

# Polycomb group proteins: Novel molecules associated with ultraviolet A-induced photoaging of human skin

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**Abstract.** Epigenetic repressor polycomb group (PcG) proteins are thought to serve a role in a number of cellular processes, including carcinogenesis, senescence, apoptosis and DNA repair. In the present study, long-wave ultraviolet A (UVA) was used to irradiate human skin fibroblasts (HSFs) and embryonic skin fibroblasts (ESFs) in order to simulate photoaging of the skin. The results of cell proliferation, apoptosis, hyaluronic acid (HA) content and reverse transcription-quantitative polymerase chain reaction assays revealed that the expression levels of genes encoding key PcG proteins (*BMI-1* and *EZH2*) were altered. In addition, the expression levels of these genes were associated with the expression of enzymes that regulate HA synthesis. Furthermore, the expression levels of PcG proteins differed between HSFs and ESFs, suggesting that PcG proteins serve a role in altering HA synthesis during the UVA-induced fibroblast aging process. This signaling pathway may represent a novel molecular mechanism regulating the photoaging of the skin. The findings of the present study provide important insights into the underlying mechanisms of photoaging of the human skin. Further studies are required to clarify the molecular mechanisms underlying skin aging and to identify targets for the clinical treatment of photoaging.

## Introduction

The skin is the body's largest sensory organ, covering the majority of the body. The primary function of the skin is the protection of body tissues and organs from physical and mechanical damage, toxic chemicals and pathogenic microorganisms; therefore, the skin is subjected to various factors that cause aging of the tissue. In addition to the body's natural aging processes, exogenous optical radiation directly promotes

skin aging. Among the various types of light affecting the skin, ultraviolet A (UVA) is the primary contributor to skin photoaging because it is not absorbed by any laminar flow and potentially penetrates the skin (1,2). Previous studies have demonstrated that the probability of skin aging is doubled in populations with high UV exposure compared with populations with lower exposure, and skin aging occurs  $\leq 10$  years earlier in such populations (3-5).

Common features of skin aging include a rough texture, dryness, reduced elasticity and wrinkles, and these features are associated with a reduction of the extracellular matrix in the skin dermis (6). Hyaluronic acid (HA), which is synthesized and secreted by the fibroblasts in the skin dermis, is an important molecule because of its strong hydrophilicity. HA controls the water content, swelling and diffusion gradient in the skin and therefore functions to maintain its structural integrity (7-9). The primary treatment to prevent or alleviate skin aging is the injection of exogenous HA (10). However, due to the rapid absorption and metabolism of HA, these treatments do not provide long-term effects. In order to slow down the process of skin aging, the underlying molecular mechanism of the reduction in HA during skin aging requires elucidation.

HA is a glycosaminoglycan that is synthesized and secreted by fibroblasts. The rate of synthesis, stability, extensibility and total amount of HA are dynamically regulated by hyaluronan synthases 1-3 (*HAS 1-3*), and hyaluronate lyases 1 and 2 (*HYAL1* and *HYAL2*) (11). *HAS* and *HYAL* proteins serve different roles in the synthesis of HA, and differentially regulate HA in response to stimulation by different types of cytokines (12). Embryonic fibroblasts (EFs) are considered the youngest fibroblasts present in the body; A previous study indicated that the *HAS* gene is differently expressed in fibroblasts during different phases of embryonic development (13). In addition, HA is differentially synthesized in human embryonic skin fibroblasts (ESFs) and human skin fibroblasts (HSFs) (14), suggesting that *HAS 1-3*, *HYAL1* and *HYAL2* serve unique and different roles in human growth, development and aging. Therefore, HSFs and ESFs were used as model cells in the present study.

The present study aimed to further investigate and elucidate the underlying molecular mechanisms regulating the synthesis of HA in fibroblasts. Multiple researchers have examined the mechanisms regulating the synthesis of HA in fibroblasts (15-17). From these studies, two mechanisms have

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been confirmed; the activation of the transforming growth factor  $\beta$  receptor (TGF- $\beta$ R)/*Smad2* and HA-mediated cluster of differentiation 44-epidermal growth factor receptor (EGFR) colocalization and signaling (15-17). The shared extracellular signals of these two mechanisms are able to regulate the activity of extracellular signal-regulated kinase 1/2 (ERK1/2) (15-17). However, the underlying regulatory mechanisms of HA synthesis at the epigenetic level in skin aging remain unclear. Accordingly, the present study aimed to identify epigenetic regulatory molecules that may be associated with photoaging of the skin, with the goal of identifying novel regulatory signaling pathways.

Polycomb group (PcG) proteins are chromatin-modifying proteins that serve important roles as transformational global epigenetic repressors in cell proliferation, senescence and tumorigenesis (18). PcG proteins include two core protein complexes, polycomb repressive complex 1 and 2 (PRC1 and PRC2). PRC1 is predominantly responsible for maintaining the stability of chromatin that are in a repressive state, and PRC2 functions during the initiation phase of cellular transcriptional repression (19,20). The core subunit of PRC2, enhancer of zeste homolog 2 (*EZH2*), and the polycomb complex protein *BMI-1* (*BMI-1*) in PRC1, are the most well studied PcG proteins. PcG proteins have been demonstrated to be associated with the regulation of hematopoietic stem cell aging; however, few studies have investigated the regulation of fibroblasts in regards to photoaging of human skin. Therefore, the present study investigated whether *EZH2* and *BMI-1* serve a role in regulating the synthesis of HA during UVA irradiation-induced skin photoaging, and to examine whether the role of HA differs in ESFs and HSFs. These data are expected to aid in the identification of novel regulatory signaling pathways underlying skin photoaging and to elucidate effective therapeutic targets.

## Materials and methods

**Cell culture.** HSFs were purchased from Shanghai EK-Bioscience Co., Ltd. (Shanghai, China) and ESFs were purchased from Shanghai Xinyu Biological Technology Co., Ltd. (Shanghai, China). Cells were cultured as monolayers in high glucose Dulbecco's modified Eagle's medium (DMEM, high glucose; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; ScienCell Research Laboratories, Inc., Carlsbad, CA, USA) and 1% penicillin-streptomycin (Gibco; Thermo Fisher Scientific, Inc.) in 25 ml culture flasks at 37°C with 5% CO<sub>2</sub>. Cells were seeded in 96-well plates to a density of  $8 \times 10^3$ /well in cell viability assay and detection of HA content due to the requirements of an ELISA kit for HA (cat. no. CEA182Ge; Cloud-Clone Corp., Katy, TX, USA), and in other detections cells were seeded in 6-well plates to a density of  $2 \times 10^5$ /well.

**UVA irradiation.** A ZF-1 Tri-Ultraviolet Analyzer (Jiangsu Qilin-Lab Co., Haimen, China) was used to produce UVA radiation at a wavelength of 365 nm. A UV radiation meter (Kühnast Strahlungstechnik GmbH, Wächtersbach, Germany) was used to detect the real-time ultraviolet irradiance; the radiation distance was set as <5 cm to prevent decay. At 24 h after seeding ( $8 \times 10^3$ /well) the cells were subjected to

UVA irradiation using the designed radiation dose (0, 1, 4 and 8 J/cm<sup>2</sup>). The present study reviewed previous studies on UVA-induced skin aging and the UVA dose in the establishment of aging model range from 7.5 J/cm<sup>2</sup> to 15 J/cm<sup>2</sup> (21-27), the majority of which selected 10 J/cm<sup>2</sup>. In Huimin's (27) study on the effect of ultraviolet radiation on the proliferation of human fibroblasts, cells were irradiated with ten doses of 1-10 J/cm<sup>2</sup>. The results suggested that the inhibitory dose (ID) of 50% was 4 J/cm<sup>2</sup> and the inhibition rate of the 10 J/cm<sup>2</sup> group was 85%. In order to establish a suitable aging model, the present study selected 8 J/cm<sup>2</sup> as the high dose group, 4 J/cm<sup>2</sup> as the medium dose group, and 1 J/cm<sup>2</sup> as the low dose group. The irradiation time was calculated based on the selected irradiation dose as follows: UVA (J/cm<sup>2</sup>) = irradiance (W/cm<sup>2</sup>) x time (sec).

**Establishment of a UVA-induced aging model.** When the cells reached a density of  $6 \times 10^5$  cells/flask, HSFs and ESFs were detached by trypsinization and then centrifuged at 160 x g at 4°C for 5 min. Cells were then resuspended in the DMEM culture medium, with 10% FBS at 37°C and 5% CO<sub>2</sub>. The cells were counted manually using a hemocytometer and an optical light microscope (Olympus Corporation, Tokyo, Japan) and each well in the 96-well plate was seeded with 8,000 cells (23-27) for inoculation into cell culture plates. The two types of cells were then divided into the following groups: Experimental group (EG; cells in culture medium, irradiated), the control group (CG; cells in culture medium, nonirradiated), the blank experimental group (BE; no cells, irradiated) and the blank control group (BC; no cells, nonirradiated). Each group was analyzed in triplicate and sterile saline was added around each well to prevent evaporation. After overnight culture and adherence to the wells, the cells were exposed to UVA irradiation at four doses (0, 1, 4 or 8 J/cm<sup>2</sup>) using the ZF-1 Tri-Ultraviolet Analyzer at 365 nm. The cells were cultured at 37°C with 5% CO<sub>2</sub> and analyzed using flow cytometry, as described in detection of apoptosis, an Epoch Microplate Spectrophotometer, as described in Cell Proliferation assay (BioTek Instruments, Inc., Winooski, VT, USA) and Real-Time PCR. SPSS statistical software, version 13.0 (SPSS, Inc., Chicago, IL, USA) was used at 0, 12 and 24 h after the irradiation.

**Cell proliferation assay.** A total of 20  $\mu$ l of Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) assay solution was added to each well under sterile conditions, followed by gentle shaking to mix the contents of the plates. The absorbance at 450 nm was then measured with an Epoch microplate spectrophotometer (BioTek Instruments, Inc.) at 25°C after 0, 0.5, 1, 2 and 3 h using a microplate reader. Cell proliferation curves were drawn using data from the time points when the cells were the most potent and stably stained, as this indicates living cells. The effect of the different radiation doses on the proliferation of the two cells types was then analyzed and compared. The cell proliferation activity of the control was calculated as follows: (EG-BE)/(CG-BC).

**Detection of apoptosis.** After digestion with EDTA-free trypsin and termination of the digestion, HSFs and ESFs were washed with PBS and transferred into 5 ml tubes. The cells were centrifuged at 566 x g at 4°C for 5 min, the supernatant

Table I. Primer sequences for reverse transcription-quantitative polymerase chain reaction analysis.

| Gene         | Primer sequence                 | Direction |
|--------------|---------------------------------|-----------|
| <i>HAS1</i>  | 5'-TACAACCAGAAGTTCCTGGG-3'      | Forward   |
|              | 5'-CTGGAGGTGTACTTGGTAGC-3'      | Reverse   |
| <i>HAS2</i>  | 5'-GTGGATTATGTACAGGTTTGTGA-3'   | Forward   |
|              | 5'-TCCAACCATGGGATCTTCTT-3'      | Reverse   |
| <i>HAS3</i>  | 5'-GAGATGTCCAGATCCTCAACAA-3'    | Forward   |
|              | 5'-CCCACTAATACACTGCACAC-3'      | Reverse   |
| <i>HYAL1</i> | 5'-CCAAGGAATCATGTCAGGCCATCAA-3' | Forward   |
|              | 5'-CCCACTGGTCACGTTTCAAGG-3'     | Reverse   |
| <i>HYAL2</i> | 5'-GGCTTAGTGAGATGGACCTC-3'      | Forward   |
|              | 5'-CCGTGTCAGGTAATCTTTGAG-3'     | Reverse   |
| <i>BMI-1</i> | 5'-TGGATCGGAAAGTAAACAAAGAC-3'   | Forward   |
|              | 5'-TGCATCACAGTCATTGCTGCT-3'     | Reverse   |
| <i>EZH2</i>  | 5'-ATGCGACTGAGACAGCTCAA-3'      | Forward   |
|              | 5'-TGGGATGACTTGTGTTGGAA-3'      | Reverse   |
| <i>GAPDH</i> | 5'-GTGAAGGTCGGAGTCAACG-3'       | Forward   |
|              | 5'-TGAGGTCAATGAAGGGGTC-3'       | Reverse   |

*HYAL*, hyaluronidase; *HAS*, hyaluronan synthase; *EZH2*, enhancer of zeste homolog 2; *BMI-1*, polycomb complex protein *BMI-1*.

was discarded and the cells were resuspended, filtered and washed with PBS. This process was repeated twice. An Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) kit (cat. no. KGA105; Nanjing Keygen BioTech Co., Ltd., Nanjing, China) and a flow cytometer (BD FACSARIA; BD Biosciences, Franklin Lakes, NJ, USA) were then used to detect apoptotic cells, which were used according to the manufacturer's protocol:  $2.0 \times 10^5$  cells and 2 ml culture medium were added to each well of six-well plates, triplicate wells were performed for each experimental group. After cells were adherent for 24 h, different doses of irradiation were administered (0, 1, 4 and 8 J/cm<sup>2</sup>). Cells and flow tubes were collected and 100  $\mu$ l buffer was added to resuspend cells within each tube, filtered through filter paper to ensure that the cells were single state. Then, 50  $\mu$ l Annexin V-FITC dilution and 50  $\mu$ l PI dilution were added to each tube. The tubes were placed in the dark at room temperature for 15 min. Detection was performed using flow cytometry, the excitation wavelength (Ex) was Ex=488 nm, emission wavelength (Em) was Em=530 nm.

**Detection of HA content.** An ELISA kit for HA (cat. no. CEA182Ge; Cloud-Clone Corp., Katy, TX, USA) was used to detect the HA content of the HSFs and ESFs according to the manufacturer's protocol. Briefly, the standard (from the kit), sample (Sample to be measured) and control wells (Reagent Diluent from the kit) were produced and their optical density at 450 nm was measured using a microplate reader.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis.** Total RNA was isolated using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol and quantified spectrophotometrically. A RevertAid First Strand cDNA Synthesis kit (cat. no. K1622; Thermo Fisher Scientific, Inc.) was used for the generation of cDNA according to the

manufacturer's protocol. The thermocycling conditions were as follows: 42°C for 60 min; 70°C for 5 min; and maintenance at 4°C. A PrimeScript™ RT Reagent kit with gDNA Eraser (Perfect Real Time; cat. no. RR047A; Takara Bio Inc., Kusatsu, Japan) was used according to the manufacturer's protocol with a CFX Real-Time PCR Detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) for qPCR. The thermocycling conditions were as follows: Initial denaturation at 95°C for 3 min; and 45 cycles of denaturation at 95°C for 15 sec, annealing at 5°C for 20 sec and extension at 72°C for 30 sec. The *GAPDH* gene was used as the internal reference, and the  $2^{-\Delta\Delta C_q}$  relative quantification method (28) was used to calculate and analyze the mRNA expression levels of the target genes and selected proteolytic-cleavage products. The qPCR primer sequences are presented in Table I.

**Statistical analysis.** Data were analyzed using Dunnett's test with SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA). Data are presented as the mean  $\pm$  standard deviation of triplicate experiments.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**HSF and ESF proliferation is altered by UVA irradiation.** Microscopic observations demonstrated that the morphologies of HSFs and ESFs exhibited several differences. Specifically, the HSFs (Fig. 1A) were relatively more elongated and regularly spindle-shaped, whereas the ESFs (Fig. 1B) were relatively flatter, larger and more irregular. The results of the CCK-8 assay demonstrated that HSF proliferation in the 1 J/cm<sup>2</sup> group increased over time, whereas the proliferation rate of HSFs in the 4 and 8 J/cm<sup>2</sup> groups tended to decrease with time (Fig. 1C). This suggests that lower UVA doses promote HSF proliferation and that increasing doses

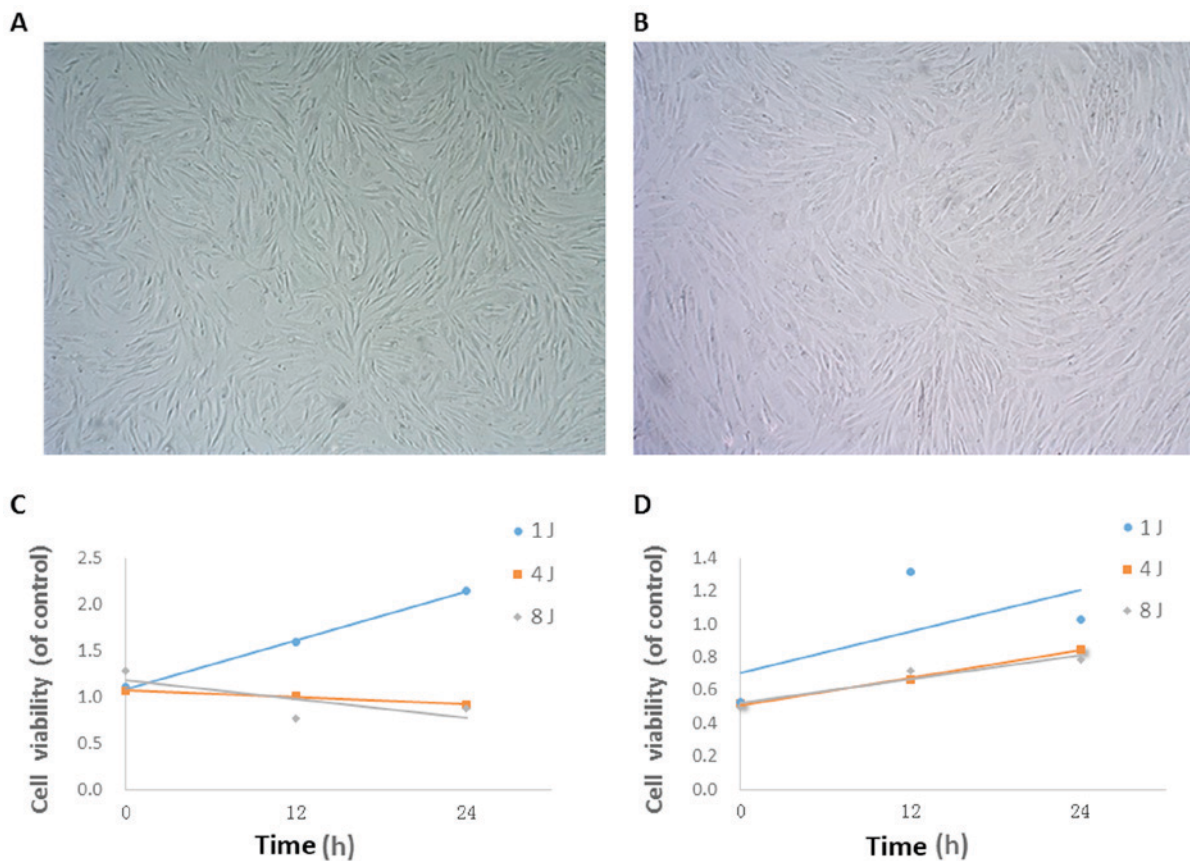


Figure 1. HSF and ESF proliferation is altered by UVA irradiation. Morphological differences between (A) HSFs and (B) ESFs. Magnification, x60. HSFs were relatively more elongated and regularly spindle, whereas ESFs were flatter and more irregular in shape. Impacts of different UVA radiation doses on the proliferative rates of (C) HSFs and (D) ESFs via the cell counting kit-8 assay. HSFs, human skin fibroblasts; ESFs, embryonic skin fibroblasts; UVA, ultraviolet A.

inhibit HSF proliferation. For ESFs, at 0 h after irradiation the cell proliferation rate of all irradiated groups was  $<1$  compared with the control (Fig. 1D). Additionally, higher UVA doses were associated with lower proliferation rates compared with lower doses, suggesting that higher doses of UVA irradiation inhibit ESF proliferation (Fig. 1D). However, as the time after irradiation increased, the cell proliferation activity gradually increased in each group (Fig. 1D).

**Apoptotic rate of HSFs and ESFs following UVA irradiation.** Flow cytometry analysis indicated that the apoptotic and necrotic HSF populations were increased in the 1 and 4 J/cm<sup>2</sup> groups compared with those in the control group (Fig. 2A and B). Additionally, the percentage of apoptotic and necrotic HSFs in the 4 J/cm<sup>2</sup> group was higher compared with those in the 1 J/cm<sup>2</sup> group, whereas those in the 8 J/cm<sup>2</sup> group were lower compared with those in the 4 J/cm<sup>2</sup> group (Fig. 2A and B) and the results tended to indicate that each dose group was consistent at different time points, compared with the control group (Fig. 2A and B). The percentage of apoptotic and necrotic ESFs were increased in a dose-dependent manner after UVA irradiation, with the percentages in the 8 J/cm<sup>2</sup> group being the largest (Fig. 2C and D).

**Alteration in HA content of HSFs and ESFs after UVA irradiation.** The HA content of HSFs and ESFs was decreased in each UVA-dose irradiated comparing with the control group, with the exception of ESFs irradiated with 1 J/cm<sup>2</sup> for 24 h

(Fig. 3). In addition, the HA content of HSFs was markedly higher compared with that in ESFs irradiated with the same dose of UVA and at the same time point.

**UVA irradiation alters mRNA expression of HAS1-3, HYAL1 and HYAL2 in HSFs and ESFs.** The results of RT-qPCR analysis revealed that in HSFs *HAS1* mRNA levels were significantly upregulated after 1 J/cm<sup>2</sup> UVA irradiation, but significantly reduced in the 4 and 8 J/cm<sup>2</sup> groups, compared with the 0 J/cm<sup>2</sup> group (all  $P < 0.05$ ; Fig. 4A). In ESFs, *HAS1* mRNA level at all irradiation doses was below the minimum detection level (Fig. 4A). Additionally, the expression of *HAS2* mRNA was upregulated by UVA irradiation in a dose-dependent manner in HSFs (Fig. 4B). However, in ESFs *HAS2* mRNA expression decreased in a dose-dependent manner with UVA irradiation (Fig. 4B). The expression of *HAS3* mRNA in HSFs was upregulated in all irradiation groups compared with the 0 J/cm<sup>2</sup> group, among which the upregulation in the 1 J/cm<sup>2</sup> group was significantly increased ( $P < 0.05$ ; Fig. 3C). Conversely, in ESFs, *HAS3* mRNA expression was downregulated in all irradiation groups (Fig. 4C). *HYAL1* mRNA expression levels were upregulated as the dose of UVA radiation increased in HSFs and ESFs (Fig. 4D). Compared with the 0 J/cm<sup>2</sup> group, *HYAL2* mRNA expression was upregulated in HSFs in the 1 J/cm<sup>2</sup> group and significantly unregulated in the 4 J/cm<sup>2</sup> group, but significantly downregulated in the 8 J/cm<sup>2</sup> group (all  $P < 0.05$ ; Fig. 4E). By contrast, in ESFs,

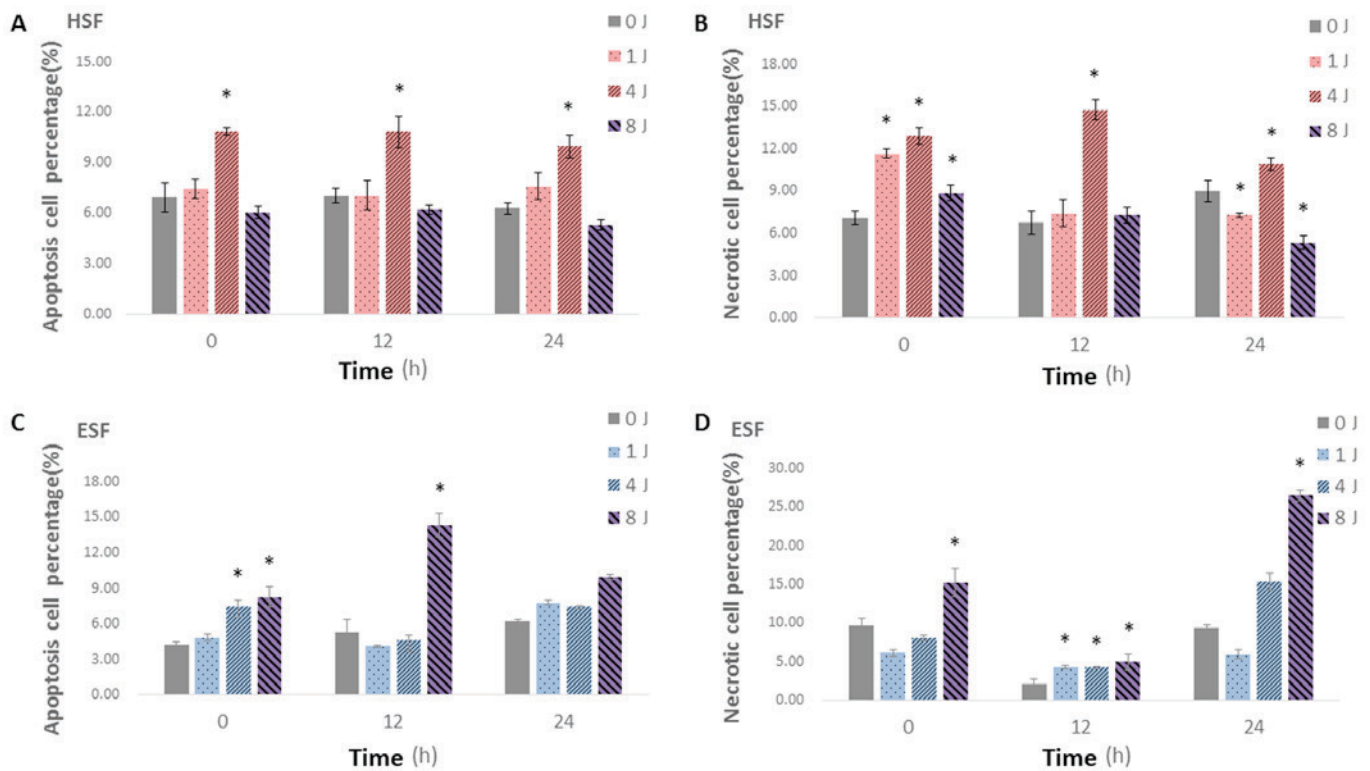


Figure 2. Apoptotic rate of HSFs and ESFs following UVA irradiation. Impacts of different UVA radiation doses on the (A) apoptosis and (B) necrosis of HSFs, and (C) apoptosis and (D) necrosis of ESFs. (\*P<0.05 vs. 0 J/cm²). HSFs, human skin fibroblasts; ESFs, embryonic skin fibroblasts; UVA, ultraviolet A.

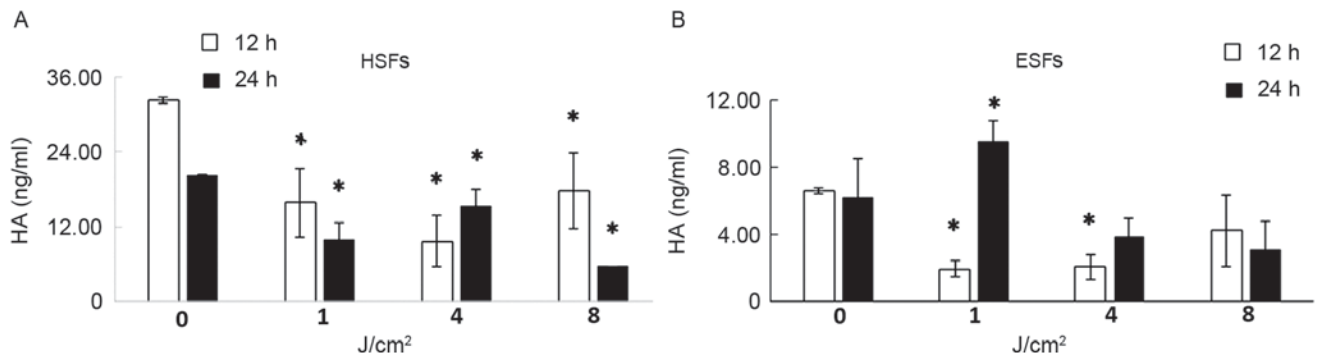


Figure 3. Alteration in HA content of HSFs and ESFs after UVA irradiation. The HA content of (A) HSFs and (B) ESFs after irradiation with UVA for 12 and 24 h. \*P<0.05. HA, hyaluronic acid; HSFs, human skin fibroblasts; ESFs, embryonic skin fibroblasts; UVA, ultraviolet A.

*HYAL2* mRNA expression levels were significantly upregulated in the 1 J/cm² group, but significantly downregulated in the 4 and 8 J/cm² groups, compared with the 0 J/cm² group (all P<0.05; Fig. 4E).

*HSF and ESF expression of BMI-1 and EZH2 mRNA is modified by UVA irradiation.* The RT-qPCR analysis results demonstrated that there were no significant differences in *BMI-1* and *EZH2* mRNA expression levels in HSFs in the 1 and 4 J/cm² groups compared with the 0 J/cm² group (Fig. 5). However, HSF mRNA levels of *BMI-1* and *EZH-2* were significantly upregulated in the 8 J/cm² group compared with the 0 J/cm² group (P<0.05; Fig. 5). By contrast, in ESFs, the expression of *BMI-1* and *EZH2* mRNA after UVA irradiation in all groups exhibited no statistical difference, compared with the 0 J/cm² group (Fig. 5).

## Discussion

Features of skin photoaging caused by long-term UVA irradiation, including wrinkle formation and reduced elasticity, are induced by changes in various molecules due to damage to dermal fibroblasts (29). Previous studies have indicated that UVA-induced skin photoaging involves a variety of signal transduction molecules and pathways, including the generation of reactive oxygen species (ROS), activation of protein kinase signaling pathways, expression and activation of metalloproteinases, and induction of epidermal growth factor expression (30-32). Photoaging of the skin at the epigenetic level remains unclear. As a transformational global epigenetic repressor, PcG proteins serve important roles in proliferation, senescence, tumorigenesis and other cellular processes (33,34); therefore, PcG proteins may be associated with UVA-induced

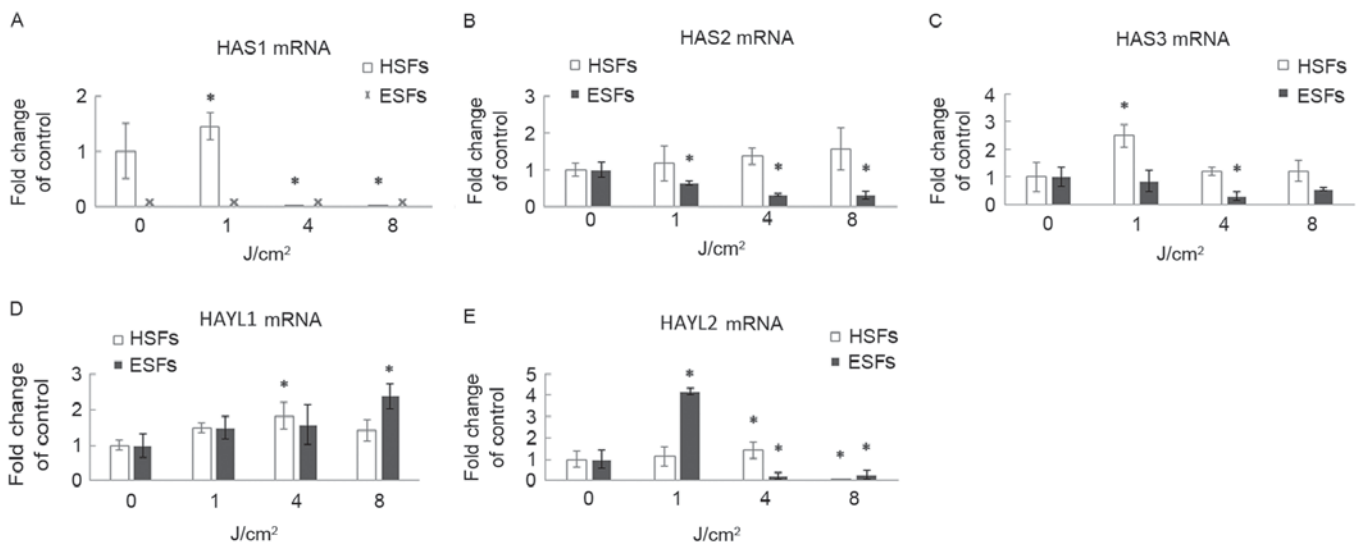


Figure 4. UVA irradiation alters mRNA expression of *HAS1-3*, *HYAL1* and *HYAL2* in HSFs and ESFs. Relative mRNA expression levels of (A) *HAS1*, (B) *HAS2* and (C) *HAS3* in HSFs and ESFs irradiated with UVA. Relative mRNA expression levels of (D) *HYAL1* and (E) *HYAL2* \*P<0.05. UVA, ultraviolet A; *HYAL*, hyaluronidase; *HAS*, hyaluronan synthase; HSFs, human skin fibroblasts; ESFs, embryonic skin fibroblasts.

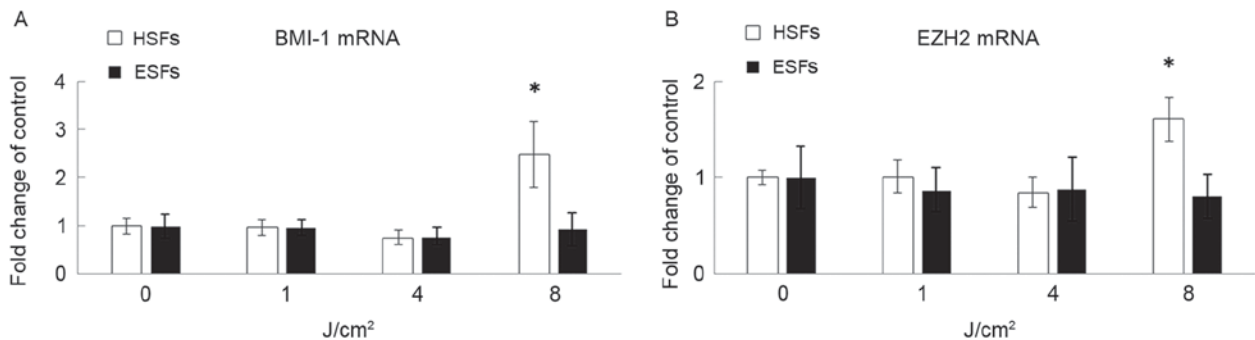


Figure 5. HSF and ESF expression of *BMI-1* and *EZH2* mRNA is modified by UVA irradiation. mRNA expression levels of (A) *BMI-1* and (B) *EZH2* in HSFs and ESFs irradiated with UVA. \*P<0.05. HSFs, human skin fibroblasts; ESFs, embryonic skin fibroblasts; *BMI-1*, polycomb complex protein *BMI-1*; *EZH2*, enhancer of zeste homolog 2; UVA, ultraviolet A.

fibroblast aging. In the present study different doses of UVA radiation were applied to HSFs and ESFs in order to establish a photoaging model. Through a variety of assays, the results of the present study demonstrated that the mRNA expression levels of two genes encoding core PcG proteins, *BMI-1* and *EZH2*, were altered in HSFs and ESFs after UVA irradiation. A similar alteration was observed in the mRNA expression of *HAS1-3*, *HYAL1* and *HYAL2*. These results suggest that PcG proteins are associated with UVA-induced fibroblast aging, and may serve different roles in ESFs and HSFs.

In the dermal layer, HA serves an essential role in the aging process, and the synthesis and secretion of HA by HSFs differs at various stages of development (35). Studies investigating ESFs were first performed in regards to wound healing, in which researchers identified that adult skin typically healed through a process involving the formation of scars, whereas fetal skin did not heal through the formation of scars (36,37). Multiple studies have demonstrated that this phenomenon is associated with differences in the amount of HA secreted by fibroblasts (38,39). In order to explore whether the roles of PcG proteins differ in fibroblasts at various phases of development, HSFs and ESFs were chosen as cell models in the present

study. The results of the present study were in agreement with previous studies, revealing significant differences in the mRNA expression levels of genes encoding key PcG proteins (*BMI-1* and *EZH2*) between HSFs and ESFs.

The effects of UVA (0, 1, 4 and 8 J/cm²) on the proliferation of human fibroblasts was evaluated in the current study. The results were consistent with previous studies, demonstrating that UVA could inhibit the proliferation of fibroblasts. For example, Lan *et al* (40) examined the effects of UVA radiation (1, 3, 5, and 10 J/cm²) on the proliferation of fibroblasts using MTT. The results revealed that at 24 h after irradiation, the proliferation activities of cells in all groups were all decreased in a dose-dependent manner; in particular, the proliferation of cells in the 10 J/cm² group was reduced by 50% (40). Furthermore, Niu *et al* (41) used CCK-8 assays to detect the effects of UVA irradiation on the proliferation activity of fibroblasts in order to investigate the underlying mechanisms of UVA-induced fibroblast photoaging. The results revealed that cell proliferation was gradually reduced on days 1, 3 and 5 after irradiation (41). However, the cell proliferation rates in groups irradiated with higher doses decreased over time in a dose-dependent manner. Thus, it was speculated

that low-dose UVA radiation may promote the proliferation of HSFs. Furthermore, the results of the present study revealed that proliferation rate of ESFs at straight after UVA irradiation was <100%, indicating that UVA irradiation inhibited cell proliferation. However, the proliferation curves increased gradually over 24 h after irradiation. The difference observed in the effect of UVA radiation on HSFs and ESFs may be associated with a difference in ROS production in. Under normal circumstances, a variety of antioxidant enzymes and nonenzymatic antioxidants in the human body alleviate or prevent ROS-induced cell damage; that is, ROS continuously produced *in vivo* are rapidly removed by antioxidant enzymes, including catalase, glutathione peroxidase or superoxide dismutase, thereby maintaining the balance between pro-oxidative and anti-oxidative factors (42,43). However, when the activities of these enzymes are reduced or when an excessive amount of ROS are produced, cells may be damaged by the accumulation of ROS (31,43), resulting in apoptosis, DNA damage, reduced enzyme activity, structural damage to the mitochondria and an increased rate of cellular senescence (30,44). This indicates that ESFs exhibit a stronger ability to repair oxidative damage compared with HSFs, and may have increased ROS-scavenging ability, explaining the increased cell proliferation rates over time.

During the oxidative damage response in fibroblasts after UV exposure, the initiation and progression of apoptosis is significant (45,46). Mirzayans *et al* (47) used flow cytometry to detect apoptosis in UV-irradiated fibroblasts and demonstrated that apoptosis was increased when the cells were exposed to a wide range of UVC influences (between 2 and 30 J/cm<sup>2</sup>), and incubated for 3 or 6 days. The results of the present study revealed that the percentage of apoptotic and necrotic ESFs was increased when the cells were irradiated with UVA. However, the amount of apoptotic and necrotic HSFs were decreased after irradiation with 8 J/cm<sup>2</sup> UVA, contradictory to the findings of other studies (48-50). The primary factors known to induce aging include replicative aging, oxidative stress, the expression of oncogenes and the activation of DNA injury signaling pathways (51). The effects observed in the present study were speculated to be associated with the expression of oncogenes that inhibit the expression of apoptotic genes, thus enabling the cells to progress towards replicative immortality. However, further studies are required to validate this theory.

The results of HA content detection demonstrated that the HA content of HSFs and ESFs was decreased in each dose group compared with the control group, with the exception of ESFs irradiated following treatment with 1 J/cm<sup>2</sup> for 24 h, indicating that UVA may inhibit the synthesis of HA in fibroblasts. However, differences were also observed in HA levels between the HSFs and ESFs. For example, the HA content of HSFs was notably higher compared with that in ESFs irradiated with the same dose of UVA. This observation may be associated with the sizes and polymer chain lengths of HA molecules secreted by these two cells. Previous studies have suggested that in fetal skin, HA tended to be present as high molecular weight polymers, whereas in adult skin, HA is present in various forms of different sizes (14,52). This may explain why the HA content in the supernatants of cultured HSFs was significantly higher compared with that in the supernatants of cultured ESFs. The reason for this difference may also be due to variations in the

level of *HAS* and *HYAL* expression levels in HSFs and ESFs. The synthesis and secretion of HA in fibroblasts is dynamically regulated by *HAS1-3*, *HYAL1* and *HYAL2*, among which *HAS2* and *HYAL1* are essential (9,53). Carre *et al* (14) studied the association between the expression levels of the Wnt-3a protein and HA, and examined differences in HA production between ESFs and HSFs. The results revealed that the addition of Wnt3a and TGF- $\beta$ 1 induced the upregulation of *HAS2* and *HAS3* in ESFs, and of *HAS1* and *HYAL2* in HSFs, further supporting the theory that there are differences in *HAS* and *HYAL* expression between these two cell types. Furthermore, the RT-qPCR analysis conducted in the present demonstrated that there were differences in the expression levels of these proteins between ESFs and HSFs. In HSFs, UVA radiation induced the upregulation of *HAS2*, *HAS3* and *HYAL1*, and the downregulation of *HAS1*. Additionally, in HSFs *HYAL2* was upregulated at 1 and 4 J/cm<sup>2</sup>, but downregulated at 8 J/cm<sup>2</sup>. By contrast, in ESFs, UVA radiation induced the downregulation of *HAS2* and *HAS3*, and the upregulation of *HYAL1*; *HYAL2* was upregulated at 1 J/cm<sup>2</sup>, but downregulated at 4 and 8 J/cm<sup>2</sup>. The upregulation of *HAS2* and *HYAL1* in HSFs, and the downregulation of *HAS2* and upregulation of *HYAL1* in ESFs, may explain the markedly increased levels of HA in HSFs compared with ESFs.

Notably, the mRNA expression of *HAS1* in ESFs was consistently below the minimum detection level, indicating a very low expression level of *HAS1* in these cells. Consistent with this, Röck *et al* (17) reported that the mRNA expression of *HAS2* accounted for 95% of all *HAS* mRNA expression, suggesting that *HAS2* was the dominant enzyme among the *HAS* family. Therefore, under normal circumstances the expression of *HAS1* would be <5% of the total *HAS* expression. Furthermore, following UVA radiation, the expression of *HAS1* would be expected to be reduced further, providing an explanation for the lack of *HAS1* detection.

PcG proteins are epigenetic regulatory inhibitors that are associated with multiple cellular processes, including carcinogenesis, senescence, apoptosis and DNA repair (54). The majority of studies investigating PcG proteins have focused on their roles in tumorigenesis and cell senescence. Notably, EFs in *BMI-1* knockout mice enter the S phase earlier compared with those in wild-type mice, causing cell progeria; however, the target gene of *BMI-1*, the tumor suppressor gene cyclin-dependent kinase inhibitor 2A (*p16*), is significantly upregulated (55,56). By contrast, when *BMI-1* is overexpressed in murine EFs, the *p16* gene is downregulated, thereby delaying replication and senescence, and promoting replicative immortality (56). Similarly, the overexpression of *BMI-1* in human fibroblasts has been demonstrated to extend the replicative life of the cells by inhibiting the p16-dependent senescence signaling pathway (57). In the present study, the expression of *BMI-1* and *EZH2* mRNA was examined in UVA-induced HSF and ESF aging model via RT-qPCR analysis. There were no significant differences in *BMI-1* and *EZH2* mRNA expression levels in ESFs in each dose group compared with the 0 J/cm<sup>2</sup> group, which demonstrated that *BMI-1* and *EZH2* didn't participate in HA regulation during UVA-induced aging ESFs. In HSFs, the mRNA expression of *BMI-1* and *EZH2* was upregulated after exposure to 8 J/cm<sup>2</sup> UVA, while the amount of apoptotic and necrotic cells were decreased at 8 J/cm<sup>2</sup>.

These results suggest that *BMI-1* and *EZH2* serve a role in HA regulation during UVA-induced aging in HSFs and also indicates that there are differences between HSF and ESF in the regulation mechanism of UVA aging. In HSFs, PcG proteins may upregulated *HAS2* and *HYAL1*, thereby suppressing HA synthesis. However, when irradiated with higher doses of UVA, PcG proteins may be overexpressed and result in inhibition of the p16-dependent aging signaling pathway, thus promoting replicative immortality. However, further studies are required to confirm this hypothesis.

The present study had two notable limitations that affected the interpretation of the results. Firstly, changes in the expression of *BMI-1* and *EZH2* were detected in the simulated photoaging cell models and indicated their participation in the aging process in fibroblasts. However, it was not examined whether these proteins function as regulatory molecules in the aging of fibroblasts. In order to investigate this concept, it will be necessary to verify the association between fibroblasts and changes in HA synthesis when *BMI-1* and *EZH2* are overexpressed or knocked down, and to evaluate changes in the expression of other known HA regulatory molecules during this process. Secondly, PcG proteins are comprised of a complex of a group of proteins (58,59). In the present study, only the expression of two core genes of PcG, *BMI-1* and *EZH2* were examined, and the changes in other PcG proteins during UVA-induced photoaging were not investigated. Therefore, further studies are required to examine other members of the PcG family in order to fully identify the roles of PcG proteins in UVA-induced skin photoaging, and to distinguish epigenetic regulatory molecules and signaling pathways associated with this process. Such studies will facilitate the identification of effective targets and aid in the identifications of novel clinical treatment approaches.

In conclusion, the mRNA expression of *BMI-1*, *EZH2*, *HAS2* and *HYAL1* in HSFs was upregulated following high-dose UVA irradiation, suppressing HA synthesis, while cell proliferation and the amount of necrotic cells both decreased. There were no significant differences in *BMI-1* and *EZH2* mRNA expression levels in ESFs in each dose group compared with the 0 J/cm<sup>2</sup> group. These results suggest that *BMI-1* and *EZH2* serve a role in HA regulation during UVA-induced aging in HSFs but not in ESFs. The current study provides a new epigenetic regulatory molecule in signaling pathways of UVA-induced skin photoaging for future studies.

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