IL-4 and IL-13 cytokines drive sex steroid hormone synthesis and lipid abnormalities in sebocyte during atopic dermatitis pathogenesis
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One of the hallmark features of atopic dermatitis (AD) is an elevation of the type 2 cytokines, IL-4, IL-13 and Th2 cytokines mainly secreted in the extracellular matrix between keratinocytes where it interacts with HA. Specifically in conditions that mimic AD and dermatophytosis. This enhanced HA leakage from keratinocytes with Atg3 mutant adenovirus abolished hBD-3-mediated activation of autophagy, as evidenced by the inhibitory effects of their specific rescence, respectively. We found that hBD-3 increased the expression of LC3, and enhanced morphogenesis with efficient barrier and typical localization of HA and differentiation markers. Our study sheds light on the role of the stem cell niche for epidermal stem cells, so we investigated its components after treatment with wild thyme (*Thymus serpyllum*). This plant is one of the most important medicinal plants and is traditionally used as a diaphoretic, antispasmodic, wound healing, and anti-inflammatory agent. The main active compound is thymol, which is known to have a Dsg1 reducing effect in the human SC. Therefore, applying an extract containing thymol and other compounds from this plant might be a therapeutic target for the treatment of skin diseases that are characterized by dysfunction of autophagy and skin barrier.

**Abnormal epithelial structure and barrier function**

Antimicrobial peptide hBD-3 improves Th2 cytokine-mediated impairment of tight junction barrier through autophagy activation
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Autophagy is a cellular degradation process that is involved in the maintenance of cellular homeostasis. One of the key players in this process is hBD-3, which is known to have anti-inflammatory and anti-proliferative effects. Our study demonstrates that hBD-3 mediates autophagy activation, as evidenced by the inhibitory effects of specific inhibitors. hBD-3 also rescued the downregulation of TJ proteins, including claudin-1 and -4 in IL-4 and IL-13-treated keratinocytes. Interestingly, autophagy deficiency in keratinocytes with Atg3 mutant adenovirus abolished hBD-3-mediated TJ barrier improvement, suggesting that hBD-3 regulates TJ barrier function through autophagy activation. Our findings provide novel evidence that hBD-3 might be a therapeutic target for the skin diseases characterized by dysfunction of autophagy and skin barrier.

**Deletion of TNAFAP6 gene in human keratinocytes by CRISPR/Cas9 edition demonstrates a role for TSG-6 to retain hyaluronan inside epidermis**
C Erard, E Faway, E De Vuyst, O Svennek, O De Backer, B Flamion, C Lambert de Rouvroit

TNAFAP6 is a gene that encodes a protein involved in the assembly of the nucleosome. We used CRISPR/Cas9 technology to delete the TNAFAP6 gene in human keratinocytes. Our results show that the TSG-6 gene, which encodes a protein involved in the maintenance of skin homeostasis, is strongly upregulated in parallel to HA production. This suggests that TSG-6 is critical for the maintenance of skin barrier function and the regulation of HA levels. Our findings provide new insights into the mechanism underlying the role of TSG-6 in skin barrier function.

**Identification of a demosgen-1 reducing component of human stratum corneum contained in wild thyme (Thymus serpyllum) extract**
A Tada and C Nakahara

The stratum corneum is the outermost layer of the skin and plays a crucial role in barrier function. We investigated the components of wild thyme extract that reduce the demosgen-1, a protein that contributes to the barrier function and mechanical strength of the skin. The histological examination of normal human skin, when observing thin skin sections with hematoxylin/eosin staining, the SC consists of two layers, an outer layer with a basket-weave (BW) structure, and an inner layer with a compact structure. Reportedly, the layer with the BW structure contributes to the barrier function and to SC flexibility. We hypothesized that by identifying the relevant component that develops the BW structure, the barrier function and flexibility of the SC might be restored, leading to healthy SC. Surprisingly, we found that a major component of this component is demosgen-1 (Dsg1), the degradation of which is a necessary process for generating the BW structure of the SC. We performed a quantitative and distribution pattern analysis of Dsg1 as a marker of the comparison of the formation in the SC. Dsg1 is involved in adhesive interactions of SC cells, so we investigated its components after treatment with wild thyme (*Thymus serpyllum*) extract, which was found to be effective in a preliminary experiment. In analyses conducted using an established Dsg1 reduction evaluation method, an 80% reduction of Dsg1 expression was observed in wild thyme-treated SC cells, which we used to investigate its components after treatment with wild thyme (*Thymus serpyllum*), containing thymol, which is known to have a Dsg1 reducing effect in the human SC. There was also a Dsg1 reducing effect with a 60% methanol eluate obtained by separation of the 80% ethanol extract using a HPLC-3 column. Subsequently, the 60% methanol eluate was purified by HPLC. When the eluates were isolated and used to treat the Dsg1-deficient cells, these six compounds showed that entriechin and salvianolic acid A have a Dsg1 reducing effect in the human SC. Therefore, applying an extract containing entriechin or salvianolic acid A to the skin can be expected to improve skin health.

**Activated Encapsulated-Grape Seed Extract (ACTIVITIS™) inhibits demethylation of PPARα promoting anti-aging benefits and barrier repair for human skin**
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Grape seed extract (GSE) is a well-known antioxidant and has been shown to have beneficial effects on skin health. In this study, we evaluated the potential of an encapsulated GSE extract, ACTIVITIS™, in promoting anti-aging benefits and barrier repair for human skin. We performed in vitro assays utilizing 3D epidermal skin tissue models and UVB irradiation. One pathway that regulates both processes involves Protein phosphatase 2A (PP2A) activity. The catalytic subunit of PP2A (PP2Ac) plays a crucial role in regulating PP2A function. Oxidative stress has been previously shown to dramatically decrease methylation of the C-terminal leucine of the PP2A catalytic subunit (PP2Ac) plays a crucial role in regulating PP2A function. Oxidative stress is a potential contributor to skin aging and barrier dysfunction. Our results demonstrate that ACTIVITIS™ significantly increases both cell proliferation (Ki67) and skin barrier (Filaggrin) marker expression after UVB-irradiation. Moreover, encapsulated ACTIVITIS™ formulation decreases DNA damage marker Cyclin B and p53, indicating that the barrier integrity and barrier repair after UVB-induced effects. Lastly, clinical results in human subjects demonstrate that encapsulated ACTIVITIS™ is well tolerated and provides anti-aging benefits such as improving fine lines and wrinkles when applied topically.