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Persistence of mature dendritic cells, Th2A and Tc2 cells characterize clinically resolved atopic dermatitis under IL-4R-alpha blockade

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Current therapeutic options for atopic dermatitis (AD) consist of either broad or targeted immunosuppressive agents. However, the natural course of AD can hardly be modified, as the disease invariably returns after cessation of treatment. Tissue-resident memory T-cells are hypothesized to be relevant players in mediating disease-specific 'immune memory', but their exact immunopathological phenotype is so far unknown. By using a multi-omics approach involving single-cell RNA sequencing combined with multiplex proteomics of skin samples, we studied AD patients undergoing short (16 weeks) and long-term (one year) treatment with the IL-4Rα blocker dupilumab. IL-4Rα blockade resulted in clearance of disease, decrease in skin immune cell counts, and normalization of transcriptomic dysregulation of keratinocytes. Interestingly, we found distinct populations of dendritic cells (DC) and memory T-cells that were largely absent in healthy control skin to persist in AD up to one year of treatment. These included LAMP3+ CCL22+ mature DC, CRTH2+ CD161+ Th2A cells, and CRTAM+ cytotoxic T-cells, expressing peak levels of CCL17 (DC) and IL13 (T-cells). Th2A cells showed a specific receptor constellation of IL17RB, IL1RL1 (ST2) and CRLF2, possibly rendering them key responders to the AD-typical epidermal alarmins IL25, IL33 and TSLP. We thus identified persisting mature DC and T-cells that maintained an inflammatory phenotype up to one year of treatment,equipped with all receptors to facilitate a keratinocyte-DC-Th2-mediated inflammatory response. These cell populations emerge as central players of a skin-intrinsic disease memory that leads to disease recurrences, and might therefore be promising targets to achieve a more sustained therapeutic response.

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The diagnostic and prognostic utility of known and emerging biomarkers in cervical and vulvar squamous cell carcinomas

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Cervical squamous cell carcinoma (SCC) and vulvar squamous cell carcinoma (VSCC) are epithelial cancers that are frequently the result of infection with high-risk human papillomavirus (HPV). The goal of this project is to determine the utility of an HPV-encoded circular RNA, circE7, as a tissue marker in cervical SCC and VSCC as well as identify novel biomarkers and correlate these with clinical and prognostic factors. We hypothesize that stage 1B cervical SCC with a recurrence are biologically distinct from those without a recurrence, and HPV+ VSCC is biologically distinct from HPV-. A retrospective case-control study was performed. Archived cases of women with cervical SCC and VSCC and available formalin fixed paraffin embedded tissue samples were collected. The samples will be analyzed by RNA-sequencing, PD-L1 immunohistochemistry, HPV in-situ hybridization, and circE7 quantification. A total of 18 cervical SCC samples, 36 VSCC, and 6 controls were identified. Chart review demonstrated improved survival of cervical SCC patients predicted by absence of recurrence (p=<0.0001) and age >36 (p=0.0033). Improved progression-free survival (PFS) was predicted by age >36 (p=.0099) and pre-op conization procedure (p=.0483). VSCC demonstrated improved survival with absence of recurrence (p=.0118), negative nodal status (p=.0180), vulvectomy procedure (p=.0151), undergoing nodal dissection (p=.0003), and infiltrating borders of tumor (p=.0041). VSCC patients had improved PFS for unifocal tumors (p=0.0211) and nodal dissection (p=.0001). Clinical data will be analyzed together with the molecular studies. These results have the potential to identify novel diagnostic tests and insights on the pathogenesis of both cervical SCC and VSCC.

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Cell therapy trial of ectopic fibroblasts to modify skin identity

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Skin identity is controlled by a combination of intrinsic features of the epidermis and dermis, as well as crosstalk between the two compartments. The modification of skin identity might have many clinical uses, such as the conversion of stump (Non-volar) skin of an amputee to pressure-responsive palmoplantar (Volar) skin in an effort to enhance prosthetic use and minimize skin breakdown. To this end, we first tested the effects of injected autologous volar (AVF) and non-volar (NVF) fibroblasts on mismatched (i.e. ectopic) locations in healthy volunteers. We measured histologic endpoints known to be greater in native volar skin to see if these were enhanced in injected non-volar skin. Greater KRT9 expression, higher epidermal thickness, larger keratinocyte cytoplasmic size, and longer collagen length are markers of volar skin. We find that these are ectopically increased in non-volar skin after AVF injection (n=31, p<0.03; n=32, p=0.0003; n=32, p=0.001; n=18 p=0.05 respectively), maintained even after 5 months. RNA seq demonstrates gene ontology categories of extracellular matrix organization and morphogenic pathways, such as FGF, Wnt, Notch, and epidermal growth factor receptor (EGFR), with confirmed immuno-histologic changes in EGFR (Y1058 n=31, p<0.0001) and Notch (RBPJ, n=31, p=0.001). Finally, single cell RNA seq demonstrates that ectopic fibroblasts have the greatest effect on transitional keratinocytes leaving the basal layer that we term Liminal Keratinocytes. The long-term engraftment of these cells and tissue changes of our model create a robust platform to test concepts of stem cell therapy toward the development of new therapeutics.

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Dupilumab associated facial and neck erythema

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Frequently reported adverse events (AE) of dupilumab treatment for atopic dermatitis (AD) in phase 3 clinical trials included conjunctivitis, injection site reactions, and herpes infections. Although not reported in randomized controlled trials, there have been increasing reports of dupilumab-associated facial and/or neck erythema (FNE) in clinical practice. A systematic review of existing literature was conducted in order to identify all reported cases of dupilumab-associated FNE and identify potential etiologies and management strategies. A search was conducted on EMBASE and PubMed databases. Two independent reviewers identified relevant studies for inclusion and performed data extraction. 101 patients from 16 studies were reported to have dupilumab-associated FNE. 52/101 (52%) had baseline involvement of the face and/or neck and 45/101 (45%) reported cutaneous symptoms differing from their pre-existing AD, possibly suggesting another etiology. Suggested etiologies included rosacea, allergic contact dermatitis, and head and neck dermatitis. Most commonly used treatments included topical corticosteroids, topical calcineurin inhibitors, and antifungal agents. 29/57 patients saw improvement, 4/57 had clearance, 16/57 had no response, and 8/57 had worsening symptoms despite treatment. 11/101 patients discontinued dupilumab treatment due to this AE. Some patients on dupilumab have developed FNE which differs from their usual AD symptoms. Educating patients on this AE prior to initiation may allow for prompt identification and early treatment, minimizing potential AE related discontinuations.

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Psoriasis patients with subclinical atherosclerosis parse into distinct endotypes by differential gene expression

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Psoriasis is an independent risk factor for atherosclerotic cardiovascular disease (ASCVD). This study aims to uncover shared molecular targets for psoriasis-ASCVD therapies and reduce diverse heterogeneous presentations into endotypes. We compared PAXgene RNA-seq of psoriasis patients with low (Agatston<100; n=21) versus moderate-to-high (Agatston≥100; n=7) coronary artery calcification scores (CACS), a surrogate for subclinical atherosclerosis. Differentially expressed genes (DEGs; α=0.05; |logFC|≥0.1; e. Bayes w/o adj.) were identified with a linear model controlling for age (56.8±13.8 yo), sex (38% F), and batch (n=3). Females were less likely to have moderate-to-high CACS than males (10% vs. 33%), although the odds ratio did not reach significance (OR=0.22; 95%CI:0.02,2.18; *p*=0.36). Pearson hierarchical clustering of the top 50 DEGs (|t|>3.3) revealed three distinct transcriptomic endotypes with median CACS values of 111 Agatston (IQR:0,189; 5 of 8 patients ≥100), 21 Agatston (IQR:0,83; 2 of 10 patients ≥100), and 0 Agatston (IQR:0,0; 0 of 10 patients ≥100), respectively (*p*=0.06, Kruskal-Wallis H test). Enriched pathways (α=0.01; e. Bayes w/o adj.) were identified using gene set variation analysis on MSigDB hallmark gene sets. The top three pathways by log-fold change were interferon-α (e.g., IFI44L), interferon-γ (e.g., IFIT1), and PI3K/AKT/mTOR signaling (e.g., E2F1). Thus, the psoriasis endotype most prone to calcifying ASCVD distinguishes itself by altered prominent inflammation pathways exhibiting interferon signatures. Identifying patients that express these signature pathways may advance personalized prediction and prevention of an ASCVD-prone psoriasis endotype.

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Pro-energetics creatine and nicotinamide prevent stress-induced senescence in human dermal fibroblasts

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Senescence is the process by which cells irreversibly avoid dividing without undergoing cell death and enter a state of irreversible growth arrest. Fibroblast senescence associated with aging is known to contribute to the increased incidence of non-melanoma skin cancer in the aged population. To that end, agents that can inhibit fibroblast senescence could be protective. Senescence can be induced by various cellular stressors such as DNA damage, oncogenic activation and oxidative stress. Hydrogen peroxide (H_2O_2), ultraviolet light, tert-butyl hydroperoxide, and hyperoxia are pro-oxidative stressors which are used experimentally to induce premature senescence. The present studies were designed to test if the pro-energetics creatine and nicotinamide can block H_2O_2-induced senescence in primary cultures of human fibroblasts in vitro. Short-term exposure of fibroblasts with H_2O_2 followed by a three day incubation resulted in senescence as denoted by increased b-galactosidase (b-gal) staining, increased P21 expression, decreased insulin-like growth factor-1 and increased expression of pro-inflammatory cytokines IL-6, IL-8 and TNFa. Pretreatment with creatine and nicotinamide blocked experimental senescence as measured by normalization of all these parameters associated with experimental senescence. Of interest, post-treatment with creatine or nicotinamide following H_2O_2 had no effect on oxidant-induced senescence. Creatine and nicotinamide pre-treatment also blocked H_2O_2-mediated increased levels of reactive oxygen species, providing a potential mechanism for their protective effects. These studies suggest that creatine and nicotinamide could have clinical use in preventing fibroblast senescence