132 Metabolic identification of an essential glucose-IRF6 axis in differentiation V Lopez-Pajares1, A Bhaduri1, A Guerrero1, Y Zhao1, L Donohue1, M Guo1,2, J Elrod1,2, A Otten1, O Amrabaya1, P Bai2, B Cheng1,2, K Qu1 and B Sun1 1 Dermatology, University of California San Diego, La Jolla, California, United States and 2 VA Palo Alto Health Care System, Palo Alto, California, United States

Advances in high throughput metabolomics enable discovery of new essential roles for biomarkers in the skin such as epidermal differentiation. Metabolic analysis of keratinocyte differentiation that detects >14,000 analytes in all major metabolite classes was performed and unexpectedly, glucose was the top increased analyte of the 614 that changed significantly. Functional studies in epidermal tissue showed that intracellular glucose elevation was required for differentiation. Metabolites in glucose catalytic pathways were unchanged in differentiation, suggesting that the accumulated pool of glucose itself was required. Consistent with this, decreasing cellular glucose levels, by restricting available glucose or by increasing intracellular glucose catalyzing enzymes, HK1/2 and GAPD, blocked differentiation. Knockout and pharmacologic inhibition studies demonstrated that 3 glucose transporters, GLUT1, GLUT1 and SLC1, were essential for glucose accumulation and differentiation. Furthermore, RNAseq analysis of glucose-depleted epidermal tissue revealed >9,000 genes whose expression was altered, 60% of which were involved in the metabolic aspect of the epidermis or keratinocytes on gene signature. ATACseq identified candidate transcription factors (TFs) that may act on glucose-regulated genes, including ZNF750, NFE2L2, and IRF6. Glucose affinity chromotography followed by mass spectrometry identified the IRF6 TF as a glucose binding protein. IRF6 was essential for epidermal differentiation and was verified to bind glucose directly at high affinity. Glucose was found to enhance IRF6 binding to its cognate DNA binding sequence, suggesting IRF6 recruitment. Interestingly, an IRF6 binding motif was found in an endothelial dysplasia and cancer disrupted diminished glucose binding. These data support a model in which epidermal differentiation requires upregulation of specific glucose transporters that enable accumulation of free intracellular glucose, which in turn binds to IRF6s and enables IRF6s DNA binding and IRF6-driven differentiation gene induction.

133 Unbound cornocyte lipid envelopes in 12R-lipoxygenase deficiency support a direct role in lipid-protein crosslinking E Genesound1, R A Tran1, Y Wu1, S Maza1, A Dick1, M Schmutz1, R Gruber1, F Radner1, S Grond1, J Wavefield1,2, T Mauro1,2 and P Elias1 1 Dermatology, San Francisco VA Health Care System, San Francisco, California, United States, 2 Dermatology, University of California San Francisco, San Francisco, California, United States, 3 Pediatrics, Univ of Erlangen-Nurnberg, Erlangen, Germany, 4 Dermatol, Vereen, Alleg, Med Univ of Innsbruck, Austria, 5 Aristotle University of Thessaloniki, Greece, 6 Graz, Austria

Loss-of-function mutations in arachidonate lipoxygenase 12B (ALOX12B) are an important cause of autosomal recessive congenital ichthyosis (ARCI). 12R-lipoxygenase (12R-LOX), the protein product of ALOX12B, has been proposed to covalently bind the cornocyte lipid envelope (CLE) to the proteinaceous cornocyte envelope (CE), thereby providing a scaffold for the assembly of barrier-providing, mature lipid lamellae. To test this hypothesis, we performed an in-depth ultrastructural examination of CLEs in ALOX12B-deficient human and mouse epidermis, extracting samples with pyridine to distinguish covalently attached CLEs from unbound (i.e., non-covalently bound) CLEs. ALOX12B-/- stratum corneum contained abundant pyridine-extractable (i.e., unbound) CLEs, compared to normal stratum corneum. These unbound CLEs were associated with defective post-secretory lipid processing, and were specific to 12R-LOX deficiency, since they were not observed with deficiency of the related ARCI-associated proteins, patatin-like phospholipase 1 (PNPLA1) or aldehyde dehydrogenase domain containing 5 (ABHD5). These results support that 12R-LOX contributes directly to CLE crosslinking, which appears to be a prerequisite for post-secretory lipid processing, and provide insights into the pathogenesis of 12R-LOX deficiency in this subtype of ARCI, as well as other conditions that display a defective CLE.

134 Correlation of 12r-lox activity and hp70 with barrier function C Hlavacek1, F Liebel1, L DiNatale1, J Ilkowick-Baldys1 and J Glynn Research and Development, Avon Products Inc, Suffern, New York, United States

The evaluation of barrier function in the skin is an essential component in the skin care industry in order to assess the safety and the efficacy of active ingredients. 12R-LOX (gene ALOX12B) is a lipoxygenase expressed in keratinocytes and is implicated in the oxygenation of the estrogenic omega-hydroxyac-sphingosine (OES) ceramides, a required process to their covalent linkage to proteins of the cornified envelope. It is an important step in establishing the water barrier by preventing unnecessary evaporation through epidermal cells. HP70 family members are among the most abundant HPs in the skin expressed constitutively within keratinocytes. HP70 expression is elevated in both episodic and dermis for cytoprotection after skin cell samples are heat shocked or after stressors like UVB. Traditionally transiperal water loss (TEWL) has been widely used as a way to evaluate skin barrier function and tape stripping as a way to evaluate the barrier function of the stratum corneum (SC) biobarriers. Our aim was to understand how HP70, 12R-LOX and HP70 could be correlated clinically. Subjects were treated with a hydrating cream for 3 weeks on their lower inner forearm. After treatment, TEWL was measured and tape strips were collected from the treatment area and untreated site. 12R-LOX enzyme activity was measured with a fluorescence-based assay and HP70 by ELISA using the protein samples isolated from the tape strips. The 12R-LOX enzyme activity had a negative correlation with TEWL as previously described and had a positive correlation with HP70. Moreover, HP70 also had a negative correlation with TEWL confirming the correlation between the two biomarkers 12R-LOX and HP70 with barrier function. The study of these two biomarkers is a new interesting and non-invasive approach for studying skin under different conditions.

135 Comparing hydration levels in healthy normals vs. atopic dermatitis and xerosis cutis using a novel wireless, non-invasive sensor D Lin1, J Chang1, A Banks1, J Rogers1, A Puller1 and S Xu1 Northwestern University, Evanston, Illinois, United States

The objective measurement of skin barrier function, essential in the management of atopic dermatitis (AD) and xerosis cutis (XC), is limited to bulky, expensive, and user-dependent tools such as comeometers. Herein, we report the development and validation of a novel, wireless, low-profile, and lightweight sensor, capable of accurately measuring skin surface water content using thermal conductivity. The aim of this study was to show a measurable difference in skin hydration of healthy normal subjects vs. subjects affected by AD and XC using this novel sensor. The novel device uses a thermal actuator and multi-sensor module to apply thermal energy to the skin and capture the corresponding temporal changes in temperature. The temperature difference between when the actuator is on and off is input to a thermal transport model, where computational modeling connects the temperature change with hydration levels of the skin. A total of 44 subjects were recruited for this study, including 25 healthy normal subjects, 5 subjects with AD, and 14 subjects with XC. Subjects were placed in the XC group if their overall dry skin score was ≥1. Measurement sites included the arm, forehead, and low-burden skin hydration sensor would support drug development, detect small but clinically meaningful changes in disease severity, and track response to treatment.

136 Physiological function of krox20 (eg2) in epithelial stem cells Q Telepnev1, C Liao2, J Eghtsahi1, A Sarid1, J Raman1 and L Le1 1 Dept of Dermatology, The University of Texas Southwester Medical Center, Dallas, Texas, United States and 2 Taipei Medical University, Taipei, Taiwan and 3 Genetics, Development and Disease Graduate Program, The University of Texas Southwester Medical Center, Dallas, Texas, United States

Resident stem cells (SCs) within tissues are important for normal homeostasis maintenance and hair development warrants the elucidation of Krox20 function in epithelial cells. We recently study, we reported for the first time a population of epithelial-derived Krox20+ SCs within tissues are important for normal homeostasis maintenance and wound repair. This is mediated by the ability of SCs to properly self-renew, maintain their identity, and differentiate; hence the importance of understanding the key mechanisms underlying SC physiology. Krox20, a zinc finger-containing transcription factor, is well known for mediating stem and progenitor cell activation and differentiation in a variety of tissues. In a recent study, we reported for the first time a population of epithelial-derived Krox20-expressing keratinocytes in the hair follicle that ultimately terminally differentiate to form the structural component of the hair shaft. These Krox20 lineage cells in the hair follicle also mediate melanocyte differentiation via Stem Cell Factor production for hair pigmentation. In light of the importance of Krox20 in other cell types, the role of Krox20 cells in epithelial and hair follicle development and the elucidation of Krox20 function. We report here that ablation of Krox20 in skin epithelial cells caused spontaneous hair loss, correlated with increased epidermal differentiation. On the other hand, the overexpression of Krox20 in epithelial cells resulted in the upregulation of the epidermal SC markers, suggesting maintenance of stemness as a potential role of Krox20 in epithelial cells. Moreover, we also observed a reduction in apoptosis in Krox20-overexpressing cells, pointing to an additional Krox20 function in regulating cell survival. Analysis of the molecular mechanisms underlying the physiological function of Krox20 sheds light into the maintenance of the epithelial SCs.

137 The long non coding RNA PRANCR regulates epidermal homeostasis and wound healing through alternative splicing of fibronectin-1 A Otten1, O Amrabaya1, P Ca1, B Cheng1,2, K Qu1 and B Sun1 1 Dermatology, University of California San Diego, La Jolla, California, United States and 2 Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, China

Most human genes undergo alternative splicing (AS), but the molecular mechanisms controlling AS are largely unknown; and the functional consequences of most AS events remain characterized. AS regulation is critical for skin development. For example, expression of specific isoforms of the epidermal transcription factor TNP1 are required for epidermal stratification. Over the recent years, long noncoding RNAs (lncRNAs) are emerging as novel regulators of AS. Here, we investigated whether PRANCR, a lncRNA that we recently discovered as essential for epidermal progenitor renewal, functions by regulation of AS. Using transcriptome-wide analysis, we demonstrated that PRANCR controls 238 AS events in epidermal keratinocytes. Specifically, we show that PRANCR promotes expression of an mRNA isoform containing extra-domain A (EDA) in the keratinocyte cell fate gene fibronectin-1 (FN1). The AS of the inclusion of EDA domain (EDA+X) is promoted by the sense/antisense-rich flanking splice sites (SRSFs) 1 and 7 and PRANCR is required for full expression of these factors. Deletion of PRANCR or the FNT1 EDA+X isoform both lead to equivalent proliferation defects and severe delays in in vitro keratinocyte migration, consistent with skin wound healing defects reported in FNT1-EDA+X deficient mice. Aberrant AS of FNT1 EDA+X isoforms have been associated with fibroblasts in psoriasis and scar formation during wound repair, and our results indicate intrinsic AS of FNT1 is also important in epidermal keratinocytes. Collectively, we identify an epidermal lncRNA that regulates epidermal proliferation and migrations by controlling AS of an important keratinocyte cell fate gene.