammation and that obesity specifically impairs this regulatory function. Taken together, obesity increases Th1/Th1-mediated ponipiao-like skin inflammation by increasing cutaneous macrophage recruitment and activation and by impairing Tregs and regulatory gamma/delta T cells causing dysregulation and exaggeration of the immune response.

**1025**

**Endoplasmic reticulum stress defines immature myeloid cell functional plasticity in leprosy**

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Leprosy is a chronic human infectious skin disease where the clinical manifestations correlate with the host response to the pathogen, Mycobacterium leprae (mLEP). In the self-limited, “tuberculoid” form (T-lep), T cells are instructed by myeloid cells, including macrophages and dendritic cells, to kill mLEP whereas, in the progressive “lepromatous” form of disease (L-lep), T cells typically do not respond well to mLEP despite the presence of numerous myeloid cells in lesions. Type I (reversal reactions; RR) and Type II (erythema nodosum leprosum; ENL) immune reactions can also occur where patients with borderline disease can either get improved (RR) or worsening (ENL) immune responses. Myeloid cells in leprosy have not been completely characterized. Herein, we identify the expansion of cells with a myeloid-derived suppressor cell phenotypes (MDSC; HLADR+CD13+CD11b+) that suppress antigen-specific T cell function in the blood of patients with L-lep and ENL. Cells with the same phenotype are present in skin lesions of leprosy patients. Using gene expression profiling of purified MDSC, we identified the presence of an ER stress signature in patients whose cells were suppressive along with increased IL-1β. Induction of ER stress in cells isolated from T-lep patients enhanced T cell suppressor activity while adding antioxidants decreased suppressive activity of MDSC from L-lep or ENL patients. Addition of IL-1β was sufficient to induce ER stress in MDSC, ex vivo. In conclusion, using leprosy as a model, we identified considerable plasticity in the function of MDSC in different clinical presentations of leprosy and identified ER stress and IL-1β as modulators of MDSC function in patients with the progressive form of the disease.

**1026**

**Immune profiling of lymphocytes in mouse model of Aloepecia Areata using single step 17 parameter polychromatic flow cytometry**

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A comprehensive immune assessment of the lymphocyte compartment is critical to identifying pathogenic lymphocyte subsets associated with inflammatory and autoimmune diseases. Here, we developed a 17 parameter immunophenotyping FACs panel to profile mouse lymphocyte subsets. We included lineage defining markers: CD3, CD4, CD8, CD19 and CD335 that identified T, B and NK cell lineages; the immune receptor NKGD2; CD127 and CD25 for identification of Treg; cell migration and homing markers CD44, CD46, CXCR3, CD62L, CD103 and CD47; the activation marker CD69 and the T cell exhaustion markers PD1 and LAG3. Using this panel, we profiled skin draining lymph node (SDLN) lymphocytes of C3H/HeJ mice with Aloepecia Areata (C3H/HeJ-aa). Compared to control non-autoimmune C3H/ HeJ mice, SDLN lymphocytes of C3H/HeJ-aa mice showed a higher frequency of memory (CD44+ CD62L-) and central memory (CD44+CD62L-) T cells, and CD4 and CD8 T cells and B cells. Within the CD4 subset, we found a higher frequency (> or =1.5 fold) of total CCRC6, CD69, CD103+ CD4 T cells and Tregs in C3H/HeJ-aa mice as compared to control mice. Notably, we found that the CD8 T cell subset in C3H/HeJ-aa mice demonstrated marked differences (>3.8 fold) in total CD69+ and NKGD2+ CD8 T cells, and identified a CD4+CD8+ effector memory CD8 T cell population that co-expressed CD103, CD69 and NKGD2 which was absent in control mice. We previously reported that this effector memory CD8 T cell population induces AA when adoptively transferred in non-alopecic C3H/HeJ mice. This immunophenotyping panel not only successfully revealed previously identified pathogenic populations (CD44+CD62L-) and central memory (CD44+CD62L-) CD4 and CD8 T cells and B cells. This panel will be useful in immune profiling lymphocytes in other mouse models of autoimmune diseases, particularly in response to immunomodulatory treatments such as JAK inhibitors.

**1027**

**Koebnerisins (S100A15) - A novel player in the pathogenesis of rosacea**

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Background: Pathophysiology of rosacea is still not completely elucidated. An aberrant innate immune response may play an essential role in the pathogenesis of rosacea. The antimicrobial peptide koebnerisins (S100A15) is an innate immune player with diverse proinflammatory functions. However, its role in rosacea has not been investigated yet. Objective of the present study was to investigate the expression, function and regulation of koebnerisins (S100A15) in rosacea. Methods: The expression of koebnerins in skin lesions of patients with rosacea (n=6) was assessed by quantitative RTPCR and immunofluorescence analysis. The regulation and function of koebnerisin in rosacea-relevant skin cells cultures (keratinocytes and fibroblasts) was assessed by RT-PCR. Results: We showed that koebnerins is overexpressed in rosacea skin lesion. It is upregulated in keratinocytes, fibroblasts by TNF-α and IL-17A, cytokines relevant in the pathogenesis of rosacea. We showed that koebnerins may exert proangiogenic and proinflammatory function by priming keratinocytes and fibroblasts for enhanced production of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9). Koebnerisins (S100A15) may emerge as a novel player in the pathogenesis of rosacea. Balancing activity of certain antimicrobial peptides might be novel goal for future therapeutic intervention in rosacea.
1030
Role of mast cells in psoriasis-associated-pruritus
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Mast cells play an important role in regulating skin inflammation. However, their role in psoriasis-associated itch remains unclear. Here, we investigated whether mast cells contribute to itch in psoriasis. We used a modified murine model of psoriasis (4AB) to investigate the role of mast cells. We found that mast cell activation was increased in psoriasis skin compared to healthy skin. Furthermore, inhibition of mast cell activity significantly reduced itch and scratching behavior in psoriasis mice. These findings suggest that mast cells play a role in mediating itch in psoriasis.

1031
Regulation of IL-13 production by human Th2 cell and Tc2 T cells from peripheral blood and atopic lesional skin by S.aureus and their cell-wall products
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S.aureus is a common skin pathogen that triggers immune responses in atopic dermatitis patients. In this study, we investigated the role of S.aureus and its cell-wall products in regulating IL-13 production by human Th2 and Tc2 cells. We found that S.aureus induced IL-13 production by Th2 cells in vitro and in vivo. Furthermore, cell-wall products of S.aureus stimulated IL-13 production by Tc2 cells. These findings suggest that S.aureus and its cell-wall products play a role in regulating IL-13 production in atopic dermatitis patients.

1032
HLA-I shield tumor skin lymphocytes from Nc-cell-mediated elimination
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HLA-I is a shield that protects tumor cells from Nc-cell-mediated elimination. In this study, we investigated the role of HLA-I in regulating Nc-cell-mediated elimination. We found that HLA-I expression on tumor cells inhibited Nc-cell-mediated elimination. Furthermore, inhibition of HLA-I expression increased Nc-cell-mediated elimination. These findings suggest that HLA-I is a shield that protects tumor cells from Nc-cell-mediated elimination.

1033
TGF-β/Smad pathway is not required for epidermal LC development
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The TGF-β/Smad pathway is a key regulator of epidermal LC development. In this study, we investigated the role of TGF-β/Smad pathway in LC development. We found that deletion of TGF-β/Smad pathway genes had no effect on LC development. Furthermore, we found that deletion of TGF-β/Smad pathway genes did not affect LC function. These findings suggest that TGF-β/Smad pathway is not required for epidermal LC development.

1034
Lipoxin A4 diminishes mast cell activation and allergic contact hypersensitivity
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Lipoxin A4 is a lipid mediator that has anti-inflammatory properties. In this study, we investigated the role of Lipoxin A4 in regulating mast cell activation. We found that Lipoxin A4 inhibited mast cell activation. Furthermore, we found that Lipoxin A4 inhibited mast cell-mediated allergic contact hypersensitivity. These findings suggest that Lipoxin A4 is a potential target for regulating mast cell activation.

1035
Loss of transcription factor Ovol1 enhances skin inflammation in animal models of atopic dermatitis and psoriasis
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Ovol1 is a transcription factor that plays a role in regulating inflammation. In this study, we investigated the role of Ovol1 in regulating skin inflammation. We found that deletion of Ovol1 enhanced skin inflammation in animal models of atopic dermatitis and psoriasis. Furthermore, we found that Ovol1 modulated the activity of immune cells. These findings suggest that Ovol1 is a potential target for regulating skin inflammation.
Necrobiosis lipoidica histopathology & inflammatory composition
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Introduction: Necrobiosis lipoidica (NL) is a chronic, granulomatous disease linked to diabetes mellitus (DM). DM is an autoimmune that causes changes in its histology and inflammatory cell feature to better understand its etiology. Methods: A retrospective study was performed in Mayo Clinic from 1992 to 2017. Inclusion into the study required biopsy-proven NL where the histopathology and inflammatory infiltrate of 93 biopsies were reviewed by a dermato-pathologist. Results: The granulomatous changes of NL were most commonly diffused (84% No DM, 97% DM-2, 60% DM-1, p=0.048). The pattern of inflammation was most often pali-saded 54% and or tiered/layered 65% (59% No DM, 80% DM-2, 40% DM-1) involving the mid dermis and the subcutaneous tissue. Results were similar to those found by others. Conclusions: Necrobiosis lipoidica granulomas were more common in those without DM (24% No DM, 3% DM-2, 0% DM-1) (p=0.025). The subunits was involved in the non-DM and DM-2 10% of the time. Mucin was present more frequently in diabetics (17% non DM, 53% DM-2, and 40% DM-1) (p=0.01). Perivascular inflammation was present in 82% of cases and composed predominantly of lymphocytes (93% No DM, 100% DM-2, 80% DM-1) (DM-2 vs DM-1 p=0.013) and plasma cells 73%. Lymphocytic infiltrates were ubiquitous and plasma cells (81% No DM, 83% DM-2, 40% DM-1) (DM-2 vs DM-1 p=0.031) were significantly different in the infiltrate. Eosinophils were present (38% No DM, 10% DM-2, 20% DM-1) (p=0.02) and were seen in differing amounts in the dermis (p=0.049). Neutrophils were present (14% No DM, 50% DM-2, 60% DM-1) (p=0.011) and were seen in differing amounts in the dermis (12% No DM, 47% DM-2, 60% DM-1) (p<0.001). Giant cells were seen in 82% of cases. Foamy histiocytes were seen in 71% of cases and were often widespread (63% No DM, 30% DM-2, 13% DM-1) (p=0.042).

Conclusion: While sharing similar histopathology, the inflammatory profiles between the NL subgroups of Non-DM, DM-2 and DM-1 are distinct. NL may have different mechanisms between groups.

Evaluation of the skin microbiota in a healthy Chinese population
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To characterize the normal skin microbiota, we recruited 50 volunteers divided into children, adolescents, young adults, middle-aged adults, and elders. A total of 9,574,365 high-quality sequences of the V3 to V4 region of the 16S rRNA gene were annotated with taxonomic information related to two archaeal phyla (Thaumarchaeota and Euryarchaeota) and five dominant bacterial phyla (Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes and Cyanobacteria). The upper backs dominated by Propionibacterium and Staphylococcus, the cheeks facilitated the survival of Betaproteobacteria, while Alphaproteobacteria were prevalent on the volar forearms. After sexual maturity the cheek microbiota became more similar to the upper back. The highest gender-specific richness. Male had a greater species richness.

Propionibacterium acneus carbohydrates from acnec associated phylotypes induce distinct inflammatory response in comparison to carbohydrates from healthy phylotypes: A potential ligand implicated in acne disease pathogenesis
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Propionibacterium acneus is a resident of the skin that is isolated from acne patients and is considered an important component of acne etiology. In the presence of lipopolysaccharide (LPS), P. acnes has been shown to induce upregulation of inflammatory cytokines, such as TNF-a, IL-1b and IL-6, and cause cellular damage to the skin. However, the carbohydrate component of P. acnes is currently unknown. The current study aimed to identify the potential carbohydrate of P. acnes that induces inflammatory responses. We isolated seven distinct P. acnes strains from acne patients and healthy controls and analyzed their carbohydrate content. We found that the P. acnes carbohydrates that induce inflammatory responses are distinct from carbohydrates from healthy controls. The carbohydrate component of P. acnes that induces inflammatory responses is a potential ligand implicated in acne disease pathogenesis.

Characterizing a nuclear hormone receptor pathway that governs parasitic nematode infection using Nipponostrangulose brasiliensis as a model
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Recent work in our lab implicates the DAF-12 nuclear hormone receptor and its pathway as potential anthelmintic drug targets. The DAF-12 receptor is one of 284 nuclear receptors in C. elegans, a non-parasitic nematode, and is a key regulator of reproductive development and dauer diapause. The dauer stage in C. elegans is similar to the infectious larval stage (1 l1) in other nematodes. Infection by C. elegans in Larval stage (1 l1) organisms is comommon. Here we demonstrate that N. brasiliensis, a rodent hookworm with a life cycle and morphology similar to human hookworms, has a conserved and functional DAF-12 receptor. We also show that in a RNA-Seq transcriptome analysis, that there is differential expression of the cytochrome P450 genes in response to different temperature and ligand conditions. One of these cytochrome P450 proteins, may be the catalytic protein that forms the endogenous ligand for the DAF-12 receptor. Discoveries in this novel pathway in nematodes offers potential therapeutic targets for the management of the deadly disease, disseminated Strongyloides stercoralis infection seen in immuno-compromised patients. Though rare, this hyper-infection could potentially affect our patients, particularly patients who grew up in countries where this organism is common. The disease is also a potential new anthelmintic drug target. The DAF-12 receptor is one of 284 nuclear receptors in C. elegans, a non-parasitic nematode, and is a key regulator of reproductive development and dauer diapause. The dauer stage in C. elegans is similar to the infectious larval stage (1 l1) in other nematodes. Infection by C. elegans in Larval stage (1 l1) organisms is common. Here we demonstrate that N. brasiliensis, a rodent hookworm with a life cycle and morphology similar to human hookworms, has a conserved and functional DAF-12 receptor. We also show that in a RNA-Seq transcriptome analysis, that there is differential expression of the cytochrome P450 genes in response to different temperature and ligand conditions. One of these cytochrome P450 proteins, may be the catalytic protein that forms the endogenous ligand for the DAF-12 receptor. Discoveries in this novel pathway in nematodes offers potential therapeutic targets for the management of the deadly disease, disseminated Strongyloides stercoralis infection seen in immuno-compromised patients. Though rare, this hyper-infection could potentially affect our patients, particularly patients who grew up in countries where this organism is common. The disease is also a potential new anthelmintic drug target.
Psoriasis vulgaris. Altered intestinal microflora may be involved in the regulation of the microflora in patients with psoriasis vulgaris have been changed before and after treatment. Skin lesion mouse model have also been investigated. We showed that the intestinal species annotation, abundance analysis and diversity analysis. The effects of changes in the development of psoriasis vulgaris. The intestinal microflora is more complex, and many distension, constipation, mouth odor, and lower abdominal discomfort. The aim of this study resistance and chronic fatigue syndrome. Our earlier epidemiological survey showed that, occurrence and/or development of systemic chronic low grade inflammation, such as insulin distension, constipation, mouth odor, and lower abdominal discomfort. The aim of this study was to determine the close relationship between the changes of the intestinal microflora and was to determine the close relationship between the changes of the intestinal microflora and the development of psoriasis vulgaris. The intestinal microflora is more complex, and many microbes in the fecal sample are difficult to cultivate normally. We extract the genomic DNA of the stool samples from patients with psoriasis, and construct small-fraction library for cluster analysis according to regional characteristics of 16S rRNA V4. We analyze the diversity of the intestinal microflora from the sample and construct the treatment through species annotation, abundance analysis and diversity analysis. The effects of changes in intestinal microflora and their metabolites on the regulation of immune cells and influence of skin lesion mouse model have also been investigated. We showed that the intestinal microflora in patients with psoriasis vulgaris have been changed before and after treatment. We demonstrate the changes in intestinal microflora and their metabolites play vital roles in the regulation of immune cells and development of psoriasis mouse model. Our preliminary study showed that changes in intestinal microflora are closely related to the development of psoriasis vulgaris. Altered intestinal microflora may be involved in the regulation of the development of psoriasis by affecting the chronic inflammatory response. However, further research is needed for specific mechanisms.

Intestinal microflora and the development of psoriasis vulgaris

Previous studies have shown that intestinal microflora disorder is closely related to the occurrence and/or development of systemic chronic low grade inflammation, such as insulin resistance and chronic fatigue syndrome. Our earlier epidemiological survey showed that, compared to the general population, psoriasis patients had high frequency of abdominal distension, constipation, mouth odor, and lower abdominal discomfort. The aim of this study was to determine the close relationship between the changes of the intestinal microflora and the development of psoriasis vulgaris. The intestinal microflora is more complex, and many microbes in the fecal sample are difficult to cultivate normally. We extract the genomic DNA of the stool samples from patients with psoriasis, and construct small-fraction library for cluster analysis according to regional characteristics of 16S rRNA V4. We analyze the diversity of the intestinal microflora from the sample and construct the treatment through species annotation, abundance analysis and diversity analysis. The effects of changes in intestinal microflora and their metabolites on the regulation of immune cells and influence of skin lesion mouse model have also been investigated. We showed that the intestinal microflora in patients with psoriasis vulgaris have been changed before and after treatment. We demonstrate the changes in intestinal microflora and their metabolites play vital roles in the regulation of immune cells and development of psoriasis mouse model. Our preliminary study showed that changes in intestinal microflora are closely related to the development of psoriasis vulgaris. Altered intestinal microflora may be involved in the regulation of the development of psoriasis by affecting the chronic inflammatory response. However, further research is needed for specific mechanisms.

Efficacy of a topical agent containing a naturally occurring lipid for alleviating drug resistant impetigo

Impetigo is a major contagious dermatosis of childhood with lifelong consequences if untreated. Mupirocin and fusidic acid are available as first line of treatment for this disease caused mainly by S. aureus and S. pyogenes. However, challenge with the current treatment modality is the development of antimicrobial resistance. Hence, novel approaches including use of lipid-based treatment strategies against antimicrobial resistance are becoming increasingly promising. Such therapy may also provide restorative effects mediating healing at the sites of infection. Based on existing understanding of the anti-microbial action of lipids on various microbes and in-house screening, we have carefully chosen a potent naturally occurring agent (VLS-001) specific in its action against S. aureus. In-vitro and in-vivo anti-microbial activity of VLS-001 against drug susceptible and resistant S. aureus revealed the scope for beneficial topical therapy. In-vitro screening indicates MIC of 16-32 μg/ml against multiple S. aureus strains with variable antibiotic resistance profiles. Interestingly, VLS-001 also showed synergistic combinations with famoxid, a naturally occurring sesquiterpene alcohol, in checkerboard assays against both mupirocin resistant and susceptible S. aureus strains. In-vitro toxicity assays with the agent on human keratinocytes confirmed its safety profile for topical use. Finally, topical formulations of VLS-001 were effective in a murine model of S. aureus wound infection. Together, the data suggests that this agent has potential benefits in the treatment of impetigo.

Molecular characterisation of a multidrug-resistant dermatophyte strain from a clinical non-responder and development of a potential therapy

Trichophyton spp. cause superficial infections in immunocompetent people and deep to systemic infections in immunocompromised patients. Though multitude of antifungal therapies are available, yet the number of non-responder to the conventional first line therapy are increasing. Interestingly, there are very few reports on drug resistance in Trichophyton spp. and most fail to directly correlate clinical non-responsiveness to microbial resistance. To comprehensively evaluate this correlation, we isolated Trichophyton spp. from time patients and studied antifungal resistance patterns in the isolates through extensive microbiological and molecular biological assays. Genomic and proteomic techniques were employed to elicit differences among recalcitrant versus wild type strains. Most isolates were identified as Trichophyton rubrum with increased fluconazole and terbinafine MIC compared to standard strain. One particular isolate (KA-01), demonstrated increased MIC for multiple azoles and allylamines. Upon further analysis of KA-01, we found interesting variations in protein expression, that may be an underlying factor for its azole resistant phenotype whereas terbinafine resistance is due to a point mutation in squalene epoxidase gene. Interestingly, this strain is very sensitive to lipid-based treatment strategies. Topical formulations containing such an antifungal lipid were found to be highly effective against KA-01 out-competing marketed antifungals or in vitro resistant strains. We have further validated the in vitro based adjunct topical therapies for clinical non-responders infected with dermatophytes displaying pan antifungal resistance.

Systemic delivery of HMGB1 peptide ameliorates imiquimod-induced psoriasis-like dermatitis

Psoriasis is an autoimmune skin disorder characterized by severe inflammation and hyper-proliferation of epidermal cells in the skin. Th17, IL-17 producing helper T, cells are known to play pivotal roles in the psoriasis pathogenesis. Although therapies targeting Th17 cells are being developed, effective treatment for psoriasis has not established. Previously, we have demonstrated that damaged tissues release high mobility group box protein 1 (HMGB1) to promote tissue regeneration by mobilizing bone marrow mesenchymal stem cells (BM-MSCs). The BM-MSCs mobilized into the damaged tissues support efficient tissue regeneration by suppressing inflammation and by differentiating into multiple cell types. We have further identified the domain of HMGB1 which is essential and sufficient for activating BM-MSCs and showed that the HMGB1 domain peptide served as a promising drug for dystrophic epidermolysis bullosa (DEB), a skin genetic disorder characterized by severe blistering at skin. Because the HMGB1 therapy promotes tissue regeneration without targeting any specific molecules, this strategy can be potentially effective for a broad range of diseases with massive inflammation. Here, we tested whether the HMGB1 peptide treatment is effective for psoriasis using imiquimod (IMQ)-induced psoriasis-like dermatitis as a model. The systemic injection of HMGB1 peptide ameliorated the inflammatory manifestations of psoriasis. Consistent with these anti-inflammation activities, HMGB1 peptide decreased the gene expression of the inflammatory cytokines (IL-17A, IL-6 and IL-1β), suggesting that the HMGB1 peptide treatment could suppress activated Th17 cells. These data provide a foundation for the development a novel psoriasis therapy using a single defined endogenous molecule, HMGB1.
Mitsubishi are highly important organelles responsible for the production of the majority of cellular energy. However, this process generates the potentially harmful by-product reactive oxygen species (ROS), which cause damage to proteins, lipids, and nucleic acids and may result in decreased biological function, cancers, and aging. The skin is the largest organ of the human body, providing protection from external insults such as UV, toxicity, and pollution, which can accelerate ROS production; therefore, maintenance of skin health is highly important. Nicotinamide, a precursor for NAD+ and NADPH, is a member of the vitamin B family and is found in various foods. It has been demonstrated previously to result in improvements in inflammatory conditions, cancer prevention, and ATP synthesis, and is highly important. Nicotinamide, a precursor for NAD+ and NADPH, is a member of the vitamin B family and is found in various foods. 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**9053**

**Systemic silver absorption following application of silver-based dressings to patients with Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis**

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There is not enough evidence available to guide wound care management in patients with Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN). Currently, the treatment is mostly based on expert opinion and mimic burn wound treatments, including the use of silver-impregnated dressings. Silver-containing dressings reduce the risk of invasive infection by minimizing the bacterial colonization of wounds. Evidence from small clinical trials in burn patients and a recent study in SJS/TEN patients confirmed the efficacy of silver in preventing skin breakdown, but not requiring daily dressing changes, which may damage the healing epithelium, and minimize patient discomfort. However, cytotoxic effects of silver have been demonstrated in human and rodent keratinocytes. Studies investigating systemic silver absorption in burn patients have yielded conflicting results, with only a few case reports warning against the use of silver due to its cytotoxic effects. Systemic silver absorption in patients with SJS/TEN treated with any type of silver-based dressing has not been addressed in previous clinical studies.

**9054**

**Siroliimus gel treatment for tuberous sclerosis complex involving facial angiomyofibroblastic plaques: A multicenter randomized controlled trial**

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**Tuberous sclerosis complex (TSC), an autosomal dominant disorder that is caused by the constitutive activation of mammalian target of rapamycin, develops hamartomas in multiple organs. Facial angiomyofibroblastic plaques develop frequently and provoke cosmetic disturbances.** We conducted a phase 3, multicenter, randomized, double-blind, placebo-controlled, parallel-group, multinational, active-controlled, double-blind, placebo-controlled, phase 3 trial to evaluate the efficacy and safety of sirolimus gel for facial angiomyofibroblastic plaques in patients with TSC.

**9055**

**Evaluation of film dressings for the prevention of intraepidermal nerve growth and allodynia (touch-evoked itch) in murine dry skin models**

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Epidermal hyperinnervation is observed in dry skin diseases such as xerosis and atopic dermatitis. Such diseases manifest in low-threshold mechanical stimuli irritation of the skin, which is known as allodynia. Increased intraepidermal nerve fibers are thought to be one of the pathogenic factors in itch hypersensitivity. Film dressings for wound treatment act as a skin barrier and provide a moist environment. However, it has not been clear whether film dressings have any preventive effects on epidermal hyperinnervation and allodynia in skin diseases. We evaluated two different thickness film dressings, TEGADERM® (TDM) and PERME ROLL Lite (PMR), for their preventive effect on epidermal hyperinnervation using an acute dry skin model. Twenty square millimeters of TDM or PMR was applied after 5-min acetone treatment to the back skin of ICR mice. Skin samples were taken after an additional 48 h. Immunohistochemically, the numbers of protein gene product 9.5-immunoreactive nerve fibers in and penetrating into the epidermis were significantly decreased by TDM or PMR treatment. We further evaluated allodynia using a chronic dry skin model. A mixture of acetone and diethylether (1:1) for 15 s followed by distilled water for 30 s (AEW) was applied to the back skin of C57BL/6NCrHsd mice twice daily for 8 days. TDM or PMR with a diameter of 8 mm was applied on the AEW-treated site on the day after the last treatment. Allodynia assays showed that TDM and PMR application significantly prevented induction of allodynia. These findings suggest that the film dressings not only prevent epidermal hyperinnervation by moisturization but also allodynia by inhibiting the transmission of mechanical stimuli.

**9056**

**Tofacitinib leads to increased infections by downregulation of antiviral immune defense**

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Tofacitinib (Tykerb) is a Janus kinase (JAK) inhibitor approved for the treatment of rheumatoid arthritis and in clinical trials for psoriasis. In clinical trials investigating the therapeutic effects of tofacitinib, the most common adverse events observed were nasopharyngitis and upper respiratory tract infections. JAKs are found downstream of the type II cytokine receptor family. Many cytokines produced by TH17 cells stimulate their target cells via the type II cytokine receptor family and these receptors use the JAK pathway for signal transduction. These cytokines are also the leading cause of antiviral and antimicrobial peptides (AMP) by keratinocytes or synovocytes. Blockage of the JAK pathway might therefore result in a diminished secretion of antiviral and antimicrobial peptides. This reduced antiviral defense might result in a higher susceptibility to bacterial infections in patients treated with JAK inhibitors. We found that gene expression of antiviral peptides (SOAT007, IL18 or BD22) or others is not affected by JAK pathway inhibition using tofacitinib. But on the other hand, gene expression of all tested antiviral peptides such as MX1 or ISG51 is markedly reduced in a dose-dependent manner even without any co-stimulatory cytokine or bacterial component added. These findings are in accordance with our hypothesis that tofacitinib has an impact on antiviral immunity such as patients treated with tofacitinib often show adverse events like upper respiratory tract infections, and zoster. To sum it up, anti-microbial immunity does not seem to be affected by tofacitinib, but the antiviral immunity in basically shut down in presence of tofacitin in vitro.

**9057**

**Vemurafenib acts as an aryl hydrocarbon receptor antagonist**

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The BRAF-inhibitor vemurafenib has been successfully established in the therapy of advanced melanoma. Despite its superior efficacy, the use of vemurafenib is limited by frequent inflammatory cutaneous adverse events that affect patients quality of life and may lead to dose reduction or even cessation of anti-tumor therapy. To date, the molecular and cellular mechanisms of the induced rash effects have largely eluded analysis. Against this background, we here demonstrate that vemurafenib inhibits the downstream signaling of the canonical pathway of aryl hydrocarbon receptor (AhR) in vitro, thereby reducing the expression of proinflammatory cytokines (e.g. IL-1β, TNFα) and angiogenic factors. With these results we observed an impaired expression of AhR regulated genes (e.g. CYP1A1) and an upregulation of the corresponding proinflammatory genes in vivo. Moreover, results of lymphoma models revealed the absence of specific T cells in vemurafenib-treated mice. Taken together, we obtained no hint of an underlying sensitization against vemurafenib but found evidence suggesting that vemurafenib enhances proinflammatory responses by inhibition of AhR signaling. Our findings contribute to our understanding of the central role of the AhR in skin inflammation and may point towards a potential role for topical AhR agonists in supportive cancer care.

**9058**

**IL36-mediated skin inflammation requires signaling through chemokine receptor CCR6**

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ChemOCentex, Inc, Mountain View, CA

Generalized pustular psoriasis (GPP) is a rare inflammatory skin disorder with an etiology distinct from common plaque psoriasis. Patients often do not respond to therapeutic agents used routinely to treat plaque psoriasis. Genetic evidence suggests that GPP arises from dysfunction in the IL13/IL13α/IL13α signaling axis, and many aspects of GPP can be reproduced in the mice by intradermal IED injection of pre-activated IL13. We have used this ID-IL36 model to study the leukocyte populations that accumulate within GPP skin. In a previous study, we found that a small molecule CCR6 antagonist, CXC2553, ameliorates IL36-mediated skin inflammation. CXC2553 reduces IL36-mediated skin inflammation while preserving cytokines such as CD4+ T cell population. Thus, although disparate T cell populations are associated with each model, inflammation is ameliorated by CCR6 antagonism in both models. These findings suggest that CCR6 can constitute a novel target for a mechanistically distinct approach towards GPP therapy.
Using the methyl nicotinate induced vasodilatation and histamine iontophoresis in vivo models for screening anti-inflammatory activity

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Skin erythema can be induced by multiple pathways. To screen actives for broad anti-inflammatory benefits, we used two different induced-inflammation models for evaluation: topical histamine iontophoresis (HI) and iontophotic application of histamine (IH). Topical HI induces a rapid vasodilatation of the peripheral blood capillaries of the skin and is mediated by prostaglandin release. While topical histamine penetration is generally poor, iontophotic application of histamine enhances skin permeability and releases neuropeptides to induce erythema (flares). After a 3 day pretreatment application of the known anti-inflammatory Tetracyclorocurcumin (THC), a metabolite of curcumin, both the HI and IH induced erythema were reduced significantly. These results demonstrate the broad anti-inflammatory activity of THC.

1063 Topically applied Nanoflares to measure gene expression in vivo

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Spherical Protein Nanospheres (Nanoflares) are topical agents that pass through the skin to monitor gene expression in vivo. We hypothesized that Nanoflares could be topically applied to measure gene expression in vivo. As proof-of-concept, we detected connective tissue growth factor expression (CTGF), known to be increased in hypertrophic scars and keloids. Using fibroblasts from normal skin, keloids, hypertrophic scars and peri-scar tissue, we found that: a) Nanoflares are specific; b) expression measured by Nanoflares mirrors that by RT-PCR; and c) Nanoflares can detect expression changes after treatment with inducers (e.g., TGF-β) or inhibitors (e.g., RepSox, rapamycin, anti-TGF-β siRNA). To test in vivo application, we confirmed by iVIS imaging that GPDH Nanoflares in Aquaphor penetrate hairless mouse and human abdominoplasty skin (8x more expression than scrambled). CTGF Nanoflares, but not controls, co-localized with labelled fibroblasts in 3-D scar models and quantification of signal showed strong correlation (r=0.95). Treatment of scar fibroblasts with RepSox reduced CTGF Nanoflare detection to that of normal fibroblasts. Finally, Nanoflares detected 2.25-fold more CTGF expression in the rabbit ear hypertrophic scar model vs unwounded skin using iVIS imaging and normalizing the Cy5 fluorescence signal to GPDH/Eccl number. These data suggest that Nanoflares can be topically applied to measure changes in gene expression, raising the possibility of adaptation to noninvasively diagnose disease based on altered gene expression or mutation, screen for new drugs, and monitor the molecular impact of disease treatment.

1064 JAK kinase inhibitors efficacious in models of murine contact hypersensitivity

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Murine models of dermal contact dermatitis have been used as preclinical screening tools to evaluate the therapeutic potential for therapy of human inflammatory diseases such as atopic eczema, psoriasis, and atopic dermatitis. All of these diseases are known to be driven by JAK/STAT and other cytokine pathways (e.g., IFNγ and IL6 pathways in humans) and have shown efficacy in multiple animal models. To understand the impact of deuterium substitution on the exposure-response relationship of CTP-543, a JAK inhibitor with a selective cytokine inhibition profile biased towards proinflammatory cytokines, PK/PD modeling suggests that CTP-543 can provide therapeutic efficacy on AA-relevant pathways (e.g., IFNγ while potentially reducing Jak1/3- and JAK2/2-dependent adverse effects. CTP-543 is currently in Phase 2 testing in AA patients. These results support further testing in other autoimmune diseases.
1065
Assessment of anti-aging properties of novel natural compounds-peptide derivatives
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Peptides generally have biocompatibility and high activity, but their cosmetic applications are often limited by susceptibility to proteolysis which was resulted to in vivo fragility. Therefore, N-terminal truncation method using small organic molecules is commonly used to prevent peptide degradation. Hereby we investigated safety and activity of the newly synthesized two peptide derivatives which were prepared by introducing 2,5-dihydroxybenzoic acid on N-terminal of peptide or pentapeptide. The results obtained by in vitro digestion studies indicated that the two new compounds have the ability to increase collagen production by about 1.5 times compared to vehicle control.

1066
Biomarkers CCL17/TARC and total IgE do not predict clinical response to dupilumab in atopic dermatitis (AD): A post hoc analysis of pooled phase 3 trials
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Dupilumab (DUP), a fully human IL-4R mAb, inhibits signaling of IL-4/IL-13, key drivers of Type 2 Th2 immune diseases such as AD/asthma. CCL17 (TARC) and IgE production are enhanced in IL-4/IL-13 KO mice, suggesting that intradermal injection of DUP weekly in minipigs may recruit lymphocytes to dermal tumoral distribution. Having established the ability of dye-loaded NNPs and BNPs to associate with dermal targets we sought to determine the safety and clinical activity of the newly synthesized two peptide derivatives conjugated to tetrapeptide and pentapeptide, respectively, we assayed cytotoxicity with MIT assay on human dermal fibroblast (ATCC, USA). Also, anti-aging properties were assessed with three independent in vitro tests: procollagen type-1 assay, matrix metalloproteinase-12(MMP-12) activity inhibition assay. As a result, both 2,5-DHBA-peptide derivatives did not show cytotoxicity at 10, 20 and 50 μM. Tetrapeptide alone did not show increased collagen production and MMP-1 inhibitory activity, but the 2,5-DHBA-pentapeptide derivative increased collagen production by about 25% and inhibited the UVB (1J/cm²)-induced MMP-1 expression by about 40% compared to control group (p<0.05). Also, pentapeptide alone did not show increased collagen production and MMP-1 inhibitory activity, but the 2,5-DHBA-pentapeptide derivative increased collagen production by about 20% and inhibited the UVB (1J/cm²)-induced MMP-1 expression by about 40% compared to control group (p<0.05). These results proved the potential use of newly synthesized two 2,5-DHBA-peptide derivatives as cosmetic ingredient for anti-aging.

1067
Fascin and Cdk2 are synthetic lethal partners with exceptional potential as joint therapeutic targets in malignant melanoma
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Malignant melanoma is the most deadly of all skin cancers. It is important to study novel combinations to improve outcomes for patients with malignant melanoma. In this work we studied the synthetic lethality and potential activity of topical products formulated with Cannabis Sativa oil produced in Italy, in Tuscany (not containing THC). Recent studies have suggested a possible moisturizing and elasticizing activity of Cannabis Sativa oil. For such characteristics Cannabis Sativa oil appears an appropriate ingredient to be used in topical formulations for the treatment of skin hydration. The oil was incorporated into EEM emulsion in a standard formulation at percent concentration of 1%, 3% and 5% respectively of Cannabis Sativa seed oil, one EEM emulsion was prepared without active ingredient as control. The investigation was carried out on 20 healthy male and female volunteers, between the ages of 20 and 40, with normal or dry skin. Each product was applied to the volar surface of the forearm at a dose of 3 mg/cm². As control, the same cream without active ingredient was used to control. The results showed that the 3 emulsions with Cannabis Sativa oil, compared to the emulsion without active ingredient, significantly increase the degree of hydration and elasticity of the skin. The 3% formulation of Hemp oil has a greater power of hydration of the skin compared to emulsions, both short and long term and is the emulsion that has produced better results.

1068
Formulation and evaluation of topical products with Cannabis Sativa oil
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The aim of this work was to develop topical formulations and the activity of topicals formulated with Cannabis Sativa oil produced in Italy, in Tuscany (not containing THC). Recent studies have suggested a possible moisturizing and elasticizing activity of Cannabis Sativa oil. For such characteristics Cannabis Sativa oil appears an appropriate ingredient to be used in topical formulations for the treatment of skin hydration. The oil was incorporated into EEM emulsion in a standard formulation at percent concentration of 1%, 3% and 5% respectively of Cannabis Sativa seed oil, one EEM emulsion was prepared without active ingredient as control. The investigation was carried out on 20 healthy male and female volunteers, between the ages of 20 and 40, with normal or dry skin. Each product was applied to the volar surface of the forearm at a dose of 3 mg/cm². As control, the same cream without active ingredient was used to control. The results showed that the 3 emulsions with Cannabis Sativa oil, compared to the emulsion without active ingredient, significantly increase the degree of hydration and elasticity of the skin. The 3% formulation of Hemp oil has a greater power of hydration of the skin compared to emulsions, both short and long term and is the emulsion that has produced better results.

1069
Minipig model of atopic dermatitis: Assessment of in vivo and in vitro activity of recombinant porcine interleukin-4 and interleukin-13
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Atopic Dermatitis is a common skin condition that clinically presents as erythematous, dry, pruritic skin. While multiple factors contribute to the pathophysiology of AD the relative and prominent Th2-mediated immune responses are characteristic of AD. Interleukin-4 (IL-4) and Interleukin-13 may contribute to the pathogenesis of AD. Minipigs are useful for toxicology in vitro and efficacy of applied products, and thus a model of AD in minipigs would be beneficial for pre-clinical efficacy tests of such medications. This study assessed sensitivity of Hanford minipigs to recombinant porcine (rp) IL-4 and IL-13. Peripheral Blood Mononuclear Cells (PBMC) isolated from female Hanford minipigs demonstrated approximately a 40% increase in IL-4 and approximately a 38% increase in IL-13. When challenged with rp IL-4, or rp IL-11. When female Hanfords received a single intradermal dose of rp IL-4 or rp IL-13, erythema and edema was not different from vehicle control dose sites. However, repeat intradermal injections for a period of fourteen days resulting in detectable levels of rp IL-4 dose above 0.1 mg/ml in vehicle control, but not rp IL-13 dose sites. The peak iritation was observed approximately 5 minutes after dose administration, similar to histamine injections in minipigs. Interestingly, perivascular eosinophils were observed in 25% of the rp IL-4 dose sites, while not observed in vehicle or rp IL-11 dose sites. This suggests that intradermal injection of rp IL-4 and rp IL-13 may recruit lymphocytes to dermal tissues. Minipigs were relatively more sensitive to rp IL-4 and rp IL-11 compared to human dermal equivalents. Minipigs, and are good candidates for further exploration in developing a porcine model of Atopic Dermatitis.
1071

Age-specific changes in the atopic dermatitis molecular phenotype

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From old king coal to novel therapeutics for inflammatory skin diseases: The aryl hydrocarbon Receptor as a therapeutic target

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Topical application of coal tar is one of the oldest dermatological therapies. Despite its unknown untill now, we discovered that coal tar activates the aryl hydrocarbon receptor (AHR). We identified three different classes of AHR ligands as novel candidate drugs with therapeutic effects in a 3D skin model for atopic dermatitis, and 3) genotoxicity by the Ames test. We found that topical application of C3H/HeJ mice at P60 (telogen phase) with 2% Voro-nosin or Entinostat (another HDAC1/3 inhibitor) promoted early entrance into anagen phase in normal mouse skin, similar to JAK inhibition. STAT proteins are substrates for HDACs and STAT3 is a known substrate of HDAC1/3. HDAC inhibitors may promote hair regrowth in AA mice via interfering with JAK-STAT signaling that governs both hair cycle and T cell activity. Given that there are no FDA-approved drugs for AA, computationally driven drug repurposing strategies based on gene regulatory networks offer a new approach in identifying new targeted therapies for patients with AA.

1077

Promotion of hair growth in normal mouse skin and alopecia areata by topical treatment of HDAC inhibitors

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Reverse-engineered regulatory networks have recently demonstrated great promise in the treatment of complex diseases, such as cancer and have been used for drug discovery. We recently conducted regulatory modeling of an autoimmune form of hair loss disease, alopecia areata (AA), from gene expression analyses of AA scalp biopsies with the goal of predicting new drug targets. Vorinostat is an HDAC1/3 inhibitor that was predicted by computational mechanism of action analysis (DeMAND algorithm) to target over 50% of the known exo-

1073

Characterization of anti-aging potential and active component of Grifola frondosa ethanol extract

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Grifola frondosa (maitake mushroom) contains abundant polysaccharides and β-glucan, and its aqueous extract has intensively been investigated. Recently, G. frondosa ethanolic, but not aqueous, extract has been reported to have an effect on hyaluronic acid production in human dermal fibroblasts (hDFs). However the bioavailabilities of the hydrophobic components derived from G. frondosa has not been sufficiently elucidated compared with those of its hydrophilic components. In this study, we identified one of its hydrophobic components, which increases hyaluronic acid production in HDFs. Initially, we isolated one active fraction by crude fractionation using thin layer chromatography. After further purification of the crude fraction by high-pressure liquid chromatography, we found a highly anti-aging active fraction using liquid chromatography - tandem mass spectrometry and nuclear magnetic resonance. We also found that G. frondosa ethanol extract increases the expression of differentiation markers, filaggrin and involucrin, in human epidermal keratinocytes (hEKs), suggesting that the hydrophobic ingredients in G. frondosa promote epidermal differentiation. We found that the ergosterol analog increases the expression of these epidermal differentiation genes as well. These results imply that G. frondosa ethanol extract might have a common role in NDFs and hEKs, although further research is needed. Our findings indicate the possibility that the hydrophobic components in G. frondosa have anti-aging and thera-

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Mimosa pudica plant cell culture extracts (mimosine free) exhibit anti-inflammatory activities and inhibit the responses in vitro

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Mimosa pudica has been used for decades in SE-Asia to treat inflammation. However mimosine, a well-known toxic molecule, was found invariably in the natural plant extracts. By plant cell culture metabolic engineering, we were able to inhibit intracellularly mimosine expression. It was under UPLC detection level 1 ng per ger dry weight (DW) of cell extract (E13) compared to 2160 ng per ger DW in natural leaves extract (E11). (ii) RAW264.7 murine macrophages were incubated with 50 μg DW/ml of E13 or E11, or 1 μM Dexamethasone for 1 H and were stimulated with LPS for 24 H. Quantifications of NO, IL-4 and TNFα were performed by Griess, ELISA and Luminescence techniques respectively. Similarly to DEX, E13 and E11 inhibit NO and IL-6. TNFα expression was reduced by E13 and DEX, in contrast it was increased by E11. (ii) In a Th2 induced model, NHEK cells were incubated with 0, 0.75 and 1.5 μg DW/ml of W01 a similarly prepared E13 extract for 1 H and were stimulated with (0 μg + TNFα + IL-4 + IL-13) for 24 h. mRNA quantification of AMP cytokines and chemokines by RT-qPCR has shown: a dose-dependent increase of 5100±77 RNASE7, DEF044A and a significant decrease of IL-1β and CXCL8. Finally, TSLP and TLR-3 both known to be involved in pruritus were highly downregulated. In conclusion, mimosine free plant cell culture extracts exhibit anti-inflammatory activities and counteract Th2 responses. This high added value eco extracts could be potentially incorporated in topical formulations for treating inflammatory skin diseases.

1076

Novel blend of vegetable ingredients protects the skin from pollution damage

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In the past years, the cosmetic industry developed products to prevent and protect skin from early aging, against environment factors specially sun exposure. However, recent researches brought new knowledge about molecular biology of extrinsic aging skin. Not only UV ra-

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1077
Anti-inflammatory and wound healing activities of calophyllolide isolated from Calophyllum inophyllum Linn

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Due to the high-cost and limitations of current wound healing treatments, the search for alternative approaches or drugs, particularly from medicinal plants, is of great importance. In this study, we report anti-inflammatory and wound healing activities of the major calophyllolide (CP) compound isolated from Calophyllum inophyllum Linn. The results showed that CP had no effect on HaCaT cell viability over a range of concentrations. CP reduced fibrosis in tissue, protected wound models from cytokine challenge without causing body weight loss. The underlying molecular mechanisms of wound repair by CP was investigated. CP markedly reduced MPO activity, and increased M2 macrophage skewing, as shown by up-regulation of M2-related gene expression, which is beneficial to the wound healing process. CP treatment prevented a prolonged inflammatory process by down-regulation of the pro-inflammatory cytokines—IL-1β, IL-6, TNF-α, and up-regulation of the anti-inflammatory cytokine, IL-10. This study is the first to indicate a plausible role for CP in accelerating the process of wound healing through anti-inflammatory activity mechanisms, namely, by regulation of inflammatory cytokines, reduction in MPO, and switching of macrophages to an M2 phenotype. These findings may enable the utilization of CP as a potent therapeutic for cutaneous wound healing.

1079
Phospho-proteomic profiling reveals distinct signaling pathways by first and third generation EGFR inhibitors in human keratinocytes: Implications for adverse dermatologic reactions

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The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase and a major regulator of cellular proliferation, differentiation, and survival. Altered EGFR signaling is a major driver of both cutaneous and non-cutaneous cancers and has also been implicated in conditions such as psoriasis and eczema. While EGFR inhibitors are commonly used to treat several malignancies, they notoriously result in adverse dermatologic effects, including papulopustular and acniform eruptions, pruritus, paronychia, xerosis, and mucocutaneous reactions. The inhibitors exhibit differential toxicities, possibly due to differences in EGFR signaling. Here, we examined whether three such inhibitors, erlotinib, gefitinib, and osimertinib, exhibit distinct signaling in human keratinocytes. Using reverse phase protein arrays of 301 proteins associated with EGFR signaling, we find that all three inhibitors equally suppressed phosphorylation of 12 proteins that are normally phosphorylated following EGFR activation. However, the inhibitors differentially regulated phosphorylation of 13 other proteins, including important translation regulators such as 4E-BP1 and eIF4E. Gefitinib most potently inhibited the 13 proteins, whereas osimertinib blocked fewer, and erlotinib blocked none. These results may have implications for adverse dermatologic reactions associated with EGFR inhibitors, and suggest that modulating phosphorylation of proteins associated with EGFR signaling may be a novel therapeutic approach to minimize adverse dermatologic effects.

1080
Preparation and characterization of ethosomal nanocarriers for transdermal delivery of cosmetic bioactive ingredient

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Key words: Transdermal delivery system, Nanocarriers, Cosmetic bioactive ingredient. TDS was used generally for delivery of active ingredient due to limitation of its topical using method. It can overcome some disadvantages of injection and oral delivery also. But efficiency of TDS is extremely low due to the nature of skin barrier. Stratum corneum functions as barrier to protect against surrounding environments. So, various active ingredients cannot easily penetrate the skin barrier. Thus many technologies have been studied for enhancing efficiency of TDS. Liposomes, nano-sized lipid vesicles, can entrap hydrophilic or lipophilic agents on the inside. These vesicles are so suitable for delivery carriers due to non-toxic and biodegradable, while they have some drawbacks such as their poor skin permeability. Ethosomes have been developed overcame these shortcomings. They are more elastic modified liposomes containing ethanol in relatively high concentration: 65-85%. Ethosomes disturb the skin barrier, increase of vesicles on skin, ethosomes more easily penetrate into the stratum corneum. The aim of this study was to develop ethosomal carriers capable of enhancing stability and skin permeability of active ingredients and evaluate them. Our new ethosomal carriers, which were mainly composed of phospholipid, ethanol and water, were prepared with varying the composition and preparation conditions and cholesterol was added to make membrane stable. These formulations were measured particle size and entrapment efficiency and skin permeability was evaluated. Since ethosomes were permeated through the intercellular route, the smaller the size, the higher the entrapment efficiency, the better the penetration of the skin. Based on these results, the preparation conditions were optimized. Cosmetic active ingredients loaded ethosomes such as niacinamide, retinol and alpha bisabolol showed more higher permeability than TDS. And ascorbic acid loaded liposomes and ethosomes were prepared and their stability was evaluated by DPPH assay to determine how well vitamin c was protected. Ascorbic acid was the most stable when it was loaded in ethosomes rather than liposomes or solution.

1081
Identification of a natural inhibitor of one-carbon metabolism in keratinocytes

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Dysregulated metabolism has long been noticed in patients of psoriasis, especially enhanced amino acid metabolic activity. We found that in epidemics of psoriasis patients down-regulated amino acid metabolism, such as serine and methionine metabolism. One-carbon metabolism is an important metabolic pathway involving not only sulfur amino acids like taurine, homocysteine and methionine et al, but also methylation metabolism. Acetyl-11-keto-β-boswellic acid (AKBA) is the most bioactive ingredient of the ancient folk medicine boswellic acids. It is been approved potentials in various inflammatory diseases including psoriasis and tumors. Here we show that AKBA can directly interact with methionine adenosyltransferase 2A (MET) and shows potential in treating psoriasis.
**1083**

Effect of RVT-502 therapy in the NC/Nga mouse model of atopic dermatitis

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The NC/Nga mouse model represents a model of human atopic dermatitis. In this study, we investigated the efficacy of RVT-502, an immunomodulator targeting the JAK-STAT pathway, in the NC/Nga mouse model of atopic dermatitis. The study was conducted using a randomized, placebo-controlled, double-blind trial design. The results showed a significant reduction in clinical signs of dermatitis, including skin lesions, itchiness, and scratching behavior, in mice treated with RVT-502 compared to placebo. These findings suggest that RVT-502 may have potential as a therapeutic agent for the treatment of atopic dermatitis in humans.

**1084**

PR022 inhibits IL-1β in vitro and in a model of dermal hypersensitivity indicating potential efficacy for treatment of psoriasis and acne vulgaris

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The aim of this study was to investigate the potential of PR022, a novel anti-inflammatory compound, in inhibiting IL-1β production in both in vitro and in vivo models. In vitro, PR022 was found to significantly reduce IL-1β production in human keratinocytes and dermal fibroblasts. In a mouse model of dermal hypersensitivity, PR022 was shown to reduce IL-1β levels in the skin, indicating its potential as a topical therapeutic agent for the treatment of psoriasis and acne vulgaris.

**1085**

Cellular mechanistic investigation on antigen delivery by Viaskin® patch for epicutaneous immunotherapy with reconstructed human epidermis

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Viaskin® patches are widely used for the treatment of food allergies. However, the mechanism of antigen delivery and the efficacy of this approach remain unclear. In this study, we investigated the cellular mechanistic processes involved in antigen delivery by Viaskin® patches using a combination of in vitro and in vivo approaches. Our results indicate that Viaskin® patches efficiently deliver antigens to the skin, leading to an immune response that can be modulated to target specific disease states.

**1086**

QR-313, an antisense oligonucleotide, restores expression of functional type VII collagen in DEB patient cells

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Dystrophic epidermolysis bullosa (DEB) is a blistering disorder caused by mutations in the COL7A1 gene, encoding type VII collagen (C7). Our study aimed to investigate the potential of QR-313, an antisense oligonucleotide, to restore C7 expression in DEB patient cells. Using a combination of transcriptome analysis and functional assays, we found that QR-313 treatment significantly increased C7 expression and improved cell adhesion and proliferation in DEB patient cells, suggesting its potential as a therapeutic agent for DEB.

**1087**

Age- and UV-induced overstimulation of mechanistic target of rapamycin activity in keratinocytes and dendritic cells

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The mechanistic target of rapamycin (mTOR) pathway is a central regulator of metabolism and cell growth, and its activation can lead to skin aging and UV damage. In this study, we investigated the activation of mTOR in keratinocytes and dendritic cells from young and aged mice, as well as in cells exposed to UV radiation. Our results showed that mTOR activity is increased in aged cells and in cells exposed to UV radiation, indicating a role for mTOR in skin aging and UV damage.

**1088**

Efficacy of AD-DER emollient in an *in vitro* atopic dermatitis model

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Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by recurrent eczematous plaques, intense pruritus, and enhanced IgE production. The complex etiology of AD is not fully understood, but it is believed to involve both genetic and environmental factors. In this study, we investigated the efficacy of AD-DER, an emollient formulation, in an *in vitro* model of AD. Our results showed that AD-DER effectively reduced inflammation and itching in cultured skin models, indicating its potential for treating AD. Future studies are needed to confirm these findings in clinical trials.
1089
Antipruritic effect of the novel kappa opioid receptor agonist CR845

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Patients with chronic kidney disease are commonly afflicted by pruritus, a condition with no treatment currently approved in the USA. The etiology of pruritus is condition is likely multifactorial, including systemic inflammation and deficiency in the endogenous kappa opioid system. CR845 is a novel and full kappa opioid receptor agonist with no off-target activities, including mu- or delta-opioid receptors. In addition to its unique receptor profile, the peptidic structure of CR845 impacts its entry into the central nervous system. CR845 demonstrated a dose-dependent anti-itch activity in mouse models of itch induced by 5-GNT1 (a selective kappa opioid receptor antagonist), or by compound 48/80 (a mast cell secretagogue). Additionally, CR845 was well tolerated at doses ranging from 0.5 to 1.5 mcg/kg. Reduction in itch NRS scores over placebo was observed at all doses, with a change from baseline ≥5 NRS points by end of Week 8 for 64% of the patients treated with CR845 0.5 mcg/kg vs 29% of the placebo patients (p=0.001). These results suggest that peripheral kappa opioid receptors play an important role in the modulation of itch signals and represent a target for the development of novel antipruritic agents. The profile of CR845 makes this compound a suitable and promising candidate for the treatment of moderate-to-severe pruritus associated with chronic kidney disease.

1090
Clinical pharmacokinetics and immunogenicity of GBR 830, a first-in-class humanized monoclonal antibody inhibiting OX40 to treat atopic dermatitis

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In order to study pruritus in vitro, we used a skin explant model that has been re- innervated by sensory neurons. This model is based on a co-culture between a human skin explant and human dorsal root ganglia of rat. After 5 days of co-culture in tranwell, we were able to confirm the presence of nerve fibers in the epidermis. Re-innervation was correlated with decrease of epidermal thickness and apoptosis compared with non-re-innervated skin. In order to demonstrate the potential of GBR 830 to prevent skin inflammation, we compared its effect to that of the co-culture model. GBR 830 was well tolerated and showed a similar PK profile in healthy volunteers and subjects with AD. A favorable linear PK profile with a long half-life, high bioavailability, and no evidence of target-mediated clearance. Six of 14 GBR 830-treated subjects were positive for anti-drug antibody (ADA), 2 of whom had neutralizing ADA. An early bioavailability (AUC) study was conducted in healthy adults with a single dose of GBR 830 by IV (600 mg/subject) or subcutaneous (SC) administration (75 or 600 mg/subject). The 5% of GBR 830 by the SC route was 76%, with Cmax achieved around 5 days post-dose. Lower AUC of ADA was observed in neutralizing ADA subjects. In vivo efficacy studies compared to lower dose (75 mg SC: 1015 subjects). PK of GBR 830 was also evaluated in subjects with moderate-to-severe atopic dermatitis (AD) (NCT02683928). Two IV doses of GBR 830 (10 mg/kg; 4 weeks apart) in subjects with AD showed minimal immunization in AD patients (SILAB Inc., Hazlet, NJ)

1091
Evaluation of the in vitro skin penetration of acyclovir cream through human cadaver skin

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Purpose: The in vitro vertical diffusion cell system with human skin and finite dose model is a valuable tool for the study of percutaneous absorption and evaluation of the pharmacokinetics of topically applied drugs. Acyclovir, the active ingredient of acyclovir cream which is used to treat infections caused by herpes viruses, was studied for the in vitro skin penetration profile using the diffusion cell system. The profile of three dosing amounts was compared for acyclovir cream.Methods: The vertical diffusion cells have a 1 cm2 surface area and approximately 8 ml receptor volume. Human skin was placed on each cell and dosed once with 5, 10 and 15 mg of the acyclovir cream product using a positive displacement pipette. Phosphate Buffered Saline buffer (1XPBS, pH 7.4) was used as the receiving media. At pre-selected times after dose application, a 500) ml aliquot of receiving media was removed through the sampling arm of the diffusion cell and replaced with an equal volume of fresh receiving media during the tested 48 hours with a flux peak before 16 h. The penetration levels of acyclovir through human cadaver skin were measured using LC-MS/MS. Results: Acyclovir effectively penetrated through human cadaver skin from acyclovir cream of three dosing amounts. Drug penetrated gradually into the receiving media during the tested 48 hours with a flux peak before 16 h. The penetration profile demonstrated a significant discrimination of acyclovir cream of 5, 10 and 15 mg of dosing amount, with 48h drug accumulation of 1.36 ± 0.08, 5.71 ± 0.09 and 7.58 ± 0.06 μg respectively. Good linearity of regression (r²=0.99) was observed between 48-hours drug accumulation and dosing amount between 5 to 15 mg. Conclusions: This study successfully established an in vitro penetration methodology to discriminate acyclovir penetration behavior among applied dose of 5, 10 and 15 mg.

1092
Mechanism of action of Oligofructans from Ophiopogon japonicus to treat atopic dermatitic

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The aim of this study was to identify the mechanism of action of Oligofructans (OF) from Ophiopogon japonicus whose efficacy was demonstrated in atopic dermatitis pediatric population. For this purpose, we analyzed the transcriptomic level of BPD in vitro model mimicking the inflammatory context of AD skin. Interestingly, OF target essential biological pathways described as modified during the course of AD: TH1- and TH2-inflammation, epithelial differentiation and water transport. More precisely, we found 9 genes whose expression is restored by OF in this set of genes. Among them, OF reduce the expression of TLR3 an immune receptor known to induce a vicious inflammatory circle involving TSLP secretion by human keratinocytes. Hence, OF could act on key genes of the inflammatory cascade of AD. OF restore the expressions of genes involved in epidermal differentiation (Loricrin and Filaggrin) and water transport (CAB). As expected, this mechanism of action was validated at morphological and functional level since OF increase the synthesis of Claudin-1, Loricrin and Filaggrin while reducing epidermal spongiosis, penetration of Lucifer Yellow and pro-inflammatory cytokines secretion. Complementary study analyzing bacterial colonization by scanning electron microscopy also revealed that OF limit the adhesion of Staphylococcus (S.) aureus and thus the formation of a biofilm at the surface of the tissue. Analysis of HBD2 and HBD3 genes expression revealed that this efficacy may be related to a stimulation of beta-defensins by OF. Through robust modeling studies, we demonstrated that OF from Ophiopogon japonicus correct at the transcriptomic level, the four major defects of atopic skin: inflammation, disruption of barrier function, dehydration and S. aureus invasion. Altogether, these results highlighted a beneficial effect of OF in the course of AD.

1093
Selective protection against bacterial adhesion by a natural second-skin film:

Maintenance of S. epidermidis/S. aureus balance

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Skin colonization with virulent strains of Staphylococcus (S.) aureus is related to cutaneous diseases such as atopic dermatitis, pruritis or acne. Physical protection of these skin would be of interest in dermatological research in order to complete existing therapeutic treatments. In this context, we transposed the powerful IPN (Interpenetrating Polymer Network) technology to biopolymers in order to develop a protectant second-skin film able to limit pathogens adhesion. For this purpose, galactomannans from Caespasilina spinosa and sulfated galactans from Kappaphycus alvarezi were interpenetrated using an ionic crosslinker. The resulting interpenetrating biopolymer network forms a resistant, flexible and non-adhesive film. As sulfates within the biopolymer lead to a negatively charged film, it may explain the observed selective bacterial protection. Additionally, CR845 demonstrated anti-inflammatory properties in rodents and human macrophages. CR845 was not detectable in the central nervous system, therefore CR845 is likely to activate kappa opioid receptors expressed in peripheral neurons and on immune cells. These data are consistent with an 8-week phase II randomized, placebo-controlled study which demonstrated the efficacy of CR845 in treating pruritus in patients with atopic dermatitis. CR845 was administered intravenously after dose application, a 500

1094
Analysis of the activation of TRPV1, TRPA1 and PAR-2 receptors with a cytokine profile and electrophysiological response in a re-innervated skin explant model mimicking the chronic inflammation of atopic dermatitis

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To study pruritus in vitro, we used a skin explant model that has been re- innervated by sensory neurons. This model is based on a co-culture between a human skin explant and human dorsal root ganglia of rat. After 5 days of co-culture in tranwell, we were able to confirm the presence of nerve fibers in the epidermis. Re-innervation was correlated with decrease of epidermal thickness and apoptosis compared with non-re- innervated skin. In order to demonstrate the potential of CR845 to prevent skin inflammation, we compared its effect to that of the co-culture model. GBR 830 was well tolerated and showed a similar PK profile in healthy volunteers and subjects with AD. A favorable linear PK profile with a long half-life, high bioavailability, and no evidence of target-mediated clearance. Six of 14 GBR 830-treated subjects were positive for anti-drug antibody (ADA), 2 of whom had neutralizing ADA. An early bioavailability (AUC) study was conducted in healthy adults with a single dose of GBR 830 by IV (600 mg/subject) or subcutaneous (SC) administration (75 or 600 mg/subject). The 5% of GBR 830 by the SC route was 76%, with Cmax achieved around 5 days post-dose. Lower AUC of ADA was observed in neutralizing ADA subjects. In vivo efficacy studies compared to lower dose (75 mg SC: 1015 subjects). PK of GBR 830 was also evaluated in subjects with moderate-to-severe atopic dermatitis (AD) (NCT02683928). Two IV doses of GBR 830 (10 mg/kg; 4 weeks apart) in subjects with AD showed minimal immunization in AD patients (SILAB Inc., Hazlet, NJ)
1095 Evaluating Ikk 16 and ACHP as anti-inflammatory agents for treatment of skin inflammation: in vitro and in vivo

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The skin faces the greatest exposure to environmental agents which contribute to the skin's susceptibility to developing acute and chronic inflammation. NF-kB and its upstream modulators, including Ikk, play a critical role in mediating inflammation. In this study, we examined the anti-inflammatory properties of two P-gp substrates that are inhibitors of Ikk kinase (Ikk), ACHP and Ikk 16, and evaluated their potential as topical anti-inflammatory drugs through inhibition of NF-kB signaling. P-gp, or MDR1, is expressed in human and mouse skin, and we hypothesized that P-gp substrates would make good candidates for transdermal delivery of topically-applied drugs because P-gp can facilitate their absorptive transport into the systemic circulation. An P-gp-mediated efflux assay revealed both Ikk 16 and ACHP are high affinity P-gp substrates. Inhibitors, and Ikk 16 and ACHP completely blocked nuclear translocation of NF-kB in human and murine keratinocytes challenged with IL-1α. Pretreating primary keratinocytes with Ikk 16 or ACHP blocked cytokine gene expression induced by IL-1α, TNFα, and PMA in a dose-dependent manner. This indicated that both drugs block the inflammatory signaling pathway downstream of NF-kB activation in vitro. However, in vivo studies showed topical ACHP treatment, but not Ikk 16, blocked PMA induced cytokine expression in KS-PKCa mice, a mouse skin inflammation model. Further studies revealed that the topical application of ACHP reduced both skin inflammation induced by Imiquimod and tumor formation in DMBA initiated and promoted KS-PKCa mice. In this model ACHP application was not effective at eliminating established tumors. Pretreatment with Ikk 16 inhibited cytokine expression induced by UVB exposure in vivo. These data indicate that ACHP is a potential candidate for a topical drug used for treating skin inflammation that is mediated by NF-kB signaling.

1096 Non-invasive tape sampling reveals a type I interferon RNA signature in cutaneous lupus erythematosus that differentiates affected from unaffected and healthy controls

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Background: Type I interferon (IFN-I) genes are upregulated in skin lesions and blood from patients with cutaneous lupus erythematosus (CLE). Punch biopsy, the standard monitoring procedure, impedes patient recruitment and follow up due to risk of infection, discomfort and cosmetic scarring. This study assessed the feasibility of an adhesive tape device to collect RNA from affected and unaffected skin and its potential to detect gene expression differences between groups. Methods: Subjects with active discoid lupus erythematosus (DLE, n=9), subacute CLE (SCLE, n=1), atopic dermatitis (AD, n=3) and healthy volunteers (HV, n=9) were enrolled. Skin tape samples from affected (CLE-A) and unaffected (CLE-U) skin were collected with whole blood samples; gene expression was quantified by qPCR on the ABI 7500 Real-Time PCR System. Results: Significant gene expression differences were observed for both CLE-A vs HV skin and CLE-A vs CLE-U skin, respectively, in IFN-I (10- and 4-fold), cytokotyi T cell (9- and 5-fold), and CLE-upregulated (4- and 40-fold) gene sets; plasmacytoid dendritic cell related genes were upregulated (3-fold) for CLE-A vs HV skin.

1097 Small molecule Wnt pathway inhibitor (SM0755) as a potential topical treatment for psoriasis

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SM0755 is a small-molecule Wnt pathway inhibitor previously demonstrated inhibition of inflammation and keratinocyte proliferation in vitro and in an in vivo Imiquimod (IMQ)-induced mouse psoriasis (PSO) model. Further studies revealed IMQ scid mouse reconstitution model with minor histocompatibility mismatched naive C57BL/6×Imj-Mj mice, more closely resembling human IMQ pathway driven JAK2/STAT3 protein type mouse were identified from F2 (BALB/c × 129Sv) by flow cytometry. CD4+CD45RB+ cells from donor mouse spleens were purified and injected intravenously into C57BL/6×Imj-Mj mice challenged with IMQ. The CD45RB+ cells were evaluated weekly for PSO-like signs, (lesions, increased thickness). PSO Mice were randomized and treated with SM0755 (400 mg/cm2) or vehicle. After 14 weeks, body and spleen weight was not significantly different between groups. Skin appearance and tumor thickness were significantly (p<0.05) and immune infiltration in the skin compared to vehicle. Immunofluorescent levels in the skin, ears, spleen and plasma using ELISA. Epidermal thickness and infiltrating cells in skin were histologically evaluated. Immune reconstitution of IMQ scid mouse resulted in PSO-like signs, with lesions and increased thickness of the skin and ears. Compared to vehicle, topical SM0755 (400 mg/cm2) significantly (p<0.01) decreased skin and ear thicknesses and improved skin appearance. Body weights were also significantly (p<0.05) higher in treated mice. SM0755 significantly reduced microscopically epidermal thickness (p<0.05) and immune infiltration in the skin compared to vehicle. In mouse model of minor histocompatibility mismatched T lymphocyte reconstitution-induced PSO, topical SM0755 inhibited inflammatory and decreased skin and ear thicknesses compared to vehicle. SM0755 has potential as a topical therapy for PSO. Clinical trials are on-going.

1098 Efficacy of selective next-generation JAK inhibitors in the treatment of alopecia areata

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In vitro and in vivo in an Imiquimod (IMQ) - and in an Imiquimod (IMQ)- treated C3H/HeJ mouse grafted model by our group and others. To further interrogate the role of individual JAKs in AA, and to produce the desired anti-inflammatory effects without unnecessary inhibition of other JAKs, we tested the comparative efficacy of several next-generation selective JAK inhibitors in the C3H/HeJ mouse model of AA. We found that JAK1/3-selective inhibitors (BMS917592, Dostinex, and AT13017) and JAK1-selective inhibitors (Filgotinib and Ruxolitinib) as well as JAK3-selective inhibitors (PF06651600 and Decomimot) robustly induced hair re growth and decreased AA-associated inflammation in AA mice. In contrast, we observed that a number of JAK2-selective inhibitors (CIPZ3779, CYT387 and AZD1480) failed to restore hair growth in AA mice, suggesting that JAK2 inhibition is not required for efficacy in the treatment of AA. Our findings indicate a dominant role of JAK1 or JAK3 over JAK2 in the pathogenesis of AA, and further, that selectively targeting JAK1 and/or JAK3 is sufficient to reverse AA. Our findings provide a conceptual framework for predicting the relative efficacy of next-generation JAK inhibitors in the treatment of AA as these compounds move into broader clinical investigation.

1099 GBR 830: An OX40 antagonist antibody with a favorable toxicity profile in non-human primates

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GBR 830 is a monoclonal antibody that targets OX40, a co-stimulatory receptor expressed predominantly on activated T cells. Ligation of OX40 by its ligand OX40L activates T cells with a lower affinity than OX40L, at a later time point, with a lower affinity. However, its apparent affinity drastically increases when GBR 830 binds bivalently. GBR 830 blocks OX40L binding and inhibits OX40-mediated T cell proliferation at a low nM concentration. Ligation of OX40L induces low levels of cytokines by dominant cellular cytokine and complement dependent cytokotyposis. Importantly, GBR 830 was evaluated for residual agonism by assessing its costimulatory effect on the proliferation of purified T cells from multiple donors. Compared to OX40L or anti-CD28 positive controls, GBR 830 did not stimulate T cells with or without addition of a crosslinking antibody. In a more sensitive experimental setup in which anti-OX40 antibodies were co-stimulated with an anti-CD1 antibody, no agonism was detected with GBR 830, whereas all other anti-OX40 antibodies tested showed agonism. When analyzed on mononuclear cells in a xenogeneic graft versus host disease model using immunodeficient mice reconstituted with human peripheral blood mononuclear cells, in vitro vaccine (tetanus toxoid) or autologous reactivation assays revealed that GBR 830 can inhibit T cell activation. In a cytokotyposis model of T cell-dependent antibody response targeting keyhole limpet hemocyanin, GBR 830 also demonstrated an effect on memory response but not on primary antibody response. Finally, in a xenogeneic grafted model of treatment of grafted mice with GBR 830 resulted in amelioration of the psoriasis phenotype when compared to the isotype control treated, as visualized by the reduction in epidermal thickness. Overall, these data suggest that GBR 830 has immunomodulatory capabilities in memory T helper cells-mediated pathological responses.

1100 Targeting OX40 with GBR 830, an OX40 antagonist, inhibits T cell-mediated pathological responses

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OX40 is predominantly on activated T cells. Ligation of OX40 by its ligand OX40L (TNFSF4, CD252) leads to enhanced T cell survival, proliferation, and effector functions. Blocking the OX40/OX40L pathway is therefore highly attractive to treat a broad range of T cell-mediated autoimmune disorders. GBR 830, a humanized IgG1 monoclonal antibody targeting OX40 with proven antigenic properties and devoid of any detectable agonistic activity, blocks OX40L binding and inhibits OX40L-mediated T cell proliferation in vitro. Functional nonclinical pharmacology studies demonstrated that GBR 830 is able to suppress T cell-mediated allergic, immune, and inflammatory responses. In a xenogeneic graft versus host disease model using immunodeficient mouse reconstituted with human peripheral blood mononuclear cells. In vitro vaccine (tetanus toxoid) or autologous reactivation reactions showed that GBR 830 can inhibit T cell activation. In a cytokotyposis model of T cell-dependent antibody response targeting keyhole limpet hemocyanin, GBR 830 also demonstrated an effect on memory response but not on primary antibody response. Finally, in a xenogeneic grafted model of treatment of grafted mice with GBR 830 resulted in amelioration of the psoriasis phenotype when compared to the isotype control treated, as visualized by the reduction in epidermal thickness.
Pharmacology and Drug Development | ABSTRACTS

1101 Canine model of chronic atopic dermatitis and staphylococcal infections
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Atopic dermatitis (AD) is a chronic skin disease characterized by pruritus and propensity for staphylococcal infections. The majority of animal models for AD involve murine although mice do not reproduce the complexity of the human disease. Many murine models involve the injection of allergens to induce a genetic or environmental pruritic dermatitis. A new canine model, defined the landscape of subtype selective vulnerabilities in MCC, discovered aurora kinase B as a novel MCC therapeutic target. An aurora kinase B inhibitor demonstrated on-target activity in all VP - MCC cell lines, supporting the viability reduction observed in the small molecule and RNAi cell lines in the context of Aurora Kinase B inhibition. Importantly, in a preclinical xenograft mouse model of VP-MCC, a novel aurora kinase B inhibitor nanofilmation shrank MCC tumors over a four-week treatment period and resulted in a robust increase in survival. Taken together, we have identified the subtype of selective vulnerabilities in MCC, discovered aurora kinase B as a novel therapeutic target and identified an aurora kinase B inhibitor nanoparticle as a new treatment for VP-MCC.

1102 Phosphatidylglycerol as a potential therapy for psoriasis
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Psoriasis is a common inflammatory skin disease that result in a poor quality of life for the patient. Many of these antimicrobial peptides have been reported to serve as damage-associated molecular patterns (DAMPs) to activate TLRs and induce an immune system response. Therefore, we examined the ability of S100A9 to serve as a DAMP and activate inflammatory mediator expression through TR2 and/or TR4 in keratinocytes and RAW264.7 cells, as well as the ability of PG, versus another phospholipid phosphatidylcholine (PC), to regulate these events. Using antagonists, we found that recombinant S100A9-induced expression of several inflammatory mediators through both TR2 and TR4 and resulted in activation of nuclear factor-kB (NF-kB). Importantly, we show that PG, but not PC, inhibited the S100A9-induced activation of NF-kB and expression of inflammatory mediators in RAW264.7 cells. Since S100A9 and other DAMPs are in psoriasis, this result suggests the possibility of using PG to treat this common skin disease.

1103 Functional genomic evaluation of targetable pathways in three metastatic cutaneous squamous cell carcinomas
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Cutaneous squamous cell carcinoma (cSCC) is increasing, paralleled by increases in deadly invasive and metastatic primary skin tumors. To date there are neither reliable predictive biomarkers of disease progression nor medical treatments as standard of care. To address these issues, we exposed primary cultured cells from invasive or metastatic cSCC tissues to 118 small molecule inhibitors as single agents and in combination with an EGFR inhibitor, these issues, we exposed primary cultured cells from invasive or metastatic cSCC tissues to 118 small molecule inhibitors as single agents and in combination with an EGFR inhibitor. In-house bioinformatics tools were used to cross analyze drug responses and DNA mutations in tumors detected by whole exome sequencing with patient-matched whole blood DNA standards. According to a paradigm that (in)activating mutations may predict sensitivity to targeted inhibitors, we associated drug sensitivities with DNA mutations predicted to be “damaging” by SIFT. Two cases sensitive to AKT inhibitor in combination with gefitinib had mutations in AKT3. One case, the only one with FLT or CSFR mutations, was sensitive to a “damaging” by SIFT. Two cases sensitive to AKT inhibitor in combination with gefitinib had mutations in AKT3. One case, the only one with FLT or CSFR mutations, was sensitive to a

1104 High-throughput small-molecule and RNAi screens identify Aurora kinase B inhibitors as a novel treatment for Merkel cell carcinoma
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Merkel cell carcinoma (MCC) is a rare and deadly skin cancer with limited treatment options. Approximately 80% of MCCs are driven by the Merkel cell polyomavirus (virus-positive MCC: VM-MCC), and the remaining 20% have a significantly higher mutational burden and are caused by UV mutagenesis (virus-negative MCC: VN-MCC). To identify small-molecules that can be repurposed for MCC treatment, we screened our library of ~4,000 clinical drugs and pre-clinical oncology compounds against three VP-MCC cell lines, three VN-MCC cell lines, and four immortalized control cell lines. Using viability as a readout across all cell lines, we identified >100 compounds as potential MCC therapeutic candidates. We then validated the hit list of compounds in MCC cell lines, consistent with their having distinct pathophysiology. We identified compound classes, including aurora kinase B inhibitors that more potently reduced VP-MCC cell viability relative to VN-MCC and control cells. An RNAi library targeting ~700 kinases screened in VP and VN-MCC cell lines independently identified aurora kinase B as a novel MCC therapeutic target. An aurora kinase B inhibitor demonstrated on-target activity in all VP - MCC cell lines, supporting the viability reduction observed in the small molecule and RNAi cell lines in the context of Aurora Kinase B inhibition. Importantly, in a preclinical xenograft mouse model of VP-MCC, a novel aurora kinase B inhibitor nanofilmation shrank MCC tumors over a four-week treatment period and resulted in a robust increase in survival. Taken together, we have defined the subtype of selective vulnerabilities in MCC, discovered aurora kinase B as a novel therapeutic target and identified an aurora kinase B inhibitor nanoparticle as a new treatment for VP-MCC.

1105 The effect of chemical penetration enhancers and dosage form on in vitro skin permeation
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Topical delivery of any drug may be facilitated by chemical penetration enhancers (CPE), agents that alter the barrier properties of the skin. In addition, dosage form design, such as ointment, cream, or gel base, can impact skin permeation based on drug partitioning between formulation and skin. The objective of this study was to evaluate the impact of CPE and dosage form on permeation of a model compound. A model compound (TREN01) with molecular weight less than 500 Daltons was formulated into gels and ointments with addition of CPEs. The in vitro skin permeation of 1% w/w formulations was tested across dermatomed (~450μm thickness) human cadaver skin using Franz type diffusion cells over 24hs. Each skin was dosed with ~ 10 mg of drug product using a positive displacement pipet. At the end of the study, the tissue samples of stratum corneum, epidermis and dermis, and receptor media samples were analyzed for drug content using LC/MS/MS. Drug permeated into the receptor media following the permeation profile of the vehicle. In all cases, receptors levels were significantly improved permeation into derm for three gels formulations (p value 0.033, 0.019, 0.007) when compared to one of the ointments. However, no statistical difference was observed within gels and ointments with and without CPEs. Dosage form design of topical formulations appear to have the biggest effect on drug product penetration. The next major factor was solubility of the molecule in the dosage form and aid in delivery to the target site. However, this effect was seen on several factors such as the molecular properties and formulation base.

1106 Successful treatment of perioral discoid lupus with cyclosporine ophthalmic emulsion (restasis)
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Perioral involvement is an uncommon manifestation of discoid lupus that is often difficult to treat despite various topical medications and the inherent difficulty of applying topical medications. It commonly affects the lower lid margin causing blepharitis and malaras as well as significant cosmetic distress. Current treatment options include oral dihydroxychloroquine, methotrexate, topical corticosteroids, topical tacrolimus or intraleonial steroids, often with an unsatisfactory response. Here we report a case of a 55 year old Hispanic female with longstanding and isolated perioral discoid lupus who was successfully treated with cyclosporine ophthalmic emulsion. The patient presented 10 years ago with lower lid lid erythema and madarosis. Antibodies including ANA by immunofluorescence, anti-Ro, and anti-La were negative. Over the course of 10 years she experienced an incomplete response with multiple therapies including anti-malarials, thalidomide, and low-dose glucocorticoids. The patient underwent treatment with cyclosporine ophthalmic emulsion with progressive resolution of perioral DLE over a course of 12 months. Cyclosporine is a calcineurin inhibitor that inhibits T cell mediated immune response, which plays an important role in lupus pathogenesis. The ophthalmic emulsion form, commercially branded as Restasis, is commonly used to treat patients with Sjogren’s disease and Sicca symptoms. Other calcineurin inhibitors including tacrolimus and pimecrolimus are established treatments for patients with cutaneous lupus. These medi- calizations are often ineffective in treating the perioral disease. Our case suggests that intraleonial T cell inhibitors such as cyclosporine may be helpful adjuivants in the treatment of recalcitrant perioral discoid lupus.

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Fibroblast culture model of dysregulated collagen homeostasis in aged human skin: Identification of natural compounds with restorative activity

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Cumulative oxidative damage is thought to play a crucial role in the pathophysiology of many chronic diseases that are associated with aging. A prominent feature of aged human skin is reduced dermal strength and function, due to fragmentation and decreased production of type I collagen fibrils, which comprise the bulk of the dermis. We have established a fibroblast culture model that mimics the imbalance in collagen homeostasis that is observed in aged human skin and employed this model to identify natural compounds that are able to restore the balance. Primary adult human skin fibroblast cultures were exposed to hydrogen peroxide (H2O2, 200 μM), for one hour on two consecutive days. This treatment resulted in time-dependent alterations in collagen homeostasis, as observed in aged human skin. For days post treatment, type I procollagen mRNA and protein levels were reduced 84% and 88%, respectively (p<0.01, n=8). Matrix metalloproteinase-1 (MMP-1), which initiates collagen fibril degradation, mRNA and protein levels were elevated 5.2-fold and 6.4-fold (p<0.01, n=6), respectively. We tested 15 natural compounds, which are present in a variety of botanicals, that have reported medicinal properties. Of these compounds, we found that the alkaloid berberine, found in a variety of herbs, was effective at restoring type I collagen expression. Berberine acted in a dose-dependent manner, increasing type I procollagen 4-fold (p<0.01, n=6) at a concentration of 50 μM. The flavonoid kaempferol, which is found in a variety of common fruits and vegetables, was effective at reducing MMP-1 expression. Kaempferol (10 μM) reduced MMP-1 levels to near baseline (p<0.01, n=6). Interestingly, the combination of berberine and kaempferol effectively restored type I procollagen and reduced MMP-1 levels. Taken together, the above data demonstrate establishment of a fibroblast cell culture model of age-related collagen dysregulation and the use of this model to identify natural compounds, that improve collagen homeostasis.

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Resveratrol-berberine combination significantly inhibits melanoma cells growth

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Melanoma is among the most life-threatening cancers. Despite recent therapeutic advances, management of melanoma remains difficult. Therefore, new mechanism-based approaches are needed. Our previous study found that SIRTs can inhibited the primary melanoma growth through an autophagy-dependent manner. It was indicated the potential of SIRTs to be a therapeutic target in melanoma. Resveratrol, a natural stilbenes, has been shown to induce chemopreventive and therapeutic effects against melanoma. A number of in vitro and in vivo studies have suggested potential usefulness of resveratrol in melanoma management. However, its rapid metabolism limited the clinical use, which indicated that it is necessary to combine with other agents to postpone its metabolism. Berberine, has been shown to inhibit melanoma cell growth. Interestingly, resveratrol and berberine, both are found in several herbal medicine and plants, indicating their natural association. In this study, we tested the hypothesis that resveratrol-berberine combination may have better efficacy against melanoma than either of the individual use, in addition to its own effect, can enhance the bioavailability of each other, thereby imparting superior anti-proliferative efficacy. We found that resveratrol-berberine combination resulted in a superior anti-proliferative response in human melanoma cell lines (WM35 and WM793B) as assessed by CCK8 assay for cell growth and viability. Further, the flow cytometry analysis revealed significant cell cycle arrest in the G1 phase in the same cell lines. However, the combination promoted the apoptosis of melanoma cells. Our data demonstrated that resveratrol-berberine combination was markedly superior to either of the individual agents, in intervention setting when treatment was started. Further studies are ongoing in our Laboratory to verify the in vitro data and determine the mechanisms associated with the observed effect of resveratrol-berberine combination.
1101 Ultraviolet B radiation upregulates the production of serine racemase in mouse keratinocytes
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Abstract: Background: Serine racemase (SR) is a non-enzymatic protein that catalyzes the synthesis of D-serine, an endogenous coagonist for N-methyl-D-aspartate (NMDA)-type glutamate receptor in the central nervous system. Recent studies have shown that the lipopolysaccharide stimulation can induce the expression of SR from microglia. This finding suggests that the induction of SR expression following pro-inflammatory stimulation highlights the important connection between neuroinflammation and excitotoxicity. We recently showed that SR protein is present in the epidermis of wild-type mice but not in SR knockout mice, and described a mechanism underlying the conversion of L-serine to D-serine in epidermal keratinocytes. However, the role of SR in the ultraviolet (UV)-induced inflammatory phase remains elusive. We examined the expression and production of SR in mouse skin after UVB radiation. UVB exposure significantly increased the expression and production of SR in the C57BL/6 mouse skin and that in human skin.

Conclusion: In this study, SR expression was upregulated in mouse and human skin exposed to UVB radiation. Our study is the first report of the expression of SR in human normal dermal fibroblasts and mouse keratinocytes. Our results suggest a predictive and functional link in gene expression regulation. Research of ceRNA network. In this study, we focused on lncRNAs which is associated with UVB-induced inflammation and excitotoxicity. We recently showed that SR protein is present in the epidermis of wild-type mice but not in SR knockout mice, and described a mechanism underlying the conversion of L-serine to D-serine in epidermal keratinocytes. However, the role of SR in the ultraviolet (UV)-induced inflammatory phase remains elusive. We examined the expression and production of SR in mouse skin after UVB radiation. UVB exposure significantly increased the expression and production of SR in the C57BL/6 mouse skin and that in human skin.

Methods: The co-culture system of normal human epidermal melanocytes-keratinocytes was used to irradiate human dermal fibroblasts. Screen emitted light has been shown to decreased ATP production, decreased type I pro-collagen synthesis and slower cell proliferation. A LED device, designed to closely mimic the irradiation of communication screens, was used to irradiate human dermal fibroblasts. Screen emitted light has been shown to damage cells on both a genomic and cellular level. After visible light exposure, fibroblasts present a phenotype representative of aged cells and show clear damage to the mitochondria and the cytoskeleton. With increasing use of LED devices, the additional stress of screen-emitted visible light should not be neglected in the fight against photo-aging.

Conclusions: Our study is the first report of the expression of opsins in HDFs and keratinocytes. Immunofluorescence assay demonstrated that the expression of Opsin1-SW, Opsin2, Opsin3, Opsin4, Opsin5 and Opsin6 mRNA were detected by quantitative real-time PCR and western blotting analysis respectively. Results: The double-labeled of immunohistochemistry staining using polyclonal antibody against MelanA and keratin was used to detect the melanocytes and keratinocytes in the co-culture system. The difference of mRNA levels and protein contents of opsins1 between those in the NHEMs-NHks co-cultures and in the NHMs monolayer cultures was measured by real-time fluorescence quantitative PCR. The results of real-time fluorescence quantitative PCR demonstrated that higher expression of Opsin3 mRNA were detected in the melanocytes co-cultured with keratinocytes than those cultured alone. Conclusions: Our research first demonstrated that overexpression of opsins1 up-regulates expressions of tyrosinase related proteins and increases tyrosinase activity in the melanocytes co-cultured with keratinocytes in vitro. Our study is the first report of the expression of SR in human normal dermal fibroblasts and mouse keratinocytes. Our results suggest a predictive and functional link in gene expression regulation. Research of ceRNA network. In this study, we focused on lncRNAs which is associated with UVB-induced inflammation and excitotoxicity. We recently showed that SR protein is present in the epidermis of wild-type mice but not in SR knockout mice, and described a mechanism underlying the conversion of L-serine to D-serine in epidermal keratinocytes. However, the role of SR in the ultraviolet (UV)-induced inflammatory phase remains elusive. We examined the expression and production of SR in mouse skin after UVB radiation. UVB exposure significantly increased the expression and production of SR in the C57BL/6 mouse skin and that in human skin.

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1112 Over expression of opsin 3 up-regulates the activity of tyrosinase in human epidermis melanocytes co-cultured with keratinocytes in vitro
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Abstract: Opsins are a group of proteins belonging to visual opsins expressed in brain, eye and tests in both vertebrates and invertebrates, including human beings. While Opsin1 are significantly more abundant than other opsins in the human epidermis melanocytes in vitro, their function remains unknown. Objective: To investigate the relationship between overexpression of opsin1 and the activity of tyrosinase in human epidermis melanocytes. Methods: The co-culture system of normal human epidermal melanocytes-keratinocytes (NHEMs-NHks) was established in vitro. The double-labeled of immunohistochemistry staining using polyclonal antibody against MelanA and keratin was used to detect the melanocytes and keratinocytes in the co-culture system. The difference of mRNA levels and protein contents of opsins1 between those in the NHEMs-NHks co-cultures and in the NHMs monolayer cultures was measured by real-time fluorescence quantitative PCR. The results of real-time fluorescence quantitative PCR demonstrated that higher expression of Opsin3 mRNA were detected in the melanocytes co-cultured with keratinocytes than those cultured alone. Conclusions: Our research first demonstrated that overexpression of opsins1 up-regulates expressions of tyrosinase related proteins and increases tyrosinase activity in the melanocytes co-cultured with keratinocytes in vitro.

1114 Voriconazole enhances skin tumour development by UVB through the mechanism its induction of inflammatory response
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We previously shown that inflammatory response induced by ultraviolet B (UVB)/reactive oxygen species/DNA damage has a pivotal role for skin tumour development. Recently, some researches have reported that UV-induced inflammation and excitotoxicity. We recently showed that SR protein is present in the epidermis of wild-type mice but not in SR knockout mice, and described a mechanism underlying the conversion of L-serine to D-serine in epidermal keratinocytes. However, the role of SR in the ultraviolet (UV)-induced inflammatory phase remains elusive. We examined the expression and production of SR in mouse skin after UVB radiation. UVB exposure significantly increased the expression and production of SR in the C57BL/6 mouse skin and that in human skin.

Methods: The co-culture system of normal human epidermal melanocytes-keratinocytes was used to irradiate human dermal fibroblasts. Screen emitted light has been shown to decreased ATP production, decreased type I pro-collagen synthesis and slower cell proliferation. A LED device, designed to closely mimic the irradiation of communication screens, was used to irradiate human dermal fibroblasts. Screen emitted light has been shown to damage cells on both a genomic and cellular level. After visible light exposure, fibroblasts present a phenotype representative of aged cells and show clear damage to the mitochondria and the cytoskeleton. With increasing use of LED devices, the additional stress of screen-emitted visible light should not be neglected in the fight against photo-aging.

Conclusions: Our study is the first report of the expression of opsins in HDFs and keratinocytes. Immunofluorescence assay demonstrated that the expression of Opsin1-SW, Opsin2, Opsin3, Opsin4, Opsin5 and Opsin6 mRNA were detected by quantitative real-time PCR and western blotting analysis respectively. Results: The double-labeled of immunohistochemistry results confirmed the establishment of co-culture system of human epidermis melanocytes and keratinocytes in vitro. The results of real-time fluorescence quantitative PCR demonstrated that higher expression of Opsin3 mRNA were detected in the melanocytes co-cultured with keratinocytes than those cultured alone. Conclusions: Our research first demonstrated that overexpression of opsins1 up-regulates expressions of tyrosinase related proteins and increases tyrosinase activity in the melanocytes co-cultured with keratinocytes in vitro.
1116 The extract of Ganoderma lucidum inhibits MMP-1 expression through suppression of ERK activation in UVB irradiated dermal fibroblast and skin equivalent model
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Ganoderma lucidum is an ancient medicinal herb used in China as a herbal medicine. Several studies suggested that Ganoderma lucidum polysaccharides have anti-tumor activities and anti-oxidation effects. This study was conducted to investigate the protective effect of G.lucidum in UVB-induced human fibroblasts and skin equivalent models. Filiblasts exposed UBV increased MMP-1 protein expression and decreased procollagen protein expression. Treatment with G. lucidum inhibited UVB-induced MMP-1 expression and increased procollagen expression. Extracellular signal regulated kinase (ERK) activation was inhibited by treatment with G. lucidum and UVB-induced collagen destruction was decreased. The results demonstrated that G. lucidum can inhibit UVB-induced MMP-1 expression by inhibiting the ERK pathways.

1117 MiRNAs involved in ultraviolet radiation induced bystander effects
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Sunlight is a widespread natural radiation source which may induce photo aging reactions. Recent researches indicate that similar responses can be found in non-irradiated cells through bystander effects. In this study, we set up a UV-induced bystander effect model in vitro by using human skin fibroblasts. Differentially expressed miRNAs in irradiated cells, bystander cells and culture medium were identified with microarray and quantitative real-time polymerase chain reaction (qRTPCR). As a result, miR-4655-3p and miR-769-5p were found and further investigated for time-related expression, as well as the expression of precursor miRNAs (pre-miRNA). The potential biological pathways of these two miRNAs were also predicted. In addition, up-regulating miR-4655-3p and miR-769-5p by miRNA mimics in non-irradiated cells can induce bystander-like effects. Taken together, our research reveals that miR-4655-3p and miR-769-5p are involved in UV-induced bystander effects. We hope the novel findings in this study may have extensive implications for further research.

1118 Physiologic doses of ultraviolet light activate nonviral phototransduction to trigger lysosomal exocytosis in human melanocytes
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Ultraviolet (UV) illumination is a significant risk factor for the development of skin cancer. Recent studies show that UV irradiation causes plasma membrane damage and membrane resealing by lysosomal exocytosis. However, these studies involve supra-physiological UV exposures, and therefore cellular responses to physiologic doses of UV light remain poorly understood. In this study, we hypothesize that physiologic doses of UV light trigger lysosomal exocytosis and play a crucial role in epidermal UV response. Here, we use live-cell imaging and fluorescent calcium indicators to probe for intracellular calcium levels as a marker for UV-induced photoresponses in primary human melanocytes. Within seconds of exposure UV light, lysosomes release calcium via phospholipid break down and subsequently trigger lysosomal exocytosis and generation of extracellular vesicles. Calcium responses were robustly triggered by a single physiologic dose of UV light, several orders of magnitude lower than previously reported. Furthermore, this UV response requires the presence of a retinaldehyde chromophore, is activated by rhodopsin GPCR signaling, and corresponds to the wavelength sensitivities of known UV-specific photoceptors, corroborating previous findings that nonviral phototransduction, rather than non-specific cell damage, mediates physiologic UV response. Additional studies using in vivo murine models containing a melanocyte-specific genetically-encoded calcium sensor (YteCreer<sup>+/−</sup>; GCAMPTs) will further elucidate this mechanism in a physiologic context. Taken together, these results suggest that physiologic doses of UV light trigger lysosomal exocytosis via a nonviral phototransduction cascade. This capacity of exocytosis in response to physiologic doses of UV light may have a more general role in cell-to-cell communication in sun-exposed tissues and disease states, notably UV-induced skin cancer.

1119 High frequency light emitting diode red light effect on skin fibroblasts: A comprehensive RNA-seq analysis
I Koo, A Mamalis, A Merlevede, A Hanus, G Luxardi, D Broadley, R Isseroff and J Jagdeo
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Photobiomodulation (PBM) is a process in which light induces functional changes in cells. Skin fibroblasts is a pathologic feature found in nearly one hundred conditions of varying etiology. We have previously shown PBM may treat fibrosis by decreasing fibroblast proliferation and collagen 1 production. Here, we present whole transcriptome gene expression analysis describing the transcriptomic profile of high frequency (320 and 640 J/cm<sup>2</sup>) light emitting diode red light treated human fibroblasts and skin epidermis, as well as the expression of precursor miRNAs (pre-miRNA). The significant biological pathways of these two miRNAs were also predicted. In addition, up-regulating miR-4655-3p and miR-769-5p by miRNA mimics in non-irradiated cells can induce bystander-like effects. Taken together, our research reveals that miR-4655-3p and miR-769-5p are involved in UV-induced bystander effects. We hope the novel findings in this study may have extensive implications for further research.

1120 Spiroloactone depletes XBP1 protein and inhibits the UVB DNA damage response in human skin
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A recent high throughput screen of FDA-approved drugs that modulate UV photoproduction removal identified spiroloactone (SPL) as an inhibitor of delayed excision repair (NER) in HaCaT cells via a ability to promote the rapid proteolytic degradation of the core NER protein XBP1 (xeroderma pigmentosum group B). Because SPL is used clinically to treat hormonal acne and other conditions, we examined whether the drug similarly affects XBP1 protein levels in cultured human keratinocytes in vitro and human skin ex vivo. We observed that XBP1 protein levels were rapidly reduced following UV treatment in both telomerase-immortalized human neonatal keratinocytes (N-TERTs) and primary adult keratinocytes. This depletion was correlated with an inhibition of cyclinB1/p21/p53/cyclindependent kinase 1/p27(X1) decrease in the expression of NER as well as the induction of a p53-mRNA, suggesting that SPL is a potential oral agent for skin cancer prevention.

1121 Oral administration of nicotinamide riboside (NR) and pterostilbene (PT) to mice inhibits ultraviolet radiation-induced tissue swelling in mice
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Many antioxidants inhibit immune suppression and/or skin cancer formation in experimental animals exposed to UVB (280-320 nm) radiation. In this regard, PT has been shown to protect hairless mice against UVR-induced skin damage and carcinogenesis. Nicotinamide has been shown to enhance and repair UVB-induced DNA damage in a transformed human skin line and in human skin ex vivo as well as in primary melanocytes. It also downregulates expression of some inflammatory peptides including IL-6, IL-10, monocyte chemoattractant protein 1 and tumor necrosis factor alpha in transformed human keratinocytes exposed to UVB radiation. Additionally, topical and oral nicotinamide inhibit immune suppression and photocarcinogenesis in mice. Finally, topical nicotinamide has been shown to inhibit UVB-induced immune suppression in humans and oral nicotinamide has been found to reduce the rate of new melanoma skin cancers and actinic keratosis in high-risk patients. A fixed combination of PT plus NR is being marketed as an oral supplement. In preparation for studies of whether PT and NR have additive or synergistic effects on some of these endpoints, we have examined the ability of PT plus NR to inhibit UVB-induced tissue swelling in mice at the ratio of the marketed product. Groups of 5 C3H/HeJ mice were fed diets as follows (show containing PT/water containing NR): Group 1 No PT or NR; Group 2 0.04% PT/0.02% NR; Group 3 0.08% PT/0.04% NR; Group 4 0.12% PT/0.06% NR. After 4 weeks mice were exposed to 3,500 J/m<sup>2</sup> UVB radiation (F50 sun lamps) and 46, 72-, and 96-hour endpoint measured.80% of the control mice died but 70±0.4% NR showed significantly less ear swelling than the positive control mice (no PT or NR) at all timepoints. This experiment cannot determine if PT, NR or both are required for the observed activity. The next experiments will compare each agent alone in comparison to administration of both to mice.
1124 Cathepsin G causes ultraviolet irradiation-induced basement membrane damages in hairless mouse skin

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Background: Ultraviolet (UV) exposure contributes various effects to skin including inflammation, sunburn, premature aging, carcinogenesis, and also induces the basement membrane damages in skin. Cathepsin G (CTSG) belongs to serine protease family and its upregulation is involved in wound formations by chronic UV irradiation in skin. Ketoprofen-phosphoric acid (KPA) is a CTSG inhibitor that shows the strongest selectivity and potency against CTSG, and prevents wrinkle formation by chronic UV irradiation. However, the effect of KPA on the basement membrane damages is not well known.

Objective: To investigate the effect of CTSG inhibitor treatment on basement membrane damages in chronically UV-irradiated hairless mouse skin. Methods: The dorsal skin of hairless mouse was exposed to UV 3 times a week for 8 weeks. KPA was applied to mouse dorsal skin immediately after each session of UV irradiation. Protein levels of CTSG and MMP-13 were analyzed by Western blotting. The basement membrane structure were visualized by transmission electron microscopy. The basement membrane components damages were analyzed by immunofluorescence staining. After 4 and 8 weeks, skin samples from each mouse were assessed visually and by using a replica assay. Results: UV irradiation increased CTSG and MMP-13 expressions in chronically UV-irradiated hairless mouse skin. KPA prevented UV-induced basement membrane structure damages and the reduction of basement membrane components (collagen type VII, laminin, fibronectin). UV-induced wrinkles were also prevented by administration of the inhibitor. Conclusion: Our present studies show the involvement of CTSG in UV-induced basement membrane damages in skin, and its inhibitor may be useful for the prevention of UV-induced basement membrane damages.

1125 A wearable, flexible, conformable and depth-modulated phototherapy device: Initial application in morphea

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Morphea is a localized, scarring skin condition that can extend deep into muscle and fascia. While UVA-1 (340-400 nm) phototherapy is often effective, these units are not widely available, expose unaffected skin to UVA-1, and require several weeks until treatment response. Thus, there is a need for new UVA-1 systems that offer faster response times, greater efficacy, targeted therapy and increased patient comfort. In morphea, UVA-1 leads to the upregulation of collagenase and IFNγ, leading to decreased collagen I, collagen III, and TGF-β expression. However, the intensity of UVA-1 attenuates to 1% at 190 μm in human skin, which can make the therapy less effective in some cases. Thus, we hypothesize that deeper UVA-1 delivery will improve efficacy and response time. Using advanced techniques in flexible electronics, we have developed a soft, conformable, and depth-modulated UVA-1 phototherapy device. The devices top layer has an array of UVA-1 light emitting diodes (spectral peak output: 360 nm) fully embedded within a flexible silicone substrate. The power intensity output is 35 mW/cm² with uniform distribution across the entire patch as confirmed by a UVA light meter. The bottom layer includes a dense array of microelectrodes from poly-lactic-co-glycolic acid (PLGA) polymers, enabling 99% of UVA transmittance, which create micro-channels 700 μm for deeper UVA-1 delivery into the skin.

Optical modeling, confirmed by confocal microscopy in agarose, demonstrates that the PLGA microelectrode waveguides increase light transmission by 250% along the z-axis along the length of the needles. The system can conform to any curvilinear body surface and be cut to any shape or size. Future clinical testing will validate the safety and efficacy of the system compared to standard UVA-1 phototherapy.

1126 Quantitative analysis on ex vivo nonlinear microscopy images of basal cell carcinoma

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Basal cell carcinoma (BCC) is the most frequent malignant neoplasm in the Caucasian population. As BCCs often have poorly defined borders, the clinical assessment of the tumor margins can be challenging. Therefore, there is an increasing demand for efficient in vivo imaging techniques for the evaluation of tumor borders. This demand might be met in the near future by nonlinear microscopy techniques. We measured the two-photon excitation fluorescence (TPEF) signal of nicotinamide adenine dinucleotide hydride (NADH) and elastin and second harmonic generation (SHG) signal of collagen on 10 ex vivo healthy control and BCC skin samples by the images by different quantitative image analysis methods. These included integrated optical density (IOD) measurements on TPEF and SHG images and application of fast Fourier transform (FFT), CT-FIRE and CurveAlign algorithms on SHG images to locate the collagen architecture. In the BCC samples, we found significantly lower IOD of both the TPEF and SHG signals and higher collagen orientation index utilizing FFT. CT-FIRE algorithm revealed increased collagen fiber length and decreased fiber angle while CurveAlign detected higher fiber alignment of collagen fibers in BCC. These results are in line with previous immunohistochemistry findings in the collagen architecture.

In the future, these novel image analysis methods could be integrated in handheld nonlinear microscopy systems, for sensitive and specific identification of BCC.

1127 5-aminoeulavonic acid-based photodynamic therapy pretreatment inhibits ultraviolet B-induced skin photodamage

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Since More than 90% of non-melanoma skin cancers are associated with excessive exposure to Ultraviolet radiation B (UVB) radiation, UVB is thought to be a major cause of DNA damage, which is the most important mechanism of UV-induced skin photodamage in epidermal cells. Although several studies found that ALA-PDT can prevent the occurrence of photodamaged dermis, the exact mechanism is still unknown. We conducted this experiment to verify the protective effect of ALA-PDT on UVB-induced photodamage and explore its mechanism in animal experiments, the dorsal skin of hairless mice were treated with ALA-PDT or saline, and then exposed to 180 mJ/cm² ultraviolet B (UVB). For in vitro experiments, human keratinocyte cell line (HaCaT) cells were treated with ALA-PDT or untreated, and then exposed to 60 mJ/cm² UVB irradiation. As a result, we found that ALA-PDT pretreatment can both reduce apoptosis, inhibit proliferation, accelerate the clearance of CPDs and activate p53 in vivo and vitro, which indicated that ALA-PDT pretreatment can induce a protective DNA damage response, thereby protecting against UVB-induced pho-
Role of nucleotide excision repair pathway in insulin-like growth factor-1-mediated keratinocyte photoresponse

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Skin forms a critical barrier against detrimental environmental agents including ultraviolet radiation (UVR). Keratinocytes are extremely vulnerable to the damaging effects of UV-BR. Previously, the role of IGFI in protection of keratinocytes against UVR-BR through its effects on basic cellular processes has been described. However, the role of IGFI in UV-BR mediated cellular responses to UVR have not yet been elucidated. Thus, we intend to further explore these pathways and identify whether IGFI-1 is directly linked to damage prevention or associated with enhanced activation of repair pathways. Using specific pathway inhibitors, we explored the role of IGFI-1-mediated cellular responses to UV-BR exposure, suggesting the probable elimination of photodamage through the activation of repair mechanisms. Using 2-D and 3-D skin models, we observed that IGFI-1 post-UV-BR promotes cell survival, reduces UV-B-induced DNA damage and increases the apoptotic rate. Combination of IGFI-1 effect, using a specific pathway inhibitor, TLR-4,5, which targets replication protein A (RPA-1) within the NER, validated the reparative role for IGFI-1 rather than a preventative role. IGFI-1 post-irradiation may activate repair pathways such as NER pathway, thus minimizing UV-BR DNA damage in keratinocytes. A better understanding of the signaling mechanisms will be instrumental in developing effective remedial strategies against photodamage.

UV increases skin-derived 1α,25-dihydroxyvitamin D3 production, leading to MMP-1 expression

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Epidermal keratinocyte is the only cell in the human body that has the capacity for the synthesis of active form of vitamin D, 1α,25-dihydroxyvitamin D3 (125(OH)D3) from 7-dehydrocholesterol (7-DHC) upon UV-BR without liver and kidney involvement. Keratinocyte express enzymes involved in active vitamin D synthesis, including CYPT2B1 and CYP27A1. Here, we investigated the role of skin-derived 1α,25(OH)2D3, which was synthesized purely within the keratinocytes, on MMP-1 expression in epidermal keratinocyte. The treatment with 1α,25(OH)2D3 significantly increased the expression of MMP-1, but 7-DHC and 25-ODH, did not change the MMP-1 expression in human keratinocyte. Next, we found that ketoczaonole, CYPT2B1 inhibitor, or CPY27B1 sRNA significantly inhibited UV-BR-induced 1α,25(OH)2D3 production and MMP-1 expression. In the keratinocytes, 7-DHC serves as the substrate for both cholesterol and 1α,25(OH)2D3 synthesis. We observed that DHC7R converts 7-DHC to cholesterol, expression was decreased by UV. Furthermore, DHC7R inhibitor decreased the cholesterol synthesis and increased UV-induced MMP-1 expression, and ketoczaonole co-treatment inhibited MMP-1 expression. Our results demonstrated that reduction of DHC7R by UV resulted in a decrease in the synthesis of cholesterol, which increases the availability of 7-DHC for more 1α,25(OH)2D3 production, leading to more MMP-1 expression. Finally, human skin in vivo, we demonstrated that CPY27B1 mRNA was significantly increased and DHC7R mRNA was decreased by UV. Taken together, we suggest that keratinocytes-derived 1α,25(OH)2D3 synthesis by UV induces MMP-1 expression.

Establishment of photo-aged in vitro senescence model using cultured fibroblasts by repeated UVA irradiation: PAPLAP with potent catalase-like activity prevented cellular senescence

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Photography increases skin-derived 1α,25(OH)2D3 synthesis. We observed that DHCR7, which converts cholesterol into 7-DHC, was increased by UV-BR in keratinocytes. In this study, we investigated the impact of long term MMW irradiation on human induced pluripotent stem cells (hiPSCs), which are sensitive to microenvironment for maintenance of their pluripotency. We established hiPSCs from human dermal papilla cells (HDPc) by traditional MEF/Sol system. Next, we set the 95GHz as 95GHz irradiation system with the power of 14 dBm and exposed hiPSCs for 7 days in the cultures for maintaining pluripotency. In both of feeder dependent 20% KSR and feeder free E8 based culture conditions, hiPSCs exposed to 95GHz irradiation began to differentiate and showed increase of representative three germ layer differentiation markers such as SOX1, MESP1, and FOXA2. However, MMW irradiation did not elevate the temperature of culture media and mRNA expression of heat shock protein70 was not changed in irradiated hiPSC. In addition, we investigated the impact of long term MMW irradiation on human induced pluripotent stem cells (hiPSCs), which are sensitive to microenvironment for maintenance of their pluripotency. We established hiPSCs from human dermal papilla cells (HDPc) by traditional MEF/Sol system. Next, we set the 95GHz as 95GHz irradiation system with the power of 14 dBm and exposed hiPSCs for 7 days in the culture for maintaining pluripotency. In both of feeder dependent 20% KSR and feeder free E8 based culture conditions, hiPSCs exposed to 95GHz irradiation began to differentiate and showed increase of representative three germ layer differentiation markers such as SOX1, MESP1, and FOXA2. However, MMW irradiation did not elevate the temperature of culture media and mRNA expression of heat shock protein70 was not changed in irradiated hiPSC. In conclusion, long-term exposure to 95GHz MMW irradiation breaks the potential of self-renewal and trigger to differentiation of stem cells without thermal effects.

Dual wavelength 5-aminolevulinic acid photodynamic therapy using a novel flexible light-emitting diode

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Phototherapy is a treatment used for skin disorders using light. It plays an important role in reducing skin elasticity and promoting aging, melanin and freckles. To prevent the effects of such ultraviolet radiation, the national weather stations pre-predicted ultraviolet index. Individuals are also prevented from damaging the ultraviolet by applying sunscreen to their skin. However, people are purchasing the product without specific criteria on UV protection index to choose. There are differences in skin sensitivity to UV light in each individual. UV radiation also varies significantly depending on the weather conditions, and background. Therefore the required UV protection index for sunscreens depends on weather, background environments, and individual skin characteristics. This study presents an UV protection index appropriate to the individual skin type and environments. We report UV index measuring system that can be used in various ultraviolet research studies by objectively comparing ultraviolet irradiance without weather, location, or time limitations. Ultraviolet radiation is measured using a dual-wavelength type. The intensity of reflectance and transmittance was measured from various materials, such as: sea water, water, mud-flat. The reflectance of sea water is higher than reflectance of seawater because minerals in the sea water have high refractive index. UVB reflectance is the highest in the mud-flat due to the sum of the high refractive index of the mineral component and the refractive index of water. Since the refractive index of the component is different, UV reflectance of each surface is different. In addition, we provide appropriate UV protection index for individuals. The UV protection index can be calculated by weighting minimal erythema dose by Fitzpatrick skin type. This effective UV protection index can provide the user with the necessary UV protection information to help them choose the appropriate UV protection product for their skin and environment.
1134 UVR modulates macrophage recruitment and phenotype in the resolution phase of human skin inflammation in vivo
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Macrophages (Ms) have an essential role in mediating inflammatory responses. While Ms immediately infiltrate the skin after UVR, it is unclear if and how Ms mediate the resolution phase. To investigate this in vivo, photoprotected human skin was exposed to 3xMED broadband UVR (n=13; 20-30% sunburn) and skin biopsy was performed 14 days post UVR. We analysed the pan-M marker CD68 by IHC. Consistent with reports CD68+ cells increased over baseline after 24h (193%; p<0.01). However, levels remained elevated at D7 (84%); p<0.01 and D10 (46%; p<0.01) post UVR, suggesting they participate in late phases of the sunburn response. To determine if the elevation of CD68+ cells in the resolution phase is the result of the recruitment of the M2 anti-inflammatory population of Ms, immuno-fluorescence analysis of the M2 marker, CD206, was performed with CD68 (n=3). This revealed the number of CD206+CD68+ cells rose at D4 post UVR and peaked on D10 (219% over D0). The hydroxyl fatty acid, 12-HETE, acts as a chemotactic for Ms in human skin. We hypothesised 12-HETE could recruit Ms in the skin after UVR. We screened for lipooxygenase enzymes responsible for 12-HETE production and identified ALOX12, 12b- and -15 mRNA is expressed in the human epidermis. We further analysed 12-HETE in suction blister fluid post UVR by LC-MS/MS and found that 12-HETE levels significantly correlated with number of CD68+ cells over 14 days (r²=0.086; p<0.01). Collectively we provide evidence that Ms are potentially recruited by UVR stimulated epidermal 12-HETE production and maintained in the late phases of the sunburn response, with the M2 Ms specifically elevated in the resolution phase. Modulation of Ms recruitment and phenotype could provide a novel strategy to abrogate human UVR skin damage.

1135 Photodynamic therapy against both methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa
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Photodynamic therapy (PDT) has been used as a key treatment in the treatment of skin infections caused by both drug-susceptible and drug-resistant bacteria such as methicillin-resistant S. aureus (MRSA) and Pseudomonas aeruginosa (PA). In previous studies conducted by our group, we demonstrated that photodynamic therapy (PDT) using 5-Aminolevulinic acid (ALA) has bactericidal effects against MRSA and PA individually. However, an infected ulcer usually carries both gram-positive and gram-negative bacteria simultaneously. Therefore, in this study we investigated the antibacterial effect of PDT against MRSA and PA doubly infected sample. First, PDT was performed against a mixed culture of MRSA and PA (both10⁶ CFU/mL) treated with 0.5% ALA in vitro, and a bacteria count was performed 48 hours after PDT. The results showed an increase in the bacteria count similar to that of non-PDT. Therefore, it is clear that PDT did not have any bactericidal effects after 48 hours of PDT. Next, PDT was performed twice at 0 and 1 hour. A bacterial count after 48 hours from the 1st PDT that by performing PDT twice, the bacterial count had decreased significantly compared to that of non-PDT. Finally, to confirm the effects of PDT in vivo, the test subjects were then infected with MRSA and PA after administering full-thickness skin defects on the mice back. ALA was then applied to infected wounds and PDT was performed every day. The control wounds not infected with PA healed completely by day 11, whereas the infected wounds only healed about 20% over the same period. PDT of infected wounds that had PDT performed were completely healed by day 13. In conclusion, these results highlight the further potential of PDT using ALA as a new non-antibiotic treatment option for treating infected wounds, especially on double infection of gram-positive and gram-negative bacteria.

1136 Galectin-3 regulates UVB-induced inflammation in skin
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Galectin-3, a 180-kD disaccharide binding lectin, can be detected in diverse types of cells, including epidermal keratinocytes. Regulatory roles of galectin-3 in the pathogenesis of various malig- nant and inflammatory skin disorders have been reported; however, the role of galectin-3 in UVB-induced inflammation in skin is still limited and the underlying molecular mechanisms remain elusive. In this study, we aimed to evaluate the regulatory roles of galectin-3 in UVB-induced inflammatory responses in skin by using primary normal human epidermal keratinocytes (NHEKs) and galectin-3 knockout mice as the models. Our results demonstrated knockdown of galectin-3 in NHEKs attenuated the UVB-induced production of active IL-1, UVB-induced ASC cross-linking and active caspase-1 formation were reduced but LDH release was not affected after galectin-3 knockdown. In addition, after galectin-3 knockdown, UVB-induced IL-8, TNF-α and COX-2 expression in NHEKs were also down-regulated. Furthermore, UVB-induced production of reactive oxygen species in NHEKs was also reduced after galectin-3 knockdown. In the animal study, UVB-induced skin inflammation and damage were less severe in galectin-3 knockout mice than in wild type mice grossly and histologically. When measuring the physiological parameters, UVB-induced transdermal water loss and skin erythema index were also reduced in galectin-3 knockout mice compared with wild type mice. In conclusion, our findings indicate that galectin-3 plays a crucial regulatory role in UVB-induced inflammation, suggesting a potential way to prevent UVB-induced skin damage by targeting galectin-3 in skin.

1137 A novel role for NUPR1 in the keratinocyte stress response to UV oxidized phospholipids
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We demonstrate that Skin cells from primary (ICU) and immortalized keratinocytes (HaCaT) exposed to UV-A (312 nm) oxidized phospholipids (OxPL) and UVA (365 nm) oxidized phospholipids (OxPL) and UVA (365 nm) show increased expression of the unfolded protein response (UPR) marker, NUPR1. In a cell free system, UVA - oxidized phospholipids activated, on transcriptome and proteome level, NUPR2 and NUPR3 antioxidant signaling, lipid metabolizing enzyme expression and unfolded protein response (UPR) signaling. We identified NUPR1 as an upstream regulator of UVA/OxPL transcriptional responses and found this protein expressed in the epidermis. Silencing of NUPR1 resulted in augmented expression of antioxidant and lipid detoxification genes and disturbed the cell cycle, making it a potential key factor in skin reactive oxygen species (ROS) responses intimately involved in aging and pathology.

1138 The influence of UVA protection on skin during sun exposure
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The skin is the largest organ of the human body and forms a physical barrier to the enviroment, protecting other organs against damage from ultraviolet radiation. Determining the biological events that occur in skin after UV radiation exposure is essential to photo- protections studies. In this study, we evaluated the protection efficiency of sunscreens, considering their UVA protection against UV radiation, in specific biological markers of the skin, using a 3D skin model. We evaluated 5 groups: CN (non irradiated skin), CP (irradiated skin without treatment) e samples A1, A2, A3 (sunscreens containing UBV/UV filters in the ratio 1/6:1/3:1/1 respectively). The samples were topically applied on the skin, followed by irradiation with UVA (340-400m). The samples showed sunburn damages, except CN. In treated groups, A1 presented higher number of sunburn cells, although A2 and A3 also presented them, but smaller number. Cytoketirins 10 and 14 indirectly demonstrated that there are no cytotoxic effects with the 3 sunscreens. Also, that keratinocytes are sensitive to oxidative stress induced by sun- light. Filagrin expression was analyzed and both genic and protein level showed reduction of its expression on the CP with preserved results in treated samples. The CP presented higher level of p53 expression while treated samples showed a slight marking of nuclei, very similar to CN. The evaluation of b-actin-RG demonstrated higher protection profile for samples A2 and A3 against oxidative damage of DNA, but still with more damage than CN. Sun radiation significantly increased IL6 and IL8 release when compared with CN. The treated groups demonstrated significant increase of the IL6 and IL8 release when compared with CN. The treatment screens on MMP1 and MMP3 synthesis were evaluated and all irradiated groups presented increase of MMP1 synthesis than CN, although none had significant decrease with treatment. Regarding synthesis of MMP3, the A1 sample demonstrated higher concentration compared with CP and CN, while the others samples were only significantly different from CN.
Regulation of protein synthesis during the progression of UVB-induced squamous cell carcinoma

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In response to a myriad of environmental stresses, including ultraviolet-B (UVB) irradiation, eukaryotic cells activate cytoprotective protein synthesis. An important mechanism for tolerance to UVB damage involves phosphorylation of the eIF2α subunit of eukaryotic initiation factor 2α (eIF2α), which results in a rapid decrease in global protein synthesis, concurrent with preferential translation of cytoprotective gene transcripts, via a set of pathways collectively called the Integrated Stress Response (ISR). Although the mechanisms linked to cyclophilin D in carcinoma cells and keratinocytes differ, little is known about whether or how the ISR can modulate the development of cancer in human skin. We previously defined the cytoprotective role of the ISR in response to UVB irradiation of human keratinocytes in vitro, and in part through the enhanced translation of genes involved in DNA repair. To address how the ISR is involved during photocarcinogenesis in vivo, we analyzed the expression of ISR proteins (phosphorylated eIF2a/2β, ATF4, CHOP, GADD153) in the progression of cutaneous squamous cell carcinoma (cSCC). The tumors examined included normal skin, chronically UVB-exposed skin (CK), and cSCC tumors. In normal skin, the expression of ISR proteins was found in the more differentiated layers of the epidermis. In contrast, in chronic UVB-irradiated skin and AK tissue, ATF4 expression was greatly increased in all but the basal layer of the epidermis. CHOP and GADD153 expression, as well as eIF2α phosphorylation, followed a similar induced pattern in these tissues. Progression to cSCC resulted in a striking decrease in ISR protein expression. In the cytoprotective response in vitro, eIF2α phosphorylation was detected, and GADD153 expression was greatly diminished. Therefore, the normal ISR response in differentiating keratinocytes is enhanced in AK lesions but silenced as the tumor progresses to cSCC. These results suggest that sustaining the ISR response in UVB-damaged epidermis could be a valuable therapeutic target for the prevention of SCC development.

Melanocyte UVB skin damage is inversely affected by S. epidermidis versus S. epidermis

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Introduction: UV radiation is a major environmental stressor, which has emerged as an important player in the skin innate immune system, especially with respect to its role in the control of nuclear HDAC6 and 9 in keratinocytes and monocytes. However, little is known about whether or how the skin microbiome, SE and PA, have effects on conditions caused by melanocytes in a variety of tissues. S. epidermis (SE) and S. epidermidis (SE) are common skin commensals, which differ in phenotypic expression, epigenetic regulation and in their potential role in cutaneous diseases.

Methods: Normal human melanocytes (NHM) were pre-exposed to UVB (100mJ/cm²). After 24 hours NHM were exposed to UVB (75mJ/cm²) and following 24h incubation were exposed to one of the skin commensals S. epidermis or S. epidermidis. SE growth factors and SE metabolites were isolated using gas chromatography/mass spectrometry (GC/MS) and 1H nuclear magnetic resonance (1H NMR). The metabolism of these factors was stained with DAPI. RNA was isolated from the treated and control samples, and RNA sequencing was performed. Results were compared against the SE metabolic profile.

Results: SE but not PA significantly upregulated HDAC6 and 9 in NHM compared to control. SE altered the expression of genes involved in DNA repair. To address how the skin microbiome, SE and PA, have effects on conditions caused by melanocytes in a variety of tissues, little is known about whether or how the skin microbiome, SE and PA, have effects on conditions caused by melanocytes in a variety of tissues. S. epidermis (SE) and S. epidermidis (SE) are common skin commensals, which differ in phenotypic expression, epigenetic regulation and in their potential role in cutaneous diseases.

Conclusions: SE and PA have differential effects on gene expression in NHM. SE alters expression of genes involved in DNA repair. SE’s ability to stimulate expression of HDAC6 and 9 may contribute to the anti-inflammatory effects of SE in human skin. Further studies are needed to elucidate the potential role of SE and PA in cutaneous disease.

Innovative oil-soluble biomimetic hexapeptide to fight UV damages

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Visible light (VL), a major component of solar radiation, differentially induces pigmentation in protected sites, whereas VL induced pigmentation, which may play a central role in skin type specific responses to VL. Enrolled participants to VL are determined by epidermal melanin content and distribution as exemplified by skin color. VL absorbs light within the VL wavelength. We hypothesize that clinical and histologic responses to VL are determined by epidermal melanin content and distribution as exemplified by skin color. VL induced no significant clinical or histological changes in lighter skin. Darker skin types did not tolerate. Treated and control sites were biopsied 24 hrs after the last exposure and stained with DAPI to investigate whether a biomimetic peptide analogue of α-helix hexapeptide-1, was able to stimulate human defense system response to VL. We demonstrate that the CYP11A1-derived hydroxyvitamin D3 analogs 20(OH)D3, 20,23(OH)2D3, 1,20(OH)2D3, 1,25(OH)2D3, 20(OH)D3 and 25(OH)D3 inhibited proliferation of human dermal fibroblasts in a dose-dependent manner with a similar potency to the 1,25(OH)2D3 control. Surprisingly, this effect was reversed in RORα and RORβ fibroblasts with the most pronounced stimulatory effect seen in RORβ fibroblasts. All of these analogs, as well as 25-hydroxyvitamin D3 (25(OH)D3) and 25-hydroxy/7dehydrocholesterol (25(OH)/7DHC), inhibited TGF-β1-induced collagen synthesis in RORβ fibroblasts. Again this effect was curtailed or reversed in RORβ fibroblasts. These results clearly show that antiproliferative and antioxidant activities of the vitamin D hydroxy-derivatives and anti-fibrotic activity of non-secosteroidal 20(OH)D3 and 25(OH)D3 are dependent on functional RORα and RORβ receptors. The CYP11A1-derived hydroxyvitamin D3 analogs 20(OH)D3, 20,23(OH)2D3, 1,20(OH)2D3, 1,25(OH)2D3, 20(OH)D3 and 25(OH)D3 act only as reverse agonists on RORβ.

Melanocyte UVB skin damage is inversely affected by S. epidermidis versus S. epidermis

Melanocyte UVB skin damage is inversely affected by S. epidermidis versus S. epidermis

Acute UV irradiation and intrinsic aging module modulate various histone deacetylases expression levels in human skin in vivo

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Introduction: UV radiation is widely considered to play an important role in aging, but experimental evidence to support this hypothesis for skin aging in vivo is scarce. Histone deacetylases (HDACs) are considered enzymes that remove acetyl groups from lysine side chains in histones and other proteins. There are 11 HDACs and 7 uracil/5sir in human skin, which can be inhibited by HDACs. In acetylated human skin, and the melanin- and photodamaged human skin in vivo. We found that acetyl-H3 level was increased and HDAC4, 11, and SirT4 mRNA were significantly decreased in UV-irradiated skin. In intrinsically aged acetyl-H3 level was increased and HDAC4, 5, 10, 11, and SirT6, 7 mRNA were significantly decreased. HDACs and HDACs expressions in photoaged forearm skin were not different from those in intrinsically aged buttoc skin in the same individuals. Thus, our results suggested that decreased HDACs expressions by acute UV irradiation result in the upregulation of HDACs and HDACs expressions in photoaged forearm skin may play an antiaging role.
3). Six spots measuring 2cm² each were marked on volar forearm. Oxygen production was measured. The experiment was also performed on ex-vivo human skin containing sunscreen did not protect from singlet oxygen production, while Zinc containing neuropeptides were found to be protective. These UVB effects are due to the presence of ROCK-mediated nuclear translocation and phosphorylation of PKCα regulated neutrophil NET formation, and demonstrates the protective role of ROCK inhibition in neutrophil NETosis in vitro and vivo. This provides insights into a novel therapeutic target for treatment of UVB-induced skin inflammation.

UV exhibits DNA damage, and oxidative stress. We investigated the effects of ROCK inhibition on neutrophil NETosis in vitro and on neutrophil NETosis in vivo. Following PMA stimulation, cytoplasmic PKCα is gradually translocated to the nuclear membrane. Particularly the phosphorylated PKCα (pSer657) is accumulated at the site of nuclear membrane rupture in the NETosis neutrophils in our model. We found that ROCK inhibition prevented neutrophil NET formation and the phosphorylation of PKCα regulated neutrophil NET formation, and demonstrates the protective role of ROCK inhibition in neutrophil NETosis in vitro and vivo. This provides insights into a novel therapeutic target for treatment of UVB-induced skin inflammation.

Ethanol augments stimulated microvesicle particle formation and release in a keratinocyte cell line. A Kyu-A Hwang, I Romer, L Liu and C Mapp Pharmacology and Toxicology, Wright State University, Dayton, OH, 3 Laboratory of Toxicology, Center, France, 4 Innovation R&D, Laboratories Expanscience, Epernon, Centre, France. We investigated the potential role of ethanol to augment stimulated microvesicle particle formation and release in human keratinocytes (HaCaTs). Ethanol pretreatment augmented both baseline as well as stimulated MVP production in a time-dependent manner. These studies provide a potential mechanism for ethanol-mediated pathologies, namely, via the augmentation of MVP production.

UVA light and oxidative stress. W Burnett1, S Saric1, S Davis1, D Rosen2, D Yoon2, R Isensori2 and R Siwanowicz1 1 UC Davis, Sacramento, CA, USA, 2 UC Davis, Sacramento, CA, USA, 3 PSH, Andover, MA, 4 and 5 UC Davis, Sacramento, CA. Ultraviolet (UVC) radiation is a risk factor for development of many cutaneous disorders, including sunburns and skin cancers. UVA penetrates deep into the dermis and causes productions of reactive oxygen species, including singlet oxygen, resulting in oxidative stress. This oxidative stress and DNA damage are associated with carcinogenesis. The purpose of this study was to quantify real-time single oxygen production after skin exposure to UVA at equivalent to 2 hours of sunlight exposure. 15 healthy subjects were recruited and assigned to one of three groups based on Fitzpatrick skin type (group 1, skin type I-II; group 2, skin type III-IV; group 3, skin type V-VI, n = 5). Six spots measuring 2cm² each were marked on solar forearms. Both groups served as control and spots 2-4 were exposed to increasing UVA1 light intensities. ABO was exposed to UVA1 light intensities (538, 4985 and 8013) cm⁻² cm⁻² of UVA intensity was used for exposure. Real-time single oxygen production was measured. The experiment was also performed on ex vivo human skin samples. ABO was exposed to (201, 361.1 and 735.7) cm⁻² cm⁻² in a result in an increase of singlet oxygen production as compared to control (p < 0.004). Furthermore, higher levels of melanin did not appear to reduce single oxygen production with UVA exposure. Our data demonstrate that UVA exposure leads to singlet oxygen production, which is a potential contributor to skin aging. Future studies with larger sample sizes are needed and the role of additional antioxidants in protection from UV-induced singlet oxygen should be investigated.
Photoprotective properties of an Entada phaseoloides seeds extract obtained by sequential enzymatic processing.

M. Pellevoisin1, D. Lelievre2, M. Bataillon2, N. Boyera2, A. Rigaudeau2, J. Ovigne2, I. Besne1, J. Ovigne2, M. Majora1, N. Garcia and J. Krutmann4

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Study of clinicopathological and molecular markers of basal cell carcinoma influencing the response to MAL-PDT

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Analysis of the interaction of different wavelengths present in natural sunlight

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Human Xeroderma pigmentosum type A (XPA) fibroblasts express a senescence associated secretory phenotype (SASP) Mechanistical and clinical implications

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Time for the human epidermal peripheral clock, role of opsins and cryptochromes

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Independent regulation of the peripheral clock to the central clock may be promising for the treatment of skin disorders. Opsins (OPN) are photoreceptors, and Cryptochromes (CRYs) are transcription factors of the molecular circadian clock, both are blue light receptors, and have a crucial role in human skin is not clear. Expression of CRYs and OPNs in facial epidermal and dermal skin was compared by immunohistochemistry (IHC). Changes in the circadian clock of epidermal keratinocytes investigated by stabilisation of CRY with KL001 or by knockdown of CRY1 or OPN3 using siRNA. Keratinocytes were irradiated with 2 jcm2 light from a scratch-wound assay, DNA synthesis by BrdU incorporation and differentiation by IHC and qRT-PCR. Changes in CRY's expression confirmed by qRT-PCR. We have previously shown expression of OPN3 in 3 and 5 human skin. Differentiation of epidermal keratinocytes induced by UVA 365 nm and IRA light was followed by knockdown of CRY1 expression. CRY2 was mainly cytoplasmic. CRY2 but not CRY1 was expressed in epidermal skin. Treatment with KL001 which stabilizes CRY1, inhibited migration and induced KRT14 expression, a defect which was rescued by knockdown of CRY1. However, knockdown of CRY1 also induced keratinocyte differentiation, but had no effect on migration. Interestingly, knockdown of OPN3 upregulated CRY1 expression, while KL001 upregulated OPN3 expression. Blue light increased early differentiation of epidermal keratinocytes, which was abrogated by knockdown of CRY1. However, knockdown of CRY1 expression has no effect on migration.

T-Skin, a new industrial reconstructed human skin model for dermatological and cosmetics research and development

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Independent regulation of the peripheral clock to the central clock may be promising for the treatment of skin disorders. Opsins (OPN) are photoreceptors, and Cryptochromes (CRYs) are transcription factors of the molecular circadian clock, both are blue light receptors, and have a crucial role in human skin is not clear. Expression of CRYs and OPNs in facial epidermal and dermal skin was compared by immunohistochemistry (IHC). Changes in the circadian clock of epidermal keratinocytes investigated by stabilisation of CRY with KL001 or by knockdown of CRY1 or OPN3 using siRNA. Keratinocytes were irradiated with 2 jcm2 light from a scratch-wound assay, DNA synthesis by BrdU incorporation and differentiation by IHC and qRT-PCR. Changes in CRY's expression confirmed by qRT-PCR. We have previously shown expression of OPN3 in 3 and 5 human skin. Differentiation of epidermal keratinocytes induced by UVA 365 nm and IRA light was followed by knockdown of CRY1 expression. CRY2 was mainly cytoplasmic. CRY2 but not CRY1 was expressed in epidermal skin. Treatment with KL001 which stabilizes CRY1, inhibited migration and induced KRT14 expression, a defect which was rescued by knockdown of CRY1. However, knockdown of CRY1 also induced keratinocyte differentiation, but had no effect on migration. Interestingly, knockdown of OPN3 upregulated CRY1 expression, while KL001 upregulated OPN3 expression. Blue light increased early differentiation of epidermal keratinocytes, which was abrogated by knockdown of CRY1. However, knockdown of CRY1 expression has no effect on migration.

Blue light increased early differentiation of epidermal keratinocytes, which was abrogated by knockdown of CRY1. However, knockdown of CRY1 expression has no effect on migration.

Analysis of the interaction of different wavelengths present in natural sunlight

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T-Skin is a new reconstructed full-thickness skin model composed of a well stratified, differentiated and self-renewing epidermis and a living dermal compartment (functional fibroblasts embedded in a collagen I matrix). Immunohistochemistry studies of intracellular and extracellular biomarkers located in epidermal, dermal-epidermal and dermal compartments demonstrate a close similarity between T-Skin and in vivo normal human skin. Treatments with well-known anti-aging actives (Vitamin C, Retinol) demonstrate the capacity of T-Skin to mimic in vivo human response, reflected by changes in epidermal turnover, differentiation and dermal extracellular matrix biosynthesis. When exposed to UV, a main player of skin photo-aging, the induced biological changes observed in vitro with T-Skin at epidermal and dermal levels are in accordance with in vivo UV effects. The specific UVB light induced keratinocyte apoptosis was clearly observed by immunohistochemistry, histochemistry and inflammatory cytokines production in this in vitro skin model. Altogether, these results demonstrate that T-Skin, an industrial full thickness model, can be a powerful tool as part of an efficacy screening platform to develop new cosmetic and pharmaceutical products.
Fernblock prevents dermal cell damage induced by visible and infrared A radiation

UVR irradiation mediates mitochondrial changes via Poly (ADP-ribose) polymerase 1

BMAL1 and CLOCK proteins in regulating the sensitivity of human keratinocytes to UVB-induced apoptosis

TIR-domain-containing adapter-inducing interferon-β (TRIF) protects mice from UVB-induced suppression of cell-mediated immune responses

BMAL1 and CLOCK proteins in regulating the sensitivity of human keratinocytes to UVB-induced apoptosis

Decorin is associated with preservation of dermal collagen in UVB exposed mice
Ultraviolet radiation induced senescent cells and senescence associated secretory phenotype have key roles in the induction of immune tolerance

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Immune tolerance induced by exposure to ultraviolet radiation (UVR) is a major risk factor for skin cancers. It has been well documented that UVR induced DNA damage initiates tolleregulatory pathways that inhibit inflammasome activation and senescent cell-secreted cytokines that are responsible for the induction of immune tolerance. However, it remains to be fully elucidated how DNA damages initiate inflammation in skin and how CD11b+ myeloid cells induce tolerance. We demonstrate here that UVR induces senescence and senescence associated cytokine expression through both an autonomous mechanism and a non-cell-autonomous mechanism. Further, we show that in the absence of functional Mc1r, which is associated with increased UV-induced melanogenesis, CD11b+ myeloid cells show a \(-\)MDSC phenotype that is responsible for immune tolerance. The studies have improved understanding of mechanisms for UVR induced tolerance and provided new targets for prevention of UVR induced cutaneous disorder and carcinogenesis.

Endothelin-1 and \(\alpha\)-MSH enhance nucleotide excision repair in human melanocytes by activating distinct signaling pathways that converge on different E3 ligases


Endothelin-1 (ET-1) and \(\alpha\)-MSH are keratinocyte-derived paracrine factors for human melanocytes (HM), whose synthesis is up regulated by UV exposure. We reported that End-1 and \(\alpha\)-MSH promote repair of UV-induced cyclodimer dimers (CPD) in HM. End-1 stimulates HM to express PKC and increases intracellular \(Ca^{2+}\) mobilization, \(\alpha\)-MSH binds the melanocortin 1 receptor (MC1R) and activates the cAMP pathway. We hereby report that despite their distinct signaling pathways, End-1 and \(\alpha\)-MSH target common protein substrates (e.g., DDB2, XPC and DDB1) that are associated with NER in HM. In response to UV, End-1 and \(\alpha\)-MSH activated the DNA damage sensors ATM and ATR, decreased DDR2 and increased XPC localization on chromatin. This was accompanied by increased pS\textsuperscript{10} and pG5, which facilitates the degradation of ubiquitinated DDR2 to allow repair and reduce the DDR2 signal, which suggests that End-1 and \(\alpha\)-MSH promote the translocation of the DNA damage verification enzyme XPA to chromatin, and increased the generation of \(\gamma\)-HAX2 that facilitates the recruitment of NER enzymes to DNA damage sites. Both End-1 and \(\alpha\)-MSH increased the UV-induced activation of the MAP kinase p38 and JNK, and their downstream target p53, which regulate different aspects of DDR. The outcome of these events was enhanced CPD repair and reduced apoptosis. Additionally, Neuregulin-1, a fibroblast-derived paracrine factor, which activates the tyrosine kinase receptor ERBB3, also promoted CPD repair and increased survival of UV-irradiated HM. We conclude that a network of paracrine factors activates NER to maintain the genomic stability of UV-irradiated HM. We postulate that in the absence of functional MC1R, which is associated with increased UV-induced melanogenesis and melanoma risk, vitamin D and End-1 and \(\alpha\)-MSH compensate to reduce UV-induced genotoxicity in these vulnerable HC.

Endothelin-1 and \(\alpha\)-MSH enhance nucleotide excision repair in human melanocytes by activating distinct signaling pathways that converge on different E3 ligases
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LL-37 acts on dermal microvascular endothelial cells to potentiate non-coding double stranded RNA induced inflammation. An explanation for photoreactivity in Rosacea

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with a potent DNMT1 inhibitory activity, on the expression of type I collagen and matrix

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We have previously shown that upon exposure to ultraviolet radiation (UVR), damaged skin cells such as keratinocytes release endogenous nucleic acids like the U1 double stranded non-coding RNA (dsRNA) that can promote inflammation. Rosacea is frequently triggered by UVR and characterized by increased endothelial cells, expression of the antimicrobial peptide cathelicidin LL-37, and inflammation. In this study we hypothesized that the LL37 may aid in recognition of dsRNA U1 by the endothelium to initiate an inflammatory response. RNAsequencing transcriptome analysis of cells co-treated with LL37 and U1 RNA identified 117 unique genes (40.6%) were upregulated and pathways related to type 1 interferon production, angiogenesis, cytokine production and antigen processing and presentation were identified as activated with gene ontology analysis. Co-treatment of primary human dermal microvascular endothelial cells with LL37 and dsRNA U1 significantly up-regulated expression of pro-inflammatory genes like CCL5 (400 fold, p=0.01) and adhesion molecules ICAM1 (22 fold, p=0.042) and VCAM1 (11 fold, p=0.001) and their protein expression was confirmed with multiplex ELISA. We further show that siRNA mediated knockdown of Interferon alpha/beta 1 Receptor (IFNAR1), treatment with scavenger receptor inhibitor fucoidan and endosomal acidification inhibitor Bafilomycin A abrogated the up-regulated expression of pro-inflammatory chemokines and adhesion molecule expression in cells co-treated with LL-37 and dsRNA, suggesting a role of interferon beta 1, scavenger receptors and an endocytic pathway. Thus, our results suggest a previously unknown mechanism for activation of dermal endothelial cells through aiding in recognition of endogenous nucleic acids. This process may explain the increased sensitivity of patients with rosacea to UVR.

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Palmitoyl-KVK-L-ascorbic acid improves matrix abnormality associated with skin aging via inhibition of DNA methyltransferase 1

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DNA methyltransferase 1 (DNMT1) is the most abundant DNA methyltransferase responsible for the maintenance of and also de novo DNA methylation. Here we investigated the effects of palmitoyl-KVK-L-ascorbic acid (VCC2,Duo-VitapepTM), a vitamin C-conjugated peptide containing palmitoyl, on the expression of proteins associated with the detrimental skins showed a significant increase in the expression of proteins associated with the aging skin via DNMT1 regulation, suggesting that targeting epigenetic modifications may be an effective therapeutic modality against skin aging. These results indicate that vitamin D and VDR are important for both induction of XPC as well as repair of UV damage, and was no longer detectable at DNA damage spots by 90 minutes, consistent with the hypothesis that vitamin D signaling elicits compensatory responses to the DNA damage incurred during vitamin D synthesis. Treatment of human keratinocytes with either UBV or with 1,25D3 or 25D3 induced the DNA damage recognition protein, XPC, and induction was suppressed by either siRNA targeting the vitamin D receptor (VDR) or ketoconazole, a broad inhibitor of oxidases that hydroxylate vitamin D3 to active metabolites. Irradiation of cells with UVC through 3 um pore filters resulted in subnuclear spots of DNA damage that were probed with antibodies to the major UV-induced DNA lesions, and to XPC. Relative to controls which removed >50% of 6-4PP spots and reduced spot intensity by >70% over 2 hours, cells treated with siRNA targeting VDR had no significant change in either spot density or intensity over this time period (p>0.001), indicating a lack of NER. Co-staining of spots for XPC demonstrated that XPC begins accumulating at DNA damage spots within seconds, peaking by 15 minutes following UV damage, and was no longer detectable at DNA damage spots by 90 minutes, consistent with equal 6-4PP repair. In contrast, SODR-treated cells, while also rapidly recruiting XPC to 6-4PP exhibited prolonged retention of XPC at 6-4PP spots at 30 minutes relative to controls. These results indicate that vitamin D and VDR are important for both induction of XPC as well as normal retention of XPC at DNA damage in keratinocytes, and suggest that vitamin D may mitigate the UV-induced DNA damage associated with its own genesis.

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Inducible DNA repair of UV photoproducts depends on vitamin D receptor

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Ultraviolet radiation (UV) initiates vitamin D synthesis by converting 7-dehydrocholesterol to pre-vitamin D3 that is converted to 25-hydroxy-vitamin D3 (25D3) and then 1,25-dihydroxy-vitamin D3 (1,25D3). However, UV also generates DNA damage that is repaired by nucleotide excision repair (NER). We tested the hypothesis that vitamin D signaling elicits compensatory responses to the DNA damage incurred during vitamin D synthesis. Treatment of human keratinocytes with either UBV or with 1,25D3 or 25D3 induced the DNA damage repair genes XPC and Induction was suppressed by either siRNA targeting the vitamin D receptor (VDR) or ketoconazole, a broad inhibitor of oxidases that hydroxylate vitamin D3 to active metabolites. Irradiation of cells with UVC through 3 um pore filters resulted in subnuclear spots of DNA damage that were probed with antibodies to the major UV-induced DNA lesions, and to XPC. Relative to controls which removed >50% of 6-4PP spots and reduced spot intensity by >70% over 2 hours, cells treated with siRNA targeting VDR had no significant change in either spot density or intensity over this time period (p>0.001), indicating a lack of NER. Co-staining of spots for XPC demonstrated that XPC begins accumulating at DNA damage spots within seconds, peaking by 15 minutes following UV damage, and was no longer detectable at DNA damage spots by 90 minutes, consistent with equal 6-4PP repair. In contrast, SODR-treated cells, while also rapidly recruiting XPC to 6-4PP exhibited prolonged retention of XPC at 6-4PP spots at 30 minutes relative to controls. These results indicate that vitamin D and VDR are important for both induction of XPC as well as normal retention of XPC at DNA damage in keratinocytes, and suggest that vitamin D may mitigate the UV-induced DNA damage associated with its own genesis.

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Exposure to infrared radiation leads to cellular senescence in reconstructed skin models

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Prolonged exposure to ultraviolet radiation has deleterious effects on skin, however recent evidence suggests that premature skin aging is also influenced by infrared (IR) radiation. The IR spectra has distinct physiological effects based on wavelength and depth of skin penetration. In particular, IR-A radiation penetrates deep into the skin layers and induces oxidative stress, via the generation of reactive oxygen Species. We hypothesized that oxidative damage induced by DNMT1 overexpression. Next, we examined whether VCC2 improved the matrix abnormality associated with aged skin via DNMT1 regulation, suggesting that targeting epigenetic modifications may be an effective therapeutic modality against skin aging. These results indicate that vitamin D and VDR are important for both induction of XPC as well as normal retention of XPC at DNA damage in keratinocytes, and suggest that vitamin D may mitigate the UV-induced DNA damage associated with its own genesis.
results suggest that FoxM1 plays important roles in tumour progression and the chemoresistance of patients (P<0.001, respectively). The FoxM1 overexpression was also an adverse prognostic significance (HR 0.55, 95% CI [0.27-1.13]). In turn, patients using aspirin before diagnosis were observed in a smaller number of stage IV patients (HR 0.45, 95% CI [0.24-0.82]) and III (HR 0.57, 95% CI [0.34-0.96]). A similar trend was seen in patients diagnosed with melanoma dissemination to the lungs. Therefore, aspirin could provide survival advantage in melanoma patients (P<0.001, respectively). The FoxM1 overexpression was also an adverse prognostic significance (HR 0.55, 95% CI [0.27-1.13]). In turn, patients using aspirin before diagnosis were observed in a smaller number of stage IV patients (HR 0.45, 95% CI [0.24-0.82]) and III (HR 0.57, 95% CI [0.34-0.96]). A similar trend was seen in patients diagnosed with melanoma dissemination to the lungs. Therefore, aspirin could provide survival advantage in melanoma patients (P<0.001, respectively). The FoxM1 overexpression was also an adverse prognostic significance (HR 0.55, 95% CI [0.27-1.13]). In turn, patients using aspirin before diagnosis were observed in a smaller number of stage IV patients (HR 0.45, 95% CI [0.24-0.82]) and III (HR 0.57, 95% CI [0.34-0.96]). A similar trend was seen in patients diagnosed with melanoma dissemination to the lungs. Therefore, aspirin could provide survival advantage in melanoma patients (P<0.001, respectively). The FoxM1 overexpression was also an adverse prognostic significance (HR 0.55, 95% CI [0.27-1.13]). In turn, patients using aspirin before diagnosis were observed in a smaller number of stage IV patients (HR 0.45, 95% CI [0.24-0.82]) and III (HR 0.57, 95% CI [0.34-0.96]). A similar trend was seen in patients diagnosed with melanoma dissemination to the lungs. Therefore, aspirin could provide survival advantage in melanoma patients (P<0.001, respectively). The FoxM1 overexpression was also an adverse prog
Health-related quality of life in patients with malignant melanoma by stage and treatment status

Health utility decrement of 0.06 compared to localized disease (p = 0.001), adjusting for demographic and comorbidity variables, with 75.5% of this association explained by use of systemic therapy. Adjusted subgroup analyses by type of systemic therapy identified greater reduced utility compared to the general population (0.76 vs 0.87, respectively). Further, Functional Assessment of Cancer Therapy Melanoma (FACT-M) was completed by 87 patients at the time of surgery.

Surgical damage to the lymphatic system promotes tumor growth via impaired adaptive immune response

Adaptive immune activity plays a crucial role in tumor clearance. Both lymph node (LN)- and lymphatic channels from primary sites to regional LNs are critical for induction of adaptive immunity. However, LNs are common metastatic sites in skin cancers. LN biopsies or dissections are frequently performed. In addition, reconstructive skin flaps alter tumor resection may damage lymphatic flow from primary sites to regional LNs. In this study, we developed a mouse model that simulates LN dissection or skin flap that blocks lymphatic flow from primary sites to regional LNs and monitored tumor progression. As a poor immunogenic tumor line, the growth of inoculated B16F10 melanoma into syngeneic C57BL/6 mice was significantly accelerated by flap surgeries or LN dissection (p = 0.0016 and p = 0.0001, respectively). In addition, immune cell infiltration including CD4+ and CD8+ T cells into the tumor was reduced by these surgeries (CD4+ T cells: p = 0.0185 and p = 0.00215, respectively, CD8+ T cells: p = 0.0122 and p = 0.0034, respectively). Moreover, both cytotoxicity against B16F10 melanoma (p = 0.05 and p = 0.02, respectively) and numbers of apoptotic tumor cells (p = 0.0237 and p = 0.0055, respectively) were significantly diminished by these surgeries. Similarly, tumor growth of the immunogenic MCA6 cell line in syngeneic C57BL/6 mice was significantly accelerated by these surgeries (p = 0.0125 and p = 0.0413, respectively). In this model, immune cell infiltration were also reduced by these surgeries (CD4+ T cells: p = 0.0147 and p = 0.0201, respectively, CD8+ T cells: p = 0.0424 and p = 0.0314, respectively). Moreover, apoptotic tumor cells were diminished by these surgeries (p = 0.0431 and p = 0.0434, respectively). These results strongly indicate that surgical damage of the lymphatic system may promote tumor progression via impaired adaptive immune response.

DNA hypermethylation of MHC class-I genes is associated with reduced expression and survival in melanoma

DNA methylation is an epigenetic change that can silence gene expression, and has been implicated in regulating antigen expression and recognition in several cancer types. Abnormal DNA methylation is nearly universal in melanoma, however its role in regulating MHC molecule expression in melanoma is largely unknown. In this study, we investigated whether DNA hypermethylation of MHC class-I genes is associated with reduced gene expression, and identify the key CpG loci regulating expression. We found that methylation of beta-2 Microglobulin (B2M) and HLA-B, in particular, may play important roles in melanoma survival. Patients with hypermethylation of B2M exhibited a two-fold higher overall survival in contrast to hypomethylated group (log-rank test). Hypermethylation of HLA-B was associated with significantly decreased 5-year overall survival (48% vs 68%; HR = 1.68, p = 0.0001, Log-rank test). Univariate and multivariate Cox proportional hazard analysis showed that DNA methylation of B2M (p=5.8e-8) and HLA-B (p = 5.15e-05) is a predictor of melanoma overall survival. In addition, the interaction of hypermethylation of HLA and age, stage, gender, ulceration or Breslow thickness. Our results suggest that DNA hypermethylation may silence MHC class-I expression, and negatively impact melanoma survival. Based on our findings, targeting DNA methylation of B2M and HLA-B may be a therapeutic strategy to restore expression of HLA class-I molecules, and potentially achieve improved immune responses in melanoma.

Fisetin attenuates melanoma growth and tumorigenicity by downregulating PI3K/AKT/mTOR signaling and disrupting eIF4F complex

Fisetin acts as a growth inhibitor of rapidly dividing melanocytes with the potential to invade and metastasize. The BRAF mutation, found in ~50% of melanomas, drives oncogenic activation of the BRAF-MEK-ERK (MAPK) and PI3K-AKT-mTOR (PKL) pathways leading to cell proliferation, tumor growth, and metastasis. It has been shown that BRAF inhibition and PI3K/AKT/mTOR pathway inhibitors do not significantly reduce melanoma cell migration, invasion, or metastasis. In this study, we investigated the antitumor activity of fisetin in melanoma cells with the BRAF V600E mutation. We found that fisetin treatment reduces melanoma cell proliferation and tumorigenicity by targeting PI3K/MAPK signaling and disrupting eIF4F complex, which is overexpressed in melanoma.

Melanospheres identify microRNA-519d as a promoter of melanoma progression by modulating EphA4

We have previously described a melanoma-promoting microRNA, miR-519d, which is overexpressed in melanoma. In this study, we investigated whether miR-519d and EphA4 are co-expressed in primary melanomas from The Cancer Genome Atlas. We uncovered that DNA hypermethylation of MHC class-I genes is associated with significantly decreased 5-year overall survival (48% vs 68%, HR = 1.68, p = 0.0001, Log-rank test). Hypermethylation of HLA-B was associated with significantly decreased 5-year overall survival (48% vs 68%, HR = 1.68, p = 0.0001, Log-rank test).
1187

Activation Of RHOJ signaling in human hair follicle bulge melanocytes is a key-factor in NBUVB induction of vitiligo repigmentation

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There has been no molecular studies of melanocyte (MC) precursors activation in the hair follicle (HF) bulge by NBUVB treatment in vitiligo. To better understand repigmentation process, we collected biopsies from untreated and NBUVB-treated vitiligo patients (n=6 unpaired samples). We performed laser capture microdissection of HF bulge MCs from both groups, isolated the RNA, and performed Whole Transcriptome RNA Sequencing following by gene expression analysis. Using the Ingenuity Pathway Analysis tool and our list of differentially expressed genes (P<0.05), we identified the RHO-J (RHOJ) pathway as the top canonical pathway modulated by NBUVB in the bulge MCs. (P<2e-02, Area under bubble curve Z-Score 4.4E-03; fold change FC=12.7). Using qRT-PCR and new patient samples, we validated induction of RHOJ and VM (P<0.05; FC=2) by NBUVB, and found a similar expression trend for BELA, C20H1 and C20H1. To study functional phenotypes associated with RHOJ depletion, we used the PI-G1 immortalized MC cell line, which we identified as good functional model for human bulge MCs. RHO-J knockout by siRNA transfection, led to a decreased PGI1 and an altered senescent senescent phenotype; b. decreased PIG1 cell migration (245%; P<3.6E-03) during the first 48h, as assessed by a scratch wound assay, c. decreased expression of cytoxins (focal adhesions and actin stress fibers) (P<5.0E-04), as analyzed by immunostaining. Our data suggest that RHOJ depletion and migration of immature MCs, and is an important activator of these cells during NBUVB treatment.

1188

Immune surveillance and evasion in the progression from common melanocytic nevus to dysplastic nevus to malignant melanoma

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Dysplastic nevi (DN) are significant risk factors for the development of malignant melanoma (MM), with the presence of one of these lesions associated with a two-fold increased risk. However, the exact nature of DN, as well as its putative role as a progressive lesion from common melanocytic nevi (CMN), is debated. We sought to elucidate the relationship of DN with CMN and MM by evaluating genes in DN with levels of expression between CMN and MM. 1383 genes were differentially expressed and upregulated in MM compared to CMN, with intermediate levels of expression in DN (FDR<0.05). Pathway analysis revealed a significant immune system and inflammatory response pathways, including Th1 and Th2 activation, innate immune system processes, and chemokine-mediated signaling pathways. By microarray, MM exhibited progressively higher expressions of both negative and positive immunomodulatory genes from a comprehensive list curated through Gene Ontology terms and literature review. Furthermore, DN contained increased Th1 (P=0.0077), Th6 (P=0.0002), and Th22 axes involvement (IL-12, IL-4, IL-23, IL-17, IL-22), innate immune system processes (IL-6, IL-8), regulatory T-cell involvement (TGF-β, IL-10), and immunomodulation (CTLA-4, PD-L1, PD-L2, IDO-1) from CMN to MM. Interestingly, while the Th1-inducing gene IL-12 progressively increased, Th1 output (IFN-γ) decreased from DN to MM, suggesting impaired immune response in MM. Histologically, progressively greater amounts of lymphocytic infiltration were appreciated from CMN to DN to MM. Holistically, our findings suggest that progressively increased negative immunomodulation and impaired type II interferon response in MM suggest that intact immune system axes and lower expression of immunosuppressive molecules in DN may provide effective immune surveillance against neoplastic cells. Greater understanding of the complex interplay between immunomodulation and malignant transformation in DN and MM could translate to further advancements in immunotherapy.

1189

Impaired activation of SIRT3 contributes to oxidative-stress induced mitochondrial dysfunction: A possible mechanism underlying the degeneration of melanocytes in vitiligo

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Changes in immune function have been implicated in the pathogenesis of vitiligo, a skin disorder characterized by the abnormal loss of melanocytes. In our previous study, we found oxidative-stress induced cell death in vitiligo is correlated with mitochondrial-dependent apoptosis. To understand the underlying mechanisms, we studied the functional roles of SIRT3, a mitochondrial deacetylase that is involved in the heterochromatin assembly and chromatin remodeling. SIRT3 deficiency leads to oxidative stress induced cell death in melanocytes. The mitochondrial dysfunction that occur in melanocytes with SIRT3 deficiency is highly associated with the dysfunction of SIRT3 in vitiligo skin lesions. We found that oxidative stress induced cell death in melanocytes is highly associated with the dysfunction of SIRT3 in vitiligo skin lesions. The impaired activation of SIRT3 contributes to oxidative-stress induced mitochondria dysfunction and subsequent melanocyte degeneration in vitiligo.

1190

Demethylation of TRPM2 induced by oxidative stress triggers mitochondria-dependent apoptosis of melanocytes

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Abnormal mitochondrial calcium homeostasis has been proved to play a critical role in oxidative-stress-induced apoptosis of melanocytes. TRPM2 is an oxidative stress sensitive calcium channels, however, whether TRPM2 participate in oxidative-stress-induced apoptosis of melanocytes has not been investigated. In the present study, we initially found that H2O2 induced demethylation of TRPM2 and increased the expression of TRPM2 in melanocytes. In addition, TRPM2 inhibitors or knockdown of TRPM2 lesions H2O2-induced calcium overload and apoptosis of melanocytes. Furthermore, we found that H2O2-induced Ca2+ flux mainly located in mitochondrial, and a specific mitochondrial Ca2+ uptake inhibitor Ru360 suppressed H2O2-induced mitochondrial reactive oxygen species (ROS) accumulation and mitochondrial membrane potential decreasing. More importantly, TRPM2 inhibitors or knockdown of TRPM2 ameliorated H2O2-induced apoptosis and mitochondrial damage of melanocytes. Taken together, we demonstrated that demethylation of TRPM2 induced by oxidative stress triggers mitochondria-dependent apoptosis of melanocytes, thus inhibition of TRPM2 may as a potential target for protecting melanocytes from oxidative stress-induced damage.

1191

Randomized, single-blinded, split-face comparison of superficial chemical peel vs. Nd: YAG laser for the treatment of melasma

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While both superficial chemical peels and Nd:YAG laser have been reported to be effective in melasma, there is an absence of side-by-side comparison studies. We prospectively compared chemical peels and laser for a melanoma at an urban, tertiary care university dermatology practice from January 2014 to January 2015. 20 women, age 18 and older, with at least a 2x2cm patch of melasma on each side of the face (forehead or cheek), were randomized to receive 30% glycolic acid peel on one side of the face and 1064nm Q-switched laser on the contralateral side. Areas with treatment were pre-treated with 4% hydroquinone and 2.5% hydroquinone cream. Procedural treatments were delivered at weeks 2 and 6. Photographs were obtained at baseline, before treatment 1 (week 2), before treatment 2 (week 6), and at 10-week follow up. 18 patients completed both treatments. Both treatments caused similar improvement in mean ASA scores at week 6 and 10. Improvement scores did not significantly differ between the two treatments at 6 weeks or 10 weeks for either ASA scores by blinded photoraters, or improvement percentage of the two blinding photoraters, or the patients. Based on mean PAV scores, the peels were more painful than the laser; this finding was significant only for treatment 2. Participants were equally pleased with both treatments. Superficial chemical peels and Nd:YAG laser appear to be equally effective in the treatment of melasma.

1192

Distinguishing melanophages from melanoma in metastatic melanoma treated with TVEC: A clinical application of quantitative multiplex immunofluorescence

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Talgomire physiological assays (T-Vec) is the first FDA-approved oncolytic virus for treatment of advanced melanoma. Melanoma persistence post-injection is difficult to determine both clinically and pathologically as melanomas, macrophages that have phagocytosed mela-noma cells are frequently found. Novel methods to evaluate cells in the tumor microenvironment (TME) post T-Vec are critically needed. Quantitative multiplex immunofluorescence (QmIF) is a novel pathology technique allowing for precise visualization and quantification of DNA and RNA in situ. We sought to determine the TME of lesions post T-Vec and to determine characteristics that distinguish melanophages from melanoma. Two pre- and 2 post-treatment biopsies were obtained. Samples were stained for DAPI, CD3, CD4, CD8, CD68, SOX10, and FOX3 and multiscopic images were acquired. QmIF successfully distinguished melanophages from melanoma. Post T-Vec samples showed only a few SOX10+ tumor cells (nuclear SOX10+) as well as a plethora of cells with cytopenic staining of CD68 and SOX10 with no nuclear expression of SOX10. Post T-Vec samples also showed increased immun infiltrate in tumors, CD4+, CD8+, CD68+, and CD66+ cells. Precise quantitative and spatial analysis of the TME is ongoing. QmIF distinguishes melanophages from melanoma and allowed for characterization of immune infiltrates post T-Vec, which would prove useful in clinical practice.
1193

DRP1 Inhibition as an adjuvant for BH3 mimetics in melanoma

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Current melanoma treatment have limitations of relapse. BH3 mimetics against BCL-2 family members have gained excitement with recent success in hematological cancers. However, single drug BH3 therapy not effective in melanoma due to escape by the anti-apoptotic protein MCL-1 and/or survival of Melanoma Initiating Cells (MiCs). Melanoma progression correlates with increase in Dynamic-related protein 1 (DRP1). DRP1 interacts with BCL-2 family members, but its potential effects on BH3 mictmetic treatment is not defined in melanoma. This study targeted the above components to develop treatment options for melanoma. We tested the efficacy of the BH3 mimetic on cell apoptosis and cell proliferation in melanoma rederived cell lines (C6) and melanoma xenografts. We further examined how inhibiting DRP1 may influence this effect. We used multiple assays (cell viability, bright field, immunoblot, and sphere formation), as well as the CRISP/Cas9 genome-editing technique. To make the study clinically relevant, we utilized patient samples of different melanoma stages, including those from non-metastatic, metastatic and CRP1-3 adenovirus infected melanoma. We found that the BH3 mimetic combination de-bulks and kills MiCs in all samples irrespective of the mutation status or relapsed state (p<0.05). Unexpectedly, cell death occurs independent of major pro-apoptotic proteins such as NORA/BIM or BID. Moreover, the combination treatment impedes the activation of DRP1 and inhibition of DRP1 further enhances the combination-induced apoptosis (p<0.05). This finding is different from those seen in other cancers. We are currently studying how manipulation of DRP1 affects BCL-2 family members in melanoma.

1194

Down-regulated TRPV1 expression contributes to melanoma growth via calcineurin-AIF3-p53 pathway

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Melanoma is the most lethal form of skin cancer with increasing incidence over the years. Despite remarkable advances, metastatic melanoma remains an incurable disease for its notorious aggressiveness. Therefore, further clarification of the underlying mechanism of melanoma pathogenesis is critical for the improvement of melanoma therapy. Ubiqutination is an important regulatory event for cancer hallmarks and melanoma development, and the deubiquitinating enzymes including ubiquitin-specific peptidase (USP) families are greatly implicated in melanoma cancer biology. Herein, we first found that the expression of deubiquitinating enzyme USP4 was significantly up-regulated in melanoma tissues and cell lines. Furthermore, although USP4 knockdown had little impact on melanoma cell proliferation, it could increase the sensitivity to DNA damage agent cisplatin. We subsequently showed that USP4 regulated cisplatin-induced cell apoptosis via p53 signaling. More importantly, USP4 could attenuate the invasive and migratory capacity of melanoma cells by promoting epithelial-mesenchymal transition. Altogether, our results demonstrate that the up-regulated USP4 plays an oncogenic role in melanoma by simultaneously suppressing stress-induced cell apoptosis and facilitating tumor metastasis.

1195

Folic acid protects melanocyte against the increased homocysteine induced apoptosis in vitro

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Melanoma is the most malignant skin cancer with increasing incidence worldwide. Although innovative therapies such as BRAF inhibitor and immune checkpoint inhibitor have gained remarkable advances, metastatic melanoma remains an incurable disease for its notorious aggressiveness. Therefore, further clarification of the underlying mechanism of melanoma pathogenesis is critical for the improvement of melanoma therapy. Ubiqutination is an important regulatory event for cancer hallmarks and melanoma development, and the deubiquitinating enzymes including ubiquitin-specific peptidase (USP) families are greatly implicated in melanoma cancer biology. Herein, we first found that the expression of deubiquitinating enzyme USP4 was significantly up-regulated in melanoma tissues and cell lines. Furthermore, although USP4 knockdown had little impact on melanoma cell proliferation, it could increase the sensitivity to DNA damage agent cisplatin. We subsequently showed that USP4 regulated cisplatin-induced cell apoptosis via p53 signaling. More importantly, USP4 could attenuate the invasive and migratory capacity of melanoma cells by promoting epithelial-mesenchymal transition. Altogether, our results demonstrate that the up-regulated USP4 plays an oncogenic role in melanoma by simultaneously suppressing stress-induced cell apoptosis and facilitating tumor metastasis.

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Up-regulated dehydrogenase USP4 plays an oncogenic role in melanoma

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Atpcitrate lyase contributes to melanoma growth and MAPK inhibitors resistance by epigenetically regulating Mitf and oxidative phosphorylation

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1199 The regulatory role of IncRNA CD27-AS1 on CD27 contributes to melanomagenesis

L Shmuylovich1, YQ u 3, LA Cornelius4 and L Wang5

In the current study, we demonstrated that CD27-AS1 knockdown in melanoma cells significantly attenuated the proliferation, invasion and migration of melanoma cells. The results of our study demonstrated that CD27-AS1 knockdown in melanoma cells blocked the release of CCL22 cytokine when repressing the expression of CD27-AS1 in melanoma cells. These results demonstrate that CD27-AS1 may function as an oncogenic RNA and the regulatory role of CD27-AS1 on CD27 contributes to melanomagenesis. In conclusion, our data for the first time identified a large group of IncRNAs dysregulated in melanoma compared with normal skin, providing a resource for investigating IncRNAs in melanomagenesis.

1201 Melanoma cell-intrinsic TNFAIP3 promotes tumor progression and immune escape by activating STAT3

J Ma1, W Guo2, S Wang1, S Guo3 and C Li4

In conclusion, our results demonstrate that melanoma cell-intrinsic TNFAIP3 promotes tumor progression and immune escape by activating STAT3.

1202 Up-regulation of melanocyte motility in UV-irradiated skin is mediated by switching between molecular species of stem cell factor

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Melanocytes play a key role in skin pigmentation. Many reports show increased melanocytes in pigmented areas indicating that both up-regulation of melanin synthesis and accumulation of melanocytes at the pigmented area are important to develop pigmentation. In our previous observations, the melanin-containing area diffused around hair follicles after UVB-exposure of hairless murine skin. This diffusion did not transcend the irradiation boundary. These observations suggested that a UVB-induced increase of melanocytes is at least partly mediated by movement from hair follicles, and that melanocyte movement is suppressed in un-irradiated areas, though it is accelerated in irradiated areas. Previous reports indicated that two molecular species of stem cell factor (SCF) have different biological functions in melanocyte behavior. The soluble SCF (sSCF) stimulates chemotaxis of melanocytes whereas the membrane-bound SCF (mSCF) stimulates chemokinesis. We hypothesized that switching between these SCF molecular species might determine the motility of melanocytes in the epidermis. First, we compared the amount of mRNA encoding each SCF species in UVB-irradiated and normal skin using the digital polymerase chain reaction with various primer sets for SCF. In the UVB-irradiated epidermis, total SCF increased by 3-fold, and sSCF by 5-fold, suggesting a relative increase in the content of sSCF mRNA by UVB-irradiation. Next, we confirmed that sSCF recruited melanocytes whereas sSCF stimulated melanocyte movement leading to scattering in a 3-dimensional skin model. These findings suggest that UVB stimulates melanocyte motility via switching the molecular species from mSCF to sSCF. In addition, we found up-regulation of sSCF as a regulatory factor for alternative splicing, in UVB-irradiated murine skin. Because SFQ knockdown by siRNA decreased sSCF mRNA in cultured murine keratinocytes, these data suggest that SFQ regulates melanocyte motility via the content of sSCF in UVB-irradiated skin.

1203 AIM2-deficient dendritic cell vaccination improves adoptive T-cell therapy via the activation of type I IFN signaling through STING

M Yamada1, R Fukuda1, RL Riding2 and JE Harris3

In conclusion, our results demonstrate that AIM2-deficient dendritic cell vaccination improves adoptive T-cell therapy via the activation of type I IFN signaling through STING.

1204 Label-free high-throughput photoacoustic imaging of circulating melanoma cells in patients in vivo

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The majority of cancer deaths are associated with metastasis, the process of cancer cells spreading from primary tumor sites and forming new tumors at distant organs. Circulating tumor cells (CTCs) are the key determinants of metastatic propensity. Detection and characterization of CTCs is critical for understanding tumor metastasis and improving cancer therapy. Here, we applied a linear-array-based photoacoustic tomography (LAPAT) system for label-free high-throughput melanoma CTC imaging in patients in vivo. By imaging CTC-mimicking flowing melanoma cells in microtubes, we demonstrated the capability of LAPAT to detect melanoma CTCs, quantify their contrast-to-noise ratios (CNRs), and measure their flowing velocities. We showed that the maximum depth of LAPAT to detect a single melanoma CTC was ~3.5 mm, which is adequate for imaging most human superficial veins. We successfully imaged 16 Stage III and IV melanoma patients with LAPAT and detected CTCs in 3 patients. We applied a line-array-based photoacoustic tomography (LAPAT) system for label-free high-throughput melanoma CTC imaging in patients in vivo. By imaging CTC-mimicking flowing melanoma cells in microtubes, we demonstrated the capability of LAPAT to detect melanoma CTCs, quantify their contrast-to-noise ratios (CNRs), and measure their flowing velocities. We showed that the maximum depth of LAPAT to detect a single melanoma CTC was ~3.5 mm, which is adequate for imaging most human superficial veins. We successfully imaged 16 Stage III and IV melanoma patients with LAPAT and detected CTCs in 3 patients. We applied a line-array-based photoacoustic tomography (LAPAT) system for label-free high-throughput melanoma CTC imaging in patients in vivo. By imaging CTC-mimicking flowing melanoma cells in microtubes, we demonstrated the capability of LAPAT to detect melanoma CTCs, quantify their contrast-to-noise ratios (CNRs), and measure their flowing velocities. We showed that the maximum depth of LAPAT to detect a single melanoma CTC was ~3.5 mm, which is adequate for imaging most human superficial veins. We successfully imaged 16 Stage III and IV melanoma patients with LAPAT and detected CTCs in 3 patients. We applied a line-array-based photoacoustic tomography (LAPAT) system for label-free high-throughput melanoma CTC imaging in patients in vivo. By imaging CTC-mimicking flowing melanoma cells in microtubes, we demonstrated the capability of LAPAT to detect melanoma CTCs, quantify their contrast-to-noise ratios (CNRs), and measure their flowing velocities. We showed that the maximum depth of LAPAT to detect a single melanoma CTC was ~3.5 mm, which is adequate for imaging most human superficial veins.
Vitiligo lesional and non-lesional skin shows polar cytokine activation
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Vitiligo is a chronic autoimmune depigmentation disorder affecting up to 1.5 million people worldwide, with increased prevalence among some skin of color (African descent) who are subject to repeated mechanical loads from food pressed against it by the tongue and during swallowing. We postulate that mechanical stress may be at least partially responsible for the observed lesions.

1207
RNF4 ubiquitin ligase drives melanoma progression
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Deciphering new molecular targets that are crucial for melanoma progression is of utmost importance. One such potential target is RNF4, a SUMO-targeted ubiquitin ligase. In most cases, RNF4-dependent ubiquitination tags SUMOylated proteins for degradation. Additionally, RNF4 enhances the stability & transcriptional activity of selected phospho-oncoproteins, hence promoting tumorigenesis. We aimed to evaluate the contribution of RNF4 to human melanoma progression and metastasis and to determine whether RNF4 is essential for melanoma progression and survival. Knockdown of RNF4 attenuated melanoma cell proliferation, migration and clonogenicity. Furthermore, conditional expression of RNF4, but not the inactive mutant, promoted tumor growth, resulting in larger and highly vascular tumors. Even more strikingly, RNF4 knockdown reduced tumor size by over 50% in vivo. These findings suggest that RNF4 is a valuable target for the development of melanoma therapies.

1208
Predominance of oral mucosal melanoma within high areas of mechanical stress
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Mechanistic evidence directly linking skin exposure to traffic-related air pollutants with skin hyperpigmentation (HP) is lacking. We therefore developed a standardized, robust human skin model which allows application of ambient relevant, toxicologically well-characterized human skin samples. We exposed these samples to fine particulate matter and soot at concentrations and doses representative of those measured at busy roadways. We observed increased melanin synthesis and deposition, accompanied by an increased transcriptional expression of genes involved in melanin synthesis, strongly suggesting that these environmental factors can trigger melanogenesis in vivo. We found that RNF4 is essential for melanoma progression and survival. Knockdown of RNF4 attenuated melanoma cell proliferation, migration and clonogenicity. Furthermore, conditional expression of RNF4, but not the inactive mutant, promoted tumor growth, resulting in larger and highly vascular tumors. Even more strikingly, RNF4 knockdown reduced tumor size by over 50% in vivo. These findings suggest that RNF4 is a valuable target for the development of melanoma therapies.

1209
Ambient relevant diesel exhaust particles cause skin hyperpigmentation ex vivo and in vivo in human skin: The Düsseldorf Pollution Patch Test
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Pathophysiology underlying this unique premelanoma lesion remains unclear. The current study aimed to determine the feasibility of a South African melanoma patient registry. The registry was established to collect epidemiologic and genetic data on South African melanoma patients, with the ultimate aim to inform future research and clinical practice.

1210
Enhancing the therapeutic efficacy of immune checkpoint inhibition and targeted therapy using anti-tumor antibodies in mouse melanoma
F. Massoudi, F. Saeedi, A. Marini, T. Jaenicke, H. Felsner and J. Krutmann
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Pathophysiology underlying this unique premelanoma lesion remains unclear. The current study aimed to determine the feasibility of a South African melanoma patient registry. The registry was established to collect epidemiologic and genetic data on South African melanoma patients, with the ultimate aim to inform future research and clinical practice.
1211 Bio-molecular profile of melanoma subtypes selected by reflectance confocal microscopy
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Reflectance confocal microscopy (RCM) allows the identification of four malignant melanoma (MM) subtypes: dendritic-cell (DC), round cell (RN), dermal-nest (DN) and combined-type (CT). The aim was to study the biomolecular profile of 144 RCM subtypes. ISM MM were evaluated by immunohistochemical and histopathological analyses. DC and RN MM were generally Radial Growth Phase in stage I and II, while CT and DN were Vertical Growth Phase in stage III and IV. Ki67, as shown by immunohistochemistry, was significantly more expressed in the epidermis of DC and RN. CD271 expression increased from DC to RN and decreased from CT to RC. This correlates with our previously published concept that CD271 has a switch on-off function in melanoma progression. ABCB5 expression was lower in DC than in CT, DC, and CT. Co-expression of BMF and RMA6608 was observed in CT, while it inversely correlated in DC, RC, and DN. In SxOx, were highly co-expressed in CT and DN, indicating a more aggressive behaviour of CT and DN, as compared to DC. RNAs extracted from paraffin-embedded melanoma tissues were analysed for the expression of 776 genes by Nanostring technology. The analysis revealed significant differences in the expression of genes involved in chemotaxis, inflammation, cell-cell adhesion, cell motility and angiogenesis. When CT melanomas were able to generate spheroids, compact and with a sphere-like structure, probably because of their proliferation capacity, as shown by MTT assay. Collagen invasion assay confirmed that DC is a less aggressive tumour, as compared to the other RCM-subtypes. These results represent a first step to develop a new method to identify and manage melanoma, reaching a more accurate patient/tumour tailored therapeutic approach.

1213 Exosome-mediated RNA delivery for melanoma therapy
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RNA interference has long been recognized as an effective method that is capable of reducing gene expression by up to 80%. Its efficacy in both animal models and humans has also been demonstrated. However, for RNAi to become clinically feasible to treat cancer, an efficient method to deliver RNAs to tumor cells has to be devised. In the present study, we explored the use of exosomes to deliver small hairpin RNAs (shRNAs) into melanoma cells. To generate gene expression by up to 80%. Its efficacy in both animal models and humans has also been sustained tumorigenicity and stemness of cancer cells. Cell Research 2017 June, 27.

1214 Palladium based nanoparticles for the treatment of advanced melanoma
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Braf and MEK inhibitors are commonly used for the treatment of melanoma; however, resistance to this treatment is encountered frequently. ICGR -1 and CD44 are usually over-expressed in most advanced Braf inhibitor-resistant melanomas. Non-Braf mutated melanomas are also resistant to Braf inhibitors. Thus, a significant need exists for the treatment of these melanomas. Tris DBA Palladium (Tris DBA pd) is a novel inhibitor of N-myristoyltransferase 1 and has proven in vivo activity against melanoma. However, its usefulness is impaired by its poor solubility. To improve its therapeutic efficacy and overcome drug resistance in genetically diverse melanomas, we synthesized Tris DBA pd hyaluronic acid nanoparticles (HANP) and have shown that these nanoparticles target LM60A, an aggressive human melanoma that is resistant to Braf inhibitors. We induced LM60A tumor growth in four groups of nude mice and then tested with Tris DBA pd HANP with ICRG-1 antibody, Tris DBA pd HANP free Tris DBA pd, and empty nanoparticle through tail vein injections. We observed that the tumors in the group treated with Tris DBA pd HANP were the most responsive to treatment. In melanoma cells treated with Tris DBA pd HANP, we noted depletion of CD44 positive cells on IHC with CD44 being the receptor for hyaluronic acid, as well as a melanoma stem cell marker. Surprisingly, the ICRG-1 containing HANP nanoparticle was less effective, possibly due to steric hindrance of ICRG-1 and CD44 binding. This may be an important factor in the design of nanoparticles. Microarray analysis of the treated tumors compared with controls indicates a B lymphocyte response that may be mediating tumor regression. HANP Tris DBA pd nanoparticles are an effective therapy for CD44 positive tumors like melanoma, and may stimulate novel immune methods of anti-tumor immunity.

1216 Multiple primary melanomas are associated with increased risk of internal malignancy
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Cutaneous melanoma is one of the most common cancers in the United States with projected increased incidence rates through 2030. Approximately 8% of melanoma patients will develop multiple primary melanomas (MPMs). However, it is unclear if patients with MPMs are at increased risk of developing other malignancies. To investigate this, we analyzed data from the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) database from 1973 - 2014 for an association of internal malignancy with MPM. We identified 221,799 individuals of European descent with 1 or more melanomas, 3,995 with 3 or more MPMs, and 481 with 5 or more MPMs. Notably, the male to female ratio was 1.22, which is consistent with previous studies. The incidence of MPMs increased from 1.22, to 2.12, to 2.51 in those with 1 or more, 3 or more, and 5 or more MPMs respectively. Additional, we found markedly elevated risks of developing additional malignancies with increasing numbers of MPMs, with the highest being observed in those with ≥5 MPMs (O:Es = 4.74, 5.54, and 6.53, respectively). The risk of developing lung cancer was increased with increasing numbers of MPMs, with the highest being observed in those with ≥5 MPMs (O:Es = 1.62, 1.71, and 1.87 respectively). Our findings support that individuals with MPMs are at increased risk of internal malignancy with increasing numbers of MPMs, with the highest being observed in those with ≥5 MPMs.

1217 RAGE mediates UVB-induced persistent DNA Damage Response (DDR) and resistance to apoptosis in human melanocytes
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The major modifiable risk factor in melanogenesis is UV exposure and mutagenesis of melanocytes. Other UV-induced events that contribute to early tumorigenesis are poorly understood. Repeated exposure of human primary melanocytes to UVB results in a sustained Senescence Associated Secretory Profile (SASP), increases in expression of STAT1, MX1, OA52 and IRF7 proteins of up to 75-fold, and resistance to subsequent UVB-induced apoptosis. In the setting of UV-induced DNA damage, we then investigated whether or not the release of damage associated molecular patterns (DAMPs) contributed to this response and to melanocyte survival. One to three days following single and repeated UVB-exposures, we detected time-dependent increases in intracellular HMGB1 by Western analysis and quantification of KIT and LEF1 expression in epidermis. Our study demonstrate the whitening effect of Lilium Candidum leaf extract on 2D human melanocytes and on UVB-exposed 3D pigmented skin model. It seems that this extract inhibits the UVB-induced pigmentation via a down-regulation of KIT and LEF1 expression.

1218 Palladium based nanoparticles for the treatment of advanced melanoma
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Lilium Candidum leaf extract prevents Ultraviolet B-induced pigmentation in an engineered skin model by down-regulating KIT and LEF1 expression. S. Heraud, X. Liu, A. Tapote, G. Liu, A. Boher, M. Albouy, B. Tang and O. Damour
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Lilium Candidum leaf extract (LCE) inhibits this UVB-effect with a decrease of melanin content and PMEL expression compared to the UVB control. Our genomic analysis identified the down-regulation of KIT and LEF1 genes expression, both implicated in melanogenesis. This inhibition was subsequently confirmed at the protein level by an immunofluorescence quantification of KIT and LEF1 expression in epidermis. Our study demonstrate the whitening effect of Lilium Candidum leaf extract on 2D human melanocytes and on UVB-exposed 3D pigmented skin model. It seems that this extract inhibits the UVB-induced pigmentation via a down-regulation of KIT and LEF1 expression.
Nicotinic acid inhibit the melanocyte migration of melanoma

1217

Nicotinic acid inhibit the melanocyte migration of melanoma
Z Dong and Y Shi
Children’s Hospital of Chongqing Medical University, Chongqing, China

MicroRNA signature distinguishing nevi from primary melanoma
1220

MicroRNA signature distinguishing nevi from primary melanoma
J Hong1, T Lee2, W Wang2 and Y Chen3

Intracellular vacuoles observed in cultured melanocytes obtained from normally pigmented skin of a vitiligo patient were vanished by the treatment of nicotinic acid.

1221

Intracellular vacuoles observed in cultured melanocytes obtained from normally pigmented skin of a vitiligo patient were vanished by the treatment of nicotinic acid.

1218

MAPK4 is critical for the resistance to BRAF inhibitor in melanoma
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E-cadherin expression correlates to nuclear receptors for vitamin D and pigment melanomas
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Multiple MC1R variants associated with extensive freckles and red hair found in a Mongolian family

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Freckles are flat, tanned circular spots that typically are multiple and may develop on sun-
exposed skin, especially on the face. In freckles, an increased number of melanocytes are not
observed, but the melanocytes overproduce melanin granules changing the coloration of
keratinocytes. The presence of freckles is related to variants of MC1R, which has been also
reported an association with red hair more strongly. We present a very interesting case of a
Mongolian boy with extensive freckles and harboring two MC1R variants as found by whole-
exome sequencing (WES). The proband, born in a non-consanguineous mongoloid family, was
a 14-year-old Mongolian boy living in the northernmost part of Mongolia. He developed
extensive freckles on the face, anterior chest, and the extensor surfaces of upper extremities
(sun-exposed area) and showed lighter skin tone in comparison to other family members. The
freckles became apparent when he was 5 years old, and the color intensity increased gradually ever since. WES was performed for the proband and his family to elucidate the genetic differences in the family. Three different variants in the MC1R were detected in the family, and two of them were evaluated as damaging. Furthermore, we confirmed these damaging variants were on different alleles respectively, indicating he was a compound heterozygote for both of the damaging variants. We concluded that the extensive freckles on the face were explained by his sun exposure and his genetic makeup, compound heterozygosity for rare variants in MC1R.

1224

Role of CCR6 in melanoma metastasis

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Melanocytes are the primary source of melanin, which is known to impart protection against UV light damage to the skin. In recent years, several studies have demonstrated that melanocytes are capable of acting as an immunosurveillance mechanism by releasing several cytokines and chemokines. These factors are essential for the immune control of melanoma. The CCR6/CCL20 axis has been identified as a critical axis in melanoma immune control. We have previously demonstrated that CCR6 is expressed on tumor-infiltrating lymphocytes and melanoma cells. In this study, we aimed to evaluate the contribution of the CCR6/CCL20 axis for the immune control of melanoma.

We used an established melanoma cell line, B16, which has been extensively used in studies on melanoma metastasis. In this study, we evaluated the role of CCR6 in melanoma metastasis by using a B16-CCL20 expressing melanoma cell line. We generated a B16-CCL20 expressing melanoma cell line and evaluated its metastatic potential in vivo and in vitro. We observed that the expression of CCR6 in melanoma cells significantly increased in mice injected with B16-CCL20 as compared to control naive or tumor-bearing animals. Both cytotoxic T cell activity (CTL) and melanoma cell-reactive antibodies were evaluated in animals injected with B16-CCL20. We observed a significant increase in the number of cytotoxic T cells and melanoma cell-reactive antibodies in mice injected with B16-CCL20. These results suggest that the expression of CCR6 in melanoma cells plays a critical role in melanoma metastasis.

1225

Paving the road to explain melanocyte loss in vitiligo: An uncovered role of matrix metalloproteinase MMP9

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Melanocyte loss is the pathological hallmark of vitiligo, the archetype of a chronic depig-
menting inflammatory skin disorder. Yet, whether such disappearance results from melanocy-
te death and/or detachment is still a matter of debate. We previously showed that vitiligo
lesions are characterized by a high density of infiltrating inflammatory cells, which are known to
be responsible for melanocyte loss in vitiligo. In this study, we aimed to study the contribution of matrix metalloproteinase MMP9 to melanocyte loss in vitiligo.

We first evaluated the contribution of matrix metalloproteinase MMP9 to melanocyte loss in vitiligo by using a B16-CCL20 expressing melanoma cell line. We observed a significant increase in the number of cytotoxic T cells and melanoma cell-reactive antibodies in mice injected with B16-CCL20. These results suggest that the expression of CCR6 in melanoma cells plays a critical role in melanoma metastasis.

1226

Cold atmospheric plasma treatment of melanoma enhances immune response

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We have shown that nanosecond pulsed nsP-plasma treatment of established B16 melanoma tumors resulted in complete remission greater than 60% of the time, and as dependent on reactive oxygen and nitrogen species (ROS and RNS). nsP-plasma is a cold atmospheric plasma (CAP) composed of ionized gases containing ions, radicals, ROS, RNS and nsP-electric fields. Strikingly, within two hours of treatment both the vasculature and the tumor volume were decreased by 50%. Histological examination revealed blood vessel thinning, down, areas of hemmorhage and cell lysis, increased cytochrome C release, a marker of mitochondrial death pathway, and HZAI, a marker for DNA damage/regap. Indirect immunofluorescent staining and nsP-plasma treated lesions at 48 h post treatment revealed a decrease in the number of blood vessels (decreased C3D1 staining) and proliferating cells (Ki-67+), in addition to increased infiltration of CD8+ T cells, CD4+ T cells and CD11c+ dendritic cells. Most importantly, treatment led to activation of B16-specific im-
munity. Both cytotoxic T cell activity (CTL) and melanoma cell-reactive antibodies were increased in nsP-plasma-treated mice as compared to control naive or tumor-bearing animals. As nsP-plasma treatment seemed to promote immune recognition of tumors, we hypothesized that a melanoma cell line, B16, which is known to be resistant to immune recognition, might be a relevant model for our study. We first evaluated the contribution of matrix metalloproteinase MMP9 to melanocyte loss in vitiligo by using a B16-CCL20 expressing melanoma cell line. We observed a significant increase in the number of cytotoxic T cells and melanoma cell-reactive antibodies in mice injected with B16-CCL20. These results suggest that the expression of CCR6 in melanoma cells plays a critical role in melanoma metastasis.
Aberant differentiation as a novel mechanism for hyperpigmentation disorders

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Melanoma is a malignant form of skin cancer that is increasing in incidence. The discovery of targeted melanoma therapeutics and immune checkpoint inhibitors has ushered in a new era of melanoma therapy. However, most patients become resistant to targeted therapies and a large number of patients never respond to immune checkpoint inhibitors, leaving these patients with limited therapeutic options. Therapies directed towards the metabolic state of melanoma are a burgeoning area of research. We recently discovered that Vitamin C (VitC), a safe natural substance, kills cancer cells that express GLUT1 and produce high levels of reactive oxygen species (ROS) by blocking GAPDH and halting tumor metabolism. Melanoma expresses GLUT1 and generates high levels of ROS suggesting that it would be susceptible to VitC treatment. We have found that melanoma from diverse mutational backgrounds are killed by low levels of VitC. In addition, VitC is highly effective for the treatment of melanoma. Surprisingly, MAPK inhibitors are known to raise ROS levels in melanoma and we have found that MAPK inhibitors synergize with VitC to induce BRAFV600 melanoma cell death. Our data suggest that Vitamin C is a potential therapeutic adjuvant for the treatment of melanoma.
**1235**

**Expression pattern of melanosomal proteins following inhibition of soluble adenyl cyclase**

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Soluble adenyl cyclase (sAC) is a non-canonical, pH-sensitive source of cAMP in mammalian cells. We previously demonstrated that loss of sAC elevates melanosomal pH and increases melanin production, both in vitro and in vivo. An ultrastructural analysis of melanosomes following loss of sAC suggested that sAC inhibition increased melanosome maturation. Since melanosome maturation and changes in pH are associated with melanosome protein processing, we have begun to explore whether sAC activity influences the expression of melanosome proteins. RNAseq analysis of wild-type and knockout sAC melanocytes did not reveal any overt changes in melanosome protein mRNA. However, Western analysis suggests that post-translational modification of melanosome proteins is affected by loss of sAC. The observed change in protein processing is consistent with previous studies using drugs that alter melanosome pH. Thus, in addition to altering pigmentation, sAC regulation of melanosome pH affects the melanosome protein profile.

**1236**

**A peptide product with a function of autophagy induction regulates skin pigmentation via facilitation of melanosome degradation in keratinocytes and melanoma cells**

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Autophagy is involved in a large variety of cell physiological processes, including anti-microbial defense, apoptosis, differentiation, tumorigenesis, immune response and etc. With regard to melanogenesis, it is assumed that autophagy and autophagy regulators can play different roles in both the biosynthesis of melanosomes and melanosome degradation. However, the exact function of autophagy in melanin production still remains unknown. In this study, we developed a lysine dimers-based peptide product which has a function of autophagy induction. We also investigated how it regulates melanogenesis in melanocytes and keratinocytes with uptake melanosomes. Human primary melanocytes and keratinocytes, which were cultured with isolated melanosomes in order to uptake melanosome within keratinocytes, were treated with a lysine dimer-based peptide product. The product increased expression of LC3-II, a marker of autophagy, while it decreased melanin production with remarkable decrease in H2A-X, a melanosome protein, on the other hand, melanosomes were trapped within autophagosome in melanocytes. Chloroquin was used to confirm whether the product increases melanosome degradation via autophagy mechanism. In addition, ex vivo skin organ culture showed decreased melanin staining after treatment of the product in Fontana Masson staining. Taken together, our data suggest that the lysine dimer-based peptide product regulates melanogenesis through melanosome degradation by autophagy induction. And autophagy regulators, though implicated mechanisms could differ depending on their forms, might become powerful therapeutic targets for the treatment of pigmented disorders.

**1237**

**Escape form adaptive drug tolerance through OGT and TET1 mediated H3K4me3 remodeling in MAPKi-resistant melanoma**

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Genetic alterations linked to acquired BRAF inhibitor resistance are absent in about 40% of relapsed melanoma patients suggesting the involvement of epigenetic changes. We investigated epigenetic remodeling in BRAF mutant melanoma upon BRAFi/MEKi inhibition. Long term treatment of more than 45 days enables the cells to escape the slow cycling state which results in proliferating cellular clusters (drug-tolerant persistent colonies) with stem-like characteristics that regain global H3K4me3. H3K4me3 CHIP-seq of colonies compared to parental cells revealed differential marking at promoters regions of several target genes involved in MAPKi resistance, including ARAF, BRAF, and CRAF. H3K4me3 remodeling corresponded to increased gene expression and susceptibility to pan-RAF inhibitors. Two enzymes, OGT and TET1 that are both linked to H3K4me3 regulation are significantly upregulated in persistent colonies and tumor tissue of FDAs from BRAF mutant melanoma patients under MEKi/2 inhibition. A shift in OGT nuclear localization and O-linked glycosylation patterns was observed in colonies compared to parental cells and OGT CHIP-PCR confirmed a set of genes with exclusively H3K4me3 marking in colonies. sRNA mediated knockdown of OGT and TET1 blocked H3K4me3 increase in colonies, prevented colony formation and delayed tumor relapse in BRAF mutant xenografts. OGT and TET1 are promising targets to combat treatment failure and prolong overall survival.

**1238**

**A BRAF inhibitor, vemurafenib, enhances insulin-induced sebum production but antagonizes 5α-DHT-mediated sebaceous lipogenesis in hamster sebocytes**

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The activation of the RAS-RAF-MEK-ERK signal pathway has been associated with tumor progression. A selective BRAF protein kinase inhibitor, vemurafenib, exhibits anti-tumorigenic activity in patients with metastatic melanoma. Many patients treated with vemurafenib develop common cutaneous disorders including dry skin and acne-like rashes, which are closely associated with the dysfunction of sebaceous glands and pilosebaceous units. However, there have been no reports to date that vemurafenib may modulate sebum production in sebaceous glands. In the present study, we examined whether or not vemurafenib directly influenced sebum production in hamster sebocytes (HamSEB) in vitro. Insulin-activated intracellular lipid-droplet formation was enhanced by vemurafenib in HamSEB, which was due to the increased expression of the triglyceride (TG), a major component of sebum. On the other hand, vemurafenib was found to suppress 5α-dihydrotestosterone (5α-DHT)-induced lipid-droplet formation and TG production in HamSEB. Thus, vemurafenib is likely to enhance or suppress sebum production, depending on lipogenic factor species in sebaceous glands. These findings may increase the clinical understanding of the side effects of vemurafenib.

**1239**

**Genome-wide scans found variants in TERT associated with pigmented spots on hands/arms but not with other skin aging signs**

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Skin aging is a health topic of growing concern to the general public. Researchers have been identifying extrinsic and intrinsic factors affecting skin aging. So far, genetic studies on skin aging have mainly focused on facial pigmentation and wrinkles. Here we are presenting the first genome-wide study on skin aging signs also including pigmentations and wrinkles on hands/arms (according to SCINEXATM). By sampling 2959 individuals from the Taizhou study, we identified a total of 83 variants in TERT but no other skin aging signs. Top hits were a synonymous variant in TERT (p=1.51×10^-07). This association was independently replicated in SALIA cohort in Germany. An associated haplotype near the TERT promoter region was identified by fine mapping. Luciferase reporter gene assay was performed to confirm that the haplotype is associated with modified expression level of TERT (p<0.01). We also showed that sun exposure is the main confounding factor that facial pigmented spots were not found to be associated with variants in TERT. Interestingly, even after controlling all the potential confounding factors, the variants are not associated with other skin aging signs, such as wrinkle and sagging, indicating that TERT is not affecting skin aging in a global aging mechanism, but rather affecting pigmented spots in a more specific yet unknown mechanism.

**1240**

**Endovascular progenitors initiate and drive de novo vascularisation in melanoma**

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The development of new vascular structures is a pre-requisite for melanoma growth and spread. We aimed to better identify and characterise the source of de novo endothelium in melanoma. Among Lin-CD34+ cells, expression levels of VEGFR2 and CD31 defined three distinct endothelial sub-populations. Lineage tracing of endothelial cells (Cdh5CreER/+) demonstrated a maturation sequence from endothoportor progenitor (EVP) via trans-amplying (TA) to fully differentiated (D) cells in B16 melanoma. Transplantation and clonal culture at limited dilution demonstrated EVP cells were the only endothelial sub-population with self-renewal and engulfment capacity. Clonal analyses of multicolour lineage tracing (Cdh5CreER/Rosa-YFP) further showed the contribution of different progenitors to venous and arterial structures within tumours. RNA-seq demonstrated significant differences between populations and pointed to Sox18, JAK/STAT and Notch signalling to be significantly upregulated in EVP cells. Sox18 reporter mice, EVP activated Sox18 expression as early as 3 days after tumour inoculation. We next sought to specifically target EVP activity. Anti-IL6R antibody and gp-130 antibodies blocked JAK/STAT and Notch signalling, significantly reducing EVP infiltration and vessel formation. Importantly, this caused a significant reduction of the vascular network and a reduction in tumour size. To validate the activity on Notch signalling within EVP, we used the Notch conditional knockout model RBPjlox/lox/Cdh5CreER/Rosa-YFP. Deletion of Notch signalling significantly reduced EVP and vessel formation in tumours. Interestingly, a significant reduction of the vascular network was also observed. In conclusion, we demonstrated the importance of EVP initiating the de novo vascular network in melanoma and blocking JAK/STAT or Notch signalling prevented EVP infiltration and vessel formation.
Pigmentation and Melanoma ABSTRACTS

1241 Pretreatment serum CTLA-4 is a potential biomarker of a risk of immune-related adverse events in metastatic melanoma
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Immune checkpoint inhibitors have dramatically changed metastatic melanoma treatment strategies; however, these new immunotherapies have some potential side effects. Identifying markers that can help predict the likelihood of these side effects can be useful and permet the development of biomarkers to predict their efficacy is needed. Second, that predicting the side effects are also required. By the immune system imbalance, these therapies also generate severe autoimmune toxicities, called immune-related adverse events that can potentially affect any tissue. Some potential candidates have reported as useful biomarkers to predict the efficacy. However, biomarkers to predict immune-related adverse events has not been gaining attention. It is also important to develop biomarkers to predict severe immune-related adverse events for better management of melanoma treatment. Therefore, we investigated the pretreatment serum levels of soluble CTLA-4 whether it can be considered as a biomarker for immune-related adverse events. 38 patients with metastatic melanoma treated with immune checkpoint inhibitors were analyzed. The serum levels of soluble CTLA-4 in patients with immune-related adverse events were lower than those of patients without them (p<0.05), especially patients with more serious events have a tendency to show higher levels of soluble CTLA-4. These results suggested the possibility that soluble CTLA-4 could be a potential biomarker for immune checkpoint inhibitors in melanoma patients.

1242 A natural compound harmine decreases melanin synthesis through inhibition of DYRK1A in human skin melanocytes
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Melanin plays important roles in protecting human skin against harmful ultraviolet light and other environmental factors. However, hyperpigmentation in specific parts of the skin such as melasma, freckles, and age spots might become an esthetic problem. While we are searching for the compounds that affect melanin synthesis in MNT-1 cells, a human melanoma cell line, we found that harmine significantly decreased melanin synthesis. Harmane is known to inhibit D7RKA. Here, we investigated whether DYRK1A is involved in harmine-regulated melanin synthesis. Inhibition of DYRK1A activity by two other chemical DYRK1A inhibitors, INDA and Roscovitine, and knockdown of DYRK1A, two reported SNAs also reduced melanin synthesis in MNT-1 cells, indicating that DYRK1A can regulate melanin synthesis. Further studies have suggested that suppression of DYRK1A activity or DYRK1A expression inhibited the expression of tyrosinase and that knockdown of NFATC1 expression alleviated the inhibition of melanin synthesis induced by suppression of DYRK1A, suggesting that NFATC1 mediates the effect of harmine/DYRK1A on melanin synthesis. Finally, we also found that harmine could inhibit melanin synthesis and NFATC1 expression in primary human primary melanocytes. Taken together, our results indicate that harmine decreases melanin synthesis through inhibition of DYRK1A and suggest that DYRK1A can be a potential target for the development of depigmenting agents.

1243 High Rab27a expression is associated with poor melanoma prognosis and promotes melanoma cell invasion and metastasis via regulation of pro-invasive exosomes
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Despite recent advances in targeted and immune-based therapies, metastatic melanoma remains a clinical challenge with a poor prognosis. Understanding the genes and cellular processes that drive progression and metastasis are critical to identify new therapeutic strategies and improve treatment strategies and patient outcomes. Here, using clinical samples, we identified that Rab27a was overexpressed in a subset of melanomas, which correlated with poor survival. To further dissect the function of Rab27a in melanoma, we performed CRISPR-knockout and shRNA-knockdown of this gene in melanoma cell lines. We demonstrated that Rab27a loss inhibited proliferation, colony formation, 3D sphere formation, and motility in vitro, as well as spontaneous metastasis in vivo. The reduced invasion phenotype observed with Rab27a knockdown was rescued by Rab27a-replicative exosomes, indicating that exosomes drive Rab27a-mediated invasion. Further studies revealed that while Rab27a loss did not alter the number of exosomes secreted, there were changes in exosome morphology and the abundance of exosomal proteins associated with cancer cell migration and metastasis. These findings expand on a growing body of literature indicating important roles for Rab27a in regulating cancer cell biology, and further highlights its potential to be utilized as a clinical prognostic marker or therapeutic target in melanoma.

1244 Activation of G protein-coupled estrogen receptor signaling inhibits melanoma and improves response to immune checkpoint blockade
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Female sex and history of prior pregnancies are associated with favorable melanoma outcomes. Here we show that much of the melanoma protective effect likely results from estrogen signaling through the G protein-coupled estrogen receptor (GPER) on melanocytes. Selective GPER activation in primary melanocytes and melanoma cells induced long-term changes that maintained a more differentiated cell state as defined by increased expression of well-established melanocyte differentiation antigens, increased pigment production, decreased proliferative capacity, and decreased expression of the oncofetal and stem marker c-Myc. GPER signaling also rendered melanoma cells more vulnerable to immune checkpoint blockade. Systemically delivered GPER agonist was well tolerated, and cooperated with immune checkpoint blockade in melanoma-bearing mice to dramatically extend survival, with up to half of mice clearing their tumor. Complete responses were associated with immune memory that protected against tumor rechallenge. GPER may be a useful, pharmacologically accessible target for melanoma.

1245 PD-1 blockade impedes tumor growth in the immunogenic YUMMER1.7 mouse melanoma model
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Introduction: Despite interest in immune checkpoint inhibitor therapies for melanoma, little is known about their mechanisms of action. To address this, we generated congenic, genetically-inducible murine melanoma models and derived syngeneic cell lines from them. The Yale University Mouse Melanoma 1.7 (YUMM1.7) model originates from a murine melanoma model that is syngeneic with the C57BL/6 strain. The melanoma cells constitutively express the oncoprotein monogenic-Cdkn2a-/- and express high levels of BrafV600E. Clonal sublines of YUMM1.7 were genetically engineered mouse melanoma. Clonal sublines of YUMM1.7 and YUMMER1.7 were injected subcutaneously into 6 to 8-week-old male C57BL/6 mice. In each group, 20 mice received biweekly injections of a PD-1-blocking antibody starting on day 7, and 20 mice received isotype control antibody. Tumors were measured until they reached 15 mm in any dimension. Results: 75% of anti-PD-1-treated YUMMER1.7 tumors exhibited partial response to treatment (slow growth relative to control), and the remaining 25% showed complete response (durable cure). 100% of untreated (control) YUMMER1.7 tumors and YUMM1.7 tumors (regardless of treatment) grew to endpoint by post-injection day 25. Further studies have shown that increased IFNγ signaling is observed in treated YUMMER1.7 tumors. Conclusions: PD-1 blockade impeded tumor growth in 100% of YUMMER1.7 tumors and 75% of YUMM1.7 tumors examined. This suggests that there is no complete T cell disfunction in this animal model. In contrast to prior mouse melanoma models, such as B16, YUMMER1.7 melanomas respond to anti-PD-1 therapy and will serve as a valuable platform for both mechanistic work and development of combination treatment strategies with anti-PD-1 therapy.

1246 An ex vivo human skin model to investigate the synergistic effects of UV and benzaldehyde
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Everyone is exposed to a complex mix of environmental pollution playing a major role in hormone health. C. Wild introduced the exposome concept, which considers environmental pollutants and biological responses throughout the life course. Exposome have the potential to enable novel insights into numerous research questions in environmental health sciences. The skin is exposed to two types of radiation (UV) and environmental air pollutants such as PAHs (e.g., B(a)P), VOCs, particulate matter etc. The prolonged or repetitive exposure may have negative effects on the skin (aging, inflammation, oxidative stress, pigment irregularities). To better understand mechanisms of UV-pollutant combination, we developed an ex vivo skin model on which have been topically and chronically exposed to UVA, B and/or B[a]P. Obviously, the ex vivo human skin model has responded to the UV impacts on increasing expression of genes related to skin pigmentation. Repetitive treatment with B[a]P combined to UV resulted in reduced pigmentation (MLANA, TYRP1, MC1R, POMC). We observed that the skin explant maintained redox homeostasis by induced antioxidant defense system transcriptionally up-regulated in response to UV doses. Combined to UV, the pollutant B[a]P decreased expression of genes related to antioxidant system (CAT, GSR, SOD1). In our model, we have shown that UV exposure has dramatic effects on barrier integrity that are related to its effects on the stratum corneum components like loricrin and SPPRs. We highlighted by IHC analysis these stratum corneum components appear altered in the stratum corneum exposed to UV and even more in skin treated with both pollutants and B[a]P. Obviously, the ex vivo human skin model to analyze the effects of UV and B[a]P combination. We highlighted the negative synergistic damage on barrier integrity, antioxidant system and skin pigmentation induced by these pollutants.
1247
Pharmacological activation of NRF2 signaling prevents UVB induced hyperpigmentation
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Nuclear factor erythroid 2-related factor 2 (NRF2) signaling is a cellular defense pathway that is upregulated by multiple cellular stressors, including ultraviolet radiation (UVR). We found that NRF2 signaling is impaired in human skin biopsies of solar lentigines. Here, we set out to determine whether NRF2 signaling was an important regulator of skin pigmentation following UV light exposure. Using a murine model of UVB induced hyperpigmentation, we exposed ears of C57BL/6 male WT (n=14) and NRF2 null (n=10) mice to 80mJ/cm² UVB once daily 5 times a week for 1 month. Prior to each UVB treatment, the NRF2 inducer sulforaphane (SF) (1 μmol) was topically applied to one ear with the other receiving vehicle or no topical treatment. Skin architecture and melanin deposition were assessed with hematoxylin and eosin and Fontana-Mason (F&M) staining, respectively. Levels of total and phosphorylated NRF2 levels and its downstream targets HO-1 and GCLC were evaluated with indirect immunofluorescence. Following UVB, WT and NRF2 null ear skin had increased in melanin deposition, skin pigmentation (1.6±0.1 fold, respectively) and in skin thickness (1.7±0.2 and 2.6±0.1 fold, respectively) (mean ± SEM, p WT ears pretreated with SF had elevated epidermal levels of total and phosphorylated NRF2, HO-1 and GCLC and had no significant change in melanin deposition, pigmentation or skin thickness relative to non-UVB controls. SF’s protective effect was not observed in UV treated NRF2 null ears. Taken together, these results demonstrate that skin pigmentation following UV light exposure is a consequence of impaired NRF2 signaling that can be prevented with the NRF2 agonist SF, providing a novel mechanism and therapeutic target for UV induced hyperpigmentation disorders.

1249
The treatment of acral vitiligo with autologous cultured epidermal grafts
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The effect of treatment of acral vitiligo is poor. We used autologous cultured epidermal grafts as a novel surgical approach for acral vitiligo. The purpose of this study was to evaluate the efficacy of a modified procedure using autologous cultured epidermal graft transplantation in the management of vitiligo lesions over acral areas and joints. Of the 200 treated lesions, 123 had regained > 75% repigmentation and 55 had regained 50-75% repigmentation. The remaining 22 lesions, which were all on the distal fingers or toes, had a poor response. Autologous cultured epidermal grafts transplantation, as a useful therapy for acral vitiligo, could produce some degree of repigmentation in our patients.

1250
Development of an ex vivo human skin explant model to examine candidate gene functions in the hair follicle and epidermis
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We have developed a novel in vitro research platform that combines a human hair-bearing skin explant (that retains the normal cellular organization interactions of the skin) with immunostaining of transverse human frozen skin sections of the hair follicle (HF) and interfollicular epidermis (IE). Fresh skin obtained from abdominoplasty from 3 healthy individuals was placed in explant standard (control) media or in media containing 12-0-tetradecanoylphorbol-13-acetate (TPA) (48 h), a compound with attributed pro-proliferative effects on melanocytes (MCs). Biopsies were evaluated by immunostaining using fluorescent markers to identify Pmel(+) MC and K16(+) proliferative cells that were quantified by microscopy and calculated. We show that skin explants incubated with standard media for 7 days reproduced the features of control skin (that was collected and frozen on day (D)0 and was not incubated with media); these reproduced features were preserved architecture and similar expression of Pmel and of its proliferative and non-proliferative phenotypes, in both HF’s and IE. On the D7-treated explants, TPA treatment was associated with remarkably increased number of Pmel(+) cells (p=0.02), and of Pmel non-proliferative phenotype (p=0.06). We observed slight increased number of Pmel proliferative cells (p=0.002) in contrast to the to D7 explants incubated in control media. The above phenotypes had a similar expression trend in the upper HF infundibulum, but no treatment changes have been observed on the overall-depth analysis of HF’s (p=0.05 for all 3 phenotypes). In summary, TPA treatment impacted the proliferation of mature MCs in the IE, but not the proliferation of the HF MC precursors. Our results suggest that ex vivo explants represent a valuable platform to reproduce the pharmacologic response of the in vivo human skin and will be a useful tool for testing candidate gene functions. Our goal is to study the effects of agonists on pathways activated in the HF and IE of vitiligo skin.

1251
Janus kinase inhibitor tofacitinib does not facilitate the repigmentation in mice model of rhododendrol-induced leukoderma
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Tofacitinib is an oral Janus kinase (JAK) inhibitor that is suggested to be effective for various skin diseases including psoriasis, atopic dermatitis and vitiligo. In vitiligo, IFN-gamma-mediated JAK/STAT1/3 pathway is involved in melanocyte loss and melanocyte hypopigmentation. To test this hypothesis, we exposed a C57BL/6J mouse with 4% rhododendrol (RH) on shaved dorsal skin. The control group was treated with an equal volume of vehicle. Mice were sacrificed 4 weeks after the first treatment. We observed that the lesion area of tofacitinib group was similar to vehicle group, whereas the epidermal thickness and dermal collagen thickness were significantly increased in tofacitinib group. Tofacitinib significantly reduced the expression of Th17 cytokines (IL-17A and IL-23) and IFN-gamma in the lesions. In conclusion, tofacitinib does not facilitate the repigmentation in mice with RH induced leukoderma.

1252
Measurement of skin pigmentation using a chromometer in a 3-dimensional epidermal model containing functional melanocytes
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Considerable interest exists in evaluating effects on skin pigmentation resulting from treatment with raw materials, skin care formulations, or as a side effect induced by medication. A convenient way to screen such effects utilizes the MelanoDerm tissue model, a highly differentiated, three-dimensional tissue culture model of human epidermis containing normal human melanocytes and keratinocytes. Use of this in vitro model can provide valuable information in early screening tool prior to the commencement of costly clinical trials. In this study, pigmentation was evaluated over the course of 2-3 weeks using a tristimulus chromometer to measure brightness (L*) in MelanoDerm tissue produced by normal human melanocytes from Black, Asian, or Caucasian donors. In parallel to measurements taken with the chromometer, total melanin content of tissues was also quantified. Over time, cultures became increasingly pigmented with retention of normal epithelial morphology with the expected pigmentation level of the donor tissue, i.e. Black>Asian>Caucasian when cultured in media containing alpha-MSH and beta-FGF. Several over-the-counter skin lightening products were also evaluated in cultures containing normal human melanocytes from Black donors. Over the 2-3 week treatment period, control cultures became increasingly pigmented while tissues treated topically with cosmetic skin lightening agents containing tiosine derivatives were significantly lower when compared to control cultures. After 14 days in culture, total melanin content was found to inversely correlate with surface reflectance (L*). The results described herein suggest that this model is useful for evaluating skin pigmentation changes in epidermal and other pigmentation phenomena of skin in vitro. In particular, this study highlights two distinct endpoints, total melanin content and skin color measurement that can be used to evaluate skin pigmentation in vitro.
**1253**

**Distribution of acral melanocytic nevi and acral melanomas on the plantar foot.**

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The pathogenesis of acral melanoma (AM) is poorly understood. The precursor lesions of cutaneous melanoma (CM) have been identified; however, the precursor lesions of AM remain elusive. Prior studies found that nevus burden correlates with CM but does not correlate with AM. This study aims to investigate the distribution of acral melanocytic nevi (AMN) and its correlation with the distribution of AM on the plantar foot. A search of the pathology records was performed at Mayo Clinic. A chart review was conducted to obtain pathology images, personal histories, and demographics for each patient. Patients were required to have all components for inclusion. We defined locations by exact location which was split into weight bearing and non-weight bearing regions. A total of 122 AM and 137 AMN were included in the study. The study population for AM and AMN was Caucasian (96.3% & 83.2% respectively), Asian (0.9% & 6.9%), African American (0.9% & 0.8%), African American (0.9% & 0.8%) and Hispanic (0.9% & 2.3%). The non-weight bearing surface represented 18.6% of the total plantar surface area and contained 51.28% of the AMN. The weight-bearing surface area contained 81.4% of the AMN. Combined with the preclinical data, these clinical results indicate that RXI-231 may impact skin pigmentation induced by UV exposure.

**1254**

**DNA methylation in malar melasma and its improvement by sunscreen, retinoid acid and niacinamide.**

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Malar melasma has a chronic and recurrent feature that may be related with epigenetic adjuvants. To recognize the DNA methylation status of the malar melanin and perilesional skin, and its change after topical application of 50 SPF sunscreen (S), 4% niacinamide (N), or 0.025% retinoid acid (RA). Fifty-six lesions of 28 female patients without treatment were clinically evaluated, as also the expression of DNA methyltransferases in melanin lesions. Environment factors such as sun radiation may induce DNA hypermethylation triggering hyperpigmentation through the activation of pathways regulated by epigenetic modifications. Thus, decreasing methylation by sunscreen protection and the genetic transcription modulation through N and RA, may allow their clinical improvement regardless its depigmenting effect.
1259
Effect of selective Gq/11-inhibition on malignant melanoma
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G-protein-coupled receptors (GPCRs) comprise a large family of cell-surface receptors that transduce signals in interaction with heterotrimeric G-proteins (α, βγ, and G). The aberrant expression, overexpression or signal reprogramming of GPCRs and G-proteins have been linked to cancer initiation, tumor cell growth, metastasis and angiogenesis. Frequent somatic mutations of Gnaq and Gna11 were found in uveal melanomas of humans thereby identifying these G-proteins as potential oncogenes in human neoplasia. As a future perspective the inhibition of wild-type and/or mutated GqGPCRs may represent an effective molecular intervention to target oncogenic signaling. Currently there are only two known selective Gq11-inhibitors, FR-900359 and YM-254890, which are of potential interest to target oncogenic signaling. Here we analyzed the role of GPCR-Gq signaling in primary and transplanted Hgf-Cdk4K435A mouse melanomas using FR-900359 in vitro and in vivo. We also examined the effect of FR-900359 and YM-254890 on human uveal melanoma cells carrying wild-type or mutated Gnaq genes. We found that oncogenic mutations in Gnaq11 appear to be selected in primary Hgf-Cdk4 mouse melanomas. All transplantable Hgf-Cdk4 mouse melanomas including Hcmel12 and Hcmel3 carried oncogenic mutations in Gna11 genes. FR-900359 inhibited the proliferation of the Gna11Q294L–mutated Hcmel12 mouse melanoma cell line and abrogated ERK activation. FR-900359 and YM-254890 both reduced the proliferation and ERK activation of Gnaq–mutated uveal melanoma cells but had no effect on Gnaq wild-type human melanoma cells. Future studies will have to address how exactly Gαq-coupled receptors transduce proliferative signals in melanoma cells and how these pathways contribute to growth and migration of tumor cells.

1261
Transcriptome profiling of lentigos identifies potential therapeutic targets
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We have previously demonstrated that human lentigo maligna melanoma (LMM) displays a distinct transcriptome compared to nevus. Frequent somatic mutations of Gnaq and Gna11 were found in uveal melanomas of humans thereby identifying these G-proteins as potential oncogenes in human neoplasia. As a future perspective the inhibition of wild-type and/or mutated GαqGPCRs may represent an effective molecular intervention to target oncogenic signaling. Currently there are only two known selective Gq11-inhibitors, FR-900359 and YM-254890, which are of potential interest to target oncogenic signaling. Here we analyzed the role of GPCR-Gq signaling in primary and transplanted Hgf-Cdk4K435A mouse melanomas using FR-900359 in vitro and in vivo. We also examined the effect of FR-900359 and YM-254890 on human uveal melanoma cells carrying wild-type or mutated Gnaq genes. We found that oncogenic mutations in Gnaq11 appear to be selected in primary Hgf-Cdk4 mouse melanomas. All transplantable Hgf-Cdk4 mouse melanomas including Hcmel12 and Hcmel3 carried oncogenic mutations in Gna11 genes. FR-900359 inhibited the proliferation of the Gna11Q294L–mutated Hcmel12 mouse melanoma cell line and abrogated ERK activation. FR-900359 and YM-254890 both reduced the proliferation and ERK activation of Gnaq–mutated uveal melanoma cells but had no effect on Gnaq wild-type human melanoma cells. Future studies will have to address how exactly Gαq-coupled receptors transduce proliferative signals in melanoma cells and how these pathways contribute to growth and migration of tumor cells.

1262
CDK1 phosphorolysates Sox2 and enhances tumor initiation and stemness in human melanoma
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CDK1 phosphorylates Sox2 and enhances tumor initiation and stemness in human melanoma

1263
Involvement of SIRT6 deacetylase in autophagy regulation in melanoma
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Recent work has shown that SIRT6 deacetylase is involved in the regulation of autophagy. The involvement of SIRT6 in autophagy regulation is well documented. However, the role of SIRT6 in melanoma autophagy has not been studied. We aimed to investigate the role of SIRT6 in melanoma autophagy. SIRT6 was consistently upregulated in this subpopulation of cells. MHC class I upregulation in proliferative advantage is abrogated. Additionally, we find that the altered actin polymerization. This tumor heterogeneity is a hallmark of cancer and drives disease progression. However, the genes associated with highly tumorigenic subpopulations and their mechanism or regulation remain elusive. We analyzed patient-derived xenografts from melanoma, colon and pancreatic cancer tissues to isolate subpopulations with increased tumor initiating potential and identify their underlying gene signature. We found a distinct subpopulation of cancer cells with high MHC class I expression, which had a high tumor initiating capacity. Interestingly, cyclin-dependent kinase 1 (CDK1), a master regulator of the cell cycle, was consistently upregulated in this subpopulation of cells. CDK1 phosphorylates Sox2 and enhances tumor initiation and stemness in human melanoma.

1264
Rac1P29S coordinates the actin cytoskeleton to mediate a mechanosensitive proliferative advantage in melanoma
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Despite the prevalence of dysregulation of the Rho family of GTPases found in melanoma, as well as the role of Rac1 in melanoma development and progression. Recent, we demonstrated that Rac1P29S is overexpressed in melanomas, and its knockdown resulted in strong anti-proliferative effects in human melanoma cells. In addition, studies have implicated SIRT6 in autophagy, a process used by cells as a response to stress and in recycling cellular components. In melanoma, autophagy is thought to play a dual role depending on the stage of the cancer, with either anti-tumor or pro-tumor signaling. Indeed, upon inhibition of actin polymerization we find that Rac1P29S-mediated proliferative advantage is abrogated. Additionally, we find that the altered actin polymerization in Rac1P29S cells promotes proliferative signaling through cyclin D1 upregulation and functions, including cell transformation, tumor invasion and movement of tumor cells. Modulation of the autophagy markers Becn1, Sqstm1, Atg3, Atg7, Atg10 and Gaa as well as the key autophagy protein LC3 were validated at protein and/or mRNA levels. Our findings reveal a novel role for CDK1 in regulating tumor initiating capacity in melanoma and suggest a strategy in cancer treatment by inhibiting CDK1’s function or its protein-protein interaction.
Primary female genitourinary melanoma: A systematic review
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Quantitative modeling to predict margin involvement for melanoma in situ excisions
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Prevalence of melanoma-in-situ (MIS) continues to increase worldwide. Current treatment is aimed at preventing recurrence and progression to invasive disease through surgical extirpation with wide local excision. Critical to this process is the histopathologic evaluation of excision margins to examine for subclinical spread. An accurate and objective method for histopathologic evaluation of excision margins would contribute to identifying high risk excisions and decrease recurrence rates of MIS. Towards that end, we have developed a novel quantitative method for evaluating melanoma excision margins. Using specific microscopic margin measurements from excision specimens, we use statistical inference to score the likelihood of an unobserved positive margin within the gap between bread-loaf sections. We test this method by retrospectively calculating scores for three MIS cases from our institution that recurred and comparing those scores with five MIS cases that did not recur after at least 5 years follow up. The average score for MIS excisions that recurred was 32.1 compared to a score of 6.1 for MIS that did not recur (p<0.02). In this pilot study, our quantitative score distinguished MIS cases that recurred from those that did not.

The role of planar cell polarity gene FZD6 in melanoma progression
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Melanoma is the malignant tumor of melanin-producing melanocytes in the skin. It is the leading cause of skin cancer deaths. Despite recent progress in melanoma therapeutics, the prognosis remains very poor due to the low response rate to drugs and the rapid distant metastasis in patients. A broader understanding of the biology of melanoma, especially how tumor cells metastasize, is needed to develop effective treatments for this devastating disease. The planar cell polarity (PCP) pathway controls tissue polarity during development by regulating the directional movement of cells and coordinating neighboring cells to the body/tissue axes. Increasing evidence suggests that it also plays active roles in cancer by promoting tumor cell migration and invasion. Although limited information is available regarding PCP pathway in certain cancers, its involvement in melanoma development and progression has not been studied. In our preliminary studies, we found that Fzrizzled6 (FZD6), one of the core PCP genes, is overexpressed in multiple human melanoma cell lines. SK-MEL28 has the highest level (over 90-fold compared to normal adult human epidermal melanocytes). To determine the role of FZD6 in melanoma cells, we made FZD6 knockout SK-MEL28 melanoma cell lines using CRISPR/Cas9. We found that knockout of FZD6 significantly reduces the migration ability of melanoma cells (using in vitro wound healing assays), while tumor cell proliferation is not affected. Current studies are underway to determine the role of FZD6 in melanoma metastasis using genetically engineered mouse models. We are also investigating the downstream mechanisms of FZD6 using both candidate approaches and non-biased transcriptome analysis. Our study will not only provide insights into the role of other PCP genes in melanoma. The information generated from these studies will substantially broaden our knowledge on the biology of melanoma and might lead to novel therapeutic approaches for melanoma.
1268
Necroptosis is a novel way of melanocyte death in oxidative-related vitiligo pathogenesis.

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Necroptosis is a type of programmed cell death that occurs when RIP1 and RIP3 are activated in cells. It is a pro-inflammatory form of cell death that is involved in various diseases. In this study, we investigated the role of necroptosis in oxidative-related vitiligo pathogenesis. We found that necroptosis is a key mechanism in the death of melanocytes in patients with vitiligo. Our findings suggest that targeting necroptosis could be a potential approach for vitiligo treatment.

1270
Fibroproliferative genes are preferentially expressed in central centrifugal cicatricial alopecia.

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Central centrifugal cicatricial alopecia (CCCA), a disease characterized by hair loss and scarring, affects black women. In this study, we identified that fibroproliferative genes are upregulated in CCCA tissue compared to control tissue. These genes are involved in fibrosis and inflammation, contributing to the development of scarring. Our findings highlight the importance of fibroproliferative genes in the pathogenesis of CCCA.

1271
The expression of phosphatidylinositol glycan, class K gene (PIGG) correlates with tyrosinase activity in human melanocytes.

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Phosphatidylinositol glycan (PIG) plays a role in melanocyte development and function. In this study, we found that the expression of the PIGK gene, which encodes phosphatidylinositol glycan, class K, is correlated with tyrosinase activity in human melanocytes. This finding suggests a potential role for PIGK in melanocyte function and could provide new insights into melanocyte biology.

1272
Differences in skin aging characteristics in women of East Asian versus European descent residing in the same geographic location.

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Skin aging is a complex process influenced by numerous factors. In this study, we compared skin aging characteristics between women of East Asian and European descent residing in the same geographic location. We found significant differences in skin aging parameters, such as fine rhytides and coarse rhytides, between the two groups. These findings highlight the importance of considering demographic differences in skin aging research.
The effect of drinking and sleep deprivation on the menopause
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Recently, men are increasingly interested in skin and beauty. However, men are exposed to various stress factors, such as smoking, alcohol, and stress. In addition, there is a report that Korean men drink more and sleep less than recommended level. Therefore, we investigated the changes of men skin after stress factors of drinking alcohol and sleep deprivation in this study. The healthy males (30-36 years, n=20) who often drink alcohol and lack of sleep participated in a study. They visited the clinical research center twice and the skin biophysical parameters including skin color, hydration, transdermal water loss (TEWL), facial pore size, skin roughness and blood flow on facial areas were measured. On the first day, they visited the morning after good nights sleep. On the second day, they visited at night, and drank 360ml of alcoholic beverage with 17.5% of alcohol content for 1 hour and they were kept awake until next morning. All protocols were approved by the Institutional Review Board (IRB, P1611-82), and all participants provided informed consent before the study. After the study, we observed that some male skin biophysical parameters changed when compared to the baseline measurements. The lightness on the cheeks and under the eyes were significantly decreased (p < 0.05), and the unevenness of skin color, skin roughness and blood flow were significantly increased (p < 0.05). Also, papule or pustule was found or worsened in some subjects. The skin hydration and TEWL did not show significant differences. Taken together, drinking alcohol and sleep deprivation could skin-stressing factors and it practically came out to the changes of skin tone and texture during one night.
1280
The role of surgery in hidradenitsis suppurativa management

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The aim of this study was to investigate surgical complications and localized recurrence rates of patients presenting with an excision of HS lesions. Duke Enterprise Data Unified Content Explorer (DEDUCE) was queried for all patients who underwent a first-time excision of HS lesion(s) from 2006-2016. Data regarding HS medications, surgical procedures, surgical complications, and localized recurrence rates were collected and presented below. McNemar test will be used to compare the correlated recurrence rates between anatomic locations. From 2006-2016, 128 patients underwent surgical excision of HS lesions at a median age of 36.4 years (range 15.1-72.5 years). Forty-one patients (32.0%) underwent excisions of multiple locations simultaneously. Sites included the axilla, inguinal region, perianal region, vulva, mons pubis, scrotum, pannus, and inframammary region. Of 9 HS locations reviewed, the most commonly excised lesions included 100 in the axilla, 40 inguinal excisions, and 19 perianal. Surgical closure techniques included 87 primary closures (67.0%), 28 secondary closures (21.9%), grafts (10.5%), and flaps (2.3%). A meta-analysis of this data showed that excisions of HS lesions are associated with long-term remission for the majority of patients. Given the recurring and chronic nature of the disease, early surgical intervention should be considered in patients with significant HS-associated morbidity.

1281
Ex-vivo evaluation of cytotoxicity and melanocyte viability of Fitzpatrick V skin after A101 hydrogen peroxide topical solution 40% or cryosurgery treatment

S Kim3 and E Kim4

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Development of effective therapeutics for this particularly in need population. Our objective was to informing AD targeted therapeutic development are largely based on studies in European patient populations. Atopy, characterized by AD, is more prevalent among African Americans than among non-African American populations, but the cost and race-oriented treatment guidelines. The prevalence of AD among African Americans is much higher than that of non-African Americans. Despite the unprecedented increase in AD prevalence among African Americans, the literature contains only a few reports on African American AD patients.

1282
Analysis of skin color parameters of facial image for perceived ages in Korean female

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The aim of this study was to investigate the factors affecting the perceived age focusing on skin color, and to find out the clues for youthful appearance skin color. Total 160 Korean female females (45.11 14.61 y.o.) participated in this study. Digital facial images were obtained using VISIA-CR and the color data (dRCB) of cheek were calculated using Image-pro plus. These skin color parameters of facial image were correlated with SKT scores of the skin, wrinkle and lips volume. However, they didn’t investigate perceived age focusing on skin color. The median time to recurrence was 1.1 year (range 0.1-7.4 years). Despite each recognizing perioperative antibiotics, 7 patients (5.5%) experienced a postoperative infection, all of which were successfully managed with additional antibiotics. Other operative complications included hematoma/seroma formation in 2 patients (1.6%) and wound dehiscence in 11 patients (8.8%). The total number of HS lesions is associated with a higher recurrence, and produces long-term remission for the majority of patients. Given the recurrent and chronic nature of the disease, early surgical intervention should be considered in patients with significant HS-associated morbidity.

1283
Somatic GNAQ mutation in different structures of Port-wine Macrocheilia

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Background: The etiology of Port-wine macrocheilia (PWM), which involves a congenital, progressive, capillary malformation that results in soft tissue hypertrophy in the lips, has yet not been fully elucidated. Objective: We sought to investigate frequencies of GNAQ mutation in different tissues from patients with PWM, including skin, mucosa, gland and muscle, using samples obtained during the excision surgery. Targeted next generation sequencing (NGS) of GNAQ was designed and performed to assess DNA extracted from 80 different affected tissues from 20 patients with PWM. Results: The GNAQ (R183Q) mutation was not detected in gland samples but was found in 90%, 95% and 99% of the skin, mucosa and muscle samples, respectively. The mutation in gland was the lowest (P < 0.0001), VS skin, mucosa and muscle respectively and the skin was the second lowest (P < 0.0262) Vs Mucosa and V=0.0116 Vs Muscle. The mutation frequency in mucosa and muscle was the highest and analogous (P=0.9179, without statistical significance). Conclusions: In PWM, GNAQ was mutated in all tissues except for glands. PWM is congenital, and all tissue layers exhibit invasive hypertrophy rather than acquired or partially related hypertrophy. Given the advantages of mucosal biopsy, including practicality, lack of scarring and rapid healing, NGS mutation in the lip mucosa may be a useful predictor for early-stage PWM in patients with PWM.

1284
Keloid fibroblasts can tolerate starvation conditions by activation of the IGF1 signaling pathway

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Keloid disease is a form of skin fibrosis where an excessive amount of collagen and extracellular matrix are deposited in a disorganized manner in a raised scar. Genetic predisposition has been suggested, with higher incidence among skin of color individuals. Current keloid treatments are non-specific and ineffective. To provide a targeted treatment, an improved understanding of keloid pathophysiology is required. Using keloid-derived fibroblasts, we uncovered a greater ability to survive under starvation conditions. Typically, serum withdrawal induces cell death in normal fibroblasts. In contrast, keloid fibroblasts can withstand starvation over a few days. In the absence of serum, we reasoned that keloid fibroblasts, could likely generate its own growth factors. We examined a number of candidate growth factors and found enhanced activity of the IGF1 signaling pathway. IGF1 and its receptor, the IGFR1, utilize the PI3K signaling pathway in mediating its signal. We found that inhibition of PI3K signaling was as compared to N-Myc in A375 cells and Ewing sarcoma, respectively. Low-PNGF receptor (PDGFR-α), effectively blocked keloid fibroblast responses such as migration, proliferation, cell growth and tolerance to starvation. Through GPCR C1 reaction, activated docking, keloid fibroblasts can utilize alternative nutrients and recycle cellular components for the generation of proteins, lipids and carbohydrates to provide biomass for cell growth. These two nodes of the pathway are sufficient to provide the keloid fibroblast with an ability to continue to bypass starvation conditions. We are currently examining patient keloid samples for the same purpose. The results from this pathway may lead to the development of treatments for keloid disease. When keloid fibroblasts can be validated, it will be possible to use available drugs to develop a keloid therapy.

1285
Atopic dermatitis in African American patients is T1/T2/T22-driven with T17 attenuation and downregulation of loricrin

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African Americans (AA) are disproportionately impacted by atopic dermatitis (AD) with a prevalence of 12% and a reportedly “treatment-resistant” phenotype. Molecular profiling data inform AD targeted therapeutic development are largely based on studies in European AD patients. Such studies are lacking in skin of color patients, hindering development of effective therapeutics for this particularly in need population. Our objective was to elucidate the molecular profile of AD in lesional and non-lesional biopsies from AA patients (n=15) and matched controls (n=9), compared with EA patients and controls. While AA AD showed increased T1 expression (FC=1.7, p<0.05) compared with EA AD, both groups showed robust upregulation of T2 (IL-4, IL-13, CCL7, TGF-β (IL-9) and T22 (IL-22, SI10A09/12 markers) (p<0.05). T17 cytokines (IL-17E, IL-2p19, IL-36G) were increased in both T1-T22 and T17 groups, while T22 markers (IFN-γ, IL12/22) were upregulated in T17 (p<0.05). Congruent with these data, T17 markers (IL-17A, IL-17F) correlated with clinical severity/SCORAD in EA only (p<0.05), while T22 markers (IL-22, IL-12p70, IL-18) were significantly correlated with SCORAD. The terminal differentiation marker loricrin was significantly downregulated and negatively correlated with SCORAD in AA only (p<0.05), while filaggrin was more significantly downregulated in EA AD. Overall, AD in AA patients present with a more pronounced M2-like phenotype in comparison to EA, with T1/T2/T22 skewing to EA AD. These data encourage a personalized medicine approach with consideration of phenotype-based differences in future trials of AD targeted therapies.
1286

Stem cell factor dictates the distribution of melanogenic melanocytes in the skin

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Stem cell factor (SCF) is developmentally critical for melanocyte migration, differentiation, and survival. Recently, SCF expression in hair shaft progenitor cells has been shown to be absolutely required for the pigmentation in hair shafts, as evidenced by the complete loss of hair pigmentation in mice without SCF in these epithelial cells. Interestingly, this fully dissection of hair pigmentation only targets melanogenic mature monocytes in the upper hair bulb, but not melanocyte precursors below. This shows an important role of SCF in the determination of mature melanocytes to their destination. Here we report that the skin pigmentation appears to follow the same mechanism for the distribution of dermal melanocytes. We employed multiple cell-type specific SCF knockout mice to characterize the sources and contribution of SCF to dermal pigmentation. Our results show that dermal melanocytes are also sustained by SCF. However, in contrast to hair pigmentation, dermal melanocytes appear to be supported by SCF expression in a selective population of dermal cells. This study reveals a faithful distribution of melanogenic melanocytes in distinct skin compartments guided by selective populations of SCF expressing cells. Our findings suggest that manipulation of SCF expression in the skin cells might be a potential target to manage hyper- or hypo-pigmented skin manifestations in clinic.

1287

Enhanced susceptibility to UVR induced damage in skin melanocytes from hispanic donors

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Despite advances in melanoma treatment over the last decade, targeted drugs have been used to treat many critical, oncogenic, skin cancer genes among the US Hispanic population to provide a framework for further study. Thus, we have developed skin of color relevant cell-based models to study melanocyte behavior as a first step toward defining differential responses to ultraviolet radiation (UVR) that is a risk factor for melanoma. In our study, primary skin melanocytes derived from ethnically distinct donors were challenged with UVR and cellular responses were observed over the course of one week. Although each cultured melanocyte line behaved in a similar manner in terms of normal proliferation, the generation of dendrites and the induction of melanin synthesis upon UVR stimulation, the number of viable UVR-exposed cells was always significantly lower only for Hispanic lines. Curiously, among Hispanic melanocytes, UVR induced a higher proportion of melanocytes with abnormal nuclei despite the production of high levels of melanin. Thus, we have identified a Hispanic donor-derived melanocyte-specific predisposition to UVR exposure that appears to be uncorrelated to melanin content and warrants further investigation. With assistance from the dermatological research community, we hope to help define the underlying cause for aggressive melanoma among Hispanic individuals to provide better advice and cancer care in the near future.

1288

Dermatosis papulosa nigra: A quality of life study survey

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BACKGROUND: Dermatosis papulosa nigra (DPN) is a skin condition characterized by pigmented papules on the body that vary in size (1-5mm). It is described by some clinicians as a variant of seborrhic keratosis. There appears to be a genetic component to the condition. DPN has the highest prevalence in people of African descent with a reported range of 10-30%. In this study, we evaluate the effect of DPN on quality of life. METHODS: A 89-item questionnaire was administered to 32 African American (AA) adults with visible DPN lesions at a dermatology clinic. We elicited information about the subjects race, income level, type(s), frequency, and duration of lesions; attitudes about lesions; family history; and prior treatment. RESULTS: 31 females and 1 male completed the survey. The mean (SD) age of participants was 49.6 (9.1) years. Most subjects reported lesions on face (84%); 87.5% reported lesions on more than one body part. 56% reported 15 lesions on body, 6% were diagnosed with DPN at time of the survey. The majority of patients reported little to no symptoms of itch, pain, soreness or burning from their DPN (82%). 34% sought a physician due to their DPN, and 28% had their DPN previously treated with electrocautery or cryosurgery. 84% reported having a 1st degree relative with DPN. 18% were willing to spend $50 or less to remove their DPN. 69% were willing to pay between $100 and $1000 to have them removed. 59% were concerned about potential adverse effects of DPN removal. Pain, hyperpigmentation, and fear of regrowth were the most commonly reported concerns that subjects had about considering DPN removal. 100% of subjects that had their DPN lesions reported improvement after treatment. CONCLUSION: Our study results strongly support the idea that there may be hereditary factors in the development of DPN in AA's. Overall, most subjects did not report any symptoms of DPN, and most subjects did not report a diminished quality of life as a result of DPN; despite this, almost 30 percent of subjects have sought treatment to remove their lesions.

1289

CD26+ FAP+ fibroblasts increase ECM expression in keloid scarring

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Keloids are fibrotic scars characterised by excessive fibroblast proliferation, extracellular matrix (ECM) deposition and growth beyond the original wound site. Fibroblasts are thought to be the main cell type responsible for keloid formation. However, dermal fibroblasts are a heterogeneous population of cells and to date, the distinct fibroblast subsets involved in keloid formation remain to be determined. Thus, our aim was to characterise the different stromal cell populations in healthy human dermis and keloid scars. We were particularly interested in CD26 as a marker of fibroblasts with intrinsic fibrogenic potential, given fibroblasts expressing CD26 are responsible for ECM deposition during wound healing in mice. We isolated keloid fibroblasts from keloid scars and healthy dermis and identified a fibroblast population directly ex vivo from healthy human dermis and keloid scars. We identified a distinctive CD26+ FAP+ CD90+ fibroblast population in keloid scars and in healthy human dermis. Multicolour immunofluorescence microscopy revealed that these CD26+ FAP+ CD90+ cells form the bulk of the keloid mass. After sorting these uncultured cells directly from healthy skin and keloid scars we analysed their transcriptomes by RNA sequencing. CD26+ FAP+ CD90+ cells in keloid scars had much higher expression of ECM genes than those cells from healthy dermis. We conclude that a distinctive CD26+ FAP+ CD90+ population of fibroblasts found in both healthy human dermis and keloid scars is likely to be the major source of ECM deposition in keloid scarring. Purification and molecular characterisation of these fibrogenic cells directly from keloid scars is likely to suggest novel strategies to treat keloid scarring.

1290

Despite abnormal vitamin D receptor expression in keloid scars, keloid keratinocytes can respond to vitamin D treatment in vitro

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Keloids are fibroproliferative scars that are more prevalent in populations with darkly pigmented skin, such as African Americans. The basis for increased keloid susceptibility in skin of color is not known. Vitamin D is produced in skin in response to UV light, and rates of vitamin D deficiency are higher in populations with darker skin. Vitamin D, through binding of the vitamin D receptor (VDR), regulates calcium homeostasis, cell proliferation, differentiation, inflammation, and fibrosis. Hypothetically, the link between pigmentation and keloid formation may involve vitamin D signaling. Because ligand-bound VDR acts as a transcription factor, ligand-dependent target gene regulation requires nuclear localization. VDR expression and nuclear localization were analyzed by flow cytometry in keloid and normal human skin biopsies. Multicolour immunofluorescence microscopy revealed that these CD26+ FAP+ CD90+ cells from healthy human dermis and keloid scars. We identified a distinctive CD26+ FAP+ CD90+ fibroblast population in keloid scars and in healthy human dermis. Multicolour immunofluorescence microscopy revealed that these CD26+ FAP+ CD90+ cells form the bulk of the keloid mass. After sorting these uncultured cells directly from healthy skin and keloid scars we analysed their transcriptomes by RNA sequencing. CD26+ FAP+ CD90+ cells in keloid scars had much higher expression of ECM genes than those cells from healthy dermis. We conclude that a distinctive CD26+ FAP+ CD90+ population of fibroblasts found in both healthy human dermis and keloid scars is likely to be the major source of ECM deposition in keloid scarring. Purification and molecular characterisation of these fibrogenic cells directly from keloid scars is likely to suggest novel strategies to treat keloid scarring.

1291

PPARγ signaling is downregulated in keloids

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Keloids are exuberant scars in which overgrowth of fibrous tissue extend beyond the borders of the original skin injury. Traumatic insults that can lead to keloids include surgery, piercings, acne, insect bites, and even mild abrasions. These lesions do not regress, and can cause pain, pruritus and psychosocial effects when they appear. Pathway analysis of RNA-seq data obtained from comparing RNA expression between matched keloid and normal tissue suggests that the PPARγ signaling pathway may be dysregulated in keloids. To investigate this possibility, matched keloid and normal tissue obtained from unrelated patients were used to compare levels of mRNA expression using real-time PCR. FN1, ADAMTS1, and POSTN – all known PPARγ target genes – were found to be dysregulated in expression between keloid tissue and normal skin. Immunohistochemistry and tissue culture experiments are currently being done to determine the location and intensity of the dysregulation of these genes and whether this dysregulation is PPARγ signaling-dependent. As this pathway can inhibit TGF signaling, which plays a critical role in fibrosis, further elucidation of this mechanism may lead to developing new forms of treatment.
1292 Comparative study of the immunological profile in stable segmental and non-segmental vitiligo patients undergoing melanocyte keratinocyte transplantation

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We established a tight-skinned porcine wound model to explore the molecular mechanism of skin pigmentation, has genetic variants with outcrosses, and the genetic structure has been studied by targeted sequencing for 22 atopic dermatitis patients. Our hypothesis is melanocytes are inefficient at producing and transferring melanosomes to keratinocytes. On the other hand, significant differences in skin pigmentation were observed in keratinocytes. In the future, we will characterize melanocyte LOF pathogenic variants. Further research is necessary to better further characterize these differences.

1293 Challenges in clinical imaging of darker skin subjects

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Proper photography of subjects is key to good clinical study conduct, especially when the photographs are being used as the basis for image analysis. The majority of facial studies are conducted on white skin subjects. In vitiligo research, imaging systems are optimized for this population. Unfortunately, when these standard settings are used on subjects with darker skin, image quality is sacrificed and data can be difficult to extract or lost altogether. We sought to optimize the camera, filter, and flash configurations for a range of skin colors in vitiligo for use in clinical studies.

1294 Pediatric atopic dermatitis: A pilot comparative study across different races and ethnicities

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While atopic dermatitis (AD) affects all children with pruritus, a chronic, relapsing course, some clinical manifestations do vary. There is, however, a paucity of literature detailing how and what other differences across different skin types, races, and ethnicities. We assessed 1030 pediatric AD patients (<10 years of age) with active inflammatory lesions and minimal or no prior/current treatment were enrolled at two academic pediatric dermatology clinics. Clinical and family history, aggravating factors, and exercise/sweating were obtained. Levels of IL-6, TNF-α, and IL-10 were measured by an enzyme-linked immunosorbent assay (ELISA). 110 subjects (median age 1.3 years) were enrolled: 31% Hispanic White (H), 20% Non-Hispanic White (W), 19% Asian/Pacific Islander (A), 12% Black (B), and 18% mixed race (M). Prevalence of AD was highest in B and W, followed by M. In B, exercise/sweat was the most common aggravating factor (78%). Stress/emotional triggers were observed in A patients. We also report lower IL-6 and TNF-α levels in B patients. These findings were confirmed by logistic regression analyses. In summary, our findings for 2 not previously reported FLG loss-of-function (LOF) mutations, we performed additional FLG targeted sequencing for 22 atopic dermatitis pediatric patients that reported either African or Hispanic ancestry. S3640X was found in two additional AD patients that self-report African ancestry. Close interaction of family backgrounds for the S1640X AD patients revealed that two out of the 3 patients reported Native American ancestry. We also report a newly discovered FLG LOF, R788X, in a Hispanic AD patient. Together, the findings suggest the emergence of new atopic dermatitis risk markers that correlate with these ancestors. Further research is necessary to better further characterize these differences.
1298
External light activates hair follicle stem cells through eyes via the ipRGC-SCN-sympathetic neural circuit.

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Changes in external light patterns can alter stem cell activities in peripheral tissues and result in both daily and seasonal cycles of tissue regeneration. Daily oscillation of cellular activities requires hierarchical and slow entrainment of cell-autonomous clocks by the central clock in the suprachiasmatic nucleus (SCN). It remains unclear whether stem cells in otherwise photosensitive tissues can bypass circadian clock to achieve rapid responses to changes in external light. Here we show that light stimulation of animals eyes results in rapid activation of hair follicle stem cells to initiate a new anagen. The light signals are interpreted by intrinsically photosensitiven retinal ganglion cells (ipRGCs) in the retina and these cells signal via the phototransduction molecule melanopsin through optic nerves to SCN. Subsequently, photic signals are transmitted to the SCN-sympathetic neural circuit to activate hair follicle stem cells. The activation of sympathetic nerves is not limited to skin only, this circuit may also facilitate rapid adaptive responses to external light in other tissues.

1300
Blood plasma levels of heart disease biomarker cardiac troponin I are significantly increased in alopecia areata affected individuals

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Epidemiological studies suggest the development of androgenetic alopecia (AGA) is associated with increased risk of developing cardiovascular diseases (CVDs). The risk of CVDs in AGA subjects is two times higher than the general population. Hence, we hypothesized that: 1) the chicken egg contains a key hair growth factor, and 2) this key hair growth factor is sufficient in AA affected subjects. To investigate the potential presence of pro-apoptotic factors in the plasma of hair loss subjects, we measured the plasma level of cardiac troponin I (cTnl) in AA affected subjects and compared the results with healthy controls. We found that the levels of cTnl were significantly higher in AA affected subjects and subjects with no hair loss (p<0.05). The highest levels of cTnl were observed in AA subjects without hair loss (p<0.05). Samples of AA plasma with high cTnl levels also induced significantly higher rates of cardiomyocyte apoptosis in cell culture assays. The results suggest increased heart remodelling may occur in AA subjects, leading to the release of cTnl into blood plasma. Close monitoring of cardiovascular health in AA subjects, as well as subsets of AGA patients, may be appropriate.

1302
Disturbed sebum and microbiome composition in sensitive scalp

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Sensitive scalp, one of the most frequent complaints among sensitive skin syndrome, has been associated with their SC barrier function. However, it remains elusive how and when these keratin filament networks change during cornification in vivo. The purpose of this study is to visualize changes of keratin networks during the SG1-to-SC transition in living mice. To visualize keratin 1 in SG1 cells in vivo, we generated knock-in mice which specifically expressed mCherry-K1 in SG1 cells under the SAspase promoter (mCherry-K1 mice). Confocal microscopic analysis of mCherry-K1 mice demonstrated that the actin filaments in SG1 cells. Fluorescent signal of these keratin filament networks became ambiguous once differentiated into cornified epithelium. To track the change of mCherry-K1 signal in single SG1 cell, we spatiotemporally expressed in SG1 cells by using in vivo electroporation. It revealed that changes of mCherry-K1 filament networks in SG1 cells occurred prior to degradation of nuclear DNA. To examine whether SC barrier function was associated with the morphology of keratin networks in SG1 cells, next observed mCherry-K1 mice under filaggrin deficiency. mCherry-K1 filament networks of filg-/-SG1 mice were more dispersed and their fluorescent signals were fainter than those of filg+/+SG1 cells. These results showed that keratin filament network in SG1 cells dynamically changed during cornification, which is associated with their SC barrier function.

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Insights gained from a chicken’s rapid hair development during hatching lead to discovery of hair growth peptide derived from egg yolk

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Stimulation of VEGF-mediated vasculature improves hair growth in broad types of alopecia, including female pattern hair loss (FPHL), whose pathogenic mechanism remains unresolved. We noticed that hair mostly grows in a precocial bird, including the chicken, before birth. Preadipocytes, hairs are developed within one day in the middle stage of hatching. Hence, we hypothesized that: 1) the chicken egg contains a key hair growth factor, and 2) this key hair growth factor is sufficient in AA affected subjects. We prepared our water-soluble peptide by serine protease treatment of chicken egg yolk and egg white. In vitro studies using cultured human hair follicle dermal papilla cells revealed that the water-soluble peptide of egg yolk and egg white improves hair growth in cultured cells. We next orally administered the water-soluble egg yolk peptide to mice. In parallel, as a positive control, we topically applied minoxidil on murine dorsal skin. Both our egg yolk peptide and minoxidil enhances murine hair growth. Moreover, when orally-administered, our egg yolk peptide improves hair growth in FPHL. Finally, we showed that VEGF expression is increased through IGF-1 receptor activation-induced HIF-1alpha transcription pathway and that our water-soluble egg yolk peptide increased IGF-1 production. Taken together, our water-soluble egg yolk peptide improves hair growth in vivo and in vitro, and increases IGF-1 and VEGF production; and we have given the name, Hair Growth Peptide (HGPTM) to this water-soluble egg yolk peptide.
1305 Clausus serves as a model of epidermal stem cell responses to mechanical forces
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The epidermis is maintained through a fine balance between the proliferation of epidermal stem cells (SCs) and the differentiation of their progeny cells. Mechanical forces are thought to affect the fate of SCs, but the role of these relationships in diseases has not been fully elucidated. As a model of SC responses to mechanical forces, we here introduce Clausus, which is the painful skin thickening of the plantar skin from intermittent pressure. Clausus samples histopathologically show hyperkeratosis with prominent parakeratosis and flattened epidermal rete ridges. The expression of keratins 1 and 10, which are keratins in the differen-
tiated layers, is absent, while that of keratin 14, a marker of the basal layer, is present in all the epidermal layers of the clausus samples. The checkerboard pattern of keratin 6 expression seen in normal human skin is lost in the clausus samples. The number of K14+/K10− ker-
atinocytes is higher in the clausus than in the surrounding epidermis. These data suggest that, with mechanical stimuli, epidermal SCs undergo hyperproliferation accompanied by immature differentiation. Mathematical modeling of the whole epidermis harboring an epidermal SC with faster proliferation and differentiation potentials recapitulates the histo-
logical features of clausus. Our study highlights clausus as the result of an interplay between SCs and mechanical stimuli.

1307 Evaluation of the effect of a combined biofunctional on a 3D-model of dermal papilla cells and its relevance to hair density
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Hair density reflects hair beauty and is a main concern in personal appearance. This feature is controlled by specialized fibroblasts located in the mesenchymal compartment at the base of the hair follicle. Called the dermal papilla. These cells determine the hair follicle entrance in the anagen phase, as well as its maintenance, by expressing several markers that influence the keratinocytes of the matrix compartment, giving rise to the growing hair shaft. In this study, we targeted markers associated with the anagen phase and introduced a method to evaluate a combination of pea extract and hair boosters, on a 3D-model of Human Dermal Papilla Cells (HDPC) in spheroid culture. Moreover, ex vivo cultures of microdissected human hair folli-
cles were used to analyze the interaction between hair follicle and hair shaft elongation. Our results showed that, in the presence of the biofunctional, versican and noggin were increased in HDPC spheroids as well as beta-1 integrin staining in the hair follicle and hair shaft elongation. In addition, an in vivo study was performed on 40 volunteers, who received a placebo or a hair serum containing the biofunctional, over a period of 12 weeks. A significant decrease in scalp sebum was observed, as well as an improvement in scalp hydration and reduced TEWL. Moreover, after 3 month application, the biofunctional seems to be associated with an increased in the A/T ratio, with an increased number of hair in anagen phase. This study takes advantage of using 3D-cultures of HDPC to evaluate the effect of a biofunctional on hair inducitivity markers. Moreover, our in vivo study revealed a healthier scalp with less visible oil lines and more hydration, a visible maintenance of the anagen phase and visible improvement of hair density.
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Aging effects of retinoic acid in mouse skin models

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Tretinoin, also known as all-trans retinoic acid (ATRA), is well-known for its anti-aging effects on human skin. However, chemical, photochemical, and environmental insults, and concerns about toxic side effects have hindered the use of ATRA in cosmetic products. Therefore, it is desirable to find new molecules that have increased retinoic acid-like activity without the negative side effects. Hydroxypinacolone retinoate (HPR) is a new crosstalk enhancer that has been shown to have innate retinoic acid activity without causing skin irritation. Here, we compared levels of gene transcription by ATRA, retinol (ROL), retinaldehyde (RAL), and retinyl palmitate (RP) in DNA using a retinoic acid response element (RARE) reporter assay. In addition, we compared the anti-aging properties of HPR to ATRA by testing the effects on collagen levels and skin irritation in organotypic skin models. Skin models were treated for 5 days with HPR and ATRA, and basal media was collected for ELISA analysis, and skins were stained with Masson's trichrome (for collagen). RARE assay results showed that ATRA had higher levels of gene transcription than either ATRA or the vehicle control. Together these data suggest that HPR is an in vivo activator of keratinocytes in the wound epithelium, nor their migration as measured and larger wound epithelium. This however, was neither due to changes in proliferation or activation also led to the faster closure of excisional wounds through the formation of a longer and larger wound epithelium. This was observed to be functionally important for wound repair, since Nrf2 activation was functionally important for wound repair; since Nrf2 acti-

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Efficacy of topical tofacitinib in promoting hair growth in non-scarring alopecia

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Topical tofacitinib also promoted more rapid hair growth rate than topical minoxidil or vehicle once daily for 21 days. Weekly photographs were taken to determine the area and rate of hair growth, and tissue samples were collected for histopathological evaluation. mRNA and protein expression of anagen-maintaining growth factors, including vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1), were determined via RT-PCR and ELISA, respectively. Tofacitinib-treated mice exhibited more hair follicles compared to minoxidil-treated mice (P < 0.05), as well as more healthy hair follicles compared to the vehicle control (P < 0.05). Topical tofacitinib also provided rapid hair growth rate more than topical minoxidil or control (P < 0.001). Histopathology showed a distinct increase in the number of hair follicles, mostly in the anagen phase, in the tofacitinib-treated group. Hair follicles in the minoxidil- and vehicle-treated groups were more often classified as catagen and anagen. VEGF mRNA and protein expression in the tofacitinib-treated group was significantly greater than those in the other groups (P < 0.05). IGF-1 mRNA expression and protein expression were also implicated in tofacitinib-treated mice. Topical tofacitinib is effective in promoting hair growth, and the possible mechanism involves increased VEGF levels and lowered inflammation. This study will help develop a new therapeutic option for non-scarring alopecia.

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Nrf2 activation enhances the healing of cutaneous wounds through the activation of hair follicle stem cells

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The transcription factor Nrf2 is a key regulator of the cellular stress response through the regulation of antioxidant enzymes, cytoprotective proteins and various transporters. Strong genetic activation of Nrf2 in keratinocytes leads to a plosebaceous phenotype, characterized by hyperplasia of the sebaceous glands and infundibula, hyperkeratosis, and seborrhea, implicating Nrf2 in several other key processes in the epidermis. Here we show that Nrf2 activation in keratinocytes promotes the proliferation and expansion of the junctional zone (JZ) and upper isthmis (UI) hair follicle stem cells, while bulge stem cells are only mildly affected. This was observed to be functionally important for wound repair, since Nrf2 activation also led to the faster closure of excisional wounds through the formation of a longer and larger wound epithelium. This was, however, due to changes in proliferation or apoptosis of keratinocytes in the wound epithelium, or their migration as measured in vitro. Instead, an increased number and proliferation of follicular JZ and UI stem cells were observed peripheral to the wound. An enhancement of wound healing in Nrf2 transgenic mice following tape stripping of the epidermis revealed a functional link between the Nrf2-mediated expansion of hair follicles stem cells and accelerated re-epithelialization. The effect of Nrf2 activation on JZ and UI stem cells resulted from the Nrf2-mediated up-regulation of the EGF family member Epigen and subsequent EGF receptor activation. These results suggest pharmacological Nrf2 activation as a promising approach for the enhancement of wound healing through expansion of hair follicle stem cell pools.

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Fzd2 controls multiple aspects of epidermal development through distinct signaling mechanisms

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Skin ligands bind to Frizzled (Fzd) receptors to activate both β-catenin-dependent (canonical) and β-catenin-independent (non-canonical) signaling. Non-canonical signaling includes planar cell polarity (PCP) and Wnt/calcium pathways. In the skin, canonical Wnt signaling is required for hair follicle development and regenerative growth, while Wnt signaling promotes hair follicle orientation, and in vivo roles for Wnt/calcium signaling have not been described. Limited information is available regarding the functions of individual Fzd receptors in these processes. Here, using constitutive and inducible epidermal deletion mouse models, we show that the Fzd family member Fzd2 is required for normal placode formation and normal postnatal hair growth, suggesting that it mediates canonical signaling in hair follicles. In addition, we find that early deletion of Fzd2 in embryonic epidermis unexpectedly causes defective stratiﬁcation, corniﬁcation and barrier formation, a phenotype that has not been previously described. Fzd2 mutant embryos display a striking reduction in expression of the desmosomal component plakophilin 1 (PKP1). Loss of function mutations of PKP1 in humans and mice cause ectodermal dysplasia and skin fragility, phenotypes that overlap with those observed in epidermal Fzd2 mutants but do not include defective epidermal stratification. These data indicate that Fzd2 plays multiple roles in skin epithelial development and homeostasis: mediating canonical Wnt signaling in hair follicles, controlling expression of PKP1, and a novel early function in regulating epidermal stratification, corniﬁcation and barrier formation independent of PKP1.

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Advanced age impairs self-renewal and biases fate choice of hair follicle dermal stem cells

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The mammalian hair follicle (HF) regeneration cycle is uniquely dependent on the timing of activation of its resident epidermal and mesenchymal progenitor cells. In aged mice, HF regeneration is severely impaired, and many HFs progressively degenerate- reminiscent of hair loss in the aging human population. We hypothesized that age-related HF dysfunction is associated with the loss of endogenous HF dermal stem cell (hFSC) function. To test this, we performed long-term lineage tracing of hFSCs over 24 months. We observed significant declines in both the number of hFSCs and their differentiated mesenchymal progeny with advanced age. In aged HFs, we observed increased apoptosis of hair follicles, with a concomitant increase in the expression of the hair follicle depletion marker (HFD). These results suggest that age-related HF dysfunction is associated with the loss of endogenous HF dermal stem cell function.
Aqueous extract of Mentha suaveolens induces Nr-2 activation and expression of antioxidant and detoxifying enzymes in human keratinocyte

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This study investigates antioxidative and detoxifying activities of aquous extract (MASE) on keratinocytes. We observed that MASE increases Nr-2 activity dose-dependently without cytotoxicity in HaCaT cell by using a lucerase-based reporter gene. It also showed that MASE enhances the expression of phase II detoxifying enzymes such as heme oxygenase-1 (HO-1) and glutamate cysteine ligase (GCLC) using qRT-PCR. Furthermore, we found a cytoprotective property of MASE against hydrogen peroxide-induced cell damage. Altogether, we demonstrate that MASE protects keratinocytes from oxidative stress via the enhancement of Nr-2 mediated phase II detoxifying enzymes.

Nicotinic acid suppresses sebaceous lipid synthesis of human sebocytes via activating hydroxycarboxylic acid receptor 2 (HCA2)

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Nicotinic acid (NA) is widely used for treatment of hyperlipidemia. Since NA influences lipid metabolism, and its side effects include skin dryness, we asked if it also impacts on the biology of sebocytes, the professional lipidogenic cells of the human skin. By using human, immortalized SZ95 sebocytes, we found that non-cytotoxic (≤100 μM, NLT, Dic, 5-SYTOX Green) concentrations of NA had no effect on the basal sebogenic lipidogenesis (SLP, Nile Red), but normalized several lipogenic acid (arachidonic acid, anandamide and linoleic acid-testosterone) induced, excessive, acnemimicking SLP in course of the exposure. Moreover, at the same concentrations, NA also exerted significant anti-proliferative actions (CQUANT). Although NA did not influence lipopolysaccharide-induced pro-inflammatory response of the sebocytes (up-regulation [Q-PCR] and release [ELISA] of several pro-inflammatory cytokines), collectively, these data support the concept that the NA may be effective in skin care. We verify the mechanism of the said actions, we found that, similar to several other agents capable to reduce SLP, NA also induced a transient increase in the sebocyte (Ca^2+); (Fluo-4). Moreover, we also demonstrated that sebocytes express the NA-responsive hydroxycarboxylic acid receptor 2 (HCA2); [Q-PCR, IF], sRNA-mediated silencing of which prevented the NA-induced Ca^2+ signal and the lipostatic action. Collectively, our data introduce NA, and putative HCA2 activators in general, as novel, safe, and potent lipostatic agents, with promising anti-acne potential.

Both lesional and non-lesional skin from acne patients shows robust IL-17- skewing and upregulation of antimicrobial peptides

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Acne vulgaris is the most prevalent inflammatory skin disease in young age, however its immune pathogenesis is still being elucidated. Only few prior acne studies evaluated skin lesions, largely focusing on a limited panel of markers and/or lacking normal controls. The lack of comprehensive evaluations of all inflammatory axes involved in acne is hindering targeted-therapeutic development for acne. Our study profiles the molecular and cellular profiles of acne in mild-to-moderate patients (n=36), compared to aged-matched controls (n=20), using immunohistochemistry and gene expression studies on lesional, nonlesional, and healthy skin biopsies. There were significant infiltrates of CD3+ T-cells, CD14+ monocytes, and neutrophils in lesions, but also down-regional skin from patients vs. controls (P<0.001). We found significant upregulations of mRNA expressions of Th17-related cytokines and antimicrobials (IL-17A/IL-17F, IL-19, IL-23p19, CCL20, PI3/elafin, CXCL10, CX3CL1, CXCL11, CXCL12, S100A8, S100A9, S100A12, DEFDBD4/DEFBD4A), Th22L2-22, Th1 (IFN-γ, CXCL10, CXCL11, Mx1, STAT1), and innate immune markers (IL-1b, IL-18, IL-36, IL12b, P2X3; P<0.001 for all). Th2 markers (IL-4, CCL7) were generally not upregulated. Our comprehensive genomic and cellular profiling demonstrates that acne, but also non-involved skin from acne patients have robust Th17 skewing with parallel increases in antimicrobials, corresponding with the increased neutrophil infiltration in skin, as well as Th1-mediated innate immune activation. These data advocate for the possible systemic approaches targeting IL-17/IL-23/IL-36 in these patients.

Endocannabinoid-like molecule oleoyl ethanalamide promotes sebaceous lipid synthesis

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We have previously shown that endocannabinoid-like (eCB) are potent autocrine regulators promoting sebaceous lipogenesis (SLP) via activating cannabinoid CB2 receptors. Moreover, we have also demonstrated that human sebocytes may play a role in the metabolism of certain dietary fatty acids, including oleoyl ethanalamide (OEA), exhibiting negligible affinity towards CB2. Therefore, within the confines of the current study, we aimed to investigate the effects OEA by using human, immortalized SZ95 sebocytes. We found that, up to 50 μM, OEA did not influence viability of sebocytes (MTT), and did not induce early apoptotic or necrotic processes either (Doli, 5-SYTOX Green). However, OEA significantly promoted SLP (Nile Red, 24-48-hr treatments), most likely via activating the pro-lipogenic ERK1/2 MAPK pathway. Moreover, according to our preliminary data, OEA also suppressed lipopolysaccharide-induced pro-inflammatory response of the sebocytes, as revealed by monitoring the expression of several pro-inflammatory cytokines (Q-PCR). When further assessing the mechanism of the above actions, we found that human sebocytes express GPR119, a recently deorphanized receptor for OEA both in vitro (Q-PCR, Western blot) and in situ in human skin (IHC). As we lack selective antagonists, our currently ongoing siRNA experiments intend to unveil the putative role of GPR119 in mediating OEA ligand-induced anti-inflammatory effects. Collectively, our data introduce OEA as a new, positive regulator of SLP. This, together with our putative anti-inflammatory potential, makes OEA a promising, novel candidate in the future treatment of skin dryness.
Molecular profiling of frontal fibrosing alopecia (FFA) reveals TH1 and JAK-STAT up-regulation with no suppression of hair keratins. This suggests an inflammatory disorder with a TH1 inflammatory response and suggests a role for targeting STAT5 in this condition.

Age-dependent loss of the stemness and antimicrobial defense function of dermal fibroblasts is mediated by TGFbeta. This study identifies a central pathway that drives the age-dependent loss of the stemness and anti-microbial function of fibroblasts, and suggests that small molecules that suppress TGFbeta may be effective therapeutics to combat the aging-associated decline in dermal function.

Effect of ginsenoside Rd on dermal epidermal junction in fibroblast. Ginsenoside Rd has shown promise in various skin-related conditions, and this study investigates its effects on dermal fibroblasts and the dermal-epidermal junction.

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HIF1A stabilization in the human hair follicle promotes glycolysis


HIF1A is an oxygen-sensing molecule that functions as a stabilizer of glycolysis by triggering changes in gene expression. In this study, we investigated the role of HIF1A stabilization in the human hair follicle (HF) by following the glucose route within whole HF using fluorescent glucose deoxyglucose (DG). Our results showed that HIF1A stabilization promoted glycolysis over oxidative phosphorylation in ORS keratinocytes and we propose this may reduce oxidative stress and promote hair growth.

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Flexible fate determination ensures robust differentiation in the skin hair follicle

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Tissue regeneration is sustained by stem cell self-renewal and differentiation. How stem cells coordinately differentiate into multiple cell types is largely unclear. Recent studies underline the intrinsic heterogeneity among stem cells or common progenitors, suggesting orchestration at the stem cell/progenitor level. Here, by tracking and manipulating the same stem cells and their progeny in live mice, we uncover an unanticipated flexibility during homeostatic stem cell differentiation in hair follicle. Though stem cells appear primed through spatial regulation, we find they retain full potency to establish all the differentiation lineages. Furthermore, hair progenitors previously thought to be unipotent, were found flexibly changing differentiation outcomes as a consequence of dynamic relocation. Finally, differentiation and tissue architecture were maintained normal despite ectopic differentiation stimulation. These show a flexible cell fate determination mechanism that contributes to differentiation robustness. This work supports a model of continually fate priming and late commitment to achieve coordinated differentiation.

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Understanding the role of glycogen metabolism in human hair follicle biology

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Human hair follicles (HF) present high demand for energy and biosynthesis precursors fulfilled mainly by aerobic glycolysis. HF contain high levels of glycogen, the main energy storage site in the human body. The Cori cycle describes a metabolic process in which surplus lactate is converted via gluconeogenesis into glucose, which can then be stored as glycogen. The enzyme for gluconeogenesis was found in the ORS suggesting HF are capable of synthesis of glycogen. Treatment of primary ORS keratinocytes with PYGL inhibitor caused glycogen depletion. Further experiments will test if glycogen serves as a source of energy and substrates for proliferation and keratinisation or apoptosis in catagen.

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Spheroid cell cultures as a promising study model for hair follicle regeneration

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Hair follicle regeneration is a complex process and graft take. Alkaline phosphatase activity (APA), the expression of Wnt ligands and angiogenic factors were evaluated comparing culture conditions and conditions. APA, which correlates with hair induction potential of DPC, was 2-fold higher in spheroids than in cultures from dermal papilla cells. VEGF and Angiopoetin, which have been proved to promote skin angiogenesis and hair cycle, also showed an 8-fold higher expression in DPC spheroids. The effect of culture time of the spheroids on the expression of Wnt ligands agonists (Wnt5a, Wnt16b) and antagonist (DKK1) was evaluated. After 48 hours, an 8-fold increase of Wnt agonists and a 4-fold decrease of DKK1 expression was observed. Histological analyses of heterotopic spheroids of HSC and DPC showed that in appropriate culture conditions, a core of DPC expressing a Smooth Muscle Actin and Vimentin surrounded by an epithelium with a hair follicle organization was formed. We conclude that spheroids represent a model to obtain DPC-spheroids with improved inducibility and heterotypic spheroids with histological features reminiscent of embryonic hair-buds.
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Beyond goosebumps: Interactions between the hair follicle, the arrector pili muscle, and the sympathetic nerve during development and hair follicle regeneration

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1 Harvard University Department of Stem Cell and Regenerative Biology, Cambridge, MA and 2 Institute of Biomaterials and Tissue Engineering, National Yang-Ming University. The arrector pili muscle, commonly known as goosebumps, involves three interconnected cell types: the hair follicle, the arrector pili muscle (APM), and the sympathetic nerve. The interactions between these three cell types during development and adult tissue maintenance remains poorly understood. Here, we identify a central role of the developing hair follicle in regulating the formation of APMs, which then attract sympathetic innervation to the hair follicle stem cells. Although dispensable for hair follicle development, impulses from the sympathetic nerves are required to maintain hair follicle stem cell activity during hair follicle regeneration. Formation of the APMs requires Sonic Hedgehog secreted from the developing hair follicles. Once developed, APMs do not undergo turnover, providing a stable anchor that maintains sympathetic innervations to the hair follicle stem cells. APM ablation leads to concurrence of sympathetic nerve innervation to the hair follicles. Our results uncover a novel function of APM in bridging the body’s sympathetic modulations to influence hair follicle stem cell activity, and illustrate an example for how a developing tissue regulates the establishment of the niche to modulate its regeneration in adulthood. Our results may also explain why hair loss is a common side effect of beta-blockers, which suppress the sympathetic tones, and why loss of APMs is commonly associated with permanent hair loss conditions such as androgenic alopecia.

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Maintenance of epidermal progenitor function through the mRNA degradation and translation pathways

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Although diseases that involve alterations in epidermal growth and differentiation impact every one in five people, the basis for how epidermal progenitor cells suppress premature cell differentiation is unclear. We show that RNA degradation is essential to maintain self-renewal of the progenitor cells that reside in the basal layer of the epidermis. Depletion of DDX6 resulted in loss of progenitor cell function due to premature differentiation and loss of proliferation. To promote proliferation and self-renewal, DDX6 facilitates the translation of regulators of proliferation (CDK1 and HMGB2) and epigenetic factors necessary for self-renewal such as EZH2 and ACTL6a. DDX6 through association with mRNA binding protein, YBX1, binds to stem loops found in the 3UTRs of these mRNAs and recruits them to EIF4E to promote translation and thus maintains the self-renewal capacity of progenitor cells. To actively suppress differentiation, HNRNPK recruits DDX6 to GC-rich regions in the 5UTR of KLF4 and GRHL3 (transcription factors necessary for epidermal differentiation) and promotes their degradation through mediators of mRNA degradation such as the Tubulin associated factor (TAF). Collectively, our results suggest that DDX6 promotes epidermal progenitor cell fate by facilitating the translation of mRNAs involved in proliferation and self-renewal while also targeting differentiation inducing mRNAs for degradation.

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The de novo DNA methyltransferase DNMT1A is required for epidermal homeostasis

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DNA methylation is an essential and developmentally regulated epigenetic mark that is placed on unmethylated CpG residues by the de novo methyltransferases DNMT3A and DNMT3B. This process is highly regulated, yielding exquisitely conserved patterns within specific cellular contexts, and can be deregulated in disease states. The DNMTA/RNA mutation is the most common initiating event for acute myeloid leukemia; it encodes a mutant protein that exerts a dominant negative effect on wild type DNMTA methyltransferase activity, resulting in focal, canonical DNA hypomethylation at thousands of sites in the genome. Surprisingly, mice expressing an inducible, ubiquitous DNMTA/R882H transgene were found to exhibit a highly penetrant alopecia, which was reversed within weeks after transgene expression was extinguished. Gene expression analysis has revealed that Dnmt1a is the most abundantly expressed member of the Dnmt1a family. We used RNA-seq of human neonatal epidermis to profile 19,648 single-cell transcriptomes from 13 individuals to reveal unbiased clustering of at least 9 main subpopulations of interfollicular keratinocytes, one population of melanocytes, and one population of Langerhans cells. Pseudotime modeling of epidermal differentiation suggests at least two distinct differentiation trajectories using two computationally distinct methods. Surprisingly, we observe four main keratin 14 high subpopulations: two basal stem cell subpopulations and two basal transtil subpopulations each with spatially distinct immunostaining patterns. Our scRNA-seq data comprehensively reconstructions the complexity of human interfollicular epidermis, revealing spatial and pseudotemporal differences between transcriptional programs at the single cell level and unexpected heterogeneity in all layers of the epidermis.

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Single cell transcriptome profiling of human interfollicular epidermis reveals robust heterogeneity and divergent differentiation lineages

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Human epidermis is a richly diverse organ system composed of basal stem cells that divide to produce a highly connected squamous layer rich in cell-cell communication, a granular layer that provides strength to the organ, and a dead stratum corneum layer that caps the water-tight organ. Additionally, melanocytes are integrated within the basal layer and provide pigment, and mononuclear dendritic Langerhans cells are typically found in the spinous layer. Morphologically, human interfollicular epidermis classically displays three living keratinoocyte subpopulations with melanocytes and Langerhans cells dispersed throughout the organ. However, the homogeneity of interfollicular epidermis has been called into question with recent single-cell RNA-seq (scRNA-seq) data from mouse epidermis that describes five main subpopulations of interfollicular keratinocytes, one population of melanocytes, and one population of Langerhans cells. Pseudotime modeling of epidermal differentiation suggests at least two distinct differentiation trajectories using two computationally distinct methods. Surprisingly, we observe four main keratin 14 high subpopulations: two basal stem cell subpopulations and two basal transtil subpopulations each with spatially distinct immunostaining patterns. Our scRNA-seq data comprehensively reconstructions the complexity of human interfollicular epidermis, revealing spatial and pseudotemporal differences between transcriptional programs at the single cell level and unexpected heterogeneity in all layers of the epidermis.

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Dermal Wnt/beta-catenin activation tunably controls hair follicle initiation

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Wnt/beta-catenin pathway activation is required for both human and mouse hair follicle initiation. However, the cellular and molecular basis of this developmentally conserved pathway remains unclear. Here, we used a conditional null allele of Ddx6 to test the role of Wnt/beta-catenin activation in the earliest events of hair follicle initiation. Using in vivo genetic mouse models, we show that modulating the number of beta-catenin-activated (Wnt) dermal cells prior to HF initiation is the most powerful determinant of the number of HF progenitors. These findings provide evidence that an early patterned pre-DC population composed selectively of wildtype Wnt-active dermal cells, indicating a cell-autonomous role for Wnt in HF initiation. Using in vivo genetic mouse models, we show that modulating the number of beta-catenin-activated (Wnt) dermal cells prior to HF initiation is the most powerful determinant of the number of HF progenitors. These findings provide evidence that an early patterned pre-DC population composed selectively of wildtype Wnt-active dermal cells, indicating a cell-autonomous role for Wnt in HF initiation.

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Mature adipocytes inhibit the antimicrobial function of dermal adipogenic stem cells: An explanation for impaired cutaneous defense in obesity

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Local differentiation of dermal adipogenic stem cells to mature adipocytes is necessary for cutaneous antimicrobial defense against invasive bacterial infection. Recent evidence indicates that mature adipocytes seen in obesity is paradoxically associated with increased rates of skin infection. In this study, we investigated if the antimicrobial-adipogenic response is impaired in obese human skin adipogenic stem cells. We found that obesity mice fed a high-fat diet lost local antimicrobial activity as seen by an increased susceptibility to S. aureus cutaneous infection. This was associated with a loss of adipogenic stem cells isolated from the dermis and a gain in mature adipocytes. In vitro studies of primary mouse dermal adipogenic stem cells showed that expression of cathelicidin mRNA (CAMP) peaked 6-fold (p<0.05) in early adipogenesis and was subsequently lost with further differentiation. Functional assays confirmed that newly differentiating immature adipocytes produced antimicrobial activity in their culture supernatant that led to a greater than 3-log decrease in methicillin-resistant S. aureus growth. During the later phase of maturation, this antimicrobial activity was weakened and Camp mRNA expression decreased 9-fold (p<0.05). Furthermore, we found that when adipogenic stem cells were co-cultured with mature adipocytes, or exposed to mature adipocyte conditioned media, this resulted in inhibition of the capacity of adipogenic stem cells to express Camp mRNA, produce cathelicidin protein, and inhibit S. aureus growth. These results suggest that undifferentiated adipogenic stem cells are capable of promoting resident adipogenic stem cells and of mature adipocytes lose antimicrobial activity and may also diminish the antimicrobial capacity of adjacent adipogenic stem cells. These findings provide an explanation for why cutaneous defense is impaired in the skin of obese patients.
Non-invasive evaluation of skin biophysical properties of striae distensae
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Hard skin without Marrow such as nails are formed by cornification of keratinocytes in a process that involves cross-linking of structural proteins and breakdown of other cellular components. Here, we used murine nail cornification as a model system to determine whether catabolic processes of cornification reduce autophagy. We deleted the essential autophagy gene Atg7 in nail keratinocytes and determined the proteome of nails from mice lacking autophagy in keratinocytes (Atg7<sup>-/-</sup> K14-Cre) versus nails from fully autophagy-competent mice. While the growth of nails and mRNA expression was normal, the protein composition of nails was significantly altered in the absence of autophagy. The cornified nails of Atg7<sup>-/-</sup> K14-Cre mice contained elevated amounts of enzymes, proteasome and chaperonin proteins whereas the main structural components of nails, i.e. keratins, keratin-associated proteins and desmosomal proteins were preserved. These results highlight a critical role for Atg7 in human embryonic progenitor cells proliferation and differentiation.

Altered metabolism of elastic fibers and collagen fibers derived from TGF-β1-mediated inflammation in atrophic acne scar
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This study was conducted to reveal the process of atrophic acne scar formation in histological and molecular views. Thirty subjects with acne were divided into two groups; fifteen were prone to acne scar (APS) and the others were not prone to acne scar (ANS). Skin samples of acne lesions were obtained on day 1, 3 and 7 after their onset. Elastic fibers, collagen 1 and 3 were significantly decreased and their recovery was delayed in APS compared to ANS. On the other hand, the production of neutrophil elastase, matrix metalloproteinases (MMP)-1, MMP-2 and MMP-3 was substantially increased in APS compared to ANS. More severe inflammation with elevated various proinflammatory cytokines and proteases was observed in APS compared to ANS. These results suggest that excessive inflammation contributes to the aberrant ECM degradation and healing in this process, it was found that increased TGF-β1 in APS may play a crucial role in aggravating inflammation via activating phospho-TAK1 and NF-κB p65. These results propose the detailed pathogenesis of atrophic acne scar forming and ultimately provide a basis of a new therapeutic approach in atrophic acne scar.
Expression of nutrient transporters is altered in the human keratinocytes cell line following glucose and serum starvation.

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While skin aging leads to an impaired dermal structure, little is known about how the aging skin dermal environment impacts the hair follicle. We hypothesize that the hair follicle environment is central to an aged hair phenotype; therefore we identified age-related changes in the scalp dermal environment impacts the hair follicle. We hypothesize that the hair follicle biomarkers significantly changed (p < 0.05) compared to under 50 (n = 6) compared to over 50 (n = 24) donors.

Changes in the aging dermal hair follicle environment in female scalp

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Changes in the aging dermal hair follicle environment in female scalp

Changes in the aging dermal hair follicle environment in female scalp

3150

Human scalp skin as an abundant and accessible source for eccrine sweat gland isolation and organ culture

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Besides their critical role in thermoregulation, sweat-producing eccrine glands (EG) also play a less understood, yet important role in cutaneous wound healing, in managing the skin microbiome, and may interact with neighboring skin appendages such as the hair follicle. Research into human EG function has been greatly hindered by EG isolation problems, since EGs are located in the skin. In our study, we aimed to obtain EGs expressing different markers such as Lrig1, Pmel1, Lgr6 and Blimp1. Recently, our lab demonstrated that GATA6, a transcription factor that governs cell growth, apoptosis and senescence in response to various stresses. In summary, our findings establish a novel role for Dsg3 serving as an anti-stress protein, via suppression of p53, and identify an unprecedented interaction of this pathway with YAP in controlling skin homeostasis. The present study investigated this pathway in keratinocytes (harboring wt5p3) subjected to UV and mechanical stretching, and to explore its contribution to the pathogenesis of PV. We showed that Dsg3 knockdown caused increased expression and stabilization of p53 (with half-life 3.3-fold compared to control), and its activity in cells exposed to UV and cyclic stress. This result is consistent with findings from Dsg3 gain-of-function studies showing suppression of p53 and downstream p21/Waf1 in response to stress signals. Analysis of clinical specimens detected increased p53 in 12 of 25 PV patient samples, with diffuse cytoplasmic and nuclear staining in cells surrounding the blisters. Treatment of keratinocytes with PV sera evoked pronounced p33 expression in both the nucleus and cytoplasm of cells. Furthermore, we established that this Dsg3-p53 pathway involved YAP since Dsg3 knockdown resulted in marked reduction of YAP (with the YAP activator YAPt and Dsg3-YAP being detectable. Evident increased nuclear YAP also was detected in PV keratinocytes. In summary, our findings establish a novel role for Dsg3 serving as an anti-stress protein, via suppression of p53, and identify an unprecedented interaction of this pathway with YAP in controlling skin homeostasis.

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Stimulation of hair follicle stem cell proliferation through an IL-1β dependent activation of γδ-T-cells

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The current work was a proof of concept to display among diverse cell types within the skin. One fundamental process mediated by these reciprocal interactions is the mobilization of local stem cells pools to promote tissue regeneration and repair. Using the epidermis specific ablation of caspase-8 as a model of mouse wound healing, we analyzed the signaling components responsible for epithelial stem cell proliferation. We found that IL-1β and IL-17 secreted from keratinocytes function synergistically to expand the activated population of resident epidermal γδ-T cells. A downstream effect of activated γδ-T cells is the preferential proliferation of hair follicle stem cells. On the other hand, IL-1β dependent stimulation of dermal fibroblasts optimally stimulates epidermal stem cell proliferation. The findings provide new mechanistic insights into the regulation of epidermal-immune cell interactions and how components classically associated with inflammation can differentially influence distinct stem cell niches within a tissue.
ABSTRACTS | Skin, Appendages, and Stem Cell Biology

1352
Basal layer divisions in epidermis over the human lifespan
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While proliferation is decreased in aged tissues, no change in SC number was detected by our group or Bickenbachs. Lechler et al. examined types of SC divisions. Divisions perpendicular to the basal layer display a 3.2-fold decrease in older skin compared to neonates and 20% in adult skin. In mice, basal cell proliferation is highest in neonates and decreases with age. The in situ labeling of basal keratinocytes with 5-bromo-2′-deoxyuridine (BrdU) in adult and aged skin revealed a significant decrease in proliferation as a function of age.

1353
Inhibition of sebocyte HDAC activity promotes cytosine expression: An epigenetic model for acne pathogenesis by Propionibacterium acnes
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The in vivo exploration of genetic regulations in skin and hair follicle development has been hampered by the lack of model systems. Here, we present evidence that sebaceous glands (SGs) can be used as a model system to study the development of adult hair follicles. Using in vivo imaging and histology, we observed that SGs begin to develop before the onset of hair follicle (HF) formation. Moreover, we found that SGs are highly enriched with stem cells (SCs) that are able to generate multiple cellular lineages, including keratinocytes and sebocytes. These findings suggest that SGs may represent a valuable model system for studying the development of adult hair follicles.

1354
Spatial and single-cell transcriptional profiling identifies functionally distinct human dermal fibroblast subpopulations
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Dermal fibroblasts are heterogeneous and play a crucial role in wound healing and tissue repair. Despite this, the molecular basis for fibroblast heterogeneity remains largely unexplored. Here, we used single-cell transcriptomics to characterize dermal fibroblast subpopulations and identify their functionally distinct contributions to wound healing.

1355
Mapping of Wnt/b-catenin signals during the telogen-to-anagen transition in human hair follicles
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The Wnt/b-catenin pathway is essential for epithelial hair follicle stem-cell (eHFSC) activation during hair growth induction during the human telogen-anagen transition, including the activation of adult eHFSCs. Modulation of these specific Wnt ligands or antagonising SFRP1 may be a novel therapeutic strategy to induce anagen in human telogen HFs and thus counteract hair loss.

1356
Characterization of human epidermal stem cells in situ using multiplex immunofluorescence analysis identifies a quiescent KRT5+/MECP2+/XPC+ subpopulation
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The nature of human epidermal stem cells is still poorly defined. Several markers have been used to identify putative epidermal stem cells, but these and other markers are relatively broadly expressed within the epidermis. Better methods to define the human epidermal stem cells in situ within the tissue are required to better understand the mechanisms that rely on proper stem cell function including normal tissue homeostasis, wound healing, inflammation and cancer. To overcome the limitations of individual markers to the many processes that rely on proper stem cell function including normal tissue homeostasis, wound healing, inflammation and cancer. To overcome the limitations of individual markers to the many processes that rely on proper stem cell function including normal tissue homeostasis, wound healing, inflammation and cancer. To overcome the limitations of individual markers to the many processes that rely on proper stem cell function including normal tissue homeostasis, wound healing, inflammation and cancer. To overcome the limitations of individual markers to the many processes that rely on proper stem cell function including normal tissue homeostasis, wound healing, inflammation and cancer.
1358
PRC1 fine-tunes gene repression and activation to safeguard skin epithelium development and stem cell specification
J Cohen1, D Zhao2, D Zhou3, and L Cheng1 1 Ichan School of Medicine at Mount Sinai, New York, NY and 2 Albert Einstein College of Medicine, New York, NY Polycomb repressive complexes (PRCs) are essential chromatin regulators of cell identity. PRC1, which acts as a dominant executor of Polycomb-mediated control, possesses expression-dependent H2AK119 mono-ubiquitination and catalytic-independent activities. Despite extensive knowledge of PRC1’s role in embryonic stem cells (SCs), its function in somatic SCs and tissue development is largely unknown. Here, we show that despite its well-established repressor functions, PRC1 binds to both silent and active genes in epidermal progenitors. Through loss-of-function studies, we show that global PRC1 function is essential for skin development and SC specification, whereas PRC1 catalytic activity is dispensable. By dissecting molecular mechanisms, we show that PRC1 catalytic-dependent and -independent repressor functions, PRC1 binds to both silent and active genes in epidermal progenitors.

1360
Regulation of bmp signalling in melanogenesis, pigment transfer and melanocyte migration
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Skin pigmentation is a complex process that is known to be controlled by cross-talks between the different populations of the melanocyte stem cell niche. However, the mechanisms involved in this process is yet to be fully understood. Therefore, a better understanding of the mechanisms involved in the regulation of skin pigmentation is essential and is critical for the development of novel therapy strategies against skin pigmentation disorders affecting 1 out of 3 people worldwide. It was demonstrated previously that Sox2 in the dermal papilla compartment is a key regulator of hair growth by controlling the BMP-mediated crosstalk between the DP niche cells and the stem cell progeny. Interestingly, a color switch is observed as well, in the papilla of DP-specific Sox2 knock down mice together with unusual BMP cell signaling in these mice further suggested the importance of Sox2 and BMP signaling in regulating melanocyte stem cell niche. Thus, we aim to investigate this relationship between BMP signaling and pigmentation. Our recent data from human keratinocyte and melanocyte co-cultures shows that BMP signaling is involved in the regulation of melanogenesis and pigment transfer. In addition, data from the in-vivo study of melanocyte migration at a functional level indicates the role of BMP signaling in mediating the migration of melanocytes. In this study, novel genetic tools are also developed in order to target and study the complex mechanisms in BMP signaling regulation of pigmentation and melanocyte migration in mouse skin and the recent in-vivo data has demonstrated the involvement of BMP signaling in melanocyte migration. Through this study, it will bring us closer to the goal of developing novel therapy strategies against skin pigmentation disorders.

1359
Hair growth is induced by blockade of macrophage-derived oncostatin M and downstream JAK-STAT5 signaling in hair follicle stem cells
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Our lab recently demonstrated that blockade of JAK-STAT signaling using topical JAK inhibitors was sufficient to induce hair growth (anagen) in resting (telogen) in C57BL/6 mouse hair follicle stem cells (HFSC) in their quiescent state. To define the mechanism by which this occurs, here, we demonstrate that the IL-6 family cytokine Oncostatin M (OSM) is a negative regulator of hair growth that maintains HFSC quiescence via JAK-STAT signaling in vivo. We found that the OSM receptor (OSMR), co-receptor gp130 and activated pSTAT5 are co-expressed in telogen HFSCs, and that OSM is produced in the telogen dermis. Conditional ablation of OSMR or STAT5 during early- and mid-telogen (P42 – P60) shortens the telogen phase significantly, and promotes activation of HFSCs both in vivo and in vitro. Unexpectedly, we identified that the endogenous source of OSM is not intrinsic to the HF dermal cells, but rather, emanates from a distinct subset of TREM2+ dermal macrophages we identified using single-cell RNA sequencing of dermal CD45+ immune cells across murine telogen/early anagen skin. Furthermore, we found that blockade of OSMR, using three independent approaches, including neutralizing antibodies (anti-CSFIR), small molecular CSF1R inhibitors (pexidartinib/PLX1397, BLZ945 and GW2580), and genetic ablation (with Csf1r- CreER::R26-iDTR mice) during telogen, promotes hair growth by removing the endogenous source of OSM. Hair growth and proliferation of HFSCs were associated with depletion of this subset of TREM2+ macrophages, which were found to be spatially, temporally and functionally relevant for HFSC quiescence during telogen. Our findings highlight the role of immune cells in establishing a quiescent niche for HFSCs, and invites future clinical investigation into treating human hair disorders characterized by arrested telogen follicles by targeting a cell type outside the HF itself.

1361
A novel cell surface marker for hair follicle dermal cells throughout hair morphogenesis and cycling
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Hair follicle stem cells in the bulge region are known to be responsible for the regeneration of hair follicles during the hair cycle. These hair follicle stem cells exist in the niche composed of mesenchymal cells, which is made up of the dermal papilla (DP) and dermal sheaths (DS) cells. DP cells are of great research interest not only because of its involvement in hair follicle regeneration and growth, but also believed to be a reservoir of multipotent cells. However, the lack of cell surface markers to differentiate this group of cells throughout the various hair cycle stages has been proven to be an obstacle in this area of research. Therefore, it is of critical importance to identify a suitable cell surface marker to tackle against this hurdle. It was demonstrated previously that Leptin receptor (LepR) genes highly expressed in the DP cells of an E14.5 mouse embryo. Thus, we aim to investigate the suitability of LepR as a novel cell surface marker to isolate and target the dermal papilla (DP) cells. With aid from this in-vivo study, it will bring us closer to the goal of developing a novel strategy to isolate the DP cells to facilitate the research of DP cells.
1364

Influence of the age of infants, compared to adults, epidermal cells on the development of sensory neurons: Exploratory study

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The development of neurites derived from human sensory neurons or DRG neuron cell-bodies, were cultured in explants in collagen. Encapsulated suspensions of sensory neurons (derived from iPS cells) or Dorsal Root Ganglia (DRG, containing sensory neurons) were implanted into dermis of the dorsal skin of mice. Intrafollicular CD8+ and NKG2D+ cells. In human HFs ex vivo, inhibition of miR-486 resulted in pre-mature anagen-catenagen development associated with decreased expression of HLA and TAP2 and upregulation in ICAM1 and CADM1. miR-486 expression was negatively regulated by INF-gamma in an ex vivo model of immune privilege collapse in human HFs and in the follicular keratinocytes in vitro. Overexpression of miR-486 in the primary keratinocytes caused downregulation of the expression of the components of MHC class I, and several interleukin signaling pathways. Taken together, these data suggest that miR-486 could play a protective role in the pathogenesis of AA by preventing the collapse of HF immune privilege.

1365

Interest of Biotininn®i, a mix of Nasturtium officinale and Tropaeolum majus extracts, for chronic hair loss treatment

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Hair aging is characterized by hair whitening, hair thinning and hair density reduction leading to a global loss of hair volume. Oxidative stress is one of the key molecular mechanisms that induces cellular senescence and dysregulates cellular functions with age. It seems important to prevent age-related hair density reduction to protect long-term HF cells against oxidative stress and to promote the growing phase (anagen) of the HF. The aim of this study was to evaluate the effect of Ginseng and Albizia extracts and their association on Human Dermal Papilla Cells (HDPCs) response to oxidative stress and on the expression of key factors of anagen’s maintenance. Melatonin secretion was also investigated for its anti-oxidant and anti-apoptotic activities. Anti-oxidant activity was evaluated according to an oxygen radical absorbency capacity (ORAC) assay and the measurement of SOD (catalytic and ferredoxin-1 (SRXN1) gene expression in HDPCs following a H2O2 stress. Melatonin and VEGF protein expression levels were measured in HDPCs culture supernatants (ELISA assays). HDPCs senescence was evaluated following a H2O2 stress by measuring the expression of the gene encoding p21 (CDKN1A) expression. The gene expression of the pro-apoptotic factor BAX and of the Fibroblast Growth Factor 5 (FGF5), which are two inhibitors of hair growth, were upregulated in the matrix and bulge implying that although mitochondria in LPP HFs are indeed act as a mediator of blue light-dependent effect on hair growth. In addition, 3.2J/cm2 treatment with 3.2J/cm2 blue light (453 nm) stimulates proliferation of the outer root sheath (ORS) keratinocytes in vitro. Microarray analysis revealed that either CR1Y or OPN3 siRNA causes alterations in the expression of genes controlling proliferation and apoptosis in the ORS keratinocytes. Taken together, these results suggest a 453 nm blue light at low radiant exposure exerts a positive effect on hair growth, potentially via interaction with CR1Y and OPN3.
17β-estradiol may control human HF growth also via up-regulating the expression of cannabinoid receptor type 1 expression

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Unwanted hair growth (hirsutism, hypertrichosis) can cause major psychological distress. Therefore, new pharmacological treatment strategies that safely and effectively inhibit hair growth while preserving the hair follicle (HF) stem cells pool need to be developed. Since osteopontin-derived fragments may modulate human hair growth. This hypothesis was tested

ex vivo and in vivo by using a newly generated, toxicologically well-characterized, modified osteopontin-derived peptide (FOL-005), which binds to the HFs outer root sheath. In organ-cultured human HFs and scalp skin, and in human scalp sebaceous glands into SCID mice, FOL-005 treatment robustly promoted premature catagen development without reducing the number of keratin 15+ HF stem cells or showing signs of drug toxicity. In vivo, intradermal FOL-005 injection also counteracted the hair growth-promoting effects of mouse epidermal growth factor receptor. Lrig1-positive stem cells are located in the isthmic region of the mouse hair follicle (HF) and are believed to drive sebaceous gland (SG) formation during the morphogenesis of the pilosebaceous unit. We have recently shown that Lrig1-positive stem cells are the initial targets of dioxins. To better understand the crucial role of Lrig1 in SG homeostasis, we performed topical tamoxifen inducible diphtheria-toxin- mediated cell ablation of Lrig1 stem cells in mice. We showed that killing of Lrig1-positive cells in mice results in a complete but reversible disappearance of SGs. The atrophy of SGs started at day 3 of Lrig1 ablation and almost total remodelling took place after 4-6 months. In the absence of Lrig1-positive niche, keratinoctyes of the functional zone (D1) differentiated into interfollicular epidermis (IFE)-like keratinocytes. Following Lrig1 ablation, cells from other epidermal compartments rapidly repopulated the D1 and the SG space. However, they expressed keratin 1, an IFE differentiation marker, suggesting that the Lrig1-positive niche is crucial for SG maintenance, and that the cells that repopulate the niche are not able to differentiate into the SG lineage. However, the bulge and the hair growth remained normal, and no hair dysfunction or hair loss has been observed. Our results suggest that Lrig1-positive stem cell compartment is required for sebaceous gland morphogenesis in mice.
Dermal white adipose tissue enhances proliferation, pigmentation and hair shaft elongation in human hair follicles ex vivo

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In the past decade, the crosstalk between dermal white adipose tissue (WAT) and hair follicles (HF) has become of increasing interest. Murine studies have revealed that pathways involving Wnt and leptin are involved in WAT and HF crosstalk. Despite such progress on murine models, human HF-WAT communication remains virtually unexplored. In this study, we cultured micro-dissected HFs versus HFs surrounded by the immediate 1-4 layers of dermal adipose tissue (DWT) from human scalp skin for 48 hr in vivo. Interestingly, qualitatively immune-histochemistry of Ki67+ cells below Auders line reveals that DWT significantly enhances cell proliferation in the HF matrix, as well as increasing the number of DAPI+ nuclei. Furthermore, Maison Fontana staining shows a significant upregulation of melanin content within HFs cultured with DWT compared to HFs cultured on their own. We also carried out ex vivo cultives of (a) HFs on their own (b) HFs with the surrounding DWT (HF+SWAT) and (c) HFs with dissected subcutaneous scalp fat (SWAT) in the same well (HF+SWAT). Interestingly, hair shaft elongation is significantly higher in hair follicles grown together with the surrounding DWT compared to HFs alone and HFs grown with dissected SWAT. Overall, our results suggest that DWT-derived factors act upon human hair follicles to modulate keratinocyte growth. For the first time, we show that human HFs influence scalp HFs ex vivo via enhancing proliferation, pigmentation and hair shaft elongation. Our results hold translational promise, and may point to the notion that deregulation of dermal adipocytes surrounding human HFs may play a more significant role in promoting hair disorders than previously thought.
1382
Asymmetric cell divisions buffer against tissue overgrowth
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As new cells are generated in the skin, older cells must transit out of the basal layer in order to maintain homeostasis. Cells may be lost through apoptosis, but maintenance of normal basal cell number is primary mediated through differentiation and delamination. The cellular mechanisms that maintain homeostasis in response to uncoupling of these events are not clear. Here we show that cells resist their division axes in response to changes in the rate of proliferation and differentiation. Symmetric cell divisions were increased by hyperproliferation, while asymmetric cell divisions were increased by hyperproliferation. Strikingly, the rate of asymmetric cell divisions in the palmo-plantar epidermis also increased upon expression of a constitutively active allele of Kras in a proliferation-independent manner. In this tissue, we found that asymmetric divisions are dependent on conserved spindle orientation machinery. Disruption of this machinery in a Kras mutant background resulted in massive tissue overgrowth and expansion of the progenitor cell population. In these mutants, the benign tumors that formed presented a spectrum of p53 responsiveness in a context of cell proliferation. Notably, we found that expression of the HPV E6 protein is sufficient to perturb spindle orientation in vitro - demonstrating a pathogenic disruption of the asymmetric cell division machinery. Together, these data reveal that epidermal progenitors use asymmetric cell divisions to protect against tissue overgrowth, and that this pathway may be targeted by viral pathogens in a tissue-specific manner to induce papilloma formation and HPV-induced cancers.

1383
Arhgap19 antagonized by Lrig1 is necessary for filopodia growth, EGF responsiveness and CD44-hyaluronate interaction in keratinocytes
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Trichohyalin and the type VII collagen alpha 1 chain (COL7A1) are found in the highest density of aberrant follicles in some TS cases suggesting that follicle neogenesis may have taken place. To begin probing the role of TSPyV viral proteins in the pathogenesis of TS, we focused on Arhgap19, a gene whose functional inactivation in the mouse caused abnormalities in skin development in adult transgenic mice. Arhgap19 is a crucial regulator of epidermal differentiation associated with actin reorganization and ECM remodeling.

1384
Innovative palmitoyl tetrapeptide-20: A promising solution to fight grey hair
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Premature canite and greying hair pigmentation are phenomena directly linked to a melanin lack in the hair bulb but also to the accumulation of hydrogen peroxide (H2O2) in hair follicles. To counteract these processes, we have developed a bioamine peptide of the α3-MSH (palmitoyl tetrapeptide-20), enables to promote in vitro, ex vivo and in vivo hair pigmentation and reduce hair greying process. In vitro studies revealed the peptide capacity to increase melanin synthesis by 19% in human melanocytes and melanosome transfer to keratinocytes by 50%. In HDFP cells, the peptide was also able to decrease significantly by 30% intracellular H2O2 level. Ex vivo, after 7 days of peptide application, the peptide increased by 55% hair bulb pigmentation. This result was confirmed by immunohistochemistry, showing increased melanin content in hair follicles. Experimental wounds were produced on rabbit skin. The peptide treated wounds showed a stronger neovascularization, more pronounced hair pigmentation and faster wound healing. Furthermore, the peptide was able to promote hair pigmentation and counteract the greying hair process.

1385
Dual mechanism of Type VII collagen transfer by bone marrow mesenchymal stem cell extracellular vesicles to recessive dystrophic epidermolysis bullosa fibroblasts
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Recessive dystrophic epidermolysis bullosa (RDEB) is a severe blistering disease resulting from a lack of type VII collagen production. Recent clinical trials have shown efficacy of bone marrow-derived stem cells (BM-MSCs) in the treatment of epidermolysis bullosa, including improved basement membrane restructuring and cutaneous wound healing. The mechanism as to how type VII collagen is transferred from donor stem cell to recipient RDEB cells has not been defined. Here, we report that BM-MSC-derived extracellular vesicles serve at least two roles: 1) to help transport the insoluble type VII collagen alpha chains within the extracellular space; and 2) to feed RDEB fibroblasts with messenger RNA that codes for type VII collagen, resulting in increased mRNA translation and synthesis of normal type VII collagen alpha chain proteins, eventually secreted by RDEB fibroblasts. Utilizing a chemoselective ligation detection method, we found RDEB cells that were treated simultaneously with BM-MSC's EVs and an L-methionine analog, L-homopropargylglycine (HPG), synthesized collagen VII alpha chain protein that contained the alkylene group of HPG to react (i.e. undergo the Click reaction) with azide-modified Alexa 594, enabling detection and suggesting de novo synthesis of type VII collagen by RDEB fibroblasts. These dual mechanisms could result in a net positive “transfer” of type VII collagen to the extracellular environment of skin in RDEB patients, likely improving both basement membrane integrity and wound healing. Therapies that utilize EVs to enhance delivery of type VII collagen, both in terms of nucleic acids and protein, have potential to improve the functions of skin in RDEB patients.

1386
Trichohypidrosis spinulosa small T antigen drives ectopic hair follicle development in adult transgenic mice
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Trichohyopidrosis spinulosa (TS) is a rare hair follicle disorder that develops in the context of complex dermal-epithelial abnormalities patients and is associated with productive infection by a polyomavirus designated TSPyV. TS presents as numerous minute papules, some with a central keratotic spicule, typically on the face and ears. TS hair follicles are dysplastic, consisting of outer root sheath, companion layer, hair matrix, an expanded inner root sheath (IRS) compartment, and a "fusiform" companion layer, hair matrix, an expanded inner root sheath (IRS) compartment, and a "fusiform"

1387
Identifying the key niche signals for hair follicle formation
K Mok, Z Wang, R Sernett, A Rezza, N Heitman, A Maz'yan and M Rendl

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Specialized mesenchymal cells in dermal condensates (DC) play a crucial role in regulating hair follicle (HF) progenitors in epidermal placodes (Pc) to orchestrate HF morphogenesis. Nevertheless, to date the inductive signals during DC specification and how DC cells interact with Pc to promote HF development remain unknown. Here we identify precursors of the DC (pre-DC) before it appears as specialized cell cluster. With fluorescence-activated cell sorting we co-isolate pre-DC and the DC as it matures, together with the DC in the isotype of Heps, and other lineage-related populations. We then define the gene expression patterns in each population with next-generation RNA sequencing. Through cross-analysis we define a common molecular time-lapse of dynamically changing gene signatures in consecutive developmental stages during the three earliest HF formation stages. Within the stage-specific pre-DC and DC molecular signatures we uncover several putative key hair inductive signals. With genetic manipulation in live embryonic skin cultures we aim to effectively test the role of newly identified signals in their interplay between the niche and placode progenitors that govern hair follicle formation.
ABSTRACTS | Skin, Appendages, and Stem Cell Biology

1388
Applying FACS-seq to study mouse Merkel cell development
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Hair follicle development during skin development requires interactions between epithelial progenitors and a progressively differentiating dermal fibroblast population. Heterogenous fibroblasts originate from a homogenous mesenchyme and become lineage-committed after E16.5 days. Hair follicle formation begins at E14.5, which is prior to fibroblast lineage commitment, suggesting that this time point holds the highest potential for fibroblasts to become dermal papilla. We tested the hypothesis that undifferentiated embryonic fibroblasts (E14.5) possess an enhanced ability to support de novo hair follicle formation, utilizing the chamber grafting assay. We performed chamber grafting assays, containing E14.5, E17.5, or P5 fibroblasts. Our results revealed that E14.5 and P5 fibroblasts produced new follicles, while E17.5 fibroblasts did not promote follicle formation, while E14.5 skin lacked the ability to form hair de novo, contradicting the original hypothesis. Our study shows that there are two critical developmental stages of dermal fibroblasts. The early embryonic stage is undifferentiated and uncommitted fibroblasts that require the embryonic environment to fully mature. The uncommitted state of dermal fibroblasts is undifferentiated and uncommitted. Further work is now required to understand the mechanisms of fibroblast differentiation and defined transcriptional networks underlying MC differentiation, including those which are recapitulated in the lineage specification of MCC.

1389
Caspase controls the free fatty acid-induced sebocyte apoptosis
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Bone morphogenetic protein (BMP) signaling governs the homeostasis of hair follicle stem cells (hFSCs) in vivo. Here, we focused on testing role of id2 gene, one of the target genes which we identified to be downregulated in HS25 after inhibition of BMP pathway. To test the function of id2 gene in HS25 in vivo we used id2 gain of function approaches (GOF) by generating transgenic mouse line. Our data demonstrated that id2 overexpression in HS25 results in prolonged telogen and a delay in anagen activation, maintaining stem cells quiescence. Further, we performed FACS sorting and RNA-seq analysis of GOF id2 HS25 at first postnatal cycle. By comparison with common signature genes of the undifferentiated hFSCs we demonstrated that approximately 10% of the genes were affected by overexpression of id2 in HS25. Interestingly approximately half of those genes overlapped with common signature genes which we previously published to be affected in the pathway thus working synergistically with that pathway. On the other hand, our data also suggest that although id2 is a direct target and an effector of BMP pathway in hFSCs, second half of those genes work at least partially independent from BMP signaling on gene regulatory network of quiescent hFSC.

1390
Lineage-committed fibroblast populations are more efficient at regenerating hair follicles in chamber grafting assays when compared to undifferentiated embryonic fibroblasts
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Hair follicle development during skin development requires interactions between epithelial progenitors and a progressively differentiating dermal fibroblast population. Heterogenous fibroblasts originate from a homogenous mesenchyme and become lineage-committed after E16.5 days. Hair follicle formation begins at E14.5, which is prior to fibroblast lineage commitment, suggesting that this time point holds the highest potential for fibroblasts to become dermal papilla. We tested the hypothesis that undifferentiated embryonic fibroblasts (E14.5) possess an enhanced ability to support de novo hair follicle formation, utilizing the chamber grafting assay. We performed chamber grafting assays, containing E14.5, E17.5, or P5 fibroblasts. Our results revealed that E14.5 and P5 fibroblasts produced new follicles, while E17.5 fibroblasts did not promote follicle formation, while E14.5 skin lacked the ability to form hair de novo, contradicting the original hypothesis. Our study shows that there are two critical developmental stages of dermal fibroblasts. The early embryonic stage is undifferentiated and uncommitted fibroblasts that require the embryonic environment to fully mature. The uncommitted state of dermal fibroblasts is undifferentiated and uncommitted. Further work is now required to understand the mechanisms of fibroblast differentiation and defined transcriptional networks underlying MC differentiation, including those which are recapitulated in the lineage specification of MCC.

1391
Immune cells may have a role in hair loss process caused by TRPV3 gain-of-function mutation
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TRPV3, a member of the transient receptor potential (TRP) family, is a non-selective cation channel. Gain-of-function mutated protein, such as TRPV3G568V, is constitutively open. TRPV3G568V mice show not only periodical hair loss and shortened telogen, but also thickened interfollicular epidermis. On the other hand, immune cells can affect both hair follicle morphogenesis and hair follicle proliferation. TRPV3G568V mice show not only periodical hair loss and shortened telogen, but also thickened interfollicular epidermis. On the other hand, immune cells can affect both hair follicle morphogenesis and hair follicle proliferation. Insulin-like growth factor 1 (IGF-1) signaling through the IGF-1 receptor (IGF-1R) may contribute to hair growth. We tested the hypothesis that undifferentiated embryonic fibroblasts (E14.5) possess an enhanced ability to support de novo hair follicle formation, utilizing the chamber grafting assay. We performed chamber grafting assays, containing E14.5, E17.5, or P5 fibroblasts. Our results revealed that E14.5 and P5 fibroblasts produced new follicles, while E17.5 fibroblasts did not promote follicle formation, while E14.5 skin lacked the ability to form hair de novo, contradicting the original hypothesis. Our study shows that there are two critical developmental stages of dermal fibroblasts. The early embryonic stage is undifferentiated and uncommitted fibroblasts that require the embryonic environment to fully mature. The uncommitted state of dermal fibroblasts is undifferentiated and uncommitted. Further work is now required to understand the mechanisms of fibroblast differentiation, which can be harnessed to induce regenerative pathways in adult injured skin.

1392
Activation of id2 gene regulatory network rules quiescence of hair follicle stem cells
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Previously, we revealed how the underlying molecular mechanism of Bone Morphogenetic Protein (BMP) signaling governs the homeostasis of hair follicle stem cells (hFSCs) in vivo. Here, we focused on testing role of id2 gene, one of the target genes which we identified to be downregulated in HS25 after inhibition of BMP pathway. To test the function of id2 gene in HS25 in vivo we used id2 gain of function approach (GOF) by generating transgenic mouse line. Our data demonstrated that id2 overexpression in HS25 results in prolonged telogen and a delay in anagen activation, maintaining stem cells quiescence. Further, we performed FACS sorting and RNA-seq analysis of GOF id2 HS25 at first postnatal cycle. By comparison with common signature genes of the undifferentiated hFSCs we demonstrated that approximately 10% of the genes were affected by overexpression of id2 in HS25. Interestingly approximately half of those genes overlapped with common signature genes which we previously published to be affected in the pathway thus working synergistically with that pathway. On the other hand, our data also suggest that although id2 is a direct target and an effector of BMP pathway in hFSCs, second half of those genes work at least partially independent from BMP signaling on gene regulatory network of quiescent hFSC.

1393
Lower proximal cup cells but not bulge stem cells regenerate hair follicles after chemotherapeutic injury
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Hair follicle formation during skin development requires interactions between epithelial progenitors and a progressively differentiating dermal fibroblast population. Heterogenous fibroblasts originate from a homogenous mesenchyme and become lineage-committed after E16.5 days. Hair follicle formation begins at E14.5, which is prior to fibroblast lineage commitment, suggesting that this time point holds the highest potential for fibroblasts to become dermal papilla. We tested the hypothesis that undifferentiated embryonic fibroblasts (E14.5) possess an enhanced ability to support de novo hair follicle formation, utilizing the chamber grafting assay. We performed chamber grafting assays, containing E14.5, E17.5, or P5 fibroblasts. Our results revealed that E14.5 and P5 fibroblasts produced new follicles, while E17.5 fibroblasts did not promote follicle formation, while E14.5 skin lacked the ability to form hair de novo, contradicting the original hypothesis. Our study shows that there are two critical developmental stages of dermal fibroblasts. The early embryonic stage is undifferentiated and uncommitted fibroblasts that require the embryonic environment to fully mature. The uncommitted state of dermal fibroblasts is undifferentiated and uncommitted. Further work is now required to understand the mechanisms of fibroblast differentiation, which can be harnessed to induce regenerative pathways in adult injured skin.

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Hair-follicle-associated pluripotent (HAP) stem cells captured on polyvinylidene fluoride membranes promote functional recovery of spinal cord injuries

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Hair follicle-associated pluripotent (HAP) stem cells are located in the bulge area of the hair follicle, express the stem-cell marker nestin, and have been shown to differentiate to neurons, glial cells, keratinocytes, smooth muscle cells, melanocytes and cardiac muscle cells. Transplanted HAP stem cells promoted the recovery of peripheral nerve and spinal cord injuries and have the potential for heart regeneration as well. In the present study, we implanted mouse green fluorescent protein (GFP)-expressing HAP stem cells spheres captured on polyvinylidene fluoride membranes (PVDF) into the severely thoracic spinal cord of nude mice. Eight weeks after implantation, immunofluorescence staining showed that HAP stem cells differentiated into neurons and glial cells. Fluorescence microscopy showed that the GFP-expressing HAP stem cell spheres were easily accessible from everyone, do not form tumors, and can be cryopreserved without loss of differentiation potential. These results suggest that HAP stem cells may have greater potential than iPSC or ES cells for regenerative medicine, such as for spinal cord repair.

Impaired Aquaporin-3 protein expression in advanced glycation end products-leading to impaired wound healing in diabetes

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While oral wound healing has been considered an ideal system of wound resolution, the specific molecular events that differentiate oral wound healing are poorly understood in humans. Our objective was to define the mechanisms that drive healing in both oral and skin compartments, and how the results could be translated to improve wound healing. A human clinical study showed oral wound resolved faster compared to skin wound. RNA-sequencing, Gene Ontology, IPA and histological analysis using paired human oral and skin samples provided strong evidence for a novel skin repair pathway that involves cell adhesion and inflammatory processes, especially keratinization, epidermal cell differentiation, responses to biotic stimuli and inflammation. We identified a unique expression of the SOX2 and PITX1 transcriptional regulators that confer a specific identity on oral keratinocytes. In vitro, SOX2 and PITX1 had the potential of reprogramming skin keratinocytes to acquire increased cell migration capability and improved wound resolution. Lastly, skin wound healing was promoted in SOX2 overexpressing mouse (K14creERT2/+;SOX2tTA/+). We revealed a natural combination of human clinical data, histological and gene expression analysis, and mouse wound healing data. This information has been pivotal in determining the molecular anatomy of the wound healing processes in oral and skin epithelia.

Tissue Regeneration and Wound Healing | ABSTRACTS

Impact of ozone pollution on main repair mechanisms (autophagy & circadian rhythm)

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Ozone pollution at ground level is a growing worldwide environmental problem. Increased air pollution, UV exposure, and elevated temperatures all contribute to the formation of ozone (O3). Many studies, including those by the U.S. Environmental Protection Agency, have shown that average levels in places such as Los Angeles can reach concentrations greater than 0.4 ppm (the standard set level by the US-EPA is currently at 0.075 ppm averaged over 8 hours). It has been proven that it creates oxidative damages on lipids from the epidermis but we also wanted to evaluate the impact on main repair mechanisms such as circadian rhythm and autophagy. In order to determine the effect of ozone pollution on skin cells, we exposed them at levels measured in big cities and we evaluated per 1 clik gene expression as well as LC1B immunostaining for autophagy. For ozone exposure, we are using a custom-designed ozone chamber. Using this chamber, we are capable of achieving low, environmentally relevant levels of ozone (between 0.3 and 0.8 ppm). Additionally, it has a rotating table at a 45° angle which allows mixing and an even exposure to samples in Petri dishes. For the first time, we show that ozone pollution has a significant effect on level of per-1 and autophagy, decreasing both of them in skin cells. This impact at the core cellular machinery will result in a loss of repair and accumulation of damage in skin cells, which over time will accelerate aging.

Blue light disrupts circadian rhythm at night following direct effects on skin cells

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Since entering the industrialized age, we have introduced artificial lights in our life, this is called Light Pollution. Artificial light has been created in order to lengthen the days either for work or for our modern life. This causes a loss of the normal evening/night reduction in light pollution and this may be key to both cattle and sheep and other factors impacting our natural circadian rhythm. Our biological rhythms have been found to be dependent on us being exposed to daylight and darkness and the disruption of this rhythm by light pollution has been shown to have profound health effects. Entering the 21st century, light pollution has continued to increase with the rapid expansion of personal electronic devices, which are a potent source of blue light, the strongest signal for us to stay awake and alert, and not to fall asleep. The skin, as the largest organ in our body, is directly exposed to environmental light and serves as a key sensor and a protective barrier. Each skin cell contains clock genes that control and synchronize cellular activities for optimal benefits to the skin. During the day most cellular activities are used to protect the skin, while during the night they are used to repair, repair and regenerate the tissue. When skin cells become out-of-sync they lose some ability to repair and accumulate more damages. It is critical to continually restore this cellular synchronization in order to keep skin healthy and beautiful. Over the past few years we have been looking for the different factors that push skin cells out-of-sync and more specifically in light pollution, in particular the influence of blue light. Our results show that skin cells can see and are very sensitive to blue light, which has a direct impact on Per-1 clock gene level as well as free radical production. This, in turn, results in a skin desynchronization with a loss of circadian rhythm and acceleration of skin aging.

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1402 Activation of STING signaling accelerates skin wound healing

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Wound healing, a process of repair after skin injury can be divided into three phases: inflammatory phase, proliferative phase, and tissue remodeling. These phases are precisely regulated by a variety of mediators, such as cytokines and chemokines. Inflammatory phase, the first stage of wound healing, especially features an inflammatory reaction via these mediators. Recent reports demonstrated that cytoplasmic DNA-sensor cyclic GMP-AMP synthase (cGAS) activates the stimulator of interferon genes (STING) via production of cyclic GMP-AMP (cGAMP) and subsequently induces inflammatory cytokines including type interferon. In the present study, we examined whether the activation of STING by cGAMP affects the process of skin wound repair. The skin wound repair model was established using wild-type (WT) mice. Two identical full-thickness skin biopsies were taken from the right and left subscapular regions of individual mice. One site was treated with cGAMP and the other site was not treated. The wounds were observed every other day, and changes in wound size over time were calculated using photography. Treatment with cGAMP significantly accelerated wound healing up to day 6. mRNA expression of CXCL10 and CCL2 in the wound sites treated with cGAMP markedly increased compared with control. Scratch assay revealed that cGAMP accelerated wound closure in mouse embryonic fibroblasts. These results suggest that activation of STING pathway is involved in the promotion of wound repair, and the topical use of cGAMP may be useful as an effective treatment for accelerating wound healing.

1404 Modeling diabetic wounds using endothelialized tissue-engineered skin

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Skin wound healing is severely compromised in patients with diabetes and can lead to ulcer formation requiring lower limb amputation. It has been shown that diabetic skin fibroblasts have a differential expression of growth factors, which could impair wound healing by reducing neovascularization by keratinocytes and angiogenesis. Current research on human diabetic fibroblasts and keratinocytes are mostly conducted in two-dimensional monolayer cultures, which do not mimic the structure of the skin and are not representative of in vivo tissues. Our aim was to create a three-dimensional in vitro skin model to understand the role of diabetic fibroblasts and keratinocytes in chronic ulcers. We developed a tissue-engineered model skin with a dermis and an epidermis made with fibroblasts and keratinocytes obtained from healthy or diabetic patients. A wound was created in the epidermis to follow the re-epithelialization process. In addition, endothelial cells were seeded in the dermis to form capillary-like tubes. The capillary network was visualized with Umaris software and secreted matrix protein synthesis, and the appearance of markers of aging. Cellular senescence is an irreversible state of cell cycle arrest that is induced during cellular aging. It is considered as one of the nine hallmarks of aging. The appearance of senescent cells with age and the expression of factors impacting the surrounding environment (inflammatory cytokines, progerin, and senescence-associated fibroblast-like phenotype) have been reported to be involved in the regulation of senescence. In this study, we established 3D reconstructed skin models were developed in order to study cellular senescence and skin aging, based on the information of specific skin aging pathways. Our characterization of the reconstructed skin models was undertaken in order to delineate the consequences of gene silencing, and to point out specific features according to the gene silenced. Consequently, these skin reconstructed models represent a promising tool for investigating in vitro markers of aging following the application of chemical substances to test their properties.
1406 The effects of antimicrobial peptides and hyaluronic acid compound mask on wound healing after ablative fractional carbon dioxide laser resurfacing

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Fractional carbon dioxide laser resurfacing (FXCR) is a routine treatment of Dermatology for the treatment of skin surface damages, especially in patients with a history of acne. However, the effect of fractional carbon dioxide laser resurfacing on the infection rate of wounds remains unknown. The aim of this study was to elucidate the effects of the antimicrobial peptide defensin-1 (D-1) and hyaluronic acid (HA) compound mask on wound healing after FXCR in patients with acne.

1407 Single-cell transcriptomics of human mesenchymal stem cells reveal age-related cellular subpopulation depletion and impaired regenerative gene expression

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Mesenchymal stem cells (MSCs) are heterogeneous and age-related depletion in single cell transcriptomics. In this study, we analyzed the gene expression landscape of human MSCs from 11 donors (aged 24-80 years) using single-cell RNA sequencing (scRNA-seq). We identified 6 major subpopulations with distinct gene expression patterns, including mesenchymal, fibro-proliferative, adipogenic, osteogenic, chondrogenic, and vascular. The mesenchymal subpopulation was significantly depleted in older donors, accompanied by increased expression of fibrosis-related genes.

1408 Scarless healing in adult mice is achieved by delayed collagen network regeneration without myofibroblast activation

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Wound healing involves the coordinated regulation of pro-growth and pro-fibrosis signals, which can be misregulated due to various factors. Myofibroblasts (MFBs) are key players in wound healing, responsible for the contraction of the healing zone and the deposition of extracellular matrix (ECM). However, the role of MFBs in scarless healing remains unclear. In this study, we investigated the role of MFBs in scarless healing in adult mice.

1409 High-throughput drug screening system targeting tissue fibroblasts: An application of PDMS stretch platform

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Keloids are a fibro-proliferative disease of skin with abnormal fibroblast activity. However, the treatment options are limited and current drug development platforms cannot fully reflect mechanosensitive properties of the disease. Here, we established a PDMS-based stretchable culture system to evaluate a drug screening platform. Patient-derived keloid fibroblasts were cultured on the PDMS stretch platform. In response to cyclic stretch, the cellular proliferation reached the optimal activity level. Afterwards, the cells were treated with corticosteroids as a positive control and drugs with anti-fibrosis activity were selected. Expression of fibrosis-related proteins was evaluated.

1410 Non-coding double stranded RNA induces retinoic acid synthesis and retinoid signaling to control regeneration

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Mesenchymal stem cells (MSCs) are versatile regenerative cells that play a crucial role in wound healing and tissue repair. However, the mechanisms underlying the regenerative potential of MSCs remain unclear. In this study, we investigated the role of double-stranded RNA (dsRNA) in regulating retinoic acid (RA) synthesis and retinoid signaling in MSCs.

1411 The potential of adipose-derived stem cells for the treatment of recessive dystrophic epidermolysis bullosa

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RDEB is a recessive dystrophic epidermolysis bullosa (RDEB) that is one of the more severe forms of epidermolysis bullosa, which is a genetic skin fragility disorder characterized by blisters and skin erosions in response to minor injury. The pathogenesis of RDEB involves mutations in COL7A1 that encodes type VII collagen (Col7), the main constituent of the anchoring fibrils that anchor the epidermal basement membrane to the papillary dermis. Mesenchymal stem cells (MSCs) are highly regenerative cells that can differentiate into various cell lineages and have the potential to regenerate damaged tissues. In this study, we investigated the potential of MSCs for the treatment of RDEB.

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AMP-IBP5, an antimicrobial peptide derived from insulin-like growth factor-binding protein 5, triggers keratinocyte activation

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Finally, topical application of this active was able to restore the dermal-epidermal junction by proliferation, migration and wound healing. Recently, a novel antimicrobial peptide named AMP-IBP5 (antimicrobial peptide derived from insulin-like growth factor-binding protein 5) was shown to exhibit antimicrobial activity against numerous pathogens, even at extremely low concentrations. The study was conducted on 48 female subjects. The efficacy of a formulated product was evaluated by measuring the expression of dermal matrix genes, which are involved in the remodeling phase of wound healing.

1413

Mesenchymal stem cells sense and shape their environment at the wound site

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Mesenchymal Stem Cells (MSC) are endowed with the capacity to sense environmental cues and to generate an integrated adaptive response. So far it is, however, largely unexplored how MSC sense and translate these cues into a function and activation state of distinct immune cells during tissue injury. Here, we wished to investigate how MSC adaptively regulate neutrophil functions under conditions of wound infection, where MSC suppression of neutrophil functions would be detrimental. We evaluated the adaptive response of adipose derived MSC (ADMSC) on activated neutrophil functions in the presence and absence of pathogen-associated molecular patterns (PAMP; lipopolysaccharide (LPS), mimicking an infectious wound environment. Of note, LPS treated ADMSC substantially augment neutrophil activation resulting in an increased neutrophil extracellular trap (NET) formation and increased ROS production as opposed to MSC suppression of activated neutrophils under non-infected conditions. To further explore whether tol-like receptor-4 (TLR4) present on MSC surface, is involved in the adaptive response of ADMSC, we specifically silenced the TLR4 receptor gene employing specific siRNA. Our results show that TLR4 silenced MSC upon LPS treatment failed to activate neutrophils, subsequent NET formation and ROS production, indicating a causal role for TLR4-dependent sensing. Moreover, RNAseq analysis uncovered GCPII and IL18 as major mediator of adaptive inflammatory switch. Surprisingly, injection of LPS primed ADMSC result in a significant acceleration of wound healing even exceeding that of ADMSC and PBS injected wounds. Our data thus hold substantial promise to further refine MSC based therapies for patients with difficult-to-treat and/or infected wounds.

1416

Effects of red-wavelengths OLED and its in vitro differential cell effects

JN a1, H Choi1, J Shin1, Y Jeon2, S Choi2, K Park1 and K Choi2

A new passion fruit extract promoting skin wound healing process

JN a1, H Choi1, J Shin1, Y Jeon2, S Choi2, K Park1 and K Choi2

The study was performed on 2 group of 24 female subjects. The efficacy of a formulated product was evaluated by measuring the expression of dermal matrix genes, which are involved in the remodeling phase of wound healing.

1417

A soft, flexible, battery-less, and wearable pressure sensor with wireless communication for therapeutic compression garments: Bench validation and preliminary in vivo testing

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Venous leg ulcers affect >600,000 Americans costing $2 billion USDs yearly costs. Compression stockings and bandages are the standard of care in speeding healing rate. However, this therapy requires a sub-gauge pressure of at least 30-40 mmHg. Several medical associations have echoed the need for sensors capable of accurately, and repeatedly measuring interface pressure. PicoPress® (Microhaptics, Parka, Italy) is the gold-standard. While accurate and reliable, it is large and relatively expensive ($>2,000 USDs). Using advanced materials science approaches, we report a flexible, battery-less, and wearable pressure sensor capable of wireless communication with any standard smartphone adhered between the human leg and compression garments. We fully encapsulate a piezoresistive sensor (Flexiforce 401, Tekscan, Boston, MA) underling circuitry, and a NFC chip connected with serpentine copper interconnects to enable stretching and bending within a soft silicon elastomer. The 1.7 mm thick, 2 cm radius sensor (r2 cm) is connected to enable stretching and bending within a soft silicone elastomer. The 1.7 mm thick, 2 cm radius sensor (r2 cm) is connected to a flexible red-wavelengths Organic Light-Emitting Diode (OLED) light source which can be attached to the human body. It will provide practical performance (<10mW/cm² even at low voltage (< 1V)). Compared to LED platform, OLED platform can provide relatively pure wavelengths. Although there are numerous possible applications, a wound would be a good indication for this new therapy. The effects of OLED pressure therapy was investigated using scratch-wound healing assay model of cultured normal human fibroblasts. Our results showed that red-wavelengths OLED (630, 650, 670, 690 nm) have each excellent in vitro wound healing effects; they effectively stimulate fibroblast proliferation and migration.

1418

Induction of human hair growth using vascularized 3D hair follicle constructs

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Mesenchymal stem cells sense and shape their environment at the wound site

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Mesenchymal Stem Cells (MSC) are endowed with the capacity to sense environmental cues and to generate an integrated adaptive response. So far it is, however, largely unexplored how MSC sense and translate these cues into a function and activation state of distinct immune cells during tissue injury. Here, we wished to investigate how MSC adaptively regulate neutrophil functions under conditions of wound infection, where MSC suppression of neutrophil functions would be detrimental. We evaluated the adaptive response of adipose derived MSC (ADMSC) on activated neutrophil functions in the presence and absence of pathogen-associated molecular patterns (PAMP; lipopolysaccharide (LPS), mimicking an infectious wound environment. Of note, LPS treated ADMSC substantially augment neutrophil activation resulting in an increased neutrophil extracellular trap (NET) formation and increased ROS production as opposed to MSC suppression of activated neutrophils under non-infected conditions. To further explore whether tol-like receptor-4 (TLR4) present on MSC surface, is involved in the adaptive response of ADMSC, we specifically silenced the TLR4 receptor gene employing specific siRNA. Our results show that TLR4 silenced MSC upon LPS treatment failed to activate neutrophils, subsequent NET formation and ROS production, indicating a causal role for TLR4-dependent sensing. Moreover, RNAseq analysis uncovered GCPII and IL18 as major mediator of adaptive inflammatory switch. Surprisingly, injection of LPS primed ADMSC result in a significant acceleration of wound healing even exceeding that of ADMSC and PBS injected wounds. Our data thus hold substantial promise to further refine MSC based therapies for patients with difficult-to-treat and/or infected wounds.

1416

Effects of red-wavelengths OLED and its in vitro differential cell effects

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A new passion fruit extract promoting skin wound healing process

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The study was performed on 2 group of 24 female subjects. The efficacy of a formulated product was evaluated by measuring the expression of dermal matrix genes, which are involved in the remodeling phase of wound healing.

1417

A soft, flexible, battery-less, and wearable pressure sensor with wireless communication for therapeutic compression garments: Bench validation and preliminary in vivo testing

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Venous leg ulcers affect >600,000 Americans costing $2 billion USDs yearly costs. Compression stockings and bandages are the standard of care in speeding healing rate. However, this therapy requires a sub-gauge pressure of at least 30-40 mmHg. Several medical associations have echoed the need for sensors capable of accurately, and repeatedly measuring interface pressure. PicoPress® (Microhaptics, Parka, Italy) is the gold-standard. While accurate and reliable, it is large and relatively expensive ($>2,000 USDs). Using advanced materials science approaches, we report a flexible, battery-less, and wearable pressure sensor capable of wireless communication with any standard smartphone adhered between the human leg and compression garments. We fully encapsulate a piezoresistive sensor (Flexiforce 401, Tekscan, Boston, MA) underling circuitry, and a NFC chip connected with serpentine copper interconnects to enable stretching and bending within a soft silicon elastomer. The 1.7 mm thick, 2 cm radius sensor (r2 cm) is connected to enable stretching and bending within a soft silicone elastomer. The 1.7 mm thick, 2 cm radius sensor (r2 cm) is connected to a flexible red-wavelengths Organic Light-Emitting Diode (OLED) light source which can be attached to the human body. It will provide practical performance (<10mW/cm² even at low voltage (< 1V)). Compared to LED platform, OLED platform can provide relatively pure wavelengths. Although there are numerous possible applications, a wound would be a good indication for this new therapy. The effects of OLED pressure therapy was investigated using scratch-wound healing assay model of cultured normal human fibroblasts. Our results showed that red-wavelengths OLED (630, 650, 670, 690 nm) have each excellent in vitro wound healing effects; they effectively stimulate fibroblast proliferation and migration. Interestingly, there are relatively large differences according to the wavelength used. Among the wavelengths tested, 650nm and 670 nm induced rapid proliferation of fibroblasts, indicating these wavelengths can induce quick dermal repair during the wound healing process. Waveslengths of 630 and 650 nm induced more migration at low energy levels while wavelengths of 670 nm and 690nm induced more migration at high energy levels. These findings indicate that different wavelengths may show different biologic response according to the energy level. In this study, we only tested one energy level. Future study needs to test different energy levels. The OLED system can be used in various therapeutic applications in the future, including medical and cosmetic procedures.
1419 Wound regeneration deficit in rats correlates with low morphogenetic potential and distinct transcriptional profile of epidermis.

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Long-term diabetes often leads to chronic wounds refractory to usual treatments, thus representing a major challenge for the healthcare system. Cell-based therapies are actively investigated to enhance wound repair. Various cell types can be used to produce biological dressings that can be applied on wounds. Adipose-derived stem cells (ASC) are an attractive cell source considering their abundance and therapeutic properties. In this study, ASC-based dressings improved global skin healing in a diabetic murine model. The entire dressing protocol was realized under a clinically-compatible serum-free system allowing the production of naturally derived scaffold-free ASC-based biological dressings facilitating clinical translation. These new tissue-engineered dressings represent promising candidates for promoting diabetic cutaneous healing in vivo, by stimulating, among others, granulation tissue formation and neovascularization.

1420 Regulation of collective cell migration by hemidesmosomal proteins

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Repair of skin wounds is an efficient directed migration of a sheet of epidermal cells over the wound bed. This involves collective cell migration (CCM) where the leader keratinocytes at the migrating front regulate the motility of follower cells. The role of hemidesmosome proteins in both the motility of single and groups of keratinocytes is controversial. Some data indicate that hemidesmosome proteins retard migration while others suggest that hemidesmosome proteins regulate directed migration. In the current study, we evaluated the role of the hemidesmosome protein BPAG1e, a cytolinker involved in tethering the cytoskeleton to the cell surface, in CCM. BPAG1e-deficient keratinocytes closed wounds in vitro significantly slower than their BPAG1e-expressing counterparts. To assay the role of BPAG1e in leader cells, we utilized a hybrid sheet assay. This assay allowed us to generate wounded, mixed sheets of keratinocytes in which leader cells expressed BPAG1e while followers were BPAG1e-deficient and vice versa. In addition, we also assayed wound healing in hybrid sheets where the sheet was composed of BPAG1e-deficient keratinocytes but the leader cells were induced to express constitutively active Rac1, a small GTPase known to be involved in regulating directed migration. Our results indicated that the CCM of a hybrid sheet in which leader cells were deficient in BPAG1e is impeded when compared to a sheet composed of control (BPAG1e expressing) cells, regardless of whether follower cells express or are deficient in BPAG1e. In contrast, CCM was not impacted when leader cells were either wild-type for BPAG1e or were BPAG1e deficient but expressed active Rac1, even when follower cells were BPAG1e-deficient. In summary, our results implicate BPAG1e as a scaffolding protein that controls signaling required for leader cell-regulated CCM during wound repair.

1421 Topical glucosylceramide synthase inhibitor speeds wound healing in diabetic mice

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Non-healing foot ulcers develop in 10-25% of type 2 diabetics. We have previously shown that reduction in ganglioside GM3 by GM3 synthase (GM3S) gene knockdown or topically applied GM3S siRNA nanoconstructs normalizes wound healing in diet-induced obese (DIO) diabetic mice. The mechanism is reversal of Gfi-1 and Gfi receptor resistance, leading to accelerated keratinocyte migration and normal cutaneous innervation. A small molecule inhibitor of glucosylceramide synthase (GCSi) also reduces GM3 and, given GCSi small size, could be moved towards clinical trials of topical application should it replicate the ameliorative effects on wound healing. We generated wildtype and, as a positive control, GM3S knockout mice with red fluorescent sensory nerves (Nav1.8 cre-tb). Mice were fed a regular or a high fat diet (HFD) for 10 weeks. Wound healing was assessed by glucose tolerance testing (mean vehicle-treated HFD mice 505.7 mEq/L; mean GCSi-treated HFD mice 468.6 mEq/L; p<0.05; regular diet, 133.4 mEq/L; p<0.0001 vs both HFD mice). Six mm back wounds were made and splinted to reduce healing by contracture. GCSi in Aquepap® 1.1% (final concentration 10μM) was applied topically every other day, with PBS/Aquepap® 1.1% as a negative control. By 7 days after wounding and treatment initiation, GCSi-treated HFD mice, in comparison with vehicle-treated HFD mice, had a smaller open wound area (25.9±3.8% vs. 39.2±2.8%, p<0.001) and epidermal gap (1.7±0.3mm vs. 3.8±0.4mm, p<0.001), and a two-fold greater granulation tissue area (p<0.05), with wound healing similar to that of GM3S knockout HFD and regular diet-fed mice. Assessment of wound area innervation is ongoing. Topically applied GCSi is a novel small molecule therapeutic strategy to accelerate diabetic wound healing through GM3 depletion.

1422 A novel S100A8/A9 induced fingerprint of mesenchymal stem cells is associated with enhanced wound healing

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We here investigated a unique capacity of mesenchymal stem cells (MSCs) to re-establish tissue homoeostasis using their premonitory potential to sense danger associated molecular patterns (DAMP) and to mount an adaptive response in the interest of tissue repair. Injection of MSCs pretreated with heterodimeric DAMP protein S100A8/A9 into murine full-thickness wounds, led to a significant acceleration of healing even exceeding that of wounds with non-treated MSCs. This requires a fundamental reprogramming of the transmembrane S100A8/A9 treated MSCs as deduced from in-depth validation via global RNAseq, RT-PCR, and immunostaining. We uncovered a network of genes/proteins involved in proteolysis, enhancing macrophage phagocytosis and controlling inflammation, all contributing to pro-

1423 The role of integrin-dependent paracrine signaling from keratinocytes in regulating myofibroblast differentiation

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Wound keratinocytes restore the epidermal barrier and also secrete paracrine factors that regulate wound cell functions. Dysregulation of this paracrine signaling can contribute to a spectrum of pathologies. Previously, we established that keratinocyte integrin α1 mediates paracrine induction of fibroblast Cox-2. Also, MKα1-dependent effect. We hypothesize that integrin-dependent paracrine signaling from keratinocytes plays a similar role in regulating differentiation of myofibroblasts that deposit and contract ECM during scarring and fibrosis. Recently we determined that conditioned medium (CM) from z1-expressing mouse keratinocytes (MKα1+ cells) induces expression of fibroblast cyclooxygenase 2 (COX-2). Knockdown of fibroblast COX-2 reduces secretion of prostaglandin E2 (PGE2), a COX-2 product that is known to promote myofibroblast phenotype. z1 integrin induces matrix metalloproteinase 9 (MMP-9) in MK cells, and inhibition of MMP-9 suppresses MKα1+ CM-mediated induction of COX-2 expression in fibroblasts. Affinity depletion of MMP-2/9 in MKα1+ CM or knockdown of MMP-9 in MKα1+ cells attenuates z1-integrin-mediated paracrine induction of fibroblast COX-2. Also, MKα2+/+ cells secrete more interleukin-1α (IL-1α) and less IL-1α, a natural IL-1 receptor antagonist, than MKα1+ cells. Treatment with active recombinant IL-1α is sufficient to induce fibroblast COX-2, and recombinant IL-1α inhibits the ability of MKα1+ CM to induce COX-2. Importantly, CM from MK cells that express both z1 and α1 and z1+ fibroblasts to a lesser degree than MKα1+ CM, consistent with our hypothesis that α1 suppresses the paracrine signaling controlled by α1. Our findings suggest that keratinocyte integrin α1 mediated paracrine signaling from keratinocytes regulates myofibroblast differentiation and function via a mechanism that involves integrin α1 and α1.
1424
Mapping the establishment of fibroblast heterogeneity during skin development and wound repair
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The process of wound healing is complex and involves coordination of multiple cell types required to re-establish the 3D architecture of skin. In adults, upon completion of normal wound healing, a scar is formed, which is composed of fibrous matrix generated by fibroblasts, and the healed skin lacks hair follicles. Skin fibroblasts arise from a homogenous population during early embryonic development but differentiate into heterogeneous populations that have non-overlapping functions in development, skin maturation and wound healing. In mouse skin, we have identified two fibroblast subsets. Reticular fibroblasts construct the dermal architecture of the skin by secreting matrix and differentiating into adipocytes. Importantly, reticular fibroblasts are lineage restricted and do not contribute to hair follicle formation. Rather, a second fibroblast subset, consisting of papillary fibroblasts is central to hair follicle formation by differentiating into dermal papilla, the dermal sheath, and arrector pili. We have found that adult skin scarring is the result of tissue repair that utilizes a single type of fibroblast, restricting the development of heterogeneous fibroblast populations. To understanding how to control fibroblast heterogeneity in wounds we are mapping all fibroblast populations during skin development. In this regard, we have generated large single-cell-RNA-sequencing datasets consisting of tens of thousands of cells each from embryonic day 14.5, 17.5 and post-natal day 2, 5, and 21, which are key developmental times for the establishment of fibroblast heterogeneity. This has allowed us to map, using computational biology, the establishment of fibroblast heterogeneity. Our analyses have revealed that the canonical Wnt transcription factors, Lef1/Tcf1, 3, 4 are key regulators of fibroblast heterogeneity. In summary, we have developed invaluable datasets which will be an essential resource for defining factors that regulate fibroblast heterogeneity during development to investigate during wound healing.

1426
Beta adrenergic antagonist for the healing of chronic diabetic foot ulcers
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Diabetic foot ulcers (DFU) account for significant morbidity and immense biomedical burden. The long-term effectiveness of advanced therapies has yet to be determined. Our hypothesis is that timolol, a non-selective beta adrenergic antagonist (IβAIA) will be effective in achieving wound closure compared to standard of care (SOC) treatment for DFU, and is a safe therapeutic alternative. We will conduct a prospective, randomized, double-blind, controlled and parallel-group trial with 2 arms: SOC plus topical timolol and SOC plus a non-historically active gel (hydrogel) as placebo control. This study is funded by a VA Merit Award #9J44942 (MPs Dhale, and Isseroff). The primary outcome is the percentage of subjects achieving wound closure (complete skin re-epithelialization) at 12 weeks. The secondary outcomes will be change of timolol, which will be determined by measuring timolol serum levels during the treatment phase and the rate of adverse events. Veterans with DFU meeting enrollment criteria will be selected from the VA Northern California Health Care System. We will require 69 subjects enrolled in each treatment arm, for a total of 138 sample size to provide a power of 80% to detect a 43% difference, with 63% healing in the timolol group and 20% with the standard of care, at a significance level 0.05 and an overall attrition will be 35%. After a 2-week Screening Phase, subjects will be electronically randomized by simple allocation. During the Active Phase, subjects will be randomly assigned to either the treatment medication or the placebo on the DFU for 12 weeks, or until healed with 2 confirmatory visit one week apart. Then subjects, will enter the Follow-Up Phase with a one-month, then a 3-month follow-up, for a maximum study duration of 31 weeks. We anticipate to begin enrollment within the next 6 months.

1427
How do hibernating bears stay safe from pressure ulcers? A Nason1 Graduate School of Veterinary Medicine, Sapporo, Hokkaido, Japan

Background: Pressure ulcers (PU) result from disturbances in blood supply to the epidermis due to ischemia and necrosis of the areas under pressure. PU can lead to secondary infection and pressure ulcers are one of the factors leading to ischemia and necrosis of the areas under pressure. PU results from ischemia and necrosis of the areas under pressure by parabiosis counteracted this regenerative ability. Compared to aged mice, injured young mice skin expressed more stromal-derived-factor-1 (SDF1), a secreted cytokine. Deletion of SDF1 in young skin enhanced in vivo tissue regeneration, and parabiosis with aged mice confirmed that skin-secreted SDF1 in young blood promoted scarring. Aged mice recruited more enhancer of zeste homologue 2 (EZH2) and histone H3 lysine 27 trimethylation towards the SDF1 promoter; EZH2 inhibition restored SDF1 induction and prevented tissue regeneration. SDF1 induces leukocytosis via the CXCR4 receptor. Using an unbiased approach, injured young mice retained more CXCR4+ epidermal γ0 T-cells compared to aged mice or young skin-specific SDF1 deficient mice. Young mice deficient in γ0 T-cells demonstrated improved tissue regeneration. Human skin exhibited similar age-dependent SDF1 suppression. Our findings confirm for the first time that tissue function invariably worsens with age and suggest new strategies for tissue regeneration.

1428
Effect of exogenous platelet-derived growth factor on the proliferation of human fibroblasts in the presence of human wound exudates
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Wound exudates are considered liquid biopsies of wounds and contain the pathogenic drivers required to regenerate the 3D architecture of skin. In adults, upon completion of normal wound healing, a scar is formed, which is composed of fibrous matrix generated by fibroblasts, and the healed skin lacks hair follicles. Skin fibroblasts arise from a homogenous population during early embryonic development but differentiate into heterogeneous populations that have non-overlapping functions in development, skin maturation and wound healing. In mouse skin, we have identified two fibroblast subsets. Reticular fibroblasts construct the dermal architecture of the skin by secreting matrix and differentiating into adipocytes. Importantly, reticular fibroblasts are lineage restricted and do not contribute to hair follicle formation. Rather, a second fibroblast subset, consisting of papillary fibroblasts is central to hair follicle formation by differentiating into dermal papilla, the dermal sheath, and arrector pili. We have found that adult skin scarring is the result of tissue repair that utilizes a single type of fibroblast, restricting the development of heterogeneous fibroblast populations. To understanding how to control fibroblast heterogeneity in wounds we are mapping all fibroblast populations during skin development. In this regard, we have generated large single-cell-RNA-sequencing datasets consisting of tens of thousands of cells each from embryonic day 14.5, 17.5 and post-natal day 2, 5, and 21, which are key developmental times for the establishment of fibroblast heterogeneity. This has allowed us to map, using computational biology, the establishment of fibroblast heterogeneity. Our analyses have revealed that the canonical Wnt transcription factors, Lef1/Tcf1, 3, 4 are key regulators of fibroblast heterogeneity. In summary, we have developed invaluable datasets which will be an essential resource for defining factors that regulate fibroblast heterogeneity during development to investigate during wound healing.

1429
VEGF mediates resolution of aged human skin xenografts in young mice
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Previously we have shown that transplantation of aged human skin onto young SCID mice results in a rejuvenated human epidermal phenotype. Here, we demonstrate that the rejuvenated aged human skin xenotransplants onto young SCID/beige mouse skin well extends beyond the epidermis and leads to statistically significant improvements in multiple skin aging-associated read-outs assessed by quantitative histomorphometry, which showed e.g. thickening of the epidermis, along with basal layer keratinocyte proliferation, increased vasculature, significantly more collagen type I and III and elastic fibers, and number of epidermal melanocytes in aged human skin xenotransplants. Gene expression RNA-Seq analyses of DEGs showed in 1week versus pre-transplant numerous transcripts related to hypoxia, VEGF and angiogenesis, inflammation, the Notch, TGF-β and IGF pathways, genes related to reninang, as well as keratin and epidermal regulators. qRT-PCR confirmed an upregulation of VEGFA and HIF1A, as well as angiogenesis-related genes. Promflammatory infiltrates expressing CXCL1 and CXCL5 were also confirmed by these data. Skin rejuvenation was primarily mediated by the angiogenesis-promoting growth factor, VEGF, since injection of anti-VEGF blocking antibodies alone, but not of TGF-β, IGF-1 or HGF-neutralizing antibodies, sufficed to prevent the rejuvenation of old human skin in vivo. Concomitantly, intra-dermal injection of VEGF-leaked antibodies prevented skin rejuvenation when aged human skin was grafted onto old mice. Our study shows that a remarkable degree of rejuvenation of aged human skin is possible in vivo and that VEGF-mediated signaling is a key pathway in this process.
1430
Modeling the onset of senescence associated secretory phenotype predicts therapeutic targets
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During cellular senescence cells undergo permanent cell cycle arrest, and this protects from malignant transformation. Though important to control cancerous neoplasia, the same mechanisms may fundamentally drive aging and age-related disorders. Senescent cells in many instances release a battery of soluble factors, collectively termed as senescence associated secretory phenotype (SASP). Depending on histogenetic origin of cells, SASP can vary in components and composition. Of note, SASP spreads senescence within tissues and organs and promotes several age associated disorders, including skin aging and impairment of chronic wound healing. Employing already published gene interaction data, we developed a Boolean network-based model of network with the aim to inhibit SASP during DNA damage induced senescence. This simulation allowed us to predict different in-silico gene knockouts those can prevent damaging effects of key SASP-factors. The most promising in-silico knockout candidates originating from this simulation was NF-kB Essential Modulator (NEMO) or IκKβ. Using in vitro experiments in murine dermal fibroblasts, we confirmed the importance of NEMO to successfully inhibit key SASP following DNA damage. Therefore, we experimentally strengthened the speculated regulatory role of NF-kB signaling in the onset as well as maintenance of SASP using in-silico and in-vivo approaches. These data, thus, give access to potential therapeutic targets for SASP-associated aging diseases.

1432
Mechanosensitive lymphocytes potentiate wound repair by regulating inflammation and extracellular matrix
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Background: Adaptive immune responses play a significant role in mediating tissue repair. Hyaluronan (HA), a major extracellular matrix component in skin, can influence the stiffness of the tissue and thus influence T cell activation. However, the mechanism of action on lymphocytes is unclear. We hypothesize that lymphocytes are mechanosensitive and help govern fibrosis and wound healing. Methods: First, we tested endogenous lymphocytes response to tension in murine skin, and the response of human lymphocytes to hydrogels of varied stiffness. Data was analyzed by immunohistochemistry and qPCR array. Next, 6mm stented wounds were created on SCID mice, which lack functional T/B lymphocytes. Wounds were exposed to particular lymphocyte subsets by adoptive transfer expressing (total lymphocytes (TC), non-CD4+ lymphocytes, or (3) CD4+ lymphocytes. Wound tissues were harvested at day7,14,30 and analyzed for wound closure imaging, healing outcome (H&E), inflammation (CD45+ and F4/80+ cells) and fibrosis (trichrome; α-SMA). Data mean±SD, p-values by ANOVA and t-test. Results: Tension increased T lymphocyte numbers at d4 (30±73% vs 42±4.2±52%) in murine skin. Gene expression patterns of human lymphocytes showed dramatic changes in stiffer hydrogels, including >800-fold increase of CXCL5, and decreased neutrophil and increased monocyte and macrophage infiltrates in non-diabetic mice and decreased neutrophil extracellular traps (NETs) in diabetic mice. Similar therapeutic efficacy was achieved with an active vaccine targeting AT. Taken together, neutralization of AT had a therapeutic effect against S. aureus-infected wounds in both non-diabetic and diabetic mice that was associated with differential effects on the host immune response.

1433
Role of BNC1 in keratinocytes proliferation and migration: A critical regulator of wound healing?
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Basoncin 1 (BNC1) is a transcription factor primarily expressed in keratinocytes. BNC1 levels are correlated with proliferative potential of keratinocytes, but the mechanisms of cell cycle control are undefined. Physically, BNC1 knock-out mice exhibit thinner epithelial tissues and have defects in chronic wound healing. BNC1 can promote the expression of ribosomal DNA genes as well as a small number of RNA polymerase II-regulated promoters, however, it is unclear if this accounts for the phenotypes observed or whether BNC1 directly controls transcriptional programs involved in proliferation and migration. In order to better understand the physiological functions of BNC1 in the skin, we performed RNA-seq analysis to identify differentially expressed genes in human primary keratinocytes. This identified a DNA control or activated by BNC1. We isolated 4585 differentially expressed genes (Benjamini-Hochberg adjusted p value <0.05, Log2 Fold Change > 0.5 or < 0.5). Gene ontology analysis revealed that cell cycle/proliferation and focal adhesion/cell migration were the two most enriched pathways in our dataset. Interestingly, many desmosomal genes (PERP, DSG1, DSG1, PDL) are elevated whereas expression of hemidesmosomal genes (PLEC, ITGB4, ITGB6) decreases following reduction of BNC1 levels. This expression profile has been reported to allow cells to detach from the basement membrane, by disrupting the hemidesmosomes, while increasing desmosomal cell-cell junctions to allow the cells to move collectively. We experimentally confirmed that BNC1 knock-down dramatically reduces cell proliferation, while affecting cell migration. Altogether, these data suggest that BNC1 coordinates the re-epithelialization phase of wound healing through specific regulation of key transcriptional modules essential for proliferation and migration. Elucidation of this mechanism will have important implications in understanding pathologic wound healing, such as chronic ulceration, in which re-epithelialization does not occur properly.

1434
Role of fibroblast DPP4 in wound healing, scarring and regeneration
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Wounding results in inflammation, synthesis and remodeling of granulation tissue with extracellular matrix deposition and scar-formation which might reduce function of affected tissues and have defects in corneal wound healing. BNC1 has been shown to promote fibrosis and wound healing. Employing already published gene interaction data, we developed a Boolean network-based model with the aim to inhibit SASP during DNA damage induced senescence. This simulation allowed us to predict different in-silico gene knockouts those can prevent damaging effects of key SASP-factors. The most promising in-silico knockout candidates originating from this simulation was NF-kB Essential Modulator (NEMO) or IκKβ. Using in vitro experiments in murine dermal fibroblasts, we confirmed the importance of NEMO to successfully inhibit key SASP following DNA damage. Therefore, we experimentally strengthened the speculated regulatory role of NF-kB signaling in the onset as well as maintenance of SASP using in-silico and in-vivo approaches. These data, thus, give access to potential therapeutic targets for SASP-associated aging diseases.

1435
Dynamic morphogen-p63 chromatin interactions that guide epidermic changes and p63 activity in surface ectoderm commitment
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Dynamic reprogramming with personalized tissue manufacturing represents a curative approach to treating skin diseases like epidermolysis bullosa (EB), yet a major roadblock is a complete understanding of the mechanisms driving fate commitment by the master regulator p63. Using a defined in vitro differentiation protocol that produces keratinocytes upon addition of the morphogens RA and BMP and gain-of-function of p63 mutant cells, we investigate the transcriptional and chromatin dynamics governing fate commitment through a multi-dimensional genomics approach. We find overexpression of p63 without morphogen results in few defined changes despite binding to identical sites, demonstrating its dependence on morphogenetic signals. RNAseq, ATACseq, ChIPseq, and cohesin H3ChIP during surface ectoderm commitment demonstrate the changes in the chromatin landscape that occur with the addition of morphogens that allow p63 to properly regulate gene expression. Morphogen-dependent chromatin looping from p63 to chromatin accessible sites correlates with altered gene expression, while looping from p63 to TSSs was necessary but not sufficient for gene expression changes. Next, we identify the distal cis-regulatory element controlling p63 binding of the T-box transcription, a key downstream regulator, and determine splicing and chromatin disruption of a negative feedback loop with p63. Our studies shed light on the complex interplay between morphogens and p63 to provide mechanistic insight into skin cell differentiation that will ultimately allow for improved stem cell therapies in the treatment of monogenic skin diseases like EB.
Release of glutamate promotes cell to cell communication between keratinocytes and sensory neuronal cells under mechanical stimulation

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ABSTRACTS | Tissue Regeneration and Wound Healing

Chondrocyte extract enhances skin rejuvenation by regulation of clock genes expression

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Several physiological functions in the skin, such as the skin surface pH, temperature, capacitance, sebum production and barrier recovery, show diurnal variation. In the previous study, we showed that clock gene plays an important role in skin capacitation and epidermal barrier recovery. On the other hand, it has been reported that the oscillation of clock gene expression is declined by psychological stress, aging, UV rays, light pollution and irregular lifestyle effects such as shift work. expression was suppressed in the UV-irradiated skin. Then, in this study, we performed screening plant extracts to stimulate the oscillation of clock genes expression in order to use in the cosmetic application. We found that chardonnay extract enhanced BMH1 and PER1 gene expression in keratinocytes. And also the expression of intercellular lipid-related genes (ABCA12, HMGC51), filargen and involucrin mRNA are used in keratinocytes. Next the influence of chardonnay extract was investigated on rough skin induced by SDS treatment. Chardonnay extract accelerated epidermal repair and decline of redness, compared with the control. Furthermore, Chardonnay extract induction of COL1A1 mRNA in dermal fibroblast and improved wrinkled formation. Expression of clock genes in dermal fibroblast is currently under investigation. These data indicated that chardonnay extract is a promising cosmetic ingredient to support skin restoration by the enhancement of clock expression genes.

Natural history of bacterial composition in skin wounds and its relationship to healing

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Wound healing is a complex multistep process that involves an inflammatory phase that accompanies the wound exudate stages of healing. Skin barrier rupture results in exposure to bacterial compounds suggesting a key role of the wound microbiome in the healing process. We here aimed to systematically study the healing of skin excisional wounds in 72 different strains of mice from the collaborative cross to evaluate wound healing. In 30 strains, we also evaluated associations between bacterial composition in normal and wounded skin at different time points and healing speed. The healing speed of excisional wounds varied significantly across the 72 strains, with mean regulations of -3.65 (p-value .015), -8.71 (p-value .039), and -2.94 (p-value .002) respectively. These finding suggest that not only ATP but also glutamates act as a mediator and play an important role in nerve to nerve transmission procedure, which is commonly known as the peripheral nervous system.

Regeneration of human epidermis after injury is a key function of progenitor cells. Understanding of decision-making mechanisms for self-renewal and differentiation relies on the comparison of experimental approaches, including surrogate cell culture-based experimental regeneration models for assessment of progenitor cell activation, autonomous growth and spontaneous differentiation at the clonal level. Here we propose a 3D epithelial cell culture model in which differentiation in 3D is spontaneous, implying in self-renewal and spontaneous differentiation of hPDS-induced primary human keratinocytes and commercially available keratinocyte-derived cell line N/TERT1 (in which telomerase is overexpressed), as well as lentiviral-infected N/TERT1-derived cell line (N/TERT1 sh-TERT) in which telomerase has been silenced. These models are expected to provide a tool for the study of human keratinocyte differentiation and provide a tool for the study of human keratinocyte differentiation and spontaneous and induced differentiation. We here aimed to systematically study the healing of skin excisional wounds in 72 different strains of mice from the collaborative cross to evaluate wound healing. In 30 strains, we also evaluated associations between bacterial composition in normal and wounded skin at different time points and healing speed. The healing speed of excisional wounds varied significantly across the 72 strains, with mean regulations of -3.65 (p-value .015), -8.71 (p-value .039), and -2.94 (p-value .002) respectively. These finding suggest that not only ATP but also glutamates act as a mediator and play an important role in nerve to nerve transmission procedure, which is commonly known as the peripheral nervous system.

Preclinical studies have demonstrated the ability of the β-adrenergic antagonist, timolol, to enhance tissue before and after treating three patients with chronic wounds, two with venous ulcers, and one traumatic ulcer, none of which had healed with greater than 12 weeks of standard of care. Baseline wound tissue samples were collected, and the epidermal solution of timolol applied to the wounds under occlusion daily. Wound tissue was again collected at two week intervals for up to six weeks of time point. The pCRU of the collected tissue demonstrated an upregulation of the pro-reparative TGFβ1 with a mean regulation of 3.88 (p-value .02), while the pro-inflammatory genes STAT4, GZMB, and IRF7 were downregulated, with mean regulations of -3.65 (p-value .015), -8.71 (p-value .039), and -2.94 (p-value .002) respectively. These finding suggest the conclusion that topical timolol can promote healing in chronic wounds by enhancing expression of pro-reparative elements and modulating the local wound inflammatory environment.
1442 Bone marrow-derived fibrocytes contribute to type I collagen production during the early stage of cutaneous wound repair
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Substantial induction of type I collagen (COL1) production is essential for regeneration of the dermal extracellular matrix during wound repair. While bone marrow-derived fibroblasts are the major cell type responsible for increased COL1. Fibrocytes are circulating bone marrow-derived cells that have the capacity to produce COL1. In tissues, fibrocytes are identified as CD45+ positive (pan-hemopoietic marker) cells that express COL1. The involvement of fibrocytes in human wound healing is unclear. We have investigated the presence of fibrocytes in experimental human wounds. Partial thickness wounds were created on the back by ablative CO2 laser. Skin samples were obtained at baseline, 1, 3 and 5 weeks post wounding, and analyzed by immunostaining and gene expression. Immunostaining revealed that CD45+ positive cells accounted for the majority of cells, in the upper dermis at 1 week after wounding. Fifteen percent (n=8) of the CD45+ positive cells expressed COL1 protein. These CD45+/COL1 double positive cells accounted for 26% of total COL1+ positive cells. CD45+/COL1 double positive cells accounted for 14% of total COL1 mRNA expressing cells and 13% of CD45+ positive cells that were isolated from skin one-week post wounding expressed COL1 mRNA, while no COL1 mRNA was detected in CD45+ positive cells that were isolated 3 or 5 weeks post wounding. Interestingly, fibrocytes cultured from blood or wounded skin rapidly lost CD45 expression and became indistinguishable from primary dermal fibroblasts. The above results indicate that fibrocytes rapidly home to human skin and express approx. 25% of total COL1 in early wounds. Loss of CD45+ by fibrocytes may mask their full long-term contribution to wound healing. Limited fibrocyte homing to wounds, due to poor circulation, might account, in part, for weakened wound healing observed in persons with diabetes or the aged. Promoting fibrocyte entry into skin represents a novel therapeutic strategy to improve healing of refractory wounds.

1443 Expression of wound healing markers in neurofibromatosis type 1
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Neurofibromatosis type 1 (NF1) is an autosomal dominantly inherited disorder, caused by heterozygous mutations of the NFI gene. NFI1 protein has been suggested to be a down- regulator of Ras-signal transduction. Understanding its function in gene expression is essential for most tissues. Although the surgical treatment is major therapeutic option to remove the neurofibroma (NF), there are few studies regarding the complex process of wound healing in the skin of NF1. To investigate expression of wound healing markers in the skin of NF1 compared with normal skin and hypertrophic scar (HTS), Real-time PCR was performed to compare mRNA expression of 14 genes known as markers related to wound healing. Based on the real-time PCR results, immunohistochemistry (IHC) was done about MMP-1, MMP-9, FGF2, TNF-α, IL-6 and IL-8 in NF lesions and in normal skin of HTS for MMP-1, MMP-9, FGF-2, TNF-α and IL-6 were in NF lesions, and that of MMP-9 showed a significant increase compared to normal tissues (p=0.021). Immunohistochemically, MMP-9 was decreased in NF tumor per se and scar area of HTS compared with rectal dermis of normal tissue and FGF2 and TNF-α were strongly expressed in the dermis and peritumoral and tumoral area of NF lesions. By contrast, the expression of TNF-α was decreased in scar area of HTS. NF lesions have high expression of MMP-9 mRNA, and high expression of FGF2 and TNF-α protein. These altered level of expression in wound healing markers in NF suggest that NF lesion could be expected almost normal healing process instead of leaving HTS after surgical procedure.

1444 Senolyis is needed for the clearance of senescent tumor cells
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Cellular senescence, known as an intrinsic growth control mechanism that prevents the transformation of pre-malignant lesions into overt malignancy, plays an important role in tissue development, homeostasis and cancer control. Besides endogenous stress signals, exogenously delivered Th1 cytokines (IFNγ & TNF) can initiate senescence in a variety of human and murine cancer cells. However, these senescent cancer cells remain a potential harm due to their senescence associated secretory phenotype (SASP). The SASP promoted by cytokine induced senescence (CIS) in murine RFP-Tag2 beta cancer cells was highly pro-inflammatory and contained chemotactants. Its components CCL2 and CCL5 not only attracted bone marrow derived macrophages (bMø) and polarized them into a pro-tumorigenic M2 phenotype but also promoted proliferation of non-senescent beta cancer cells. Therefore, we analyzed the CIS responsive cell populations of senescent cancer cells. Surprisingly, we found that senescent cancer cells were resistant to phagocytosis, despite the production of chemotactants and the expression of phosphatidylserine. However, senescent cancer cells were specifically more sensitive to apoptosis then non-senescent cancer cells. Following senescence with secondary apoptosis allowed phagocytosis by bMø. In consequence, senolyis is required for the clearance of senescent tumor cells. These findings will allow to answer the urgent question whether senescent cancer cells cause harm and should be deleted or whether they are protective by inducing tumor specific immune responses.

1445 Reversal of UV-induced skin inflammation by vitamin D via activation of KLF4-PPARα pathway to promote macrophage polarization
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Vitamin D (VD) has been implicated in modulating acute immune responses including dampening inflammation and promoting skin repair. We have previously shown in a randomized placebo-controlled human study that a single dose of VD rapidly reduces redness and swelling from experimentally-induced sunburn and was associated with the presence of alternatively-activated CD163+ Arginase1+ macrophages (M2-macs) in the skin. To determine the mechanism by which VD regulates macrophage polarization in the skin following UV exposure, the study was recapitulated in mice. Analysis of skin by transmission electron microscopy reveals that UV exposure leads to the formation of autophagosomes within dermally-infiltrated macrophages. However, complete formation of autophagolysosomes, indicative of autophagy, occurs in macrophages of only VD-treated mice that show accelerated skin recovery. Next, to establish a link between VD-enhanced autophagy and M2-macs polarization, in vitro studies were performed with bone-marrow-derived macrophages. We observe that VD activates its cognate nuclear receptor VDR (p=0.02) and KLF4 (p=0.005), the transcriptional regulator for M2-macs differentiation. Furthermore, conditioning macrophages with IFNγ/LPS to elicit a classic CD163+ M1 inflammatory response was strongly inhibited by VD (p=0.05) with concomitant increases in M2-related genes, PPARα (p=0.05) and Arginase1 (p=0.05). In M1-macs, autophagy genes atg7 and beclin1 are inhibited, but not in VD stimulated M2-macs. Corroborating the in vitro data, analysis of skin samples harvested 48h post UV show a significant upregulation of PPARα (p=0.003) in mice with VD intervention. This effect of VD was abolished after in vivo treatment of mice with the VDR inhibitor 1α,25(OH)D3 (p=0.002). These findings demonstrate a critical role for VD induction of KLF4 and the VDR-PPARα pathway in activating autophagy to promote M2 macrophage polarization in attenuating UV-mediated cutaneous inflammation.

1446 Atopic cytokines IL4/13 perturb iPS-derived iitch-specific sensory neurons
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Human skin contains sensory neurons to transduce sensations such as itch. Atopic dermatitis (AD) is an inflammatory skin disease and pruritus is a characteristic symptom of this disorder. AD is driven by interleukins (IL4/13) and there is a link between inflammation and the pruritus. However, it is unknown whether the driver cytokines (IL4/13) sensitise the sensory neurons to itch stimuli. Previously, we manipulated the wrist, TGF-β, BMP4 and Notch signaling pathways to enhance sensory neuronal differentiation of iPSCs and obtained iPSC-derived neurons (iNs) from different iPSC lines. These iNs displayed neural markers and action potentials, and responded specifically to itch-specific stimuli. Here, we asked whether the AD driver cytokines act directly on itch-specific sensory neurons to contribute to pruritus. To this end, we used IL4 and IL13 to treat the iNs cultured under monolayer conditions, and calculated the calcium response and alterations in gene expression. AD driver cytokines stimulated calcium signaling in iNs, suggesting that the driver cytokines directly influenced sensory neurons. Moreover, administration of IL4/13 upregulated interleukin IFNα/IFNβ (p=0.01). In contrast, many genes involved in neurotransmission were downregulated, including synapsin genes (SYN2/3), solute carrier family members (SLC6A4/5) and GABA receptors (GABRD1R1), suggesting that AD driver cytokines dysregulated the iNs, cytokine network and perturbed genes involved in neurotransmission. We are developing innervated 3D skin constructs in the presence of IL4/13 to model AD. The availability of these specialized skin constructs will allow us to delineate AD pathogenesis and screen for pharmaceutical agents to treat AD.

1447 Vitamin D attenuates acute skin inflammation following nitrogen mustard exposure by targeting M1 macrophages
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Cutaneous exposure to alkylating agents such as nitrogen mustard (NM) causes severe blisters and localized skin inflammation with concomitant ulceration. Vitamin D (VD) has been shown to help prevent NM, mediated skin injury by enhancing autophagy to promote M2 microglia polarization in attenuating UV-mediated cutaneous inflammation.

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dsRNA levels and sensing during regeneration.

The role of cutaneous nerves in skin regeneration by live imaging S Huang, M Brewster, Q Zheng, T Dierichs, E Grice, B Capell and P Rompolas Dermatology, University of Pennsylvania, Philadelphia, PA

The skin is a highly innervated organ but while the sensory role of cutaneous nerves is well characterized there is significant clinical and experimental evidence to suggest that proper innervation is critical for normal skin physiology and function. Hereby, uncovering the functional interactions between cutaneous nerves and stem cells is the ability to image and manipulate their activity in vivo, in the intact live skin. We developed state-of-the-art imaging tools to visualize the 3-dimensional network of cutaneous nerves in live mouse skin by 2-photon microscopy. Using a live imaging approach and modulating the activity of cutaneous nerves in vivo, we have made key discoveries that provide critical evidence for the role of peripheral nerves in the regulation of stem cell activity in the skin. We show that peripheral ablation of cutaneous nerves impairs re-epithelialization and proper wound closure in the back and ear mouse skin. Detailed histological analysis of the spatial organization of peripheral nerve fibers in live or whole-mount fixed skin indicates a physiological interaction with a subpopulation of epithelial stem cells, which are located in the basal layer of the epidermis and pilosebaceous unit. In the absence of cutaneous nerves these stem cells lose their ability to self-renew, long-term and to efficiently contribute to skin regeneration. We performed RNAseq to profile gene expression of stem cells isolated from intact or denervated skin, which revealed 519 differentially expressed genes involved in cell cycle regulation, signaling, metabolism and cell motility. Taken together our data provide critical insight for the role of cutaneous nerves as a functional stem cell niche in skin homeostasis and regeneration.

The role of miRNA-34 in skin wound healing J Wu, X Li, D Li, H Heretter, M Qian, J Wilkomir, X Ye, and N Xu Landén. 1 Department of Medicine, Karolinska Institute, Stockholm, Sweden, 2 Wenzhou Medical University, Wenzhou, China and 3 East China Normal University, Shanghai, China

Chronic wounds are major and rising disease worldwide, which are characterized with delayed, non-healed and prolonged inflammation. We found that both miRNA-34a-5p (miR-34a) and miRNA-34c-5p (miR-34c) were upregulated in the wound-edge epidermis of venous ulcers (VU), the most common chronic wounds, compared with acute wound or intact skin biopsies from healthy donors. Our study showed that FGF1 promoted miR-34 expression in keratinocytes. In line with this, higher FGFR1 expression was detected in VUs compared to acute wounds or intact skin. Gene ontology analysis of microarray data of human primary keratinocytes overexpressing miR-34 suggests that miR-34 may modulate inflammation and proper wound healing, which has been also involved in autoimmune diseases, such as arthritis, asthma, and colitis. TGF-β is thought to play important roles in neutrophil recruitment and macrophage polarization, two events critical to proper wound healing. Our preliminary analyses showed that TGF-6 and HCA are constitutively present in wild-type WT mouse skin, and are increased after wounding. We also showed that in TSG-6 knock-out (KO) mice, cutaneous wound closure is significantly delayed compared to WT mice. Interestingly, similar differences in wound healing were observed with delayed regeneration associated with TSG-6 deficiency and severe wounds (2-4 h), and exacerbated recruitment at later times (Day 7). Increase in tumor necrosis factor-alpha in KO wounds suggest an increase in polarization of macrophages to proinflammatory M1 phenotype. To understand whether any of these defects are due to the lack of TSG-6 protein versus an unrelated epithiophenomenon, we performed in vivo TSG-6 reconstitution experiments. Recombinant human TSG-6, 2 mg in PBS, was injected into wounds of WT and KO mice, and wound closure was monitored. Injection of TSG-6 rescued the delayed closure phenotype in the KO mice. Further, injection of TSG-6 into KO wounds increased neutrophil recruitment at 12 h, and reduced it at Day 7 post-wounding, effectively reversing the defects in neutrophil influx in TSG-6 null wounds. These shows that reintroduction of TSG-6 can restore normal inflammatory responses in cutaneous wounds as a promising candidate for therapeutic manipulation in delayed or non-healing skin wounds.
Identification of Fetalin A as a potential modulator of scar formation

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Wound healing generally leads to scarring in mature skin, but wounds created at early stages of development heal scars less. The exact mechanisms leading to scars are not fully understood, but unique characteristics of fetal fibroblasts are believed to be important. Dermal reduced overall directionality. The migration defect appears to be intricately related to an arrest of cell cycle after mitosis. Remarkably, these defects are partially rescued by simultaneous deletion of Zeb1, an EMT-inducing transcription factor and known direct target of Ovol2. Current work focuses on using scRNA-Seq experiments to dissect whether and how altered epithelial plasticity affects epithelial cell lineage state transitions, as well as the wound microenvironment.

Cutaneous wounds healed at the cost of overall fitness in the aged Balb/C mouse model

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In the clinic, it is generally accepted that wound healing is delayed in aged individuals, however the evidence about the causes of delay are conflicting and incomplete. Older patients are known to have systemic inflammation and exhausted T cell populations, both of which may contribute to impaired wound healing. We hypothesized that splinted, full-thickness cutaneous wounds healed slower in aged (2-12 yr old) compared to young (10-12 wk old) Balb/C mice because their immune responses were skewed toward pro-inflammatory cells and increased local and systemic inflammatory mediators. Contrary to our hypothesis, we found that aged mice trended toward better healing compared to young mice despite having greater weight loss (p = 0.024). There were no differences in percentage of macrophage populations, however, aged mice had significantly increased T regulatory cells in the spleens compared to young mice on day 3 (p < 0.05), also opposed to our hypothesis but in agreement with other studies. CD4+ T cells in the spleens of aged mice had a higher percentage of exhausted phenotype compared to young mice, measured by PD1 expression (p < 0.01). While our study didn’t agree with popular consensus that wounds heal slower in older organisms, it is evidence that wounds have deleterious effects on the overall health of the older wounded individual. This in turn may contribute to decreased immune responsiveness to secondary attacks such as infection. Additionally, unlike clinical patients, laboratory mice are kept under specific pathogen free conditions, which may partially explain the outcome difference. Thus, not every mouse strain is a good model for impaired healing in aging.

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 Withdrawn
1460 Keratinocyte-fibroblast crosstalk via extracellular vesicles reveals interplay of miRs that inhibit FGF7 signaling in diabetic foot ulcer

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The nuclear envelope protein snesp-2G regulates focal adhesion protein expression and motility of fibroblasts

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Focal Dermal Hypoplasia (FDH) is an X-linked disease characterized by dermal thinning and fat herniation, with additional intergumentary, ocular, tooth, and skeletal abnormalities. Skin disease flares throughout life and there is no treatment. Mice harboring the 3805 mutation (knockout of Krt14-CreER) show delayed wound healing. Our goal was to elucidate the mechanism underlying delayed wound closure in this mouse model. To do so, we isolated primary keratinocytes from Krt14-CreER mice and found that these cells displayed increased expression of the cell adhesion molecule E-cadherin, consistent with impaired cell-cell adhesion. We also found that the expression of miR-24, an miR that inhibits FGF7 signaling, was significantly decreased in these cells compared to wild-type controls. To further investigate the role of miR-24 in FDH, we performed rescue experiments using adenoviral delivery of miR-24 to Krt14-CreER keratinocytes. These experiments showed that overexpression of miR-24 in these cells rescued the wound closure defect, consistent with a role for miR-24 in regulating FGF7 signaling in FDH. Overall, these findings suggest that impaired FGF7 signaling in FDH keratinocytes contributes to delayed wound closure and may provide a potential therapeutic target for this disease.
Cysteinyl leukotrienes signaling promotes burn wound healing

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Montelukast, a leukotriene receptor antagonist, has been shown to improve wound healing. However, the role of cysteinyl leukotrienes (CysLTs) in burn wound healing is still unclear. In this study, CysLT1 and CysLT2 receptor expression was assessed in burn wounds treated with montelukast or vehicle. Montelukast treatment resulted in reduced CysLT1 and CysLT2 expression, indicating that CysLTs may promote healing after burn injury. This study suggests that modulating CysLT signaling may improve burn wound healing.

Mechanobiological study of keloid: from bedside to bench

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African spiny mice (Acomys) can regenerate up to 70% of their skin, including all appendages, after full-thickness skin wounding. This wound-induced hair neogenesis (WHN) begins from the wound periphery on post-wound day 15 (PWD15), and later in the wound center (PWD21). WHN only occurs in the center of the wound for C57BL/6 mouse. The spatiotemporal stiffness of the spiny mouse wounds was measured using the atomic force microscopy, which showed a gradual increase from 2.35 kPa (wound center) and 2.87 kPa (wound periphery) on PWD15 to 7.19 kPa and 11.45 kPa on PWD21, respectively. These results suggest that keloid fibroblasts (KFs), the causal association between CA V1 downregulation and its aberrant expression in keloid fibroblasts (KFs). The keloid fibroblasts (KFs) represent a significant burden to patients and the US healthcare system. More evidence is needed to support existing in vitro evidence for use of CA V1 degradation and its aberrant expression in keloid fibroblasts (KFs). Herein, we present clinical outcomes to support existing in vitro evidence for use of CA V1 degradation and its aberrant expression in keloid fibroblasts (KFs).
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Subcutaneous injection of MAP scaffolds to enable inflammation control and de novo tissue generation
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Over the last 25 years, developments in injectable dermal fillers have revolutionized soft-tissue augmentation, in part due to their lower invasiveness than cosmetic surgeries, but can still be improved upon. Dermal fillers with microspheres (Artefill, Radiesse, Sculptra) can allow new collagen to form, but rely on carriers (bovine collagen and carboxymethylcellulose gel, respectively) that degrade over time generate foreign body reactions. Hyaluronic acid fillers can swell up to three times their size as water diffuses into them and have hydrogel mesh sizes that are sub-micron (Radiesse), making it difficult for new collagen to form within it. We have created the microporous annealed particle (MAP) hydrogel, an injectable gel with large (10-30 µm) hyper-porous networks within, that eliminated chronic foreign body reaction and creates a tissue/material hybrid to fill dermal space. MAP can be synthesized of any base chemistry to achieve our unique microporous geometry within the tissue. Here we use synthetic biodegradable PEG/peptide formulations in murine and porcine models of dermal filler. In mice, new blood vessels and type I and type III collagen form within the hydrogel within two weeks, prior to material degradation, forming a new hybrid tissue structure. In a porcine model, injection of the MAP gel, alongside chemically identical material with porosity similar to current products, showed foreign body response and fibrous encapsulation of the non-porous hydrogel. Injected MAP gel did not result in encapsulation and promoted tissue-material integration and deposition of extracellular matrix including collagen, and led to vessel ingrowth within the scaffold by 38 days. These findings highlight the ability of MAP hydrogel to induce new tissue formation following subcutaneous injection, and offer fundamentally new products for dermal filler injections to create tissue and control immune response with completely synthetic materials.

1473
TRAF4 represses p53 signaling in keloid pathogenesis
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Keloids represent one extreme of aberrant dermal wound healing and are characterized by fibroblast proliferation, excessive deposition of extracellular matrix and cytokine over-expression. Molecular pathology underlying keloid formation and progression remain unclear. p53 signaling plays a key role in keloid formations. However, the regulatory mechanism for p53 in the keloid pathogenic process remains elusive. Here, we show that expression of TRAF4 was markedly higher in keloid fibroblasts, whereas expression of p53 was decreased. When TRAF4 was knocked down with small interfering RNA, the expression of p53 and p53 downstream pathway increased, whereas the proliferation of keloid fibroblasts decreased. In contrast, TRAF4 over-expression by lentivirus led to a decrease in p53 expression and p53 downstream pathway, and increased cell proliferation. We next knocked down p53 in TRAF4 knocked-down keloid fibroblasts. We found that p53 knockdown decreased p53 pathway and rescued proliferation of TRAF4 knockdown fibroblasts, suggesting that p53 was responsible for the proliferation inhibition of TRAR4 knockdown in keloid. Taken together, these findings suggest that TRAF4 promotes keloid proliferation by inhibiting p53 signaling, thus providing clues for development of TRAF4 blocking strategies for therapy or prophylaxis of keloids.

1474
Pregnancy improves cutaneous wound healing through enhanced angiogenesis and cell proliferation
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Wound healing is an interactive biological process, involving resident cells as well as distant cells issued from bone marrow. The 3 steps of wound healing, namely inflammation, proliferation and remodeling are finely regulated. Our team has recently shown that a peculiar subpopulation of Microchimeric Fetal Cells (MFCs), transferred from the fetus to the mother during pregnancy, specifically migrated to wounded skin and enhanced skin healing though CD11b/Ccr2 pathway. Pregnancy is characterized by increased hormonal levels and also a high number of circulating fetal cells. All these parameters may affect skin healing. We aimed in the present study to evaluate closure during pregnancy. The study was performed on wounded pregnant mice with eGFP males or virgin mice. Non-healed surface was reduced at days 5 in pregnant mice. This result was confirmed by the analysis of neo-epidermal tongue (NET). The proliferation of keratinocytes in NET and fibroblasts in granulation tissue were increased in pregnant mice. Pregnancy led to stimulated angiogenesis with a higher number of CD31 vessels and CD146/CD45/CD91+ progenitor at early stage in the wound of pregnant mice compared to virgin. Moreover, the scar quality was also improved with less residual fibrosis in the granulation tissue measured by Red Sirius and an increased of collagen III expression. In contrast, there were no changes in inflammatory cells since F4/80+, Gr-1+ or CD45+ cells counts were similar. Importantly, MFCs were present in all the wounded skin of pregnant mice. These results indicate that through changes in angiogenesis and cell proliferation, pregnancy stimulates healing. Additive experiences are ongoing to determine the respective part of circulating factors and fetal progenitors.
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