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Maintenance of skin physical and immune barrier synergy via CLASP2 and JAK-STAT pathway interaction

Poster 420

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Introduction:

Lifetime exposure to different stressors leads to the accumulation of chronic inflammation, leading to an accelerated aging process. As the outermost layer of the body to the surrounding environment, the integrity of cutaneous barrier is particularly important for healthy aging. As a major cell source of skin development, function and regeneration, keratinocyte stem cells are important for cutaneous barrier homeostasis [1, 2, 3]. CLASP2 is involved in cell-cell adhesion preservation in epidermal stem cells [4] and prevents keratinocyte differentiation disorders [5]. The JAK-STAT pathway is one of the central mechanisms that regulates the production of proinflammatory cytokines, which play a main role in cellular proliferation, differentiation and immune homeostasis [6]. The activation of STAT has been implicated in the expression of NLRP3 inflammasome [7]. The purpose of this study was to understand the possibility of maintaining the synergy of skin physical and immune barrier systems through CLASP2 and JAK-STAT pathway interaction.

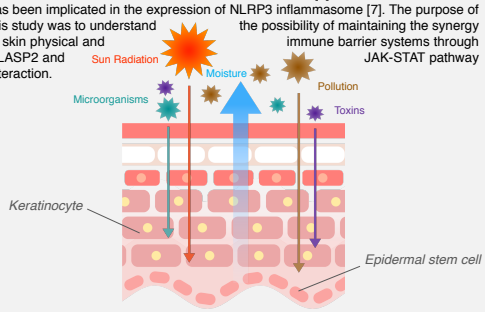

Figure 1: Importance of skin barrier

Materials & Methods:

Preparation of the natural skin protectant
An geographical indication tagged mushroom traditionally used for healthy food and skin protection has been identified. A Mushroom-Based Skin Protectant (MBSP) is obtained by an optimized water extraction of the dried sporocarp of this mushroom.

Epidermal stem cell culture and cell viability measurement
Primary keratinocyte (NHEKs) culture were enriched in epidermal stem cell following the method described by Goodell et al. Cells were pre-incubated for 24 hours in absence (control) or in presence of reference product or the MBSP. Cell viability has been measured using Alamar blue, based on resazurin reduction by mitochondria.

Primary keratinocyte culture and quantification of target proteins
NHEKs were pre-incubated during 24 hours in absence (control) or in presence of reference product or the MBSP. C. acnes lysate at a final concentration of 2.10^8 UFC was used as LPS. At the end of the pre-incubation period, CLASP2, p-STAT3, NLRP3 inflammasome and IL-1 beta protein levels were quantified in cell lysates.

Conclusions:

As the outermost layer of the body to the surrounding environment, the integrity of cutaneous barrier is particularly important for healthy aging. The skin barrier is mainly composed of the epidermis-based physical barrier and the immune barrier formed by various immune response networks. Our findings are consistent with the possibility that CLASP2 interact with the JAK2-STAT3 pathway in cutaneous cells to preserve epidermal architecture and barrier function. The CLASP2 and JAK-STAT pathway interaction through inflammasome may become a novel approach for maintaining skin physical and immune barrier synergy.

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Results & Discussion:

SKIN PHYSICAL BARRIER PROTECTION

Both skin protection and wound healing are based on intact epidermal architecture and barrier function. Quercetin and the MBSP significantly protected epidermal stem cells from UVB stress. Multiple mechanisms of stem cells and cell adhesion allow the formation of skin tissue with well-defined structure. CLASP2 has been reported to target microtubule with potential functions in maintaining cell–cell adhesion homeostasis in epidermal stem cells [4] and may prevent keratinocyte differentiation disorders [5]. The CLASP2 synthesis was significantly increased by the MBSP versus control, which coincided with the trend towards preserved epidermal stem cells under UVB condition. Exogenous CLASP2 has been shown to increase cutaneous cell migration to enhance wound healing [8]. Our results indicate that endogenous CLASP2 may also promote wound healing via epidermal keratinocyte migration.

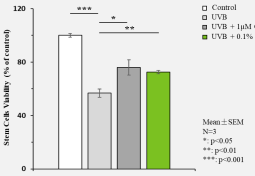

Figure 2: Effect of the MBSP on epidermal stem cell protection against UVB.

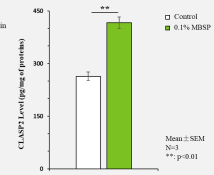

Figure 3: Effect of the MBSP on CLASP2 synthesis in NHEKs.

SKIN IMMUNE BARRIER PROTECTION

CLASP2 has also been described to be essential for the inhibition of the JAK2-STAT3 pathway [9]. The MBSP significantly reduced the LPS-induced STAT3 phosphorylation. JAK2 inhibition reduces STAT3 phosphorylation, decreases the activation of NLRP3 inflammasome, resulting in the downregulation of IL-1 beta expression [10].

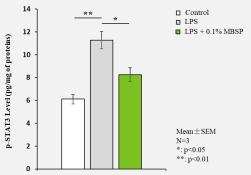

Figure 4: Effect of the MBSP on STAT3 phosphorylation in NHEKs.

The LPS-induced NLRP3 inflammasome activation and IL-1 beta production in NHEKs were statistically counterbalanced by dexamethasone and the MBSP, suggesting that the MBSP displays potent immunomodulatory effects in NHEKs. IL-1 beta is vital for the skin inflammation progression and wound repair via keratinocyte proliferation [11], constituting an important component in the crosstalk between skin physical and immune barriers.

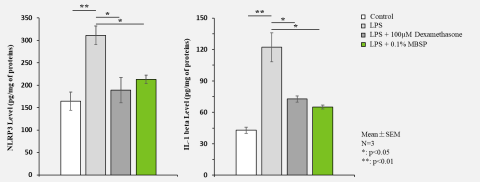

Figure 5: Effect of the MBSP on NLRP3 inflammasome activation in NHEKs.

The cutaneous cell migration can intuitively reflect the wound healing ability of the skin barrier. Both TGF-β and the MBSP significantly improved cell migration and promoted injured area healing at 72 hours compared to the control group. This result is consistent with the above regulatory trend of CLASP2 and JAK2 pathways.

References:

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