052 Targeting keratinocytes to potentiate non-viral DNA skin immunization

J You1, R Hao1, A Hao1, LD Falco1, I Kim1, CD Carey1, C Erdos2, A Gambotto2, Z You5 and LD Falco1

1 School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, United States; 2 Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, United States; 3 Dermatology, Univ of Pittsburgh, Pittsburgh, Pennsylvania, United States; 4 Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania, United States; 5 Department of Internal Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, United States and 6 Hillman Cancer Center, UPMC, Pittsburgh, Pennsylvania, United States.

Skin is a uniquely accessible and responsive target for vaccine delivery. Emerging evidence suggests that keratinocytes can modulate skin immunity in response to diverse stimuli, producing either proinflammatory or immune suppressive mediators depending on the nature of the exogenous stress. To improve the immunogenicity of skin targeted vaccines, we engineered keratinocytes to support a proinflammatory local environment. Keratinocytes were genetically engineered to express the stress response transcription factor X-box binding protein 1 (XBP1). In a mouse model, keratinocyte-specific overexpression of XBP1 was transient and induced a proinflammatory skin microenvironment characterized by increased expression of proinflammatory mediators, localized inflammatory infiltrates, including localized increases of dermal CD103+ DCs, XCR1+DCs, plasmacytoid DCs, γδ T cells, and group 1 innate lymphoid cells. Simultaneous non-viral delivery of plasmids driving expression of XBP1 and antigen OVA resulted in increased antigen expression and increased induction of antigen-specific cellular and humoral responses, including durable antigen-specific skin immunity. These findings support the feasibility of keratinocyte-targeted DNA vaccines to induce a proinflammatory skin microenvironment for effective immunization.

053 The link between N6-methyladenosine modification and psoriasis

L Cui, J Cai, C Hu and Y Shi

Department of Dermatology, Shanghai Tenth People’s Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China.

Psoriasis is a complex, chronic inflammatory skin disease characterized by inflammation and hyperproliferation of the epidermis. In recent years, the epigenetic mechanism has been increasingly recognized to play vital roles in the pathogenesis of psoriasis. N6-methyladenosine (m6A) is the most prevalent internal modification of messenger RNA (mRNA) in eukaryotes, and it is involved in gene expression regulation and various biological processes. To reveal the relationship between m6A methylation and psoriasis, we analyzed the data from the GEO database and found that the expressions of m6A regulatory enzymes have changed in psoriatic lesions compared to the healthy controls, which was also confirmed by performing real-time PCR. The m6A methylation levels are significantly reduced in psoriatic lesions compared with the skin of healthy controls. The results of immunostaining showed that the expression of METTL14, which plays a central role in catalysis of m6A, showed a declining tendency in psoriatic lesions, while the expression of ALKBH5, a main demethylase of m6A, was upregulated in psoriatic lesions compared to the healthy controls. In the back skin of imiquimod-induced psoriatic mice as well as normal mice, and we found that both of the reduced expression of METTL14 and increased expression of ALKBH5 mainly exits in the epidermis instead of the dermis. This study demonstrates the link between m6A and its regulatory enzymes with psoriasis for the first time, and it provides a solid foundation for further research on the effect of m6A modification in the pathogenesis of psoriasis.

054 Highly Multiplexed Immunophenotyping of Dermatomyositis Skin Lesions

J Patel1, S Madukuri1, C Bax1,2 and V Werth1,2

1 CMV/AMC, Philadelphia, Pennsylvania, United States and 2 Derm, UPenn, Philadelphia, Pennsylvania, United States.

Dermatomyositis (DM) is an autoimmune systemic disorder that most often affects skin and muscle. The pathogenesis of cellular skin inflammation has yet to be investigated. Previous work revealed a type I interferon gene signature characterized predominantly by interferon-β (IFN-β). To investigate the type I interferon signature, we identified pathways and cellular phenotypes in a subset of DM patients. Highly multiplexed, paraformaldehyde-fixed, paraffin-embedded (FFPE) samples obtained from trunk, arm, or leg were stained with a panel of 35 metal conjugated antibodies. Regions of interest (ROI) of 500x800 μm2 were analyzed using the App-based algorithm on the ImageMark TM platform to segment each channel. Staining intensity was compared to normal skin from HC patients (n = 20). A significant increase was identified that produced higher IFN-β levels in DM skin compared to normal skin (p = 0.003). The function of these cells is uncharacterized. Our results suggest that high IFN-β production in DM skin can be associated with a pro-inflammatory phenotype in DM patients.

055 Assessment of fidelity to the desmoglein compensation hypothesis in a large pemphigus cohort

J Selski, J D Barker, M DePaunale, K Seiffert-Sinha and A Sinha

Department of Dermatology, University at Buffalo, SUNY, Buffalo, New York, United States.

Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are autoimmune blistering diseases characterized by oral or mucosal lesions in the presence of autoantibodies (autoAb) targeting the cell-adhesion proteins desmoglein (Dsg)1 and Dsg3. Lesion location has been elegantly explained by the Desmoglein Compensation Hypothesis (DCH), which utilizes the epidermal distribution of Dsg subtypes as well as autoAb profiles. According to this theory, PF presents with subcorneal lesions in the presence of anti-Dsg1 Abs only, while lesions in PV are suprabasilar and accompanied by anti-Dsg1 only in mucosal PV, or anti-Dsg1 and -Dsg3 in mucocutaneous PV. While the validity of this hypothesis has been supported in the literature, logical inconsistencies have been noted and exceptions have been published in several small-scale studies. We sought to comparatively assess how often patients contradict the DCH and characterize these contradictions in a large sample size of 289 pemphigus patients. We find that roughly half of the PV and PF patients with active disease at time of visit present with a combination of lesion morphology and anti-Dsg levels that contradict the DCH. The most common contradiction is cutaneous only PV at time of enrollment (n = 14), including 7 patients who report no mucosal lesions at any time in their history. Other categories in which lesion morphology does not align with the predicted autoAb status include mucocutaneous disease in the absence of either Dsg1, Dsg3, or both (n = 11) and mucoidal disease in the absence of Dsg3 or presence of Dsg1 (n = 23). We find stark differences based on ethnicity, with the highest proportion of patients that follow the DCH among the Ashkenazi Jewish population (63.5%) and the lowest for African Americans (23%). These findings demonstrate clear racial differences which, if further explored, could expand our understanding of pemphigus morphology beyond the DCH, in particular for populations that have not been the focus of previous studies.

056 System approach to evaluate disease factors in pemphigus

L Liu, K Seiffert-Sinha, Y Sun and AA Sinha

1 Dermatology, University at Buffalo, Buffalo, New York, United States and 2 Computer Science and Engineering, University at Buffalo, Buffalo, New York, United States.

Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are autoimmune blistering diseases characterized by oral or mucosal lesions in the presence of autoantibodies (autoAb) targeting the cell-adhesion proteins desmoglein (Dsg)1 and Dsg3. Lesion location has been elegantly explained by the “Desmoglein Compensation Hypothesis” (DCH), which utilizes the epidermal distribution of Dsg subtypes as well as autoAb profiles. According to this theory, PV presents with suprabasilar lesions in the presence of anti-Dsg1 Abs only, while lesions in PV are suprabasilar and accompanied by anti-Dsg1 only in mucosal PV, or anti-Dsg1 and -Dsg3 in mucocutaneous PV. While the validity of this hypothesis has been supported in the literature, logical inconsistencies have been noted and exceptions have been published in several small-scale studies. We sought to comparatively assess how often patients contradict the DCH and characterize these contradictions in a large sample size of 289 pemphigus patients. We find that roughly half of the PV and PF patients with active disease at time of visit present with a combination of lesion morphology and anti-Dsg levels that contradict the DCH. The most common contradiction is cutaneous only PV at time of enrollment (n = 14), including 7 patients who report no mucosal lesions at any time in their history. Other categories in which lesion morphology does not align with the predicted autoAb status include mucocutaneous disease in the absence of either Dsg1, Dsg3, or both (n = 11) and mucoidal disease in the absence of Dsg3 or presence of Dsg1 (n = 23). We find stark differences based on ethnicity, with the highest proportion of patients that follow the DCH among the Ashkenazi Jewish population (63.5%) and the lowest for African Americans (23%). These findings demonstrate clear racial differences which, if further explored, could expand our understanding of pemphigus morphology beyond the DCH, in particular for populations that have not been the focus of previous studies.

057 Distinct Chromatin Accessibility Profiles of CD8+ Tissue Resident Memory T Cells

J Zhao, Y Pan, K Wu, CA Singley, Y Yan and TS Kupper

Dermatology, Brigham Health/ Harvard Medical School, Boston, Massachusetts, United States.

Tissue-resident memory T cells (Tmem) differ fundamentally from their circulating counterparts, central and effector memory, T cells (Tcm, Tem). The epigenetic mechanisms and transcription factor networks by which they maintain their distinct differentiation states remain obscure. Here we compared genome-wide maps of chromatin accessibility of CD8+ Tem, Tcm, Tmem generated by skin vaccinia virus (VACV) infection using Assay for Transposable-Accessible Chromatin sequencing (ATAC-seq). Principal component analysis (PCA) segregated Tmem, Tem, Tcm into 3 clearly separate clusters which were also distinct from naive CD8 T cells. We found 9627 and 9042 differentially accessible chromatin regions in Tem compared to Tcm and Tmem, respectively. The top 250 reciprocally regulated regions include Cdh2, Cxcl11, Itgb2 and Il17a. In addition, Igf2 and Cd46 were exclusively upregulated in Tmem, which is consistent with their gene expression in these cells. Conversely, Smyt1, Ccr7, Sell, Klf2 loci were closed in Tem, demonstrating selective recruitment of these factors. Transcription factor networks controlling Tem and Tmem differentiation were inferred from differentially accessible motifs using HOMER motif analysis software. We found motifs for several members of the Basic Leucine Zipper Domain (bZIP) family as most significantly enriched at open chromatin regions in Tem, top hits included Foxa2, JunB, Atf3, and Baf4. In contrast, Zinc Finger (ZF) and E-26 Transformation Specific (ETS) motifs were enriched at chromatin sites more open in Tem and Tcm. Taken together, our data indicated that Tem have a unique epigenetic framework that facilitates their development and function, which is distinct from Tcm and Tmem. The factors responsible for this epigenetic signature are under investigation.