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Intense Pulsed Light Increases Hyaluronan and CD44 in Epidermal Keratinocytes and Improves Age-Related Epidermal Structure Defects in Mice

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Dear Editor:

Aged skin exhibits epidermal thinning with decreased keratinocyte proliferation and differentiation, delayed wound healing, and decreased lipid synthesis[1]. Recently, age-dependent change in the metabolism of epidermal hyaluronan (HA) is suggested to be responsible for these epidermal dysfunctions by activating CD44 signaling. A decrease in epidermal HA has been found in aged human and murine skin[2,3], and the application of HA restored permeability barrier structure and function in aged murine skin, suggesting a role in cutaneous aging[2]. Expression of CD44 and hyaluronan synthases (HAS) was also shown to be reduced in aged skin[2,4].

Intense pulsed light (IPL) has been reported to be effective for improving aged skin, and nonablative dermal remodeling is an important mechanism underlying its photorejuvenation effect[5]. However, the efficacy and mechanisms of IPL in improving age-related epidermal dysfunctions remain largely unknown. We hypothesized that IPL treatment can induce epidermal HA production in keratinocytes, which in turn contributes to overcome the age-related epidermal dysfunctions.

The half of dorsal skin of thirty 8 week-old (young) hairless mice and fifteen 47 week-old (aged) C57BL/6J mice was irradiated by IPL (SOLARI™; Lutronic Corporation, Goyang, Korea, 570 nm cut-off filter, 12 mm×40 mm spot size); triple pulses of 20 ms, pulse interval 20 ms, fluence 13 J/cm^2. Skin specimens were obtained on days 1, 3 and 7 of postirradiation. HA levels were measured using the QnE HA ELISA (Biotech Trading Partners, Encinitas, CA, USA).

Normal human keratinocytes (NHEK) were derived from neonatal foreskin (Clonetics, San Diego, CA, USA). The epidermis was isolated by incubation in dispase, and a suspension of keratinocytes was obtained by incubation in 10 mM EDTA and subsequent trypsinization. Obtained cells were cultured with serum-free keratinocyte growth medium and used for experiments after 2 or 3 passages. Cells were irradiated with IPL using two different cut-off filters (510 nm or 570 nm); triple pulses of 20 ms, pulse interval 20 ms, fluence 12 J/cm^2. The distance between the handpiece and cells was approximately 1 cm. After irradiation, medium was immediately replaced with fresh medium and left for another 24 hours and 48 hours.

Increased HA expression in the epidermis of both young and aged mouse skin was observed after IPL treatment, which was confirmed by immunofluorescence staining of biotin-conjugated HA binding protein (HABP), which was noted from day 1 and remained high for at least 7 days (Fig. 1A). HA enzyme-linked immunosorbent assay (ELISA) confirmed a significant increase of epidermal HA content in both young and aged mice after IPL treatment. The aged mice showed greater increase in epidermal HA content compared to young mice (1.8-fold increase in young mice vs. 4.2-fold increase in aged mice on day 1) (Fig. 1B). HABP4 mRNA level was also significantly increased in the

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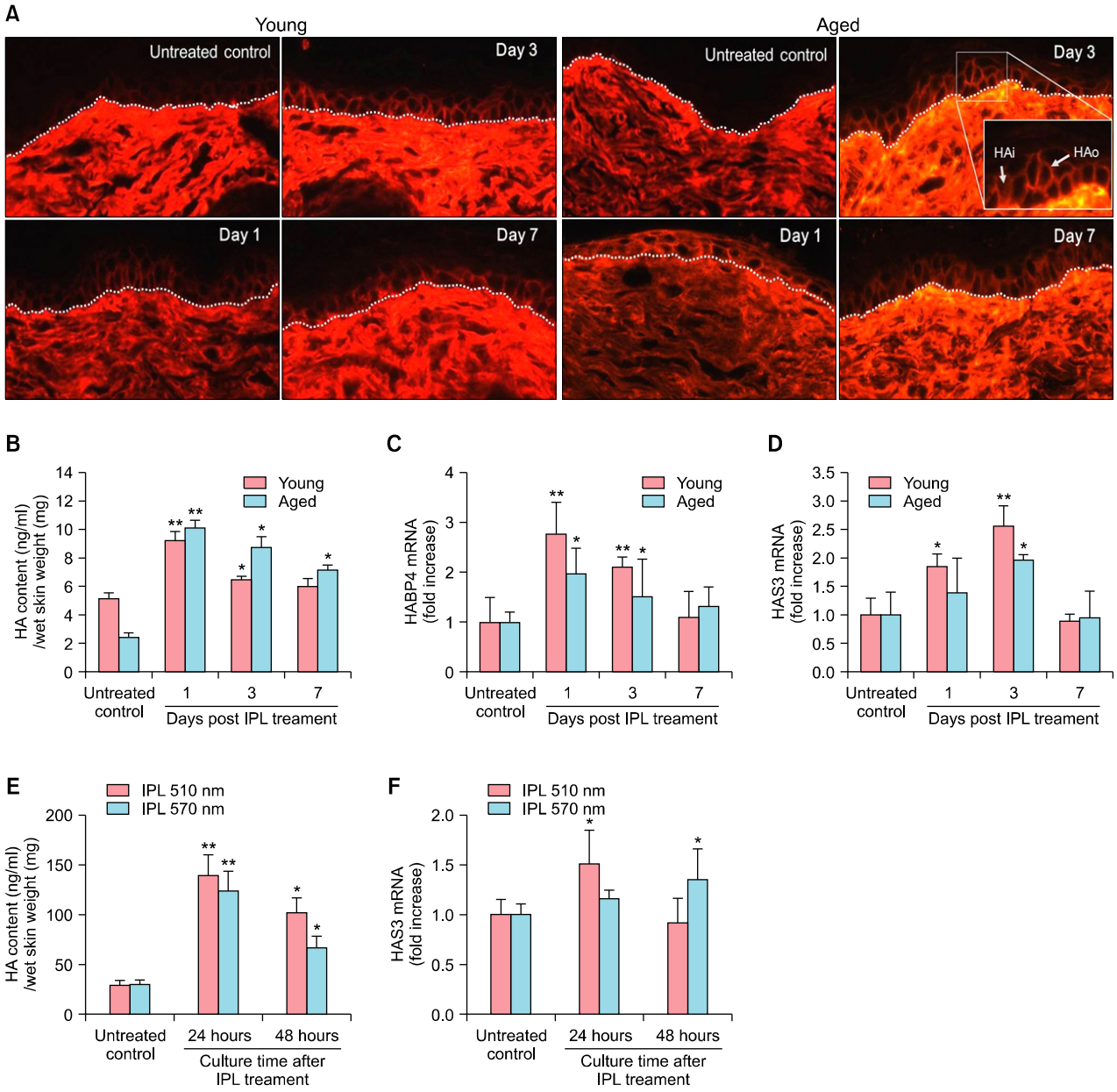

Fig. 1. Intense pulsed light (IPL) treatment increases hyaluronan (HA) production and hyaluronan synthases (HAS) 3 expression in young and old aged mouse epidermis and cultured human keratinocytes. (A) Sections of young and aged mouse skin treated with IPL were stained for HA binding protein (HABP) 4 and examined by confocal microscope. Dashed line is the dermal-epidermal junction. Magnified image shows example of accumulated HA in the intercellular (HAo) and intracellular space (HAi) (×400). After epidermal separation, HA content was measured by enzyme-linked immunosorbent assay (ELISA) (B) and the mRNA level of HABP4 (C) and HAS3 (D) was analyzed by quantitative real-time polymerase chain reaction (RT-PCR). Normal human epidermal keratinocytes (NHEK) were irradiated with IPL using either 510 nm or 570 nm cut-off filter. (E) ELISA for HA content and (F) quantitative RT-PCR analysis of HAS3 mRNA in conditioned medium from NHEK irradiated with IPL. Data represent the means±standard error. Data were analyzed by Student's t-test (*$p<0.05$, **$p<0.01$).

epidermis of both group after IPL treatment (Fig. 1C). HA is synthesized by several HAS (HAS1, 2, and 3) and recent reports have described that HAS1 is responsible for HA production in keratinocytes under normal conditions, whereas HAS3 acts mainly in pathological conditions[6]. We analyzed HAS3 mRNA levels and observed a significant increase in HAS3 mRNA expression in the epidermis of both young and aged mice after IPL treatment (Fig. 1D). In addition, IPL treatment increased epidermal CD44 immunoreactivity in both young and aged mice

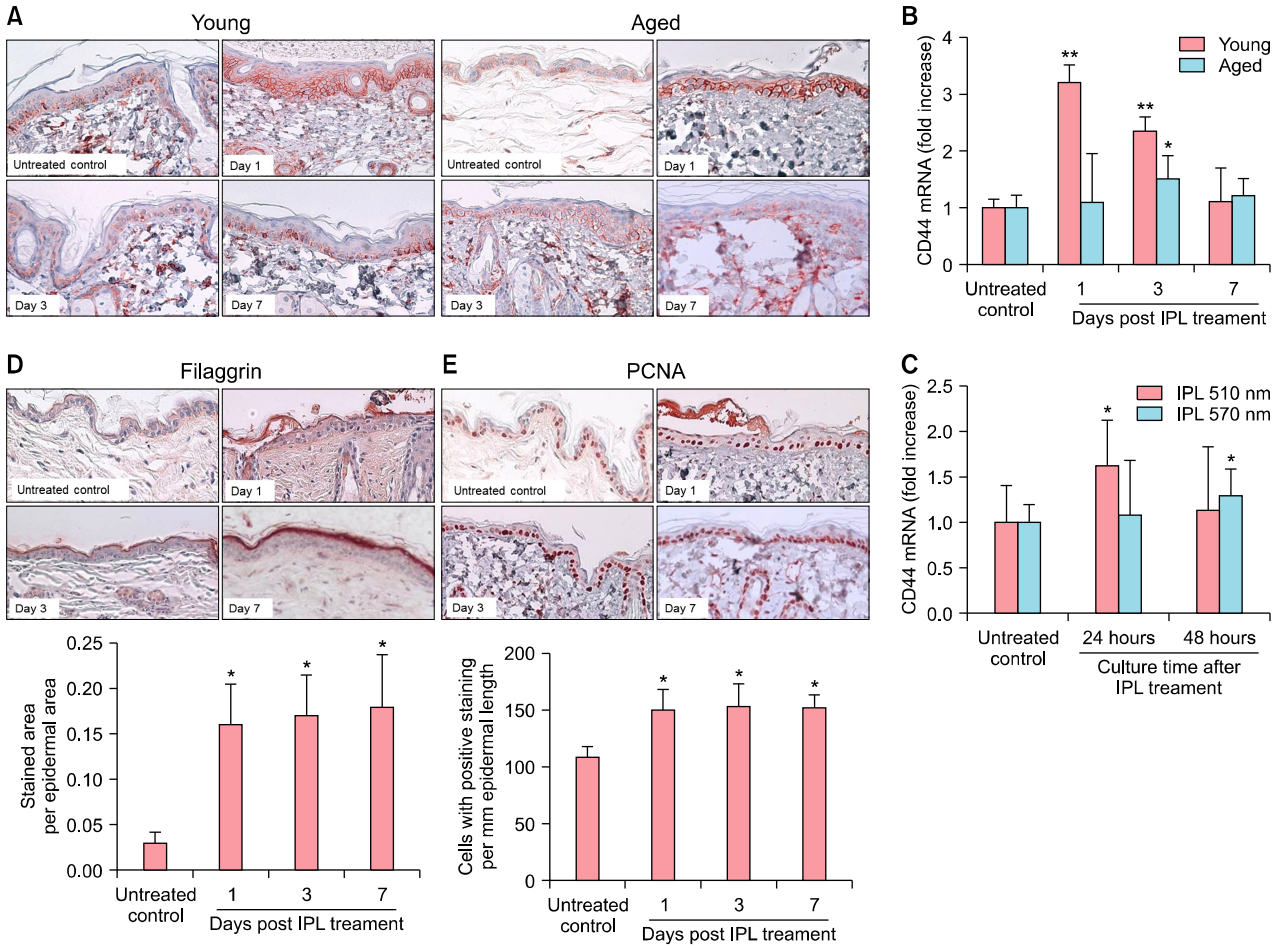

Fig. 2. Intense pulsed light (IPL) treatment increases CD44 expression in young and old aged mouse epidermis and cultured human keratinocytes and improves epidermal structure in aged mouse skin. (A) Immunohistochemical staining of CD44 in young and old aged mouse skin treated with IPL (×200). (B) After epidermal separation, CD44 mRNA level was analyzed by quantitative real-time polymerase chain reaction (RT-PCR). (C) CD44 mRNA level of conditioned medium from normal human epidermal keratinocytes (NHEK) irradiated with IPL using either 510 nm or 570 nm cut-off filter was analyzed by quantitative RT-PCR. Expression of differentiation marker, filaggrin (D) and proliferation marker, proliferating cell nuclear antigen (PCNA) (E) in aged mice skin treated with IPL was examined by immunohistochemical staining (×200). The stained area per epidermal area for filaggrin was measured (D) and cells with positive staining for PCNA per mm epidermal length were counted (E) by digital image analysis. Data represent the means±standard error (*$p<0.05$, **$p<0.01$).

(Fig. 2A). CD44 mRNA expression was also upregulated with statistical significance in both groups upon IPL treatment (Fig. 2B).

We further investigated the effect of IPL on the HA metabolism in NHEK. Compared with the untreated control, there was a significantly increased HA content in culture supernatants of NHEK following IPL. The largest increase of HA amount was observed at 24 hours and remained increased at 48 hours after IPL irradiation with 510 or 570 nm filter (Fig. 1E). Moreover, mRNA expression of HAS3 and CD44 was significantly increased by IPL treatment in NHEK (Fig. 1F, 2C). In general, the depth of penetration of laser energy increases with wavelength. However, regarding the influence of IPL on HA metabolism in the epidermal level, both cut-off filters were able to increase HA production with no significant difference.

We further investigated the effect of IPL irradiation on epidermal differentiation and proliferation in both young and aged mice. IPL treatment increased both filaggrin and proliferating cell nuclear antigen (PCNA) expression only in the aged mice epidermis, as compared with those detected in the untreated aged mice (Fig. 2D, E). Increased epidermal thickness was also detected in aged mice after IPL treatment.

These results indicate that IPL increases HA synthesis and CD44 expression in epidermal keratinocytes and stimulates epidermal proliferation and differentiation in aged mouse skin, providing evidence for the action of IPL on

HA metabolism, which may further explain the mechanism involved in the photorejuvenation effect of IPL. Based on our results, it can be postulated that increased HAS3 after IPL treatment may lead to epidermal HA synthesis. Proinflammatory cytokines such as IFN-γ, transforming growth factor-β (TGF-β)[7], interleukin (IL)-4, and IL-13, growth factors such as epidermal growth factor or keratinocyte growth factor[8], and retinoic acid[9] have been demonstrated to upregulate HAS2 and HAS3 in keratinocytes. Although comprehensive cytokine analysis studies following IPL treatment in keratinocytes are lacking, it was shown that IPL irradiation increases the protein and mRNA levels of TGF-β1. Thus, we can postulate that IPL treatment-induced TGF-β might contribute to HAS3 induction in keratinocytes.

In conclusion, we demonstrated that IPL treatment induces a significant increase of epidermal HA contents and improves the structural abnormalities in aged epidermis, suggesting that increased epidermal proliferation and differentiation in aged mice skin may be related to the skin rejuvenation effect of IPL in the context of restoring permeability barrier homeostasis at least partially through HA and CD44 interaction in the epidermis. A recent study demonstrated that large HA induces keratinocyte differentiation via Rac-PKNγ signaling, while small HA promotes keratinocyte proliferation via RhoA-ROK activation[2]. Further studies evaluating HA size distribution following IPL treatment and the biomolecular mechanism of IPL treatment on epidermal HA metabolism are needed.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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