Antimicrobial peptide hBD-3 improves Th2 cytokine-mediated impairment of tight junction barrier through autophagy activation

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Antimicrobial peptide hBD-3 improves Th2 cytokine-mediated impairment of tight junction (TJ) barrier function in keratinocytes; however, its effect on Th2 cytokine-mediated impairment of TJ barrier function remains unclear. We aimed to evaluate the effects of hBD-3 on IL-4 and IL-13-mediated impairment of TJ barrier in human keratinocytes and explore the underlying mechanism. We assessed the expression of autophagy marker LC3, and TJ-related proteins and the signaling pathways in keratinocytes by western blot, autophagosome/autolysosome formation by immunofluorescence and electron microscopy, expression and distribution of TJ proteins, and HA immunofluorescence, respectively. We found that hBD-3 increased the expression of LC3, and enhanced the formation of autophagosomes/autolysosomes in keratinocytes. Besides, hBD-3 induced activation of mTOR and MAPK signaling pathways, which were required for the hBD-3-mediated activation of autophagy, as evidenced by the inhibitory effects of their specific inhibitors. hBD-3 also rescued the downregulation of TJ proteins, including claudin-1 and -4 in IL-4+IL-13-treated keratinocytes. Interestingly, autophagy deficiency following infection of a triple deletion mouse further supported the role of autophagy in the formation of autophagosomes/autolysosomes in keratinocytes. Besides, hBD-3 induced the formation of autophagosomes/autolysosomes in keratinocytes. We further demonstrated that the antimicrobial peptide hBD-3 regulates TJ barrier function through autophagy activation. Our findings provide novel evidence that hBD-3 might be a therapeutic target for the treatment of skin diseases that are characterized by dysfunction of autophagy and skin barrier.

Deletion of TNAIP6 gene in human keratinocytes by CRISPR/Cas9 edition demonstrates a role for TSG-6 to retain hyaluronan inside epidermis

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TNAIP6-fused cell (TSG-6) protein is found in human skin, where it exerts anti-inflammatory properties and contributes to wound healing. In human epidermis, TSG-6 is mainly secreted in the extracellular matrix between keratinocytes where it interacts with HA. This work maps out better understanding of TSG-6 and HA functions in epidermal physiology. Reconstituted human epidermis (RHE) incubated with Th2 interleukins to mimic atopic dermatitis (AD) or RHE infected with Trichophyton rubrum dermatophytes were compared with RHE cultured in normal conditions. In both pathological conditions, mRNA expression levels and protein release of TSG-6 were strongly upregulated in parallel to HA production, suggesting that they might play a role together in challenged epidermal tissues. Potential role was investigated by using TSG-6 null mice cells using the CRISPR-Cas9 system to edit TNAIP6 gene in NTERT keratinocytes, an immortalized human cell line which produces keratinized layers in tissue reconstruction. Two TSG-6- clones harboring major deletions in both alleles of the target gene were used to reconstitute RHE. TSG-6- RHE exhibit normal epidermal morphology with efficient barrier and typical localization of HA and differentiation markers. Their phenotype was further analyzed through RNA sequencing. Despite no alteration in the expression of genes involved in HA metabolism, an increased amount of HA was detected in medium underneath TSG-6- RHE in concomitance with a reduced epidermal HA content, especially in conditions that mimic AD and dermatophytosis. This enhanced HA leakage from either challenged and untreated TSG-6- epidermis is reversed when TSG-6 expression is reintroduced in the tissue, suggesting TSG-6 critical involvement to cross-link and thus retain HA in epidermal extracellular matrix. In addition to other organs and tissues, this work demonstrates overexpression and function of TSG-6 in challenged epidermis.

Identification of a desmoglein-1 reducing component of human stratum corneum contained in wild thyme (Thymus serpyllum) extract

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The outermost layer of the skin, the stratum corneum (SC) has the very important function of preventing excessive transepidermal water loss (TEWL) between outer-outer or inner-inner barrier. SC also contributes to mechanical strength and mechanical strength of the skin. In 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 skin. Structural data suggest that a major component of desmosomes is desmoglein 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 compaction process and loss of formation in the SC. Dsg1 is involved in anchoring 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 with a well-known Dsg1 reduction evaluation method, an 80% reduction was prepared from wild thyme was found 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-2 column. Subsequently, the 60% methanol eluate was purified using silica gel and ODS columns, and six compounds were isolated, one of which was prepared from wild thyme was found to have a Dsg1 reducing effect in the human SC. Therefore, applying an extract containing estrinich or salvianolic acid A have a Dsg1 reducing effect in the human SC. Therefore, applying an extract containing estrinich or salvianolic acid A to the skin can be improved to enhance skin health.

Encapsulated Activated-Grape Seed Extract (ACTIVITIS™) inhibits demethylation of P2A promoting anti-aging benefits and barrier repair for human skin

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Cumulative oxidative stress and chronic inflammation are critical during skin aging. One pathway that regulates both processes involves Protein phosphatase 2A (PP2A), a serine/threonine phosphatase. Reversible methylation of the C-terminal leucine of the PP2A catalytic subunit (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) in dermal fibroblasts. Previously, we developed a novel, pseudo-cosmetic seed extract called Activated-Grape Seed Extract, or ACTIVITIS™ (INCI name: anthocyanins and hydrolyzed proanthocyanidins) which is enriched for PP2A-activating flavonoids with increased potency in preventing PP2A demethylation when compared to its parent form. This added specificity underlines the potential underlying mechanism for the observed anti-aging benefits and barrier repair for human skin. 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 skin. Structural data suggest that a major component of desmosomes is desmoglein 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 compaction process and loss of formation in the SC. Dsg1 is involved in anchoring 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 with a well-known Dsg1 reduction evaluation method, an 80% reduction was prepared from wild thyme was found 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-2 column. Subsequently, the 60% methanol eluate was purified using silica gel and ODS columns, and six compounds were isolated, one of which was prepared from wild thyme was found to have a Dsg1 reducing effect in the human SC. Therefore, applying an extract containing estrinich or salvianolic acid A have a Dsg1 reducing effect in the human SC. Therefore, applying an extract containing estrinich or salvianolic acid A to the skin can be improved to enhance skin health.