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

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Abstract

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. The skin barrier is mainly composed of the epidermis-based physical barrier and the immune barrier formed by various immune response networks. 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. In NHEKs, the CLASP2 synthesis was significantly increased by the MBSP. The latter significantly protected epidermal stem cells from UVB stress, which coincided with the trend towards increased CLASP2. The LPS-induced STAT3 phosphorylation, NLRP3 inflammasome and IL-1 beta production in NHEKs were statistically counterbalanced by MBSP, suggesting that MBSP displays potent JAK2 inhibition in NHEKs. A significant increase in wound healing at 72 hours was observed on scratched monolayer of NHEKs in the presence of MBSP. Our findings are consistent with the possibility that CLASP2 interact with the JAK2-STAT3 pathway through inflammasome in cutaneous cells to preserve epidermal architecture and barrier function, which may represent a novel approach to maintain skin physical and immune barrier synergy.

Keywords: Skin barrier; Epidermal architecture; CLASP2; JAK-STAT; Inflammasome; Metaflammaging

Introduction

In recent years, climate change and modern life behavior have become a growing concern, affecting every aspect of our lives. Lifetime exposure to different stressors leads to the accumulation of chronic inflammation, leading to oxidative and metabolic damage, which are thought to be essential factors in the aging process. 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.

The maturation and differentiation of keratinocytes as well as the regulation of cell-cell contacts are critical for the skin physical barrier [1]. As a major cell source of skin development, function and regeneration, keratinocyte stem cells are important for cutaneous barrier homeostasis [2, 3, 4]. The microtubule plus-end binding protein CLASP2 is involved in cell-cell adhesion preservation in epidermal stem cells [5] and prevents keratinocyte differentiation disorders [6], which are essential for epidermal architecture maintenance.

Skin immune barrier represents the last link of the cutaneous barrier [1]. The Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling 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 [7]. The activation of STAT has been implicated in the expression of NLRP3 inflammasome [8]. The latter has been identified as a key element in metabolic inflammation or metaflammation [9].

Various barrier-related factors are closely linked to maintain skin barrier homeostasis, but the interplay between CLASP2 and JAK-STAT pathway in cutaneous cells remains unclear. 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.

Materials and Methods

Preparation of the natural skin protectant

A Chinese edible mushroom traditionally used for healthy food and skin protection has been identified. This geographical indication tagged mushroom from organic substitute cultivation has been shown to have higher immunomodulatory activity than other origins. A

Mushroom-Based Skin Protectant (MBSP) is obtained by an optimized water extraction of the dried sporocarp of this mushroom. The *in vitro* tests are conducted with the pure active matter of MBSP at 0.1%.

Epidermal stem cell culture and cell viability measurement

Human Normal Epidermal Keratinocytes (NHEKs) obtained from a 49 years old female donor were cultured in Keratinocytes Growth Medium 2 Kit (C-20111, PROMOCELL). The cultures were maintained in an incubator at 37°C, 5% CO_2 with an atmosphere saturated in humidity. NHEKs have been cultivated in monolayer until reaching 80% of confluency. Cell culture were then enriched in epidermal stem cell following the method described by Goodell et al. Quercetin at 1µM from Sigma Aldrich has been used as reference product in this study. Cells were pre-incubated for 24 hours in absence (control) or in presence of reference product or MBSP. At the end of the pre-incubation period, cells were irradiated by UVB (30mJ/cm^2) and then incubated for 6 days at 37°C in absence (control) or in presence of reference product or MBSP. Cell viability has been measured using Alamar blue, based on resazurin reduction by mitochondria.

Primary keratinocyte culture and quantification of target proteins

NHEKs have been cultivated in monolayer until reaching confluency. Cells were pre-incubated during 24 hours in absence (control) or in presence of MBSP. At the end of the pre-incubation period, CLASP2 protein was quantified in cell lysates. *Cutibacterium acnes* (C. acnes) lysate at a final concentration of 2.10^8 UFC was used as lipopolysaccharide (LPS). Dexamethasone at 100µM has been used as reference inhibitor of C.acnes lysate induced NLRP3 production. Cells were then incubated with LPS for 24 hours in absence (control) or in presence of reference product or MBSP. At the end of the incubation period, p-STAT3, NLRP3 inflammasome and IL-1 beta protein levels were measured in cell lysates using Bradford method.

Scrap test

In order to evaluate the wound healing effect, a scratch of around 1mm of width has been performed in the NHEK monolayer. Transforming Growth Factor β (TGF- β) at

10ng/ml has been used as reference compound increasing wound healing. Cells were then incubated at 37°C, under humid atmosphere at 5% of CO_2 during 72 hours, in absence (control) or in presence of reference compound or MBSP. The area of the injury at Day 0 and the non-recolonized area at Day 3 were measured by image analysis using the Image J software. Photographs were taken using a microscope objective with 4-fold magnification.

Results

Skin physical barrier protection

As the main cell source of keratinocytes, the viability of epidermal stem cells was evaluated. Even though epidermal stem cells are more photo-resistant than their direct progeny [3], UV damage to these totipotent cells should not be underestimated. Under the experimental conditions, UVB radiation induced a loss of cell viability of more than 40% (*p<0.001*) (*Fig.-1*). When quercetin was added to the culture medium, the cytotoxic effects of UVB radiation were partially inhibited (*p<0.05*) (*Fig.-1*). The MBSP also significantly (*p<0.01*) protected epidermal stem cells from UVB stress (*Fig.-1*). Since normal skin architecture is mainly dependent on the coordinated proliferation and differentiation of epidermal keratinocytes, we then evaluated the stimulation of CLASP2 protein expression in NHEKs. The CLASP2 synthesis was significantly increased by the MBSP versus control (*p<0.01*) (*Fig.-2*), which coincided with the trend towards preserved epidermal stem cells under UVB condition.

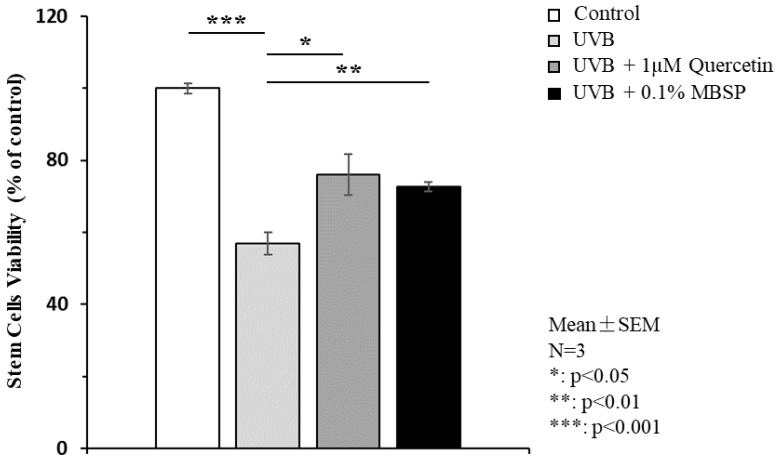

Fig.-1 Effect of MBSP on epidermal stem cell protection against UVB. *Results are presented in percentage of cell viability in comparison with the control condition. The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using T-test.*

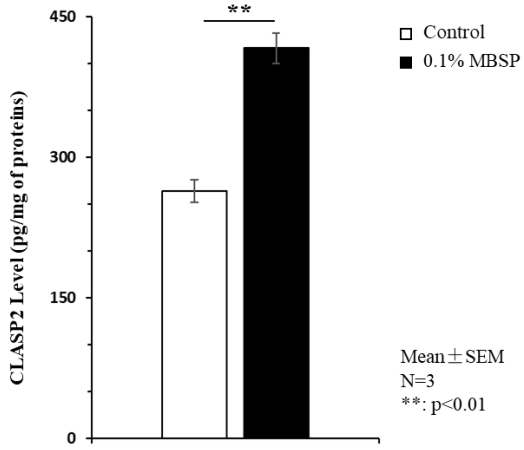

Fig.-2 Effect of MBSP on CLASP2 synthesis in NHEKs.
Results are expressed as pg of CLASP2 per mg of total proteins measured from cell monolayer lysate. The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using T-test.

Skin immune barrier protection

Pathogen-associated molecular pattern molecules (PAMPs), such as bacterial LPS, are a diverse group of microbial molecules that may induce immune responses in cutaneous cells. LPS has been described to be involved in both priming and activation signals during NLRP3 inflammasome activation [10]. Under the experimental conditions, LPS from C. acnes lysate induced a significant (*p<0.01*) rise of p-STAT3 (*Fig.-3*), NLRP3 inflammasome (*Fig.-4a*) and IL-1 beta production (*Fig.-4b*) in NHEKs compared to control. The MBSP significantly (*p<0.01*) reduced the LPS-induced STAT3 phosphorylation (*Fig.-3*). Dexamethasone at 100µM significantly (*p<0.05*) decreased NLRP3 inflammasome and IL-1 beta released induced by LPS (*Fig.-4*). The LPS-induced NLRP3 inflammasome and IL-1 beta production in NHEKs were also statistically (*p<0.05*) counterbalanced by MBSP (*Fig.-4*), suggesting that MBSP displays potent immunomodulatory effects in NHEKs.

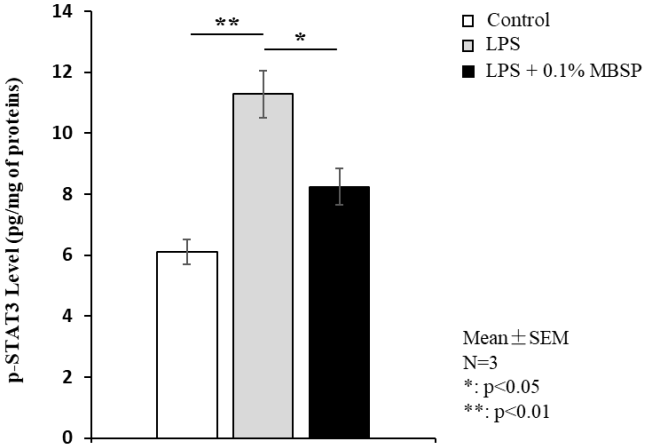

Fig.-3 Effect of MBSP on STAT3 phosphorylation in NHEKs.
Results are expressed as pg of p-STAT3 per mg of total proteins measured from cell monolayer lysate. The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using T-test.

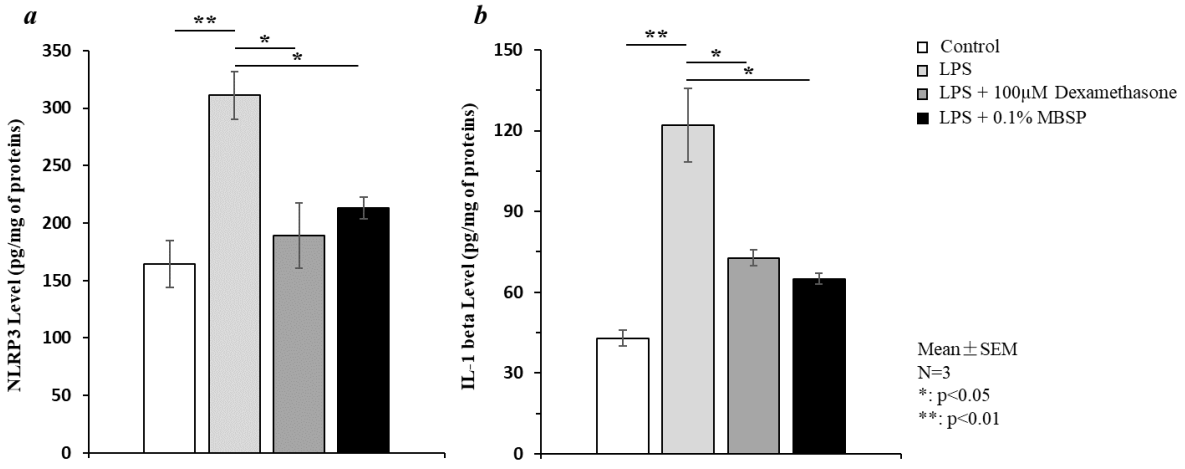

Fig.-4 Effect of MBSP on NLRP3 inflammasome activation in NHEKs.

(a) *NLRP3 protein level measurement in NHEKs;* ***(b)*** *IL-1 beta protein level measurement in NHEKs. Results are expressed as pg of NLRP3 or IL-1 beta per mg of total proteins measured from cell monolayer lysate. The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using T-test.*

Wound healing evaluation

The cutaneous cell migration can intuitively reflect the wound healing ability of the skin barrier. As shown in the image below (*Fig.-5a*), both TGF-β and MBSP significantly improved cell migration and promoted injured area healing compared to the control group. The quantified results are expressed as percentage of injured area recolonized by cells at 72 hours (day 3). In our experimental conditions, TGF-β at 10ng/ml significantly ($p<0.05$) increased wound healing (*Fig.-5b*) versus control. A significant ($p<0.05$) increase in wound healing at 72 hours was also observed on scratched monolayer of NHEKs in the presence of MBSP (*Fig.-5b*).

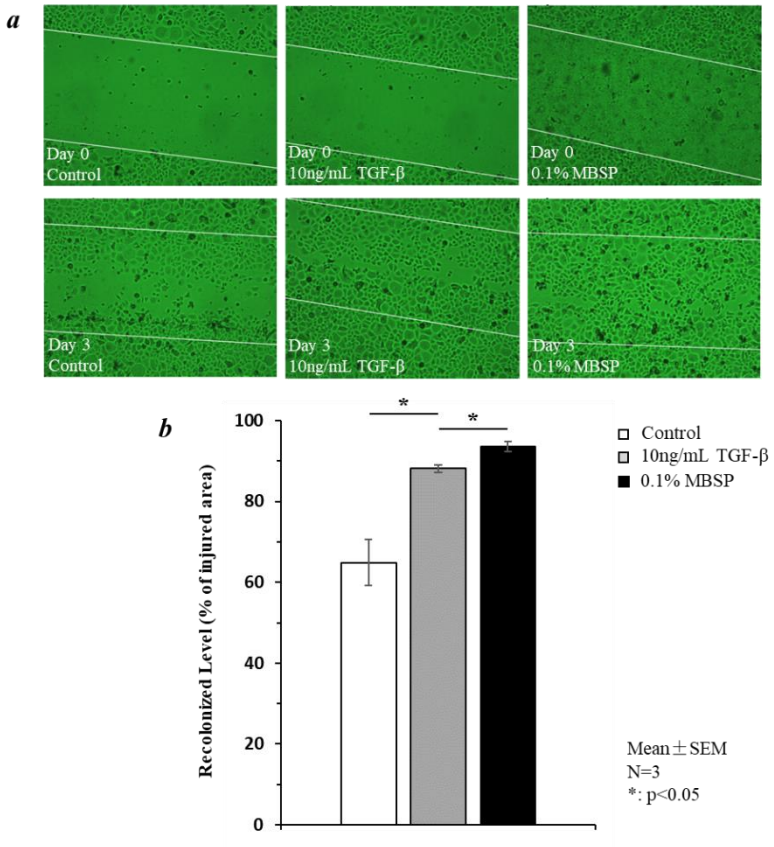

Fig.-5 Effect of MBSP on epidermal wound healing.

(a) *Representative images of the injured area at day 0 and day 3;* ***(b)*** *Recolonized level at day 3. Results are expressed as percentage of injured area recolonized by cells. The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using T-test.*

Discussion

As the skin surface is in direct contact with the external environment, external and internal factors may severely impact microbiome balance and skin health. Both skin protection and wound healing are based on intact epidermal architecture and barrier function. The epidermis is a self-renewing skin layer composed primarily of keratinocytes in various stages of terminal differentiation. Epidermal stem cells are responsible for the daily regeneration of the different layers of the epidermis, their viability and homeostasis are essential for maintaining cutaneous barrier structure, including skin repair, metabolism and regeneration [4]. Multiple mechanisms of stem cells and cell adhesion allow the formation of skin tissue with well-defined structure. UVA and UVB can penetrate the epidermis and induce DNA damage in cells of the basal layer of the epidermis where stem cells are located. Recent evidence suggests that keratinocyte stem cells are more resistant to UVA irradiation than their direct progeny [3]. Therefore, effectively fighting UVB appears to be an indispensable part of preserving the normal function of epidermal stem cells.

As mentioned before, normal skin architecture relies mostly on the coordinated proliferation and differentiation of epidermal keratinocytes. Cell differentiation is associated with microtubule reorganization [11]. CLASP2 has been reported to target microtubule with potential functions in maintaining cell–cell adhesion homeostasis in epidermal stem cells [5] and may prevent keratinocyte differentiation disorders [6]. Cell-cell adhesion is critical for the formation and maintenance of multicellular tissues like skin physical barrier [12]. Exogenous CLASP2 has been shown to increase dermal fibroblast migration to enhance wound healing [13]. Our results indicate that endogenous CLASP2 may also promote wound healing via epidermal keratinocyte migration.

CLASP2 has also been described to be essential for the inhibition of the JAK2-STAT3 pathway via the protein Suppressor of cytokine signaling 3 (SOCS3) [14], the latter is a negative regulator of JAK-STAT. Overexpression of SOCS3 may protect against cell injury by inhibition of the JAK2-STAT3 pathway [15]. This JAK2 pathway contributes to skin barrier dysfunction in some cutaneous immune diseases, such as atopic dermatitis [16] and psoriasis [17]. JAK2 inhibition reduces STAT3 phosphorylation, decreases the activation of NLRP3 inflammasome, resulting in the downregulation of IL-1 beta expression [18]. IL-1 beta is vital for the skin inflammation progression and wound repair via keratinocyte proliferation [19], constituting an important component in the crosstalk between skin physical and immune barriers.

The synergistic connection between various skin barrier systems has received increasing attention, but their linkage with the metabolic system is still poorly understood. Over the past few decades, increased life expectancy and unhealthy modern lifestyles have exposed the general population to emerging metabolic health problems [20]. The NLRP3 inflammasome is recognized as a novel target linking metabolic inflammation (metaflammation) and age-related inflammation (inflammaging) [9]. Incorporating systemic metabolism into skin homeostasis maintenance may be a promising strategy in skin care research.

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. JAK2 inhibition also has potential skin neuroprotective effects that require further investigation. The CLASP2 and JAK-STAT pathway interaction may become a novel

approach for maintaining skin physical and immune barrier synergy. In addition, by observing the interaction between the two major skin barrier systems through the NLRP3 inflammasome, we may provide a glimpse into a new field of skin aging, also known as metabolic inflammaging or metaflammaging.

Conclusion

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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Conflict of Interest Statement.

There is no any conflict of interest by the authors competing interests.

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