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Adiponectin Promotes Caspase-14 Expression in Normal Human Epidermal Keratinocytes

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Dear Editor:

Filaggrin (FLG) and its high molecular-weight precursor profilaggrin (proFLG) are filament-associated proteins that aggregate keratin fibers in keratinocytes. The cellular processing of proFLG and FLG provides an important material source of natural moisturizing factors (NMF), and multiple proteolytic enzymes including peptidylarginine deiminase (PAD) 1, PAD 3, caspase-14, calpain 1 and bleomycin hydrolase have been implicated in their proteolytic processing[1]. Among these proteases, caspase-14 is considered the key enzyme, as it is thought to directly cleave the FLG repeat in preparation for complete breakdown by other enzymes[2]. Adiponectin, an adipokine secreted from adipocytes, has primary effects on energy metabolism and anti-diabetic in nature. It has been well known that adiponectin also has anti-inflammatory effects[3].

Recently, there are a few reports investigating the effects of adiponectin on skin. It has been shown that adiponectin

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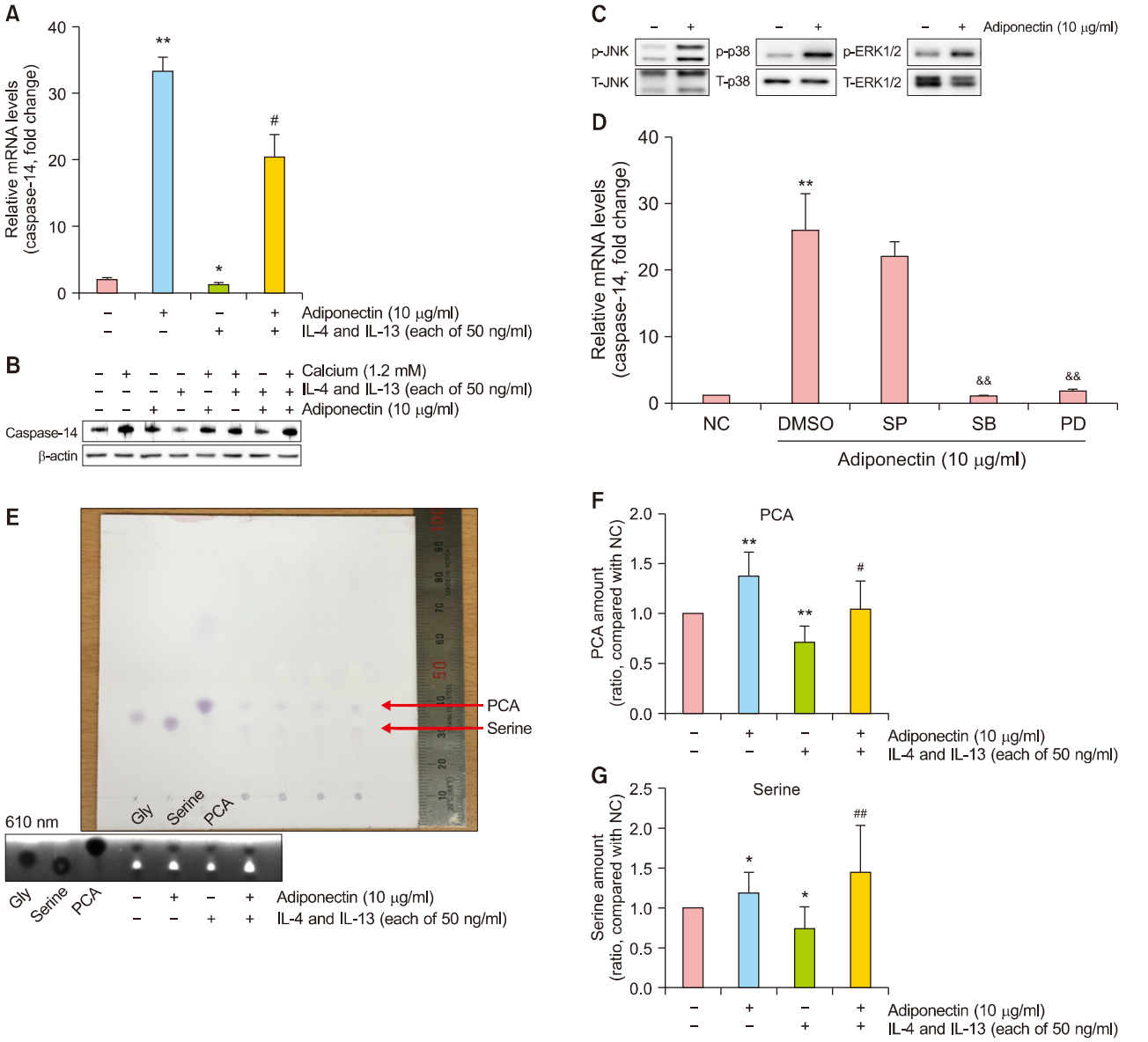

Fig. 1. Protein and mRNA levels of caspase-14 were measured by (A) real time reverse transcription polymerase chain reaction (RT-PCR) and (B) Western blot. β-actin expression was used as an internal control. The interleukin (IL)-4, IL-13 and calcium were treated with or without adiponectin for 72 hours. Data are represented in graphical form and show the fold change compared with sub-confluent normal control (NC) cells. (C) The phosphorylation of mitogen-activated protein kinases (MAPKs) (p38, JNK/SAPK and ERK1/2) were induced by adiponectin and (D) the caspase-14 mRNA expression after MAPKs inhibitors analyzed by real time RT-PCR. Normal human epidermal keratinocytes (NHEKs) were treated with MAPKs inhibitors; SP600125 (SP), SB203580 (SB) and PD98059 (PD) before adiponectin treatment. For the analyzed of amino acid, cells were harvested 72 hours after the start of adiponectin and interleukin treatments. One microgram of each cell lysate was loaded onto the high-performance thin-layer chromatography plate coated with cellulose and moved by mobile phase including 1-butanol, acetic acid and water (12:3:4). (E) Amino acid spots were detected by ninhydrin and compared with serine and pyrrolidone carboxylic acid (PCA) retention factor (R_f) values. The concentration of (F) PCA and (G) serine was measured using the intensity of amino acid spots and the values of measured spots calculated from 10 independent replicate experiments. Data are presented as the mean ± standard deviation of at least three independent replicate experiments (n = 3). DMSO: dimethyl sulfoxide, Gly: glycine. $^{*}p<0.05$, $^{**}p<0.005$ vs. NC, $^{\#}p<0.05$, $^{\#\#}p<0.005$ vs. IL-4 and IL-13 treated group, $^{\&\&}p<0.005$ vs. adiponectin treated group.

modulates proliferation, migration and cytokine secretion of keratinocyte, and then regulates cutaneous wound healing process[4-6]. However, little is known about the effect of adiponectin on skin barrier function. Therefore, we examined the effect of adiponectin on expression of caspase-14 and FLG breakdown products in normal human epidermal

keratinocytes (NHEK).

NHEK were serum-starved for 6 hours before being treated with adiponectin. For the comparative control, we used calcium, and interleukin (IL)-4 and IL-13 as the positive and negative reaction, respectively. Afterward, gene mRNA expression was quantified using real time reverse transcription polymerase chain reaction (RT-PCR) and protein expression was evaluated using immunofluorescence and Western blot. To quantitatively assess the NMF amino acid, we used high performance thin layer chromatography. To evaluate the relationship between mitogen-activated protein kinases (MAPKs), activator protein 1 (AP-1), FLG and caspase-14, we also treated cells with inhibitors for MAPKs; JNK, p38 and ERK1/2.

As shown in Fig. 1A, caspase-14 mRNA expression was markedly increased by adiponectin treatment, even while the IL-4 and IL-13 work as a caspase-14 suppressor.

Similar to those findings, Western blot showed that adiponectin also induced protein expression of caspase-14 (Fig. 1B). Hsu et al.[7] reported that MAPK pathways induced caspase-14 expression in epidermal keratinocytes. When analyzing the protein expression of MAPKs after adiponectin treatment, adiponectin up-regulated of phosphorylation of MAPKs; p38, JNK/SAPK and ERK1/2 (Fig. 1C). The inhibitor study results indicated that the p38 and ERK, MAPK pathways are required for adiponectin induced expression of caspase-14 in NHEK (Fig. 1D).

We next sought to measure the effect of adiponectin on the level of FLG breakdown products including NMF. In order to quantitatively determine the level of FLG breakdown products, the amino acid spots were detected by spraying samples with ninhydrin solution and the calculated the retention factor (R_f) values of serine and pyrrolidone carboxylic acid (PCA) were 0.253 and 0.337, respectively (Fig. 1E). The separated spots which matching with PCA and serine were measured by using densitometry program (Image J). The amount of serine and PCA were significantly increased by adiponectin. When NHEK were simultaneously treated with adiponectin as well as IL-4 and IL-13, we found that the production of FLG breakdown products, normally inhibited by IL-4 and IL-13, were restored (Fig. 1F, G).

Regarding the effect of adiponectin on the expression of FLG proteolysis enzyme, as well as FLG breakdown products, our data suggests that adiponectin has the ability to promote caspase-14 expression via p38 and ERK1/2 and thereby increase the rate at which FLG is broken down. Additionally, we found that in addition to their inhibition of production of FLG breakdown products, IL-4 and IL-13, also suppress the expression of caspase-14, and thereby reduce the rate at which FLG breakdown products accumulate, effects that are to be expected given the Th2 cytokine milieu. However, treatment with adiponectin restores caspase-14 expression and the level of FLG breakdown products. The increase in FLG breakdown products induced by adiponectin is likely to be the accumulated result of both upregulating the expression of FLG itself as well as that of an important FLG-processing enzyme[8].

In conclusion, our findings suggest that adiponectin acts to promote the production of FLG breakdown products including NMF by inducing the expression of the FLG processing enzyme caspase-14 in NHEK. The present study demonstrated for the first time that adiponectin plays a role regulating the processing of FLG in NHEK. Therefore, adiponectin may be considered as a potential therapeutic agent to control skin diseases that alter the skin barrier.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

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A Case of Suggested Pigmented Condyloma Acuminatum

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Dear Editor:

Human papillomavirus (HPV) infection of the genital skin is highly prevalent and showed variable presentations. Low-risk HPV type 6 or 11 is usually detected in cases of condyloma acuminatum (CA), while high-risk HPV type 16 or 18 is detected in cases of bowenoid papulosis (BP)[1]. Expression of p16 protein (p16) has also been identified as a marker for HPV infection and is typically associated with neoplasia of the genital lesion[2].

We received the patient's consent form about publishing all photographic materials. A 29-year-old male patient presented with multiple brownish verrucous papules on his penis that had been present for 1 year and had been progressively increasing in size. He had no subjective symptoms including pruritus or pain. He had no family or personal history of skin cancer or other medical diseases. Physical examination revealed multiple, 0.7×0.8 cm sized brownish verrucous papules around the penile shaft (Fig. 1). Histopathological examination revealed parakeratosis, acanthosis, papillomatosis, vacuolated keratinocytes and a slightly disordered arrangement of keratinocytes without atypia throughout the thickened epidermis (Fig. 2A, B). Focal positive p16 expression was present in the lesion (Fig. 2C). HPV DNA chip™ microarray analysis

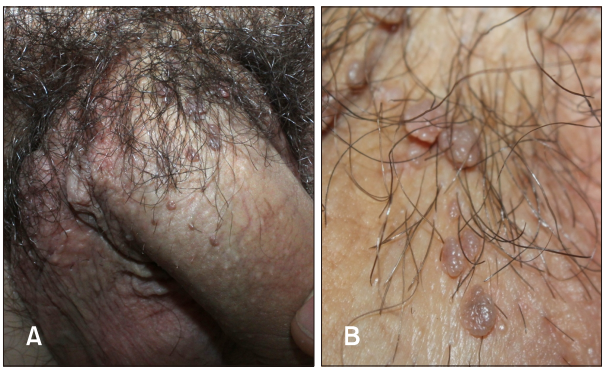

Fig. 1. (A, B) Multiple, 0.7×0.8 cm brownish verrucous papules around the penis shaft.

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