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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-nucleic 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 immune tolerance. Proximity ligation assay (PLA) revealed that LL37 facilitated 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 demonstrate that the inflammatory activity of LL37 is mediated by a cell surface, receptor-dependent interaction that is potentially therapeutically targetable.

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***Trichophyton rubrum* infection on reconstructed human epidermis induces simultaneous epidermal barrier disruption and keratinocytes activation**

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Dermatophytosis is a superficial fungal infection of keratinized structures, with a prevalence around 25% in humans. Consequences of dermatophytosis on epidermal barrier functions, and cell responses elicited in keratinocytes remain unclear. To gain mechanistic insight, an *in vitro* model of reconstructed human epidermis (RHE) infected by arthroconidia of *Trichophyton rubrum* has been investigated. Assays of trans-epithelial electrical resistance and dye permeation through infected RHE revealed a sudden loss in barrier function after four days of infection. The disruption of this barrier could result from disorganized tight junctions, since simultaneous internalization of claudin-1 immunoreactivity was monitored. Electron microscopy illustrated that fungal hyphae progressively invade the *stratum corneum* of RHE by sneaking into intercellular spaces, down to the granular layer. Also on the fourth day of infection, pro-inflammatory cytokines (IL-1α, IL-1β, TNFα, IL-8, TSLP) and antimicrobial peptides (β-defensin-2, β-defensin-3, S100A7) were increasingly expressed and released by keratinocytes. Simultaneous observation of barrier disruption and keratinocyte activation triggered investigation towards prospective causal relationship. While assessing potential role of p38 MAPK activation in consequence of fungal infection, the use of inhibitor PD169316 indicated that fungal homolog of p38 might be involved in growth of arthroconidia. Indeed, RHE treated with PD169316 were protected against invasion and growth of *T. rubrum* colonies on Sabouraud agar was altered, as observed by SEM. Altogether, these data identify fungal p38 MAPK signaling as a potential target to counteract dermatophytosis, while supporting requirement for improved knowledge in biology of such infecting species.

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β-Defensin 103 characterizes a distinct molecular phenotype of human acral melanoma, by its correlated expression with IL-17A & IFNγ-mediated immune genes, as well as MC1R-mediated pigmentation signatures

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β-Defensin 103 (DEFB103B), highly expressed in stratum corneum and regulated by IL-17A and IFNγ, was recently identified as an MC1R receptor agonist, which increases levels of pigmentation in mammals, and regulates melanoma cell migration. Our human melanoma research showed that high expression of DEFB103B characterizes acral melanoma, which is associated with a poor prognosis, and is genetically different from other subtypes of melanoma. We first compared gene expression profiles of acral melanoma (n=10 for array, n=20 for PCR) to non-acral melanoma (n=21 for array), acral skin (n=4 for array, n=7 for PCR) and non-acral skin (n=12 for array and PCR). Compared to non-acral melanoma, acral melanoma was characterized by higher expression of antimicrobial peptides (DEFB103B, DEFB4A, and S100A7A) and activation of IL-17 and IFNγ immune pathways (FCH>2, FDR0.05). The enrichment of melanoma signatures was not different between acral vs. non-acral melanoma ($p>0.05$). Compared to normal skin, the expression of DEFB103B, IL-17A, IFNγ, MC1R, and TYR was increased in acral melanoma ($p<0.05$). In addition, the expression of DEFB103B in acral melanoma was correlated with the number of $CD11c^+$ dendritic cells in the tissue (=0.77), the expression of IL-17A pathway genes (IL-17A (=0.83), IL-17F (=0.50), IL-23 (=0.49), IL-19 (=0.86), IL-20 (=0.97)), IFNγ pathway genes (IFN (=0.49)), and MC1R-mediated signatures (MITF (=0.62), TYR (=0.49)) ($p<0.05$). Those correlations were not significant in normal skin ($p>0.05$). Thus, we propose that DEFB103B is a novel access of keratinocytes to melanocytes via MC1R, which could be a potential mechanism for abnormally dark pigmentation and invasive tumor progression of acral melanoma.

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Differential profiles of pro-inflammatory and specialized pro-resolving lipid mediators in PMA-treated skin biopsies from young and old donors

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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±9 yo) and old (58±3 yo) donors, we have performed a metabololipidomic study using LC/MS-MS. We have shown that prostaglandinE2 (PGE2), a pro-inflammatory mediator as well as the lipoxinA4 (LxA4), a pro-resolutive biomarker, 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-inflammatory mediators (PGE2, TXB2) and pro-resolving mediators (LxA4, LxB4). In old skins, the EPA/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 dysregulation and/or absence of pro-resolving mediators. Taken together, the present results seem to indicate a role for lipoxins to rebalance cutaneous inflammation during aging and to rescue failed resolution in aged skin.

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MicroRNA-17-92 cluster promotes the proliferation and the chemokine production of keratinocytes: Implication for the pathogenesis of psoriasis

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Keratinocytes are the main epidermal cell type that constitutes the skin barrier against environmental damages. Aberrant proliferation of keratinocytes and the secretion of inflammatory factors from keratinocytes are related to the formation of chronic inflammatory skin diseases like psoriasis. MicroRNA-17-92 (miR-17-92) is a miRNA cluster that regulates cell growth and immunity, but the role of miR-17-92 in keratinocytes and its relation to skin diseases have not been well investigated. In the present study, we initially found that miR-17-92 cluster promoted the proliferation and the cell cycle progression of keratinocytes via suppressing cyclin dependent kinase inhibitor 2B (CDKN2B). Furthermore, miR-17-92 cluster facilitated the secretion of C-X-C motif chemokine ligand 9 (CXCL9) and C-X-C motif chemokine ligand 10 (CXCL10) from keratinocytes by inhibiting suppressor of cytokine signaling 1 (SOCS1), which enhanced the chemotaxis for T lymphocytes formed by keratinocytes. In addition, we detected increased expression of miR-17-92 cluster in psoriatic lesions and the level of lesional miR-17-92 cluster was positively correlated with the disease severity in psoriasis patients. At last, miR-17-92 cluster was increased in keratinocytes by cytokines through the activation of signal transducers and activators of transcription 1 (STAT1) signaling pathway. Our findings demonstrate that cytokine-induced overexpression of miR-17-92 cluster can promote the proliferation and the immune function of keratinocytes and thus may contribute to the development of inflammatory skin diseases like psoriasis, which implicates miR-17-92 cluster as a potential therapeutic target for psoriasis and other skin diseases with similar inflammatory pathogenesis.

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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 functions including skin barrier maintenance and protection against pathogenic microorganisms. It is postulated that skin microbiome communicates with skin cells regulating protein turnover and microorganism 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 trigged 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. Altogether our results suggest that prebiotics and the microbiome are important to maintain skin health and that these technologies can be addressed to preserve skin integrity.