

Original Article



Topical Application of Probiotic-Derived EGF From *Pediococcus pentosaceus* Improves UVB-Induced Photoaging in Hairless Mice

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ABSTRACT

Background: Epidermal growth factor (EGF) is widely applied in dermatology for its regenerative effects; however, its clinical utility is limited by low stability and high production costs. To overcome these limitations, we developed a novel probiotic-derived epidermal growth factor (pEGF) by engineering *Pediococcus pentosaceus* CBT SL4 to express and secrete EGF.

Objective: To investigate the anti-photoaging effects of topical pEGF in a ultraviolet B (UVB)-irradiated mice by assessing histological and molecular changes related to collagen synthesis, extracellular matrix (ECM) remodeling, oxidative stress, and inflammation.

Methods: SKH1 hairless mice were exposed to UVB for twelve weeks to induce photoaging and treated with topical 10% pEGF cream from week 9 to 12. Skin samples were analyzed using dermoscopy, histology, immunohistochemistry, quantitative real-time polymerase chain reaction, western blotting, and proteomics to evaluate morphological and molecular alterations in ECM components and inflammatory markers.

Results: Topical pEGF treatment improved skin texture and reduced wrinkles. Histological evaluation revealed that EGF significantly attenuated UVB-induced epidermal thickening and restored collagen density, with the strongest effects observed in the pEGF group. Molecular analysis showed downregulation of matrix metalloproteinases (MMPs; MMP-1, MMP-3), upregulation of collagen (type I collagen, type III collagen) and tissue inhibitors of metalloproteinases (TIMPs; TIMP-1, TIMP-2). Additionally, pEGF reduced pro-inflammatory cytokines (tumor necrosis factor- α , interleukin-1 β) and oxidative stress markers.

Conclusion: pEGF demonstrated potent anti-photoaging effects through ECM restoration, inflammation modulation, and barrier reinforcement. These findings support the therapeutic potential of pEGF as a stable, cost-effective alternative to conventional EGF in dermatological applications.

Keywords: Epidermal growth factor; Extracellular matrix; *Pediococcus pentosaceus*; Skin aging

INTRODUCTION

Skin aging is a multifaceted process influenced by intrinsic factors, such as genetics and hormonal changes, and extrinsic factors,

including ultraviolet (UV) radiation, environmental pollutants, and oxidative stress. Chronic ultraviolet B (UVB) exposure is the primary driver of photoaging, which leads to visible signs of skin aging such as fine wrinkles, pigmentation, decreased elasticity,

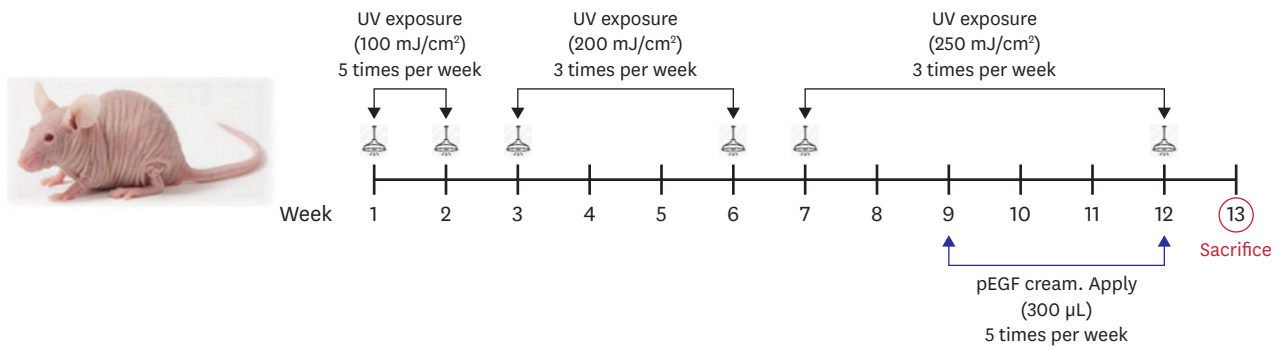


Fig. 1. Experimental design and timeline of UVB-induced photoaging and topical pEGF treatment in SKH1 hairless mice. SKH1 hairless mice were subjected to gradually increasing doses of UVB irradiation for 12 weeks to induce photoaging, followed by daily topical application of pEGF cream from week 9 to 12. Mice were sacrificed at week 13 for tissue collection and analysis. Blue arrows indicate the treatment phase. Sacrifice and sample harvesting were performed after the final treatment.

UVB: ultraviolet B, pEGF: probiotic-derived epidermal growth factor, UV: ultraviolet.

and a compromised skin barrier. These effects are mediated through the excessive generation of reactive oxygen species (ROS), activation of matrix metalloproteinases (MMPs), and inflammatory signaling pathways that disrupt extracellular matrix (ECM) integrity¹.

Epidermal growth factor (EGF) is a low-molecular-weight polypeptide that accelerates keratinocyte and fibroblast proliferation and stimulates ECM synthesis. EGF is also pivotal in wound healing and skin regeneration^{2,3}. Recent studies have demonstrated the potential of EGF not only as a topical agent but also as a functional filler. For instance, a novel hyaluronic acid filler incorporating EGF exhibited enhanced rheological stability and improved collagen production in a UVB-induced aging model, suggesting dual benefits in structural support and biological rejuvenation⁴. Despite these promising effects, the clinical application of recombinant human epidermal growth factor (hEGF) is limited by its instability and high production costs.

Microbial engineering emerged as a promising alternative to overcome these limitations. Genetically modified probiotics can serve as delivery platforms for bioactive molecules such as EGF, offering improved stability, cost-efficiency, and postbiotic synergism^{5,6}. This study developed a probiotic-derived epidermal growth factor (pEGF) by engineering *Pediococcus pentosaceus* to express and secrete EGF into culture supernatants. We evaluated the anti-photoaging effects of topically applied pEGF in a UVB-irradiated mouse model by assessing histological, molecular, and proteomic changes related to collagen synthesis, ECM remodeling, oxidative stress, and inflammation.

MATERIALS AND METHODS

Animal model and study design

This study evaluated the therapeutic potential of a topical cream containing *Pediococcus pentosaceus*-derived epidermal growth factor

(pp-EGF) for mitigating UV-induced skin aging.

Twenty-eight SKH1 hairless mice were randomly assigned to five groups: four UVB-exposed experimental groups that received either no treatment, vehicle, hEGF, or pEGF ($n=6$ per group), and a non-UV-exposed control group ($n=4$).

This study did not involve human participants; therefore, Institutional Review Board approval was not required. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Chung-Ang University (IACUC No. 202301020105).

The study comprised three phases. In the UV exposure phase (weeks 1–12), photoaging was induced by gradually increasing the dose of UVB irradiation, simulating chronic sun exposure. During the treatment phase (weeks 9–12), mice received daily topical applications of either 10% pEGF cream (25 ng/ml), hEGF cream (10 ng/ml), or a vehicle cream.

Finally, during the sacrifice and sample collection phase (week 13), all animals were euthanized. Dorsal skin samples were harvested for histological evaluation, molecular analyses, and proteomic profiling to comprehensively assess the anti-aging effects of pEGF treatment (**Fig. 1**).

Preparation of pEGF cream

P. pentosaceus SL4 (KCTC 10297BP), originally isolated from kimchi, was obtained from the culture collection maintained at Cell Biotech Co., Ltd. (Gimpo, Korea) and used for the production of pEGF. The hEGF gene was cloned into the plasmid pCBT24-2-PK-EGF-PK-EGF (accession number: KCCM13348P), and the recombinant plasmid was transformed into *P. pentosaceus* SL4 to generate pEGF-producing transformants.

Transformants grown on De Man, Rogosa, and Sharpe (MRS) agar plates were inoculated into 10 ml of MRS broth supplemented

with erythromycin (10 µg/ml) and cultured at 37°C for 15 hours without shaking. After cultivation, the cultures were centrifuged at 12,000 rpm for 20 minutes at 4°C, and the resulting supernatants were collected and sterilized by filtration through a 0.22 µm membrane. To improve the stability of the pEGF-containing culture medium supernatant as a cosmetic ingredient with biological activity, the supernatant was immobilized with 5% maltodextrin and subjected to freeze-drying. Through this powdering process, a brown pEGF powder was obtained. The pEGF content of the resulting powder was 164 ng/g. The pEGF powder was dissolved in distilled water at room temperature to prepare a pEGF solution (35.71 g in 50 ml; equivalent to 250 g in 350 ml). The solution was then thoroughly mixed with a natural essence base at room temperature until a homogeneous mixture was obtained. Carbomer 980 was added and completely dissolved under gentle stirring, followed by gradual addition of triethanolamine to achieve the desired consistency and pH. Based on these measurements, the 10% pEGF cream contained approximately 25 ng/ml of EGF protein.

Preparation of hEGF cream

Recombinant hEGF was obtained from Sigma-Aldrich (St. Louis, MO, USA). To ensure comparability with the pEGF formulation, an hEGF cream (final concentration, 10 ng/ml) was prepared using the identical base composition and preparation method, substituting hEGF for pEGF. Briefly, hEGF was dissolved in distilled water at room temperature to achieve the target concentration and then thoroughly mixed with the natural essence base until a homogeneous mixture was obtained. Carbomer 980 was subsequently added and completely dissolved under gentle stirring at room temperature, followed by gradual addition of triethanolamine to adjust the consistency and pH. The hEGF-containing cream was prepared under the same formulation components and processing conditions as the pEGF cream, allowing a direct comparison of biological effects attributable to the active ingredient.

Preparation of vehicle cream

The vehicle cream was prepared using culture-derived materials obtained from unmanipulated *P. pentosaceus* SL4. A non-transformed *P. pentosaceus* SL4 strain was cultured under the same conditions as the pEGF-producing strain, including identical culture medium composition, incubation time, and temperature. Following cultivation, the cultures were centrifuged under identical conditions, and the resulting supernatants were collected and sterilized by membrane filtration. The sterile supernatants were subsequently subjected to the same powdering process as that used for the pEGF preparation. The resulting powder was dissolved in distilled water to prepare a vehicle solution, which was then formulated into a cream using the identical

base composition and preparation procedure applied for the pEGF cream.

UVB irradiation protocol

The mice were exposed to UVB irradiation to induce photoaging using a UVB lamp (wavelength range: 280–320 nm, peak emission at 312 nm). The irradiation protocol followed a gradual dose escalation model to simulate chronic UV exposure and progressive skin damage.

During weeks 1–2, mice were exposed to a low UVB dose of 100 mJ/cm² to allow for initial adaptation and to minimize acute stress responses. In weeks 3–6, the dose was escalated to 200 mJ/cm², promoting early epidermal responses such as keratinocyte proliferation and mild inflammation. From weeks 7–12, the dose was maintained at 250 mJ/cm², a level known to induce significant histological and molecular changes characteristic of photoaging, including epidermal thickening, oxidative stress, and ECM degradation.

The irradiation distance and duration were standardized for all mice to ensure uniform UV exposure. Throughout the experiment, mice were housed under controlled environmental conditions, including a 12-hours light/dark cycle, an ambient temperature of 22±1°C, and a relative humidity of 50±5%, with ad libitum access to food and water. These conditions were maintained to minimize confounding variables and ensure the reproducibility of UV-induced skin damage.

Dermoscopy and histological analysis

Weekly dermoscopic and macroscopic skin imaging using a high-resolution dermoscope assessed visible skin changes over time (Illuco IDS-1100®; Illuco Corporation, Gunpo, Korea). Skin conditions, including pigmentation, erythema, scaling, and texture, were documented and compared across the groups.

Skin tissue samples were collected and fixed in 10% neutral-buffered formalin before being embedded in paraffin for histological analysis. Tissue sections of thickness of 5 µm were prepared and subjected to various staining techniques to evaluate structural and molecular changes. Hematoxylin and eosin (H&E) staining assessed the epidermal thickness and overall tissue morphology. Masson's trichrome (MT) staining examined the collagen fiber distribution and dermal remodeling. Additionally, immunohistochemistry (IHC) analyzed the expression of ECM degradation markers (MMP-1 and MMP-3), collagen markers (type I collagen [Col1] and type III collagen [Col3]), and tissue inhibitors of metalloproteinases (TIMPs; TIMP-1 and TIMP-2). All stained sections were observed under a light microscope (Leica DM750; Leica Microsystems, Wetzlar, Germany). Quantitative analysis using the ImageJ software (National Institutes of Health, Bethesda, MD, USA) ensured objective evaluation of histological and immunohistochemical findings.

RNA extraction and reverse transcription quantitative real-time polymerase chain reaction (qPCR)

Total RNA was extracted from skin tissues using the TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA).

qPCR assessed the expression of genes associated with collagen synthesis (Col1 and Col3) and ECM remodeling (MMP-1 and MMP-3). The reactions were performed in a real-time thermal cycler (Applied Biosystems, Thermo Fisher Scientific) using PowerUp SYBR Green Master mix (Applied Biosystems). All results were normalized to the levels of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Relative gene expression was calculated using the comparative Ct method ($\Delta\Delta C_t$) after normalization to an appropriate housekeeping gene.

Western blot analysis

Proteins were extracted from the skin tissue homogenates using 1% Triton X-100 in radioimmunoprecipitation assay buffer supplemented with a protease inhibitor cocktail (Thermo Fisher Scientific). The protein concentration was measured using a bicinchoninic acid assay (Pierce, Thermo Fisher Scientific, Rockford, IL, USA). Equal amounts of total protein (20 μ g) were separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membranes, and probed with primary antibodies against Col1, Col3, MMP-1, MMP-3, TIMP-1, TIMP-2, and GAPDH (Cell Signaling Technology, Danvers, MA, USA). Protein bands were visualized using the ChemiDoc MP Imaging System (Bio-Rad Laboratories, Hercules, CA, USA) and detected using the EzWestLumi Plus system (ATTO Corporation, Tokyo, Japan).

Protein array

Skin samples from control, UVB-exposed and pEGF-treated SKH1 hairless mice were collected, immediately snap frozen, and stored at -80°C . Protein extracts from these samples were then prepared and incubated with the Mouse L1308 Array Glass Slide kit (RayBiotech, Cat# AAM-BLG-1-4, Peachtree Corners, GA, USA) according to the manufacturer's instructions. Fully processed glass slides were imaged using the LI-COR Odyssey system (Software Version 2.1.12; LI-COR, Inc., Lincoln, NE, USA) and processed using the LI-COR Image Studio. The intensities of the array spots were calculated according to the manufacturer's instructions. Each antibody target was printed in duplicate on the array, and the average of the two spots was used for all subsequent analyses. Signal intensities from the pEGF-treated samples were normalized to their corresponding control values by multiplying each signal by the ratio of the geometric means of the internal positive controls from the two arrays. To obtain the final signal

intensities, the mean intensity of all negative control spots was subtracted from the normalized values.

Statistical analysis

All experiments were performed in triplicate, and data are expressed as mean $\pm$ standard deviation. Statistical comparisons were performed using one-way analysis of variance and Tukey's *post hoc* test for multiple comparisons. A *p*-value <0.05 was considered statistically significant. Data were analyzed using the GraphPad Prism software (version 8.0; GraphPad Software, San Diego, CA, USA).

RESULTS

Dermoscopic and histological assessment

1) Effect of pEGF treatment on photoaged skin

Dermoscopy analysis demonstrated improvement in skin texture, hydration, and wrinkle reduction following pEGF treatment. The pEGF-treated group exhibited the greatest reduction in scaling, roughness, and fine wrinkles among all groups at week 13. No-treatment group and vehicle-treated groups exhibited visible epidermal roughness, increased scaling, and accentuated wrinkle patterns, indicating UV-induced photoaging and impaired barrier function. In contrast, hEGF and pEGF-treated groups exhibited smoother epidermal surfaces, reduced scaling, and enhanced skin elasticity, with the pEGF group demonstrating the most significant improvement (**Fig. 2A**).

2) Epidermal thickness (H&E staining)

H&E staining revealed significant UVB-induced epidermal thickening. (**Fig. 2B**) The vehicle-treated-group showed persistent epidermal hyperplasia ($77 \pm 3 \mu\text{m}$). In contrast, hEGF ($50 \pm 5 \mu\text{m}$) and pEGF ($27 \pm 5 \mu\text{m}$)-treated groups exhibited reduction in epidermal thickness, suggesting that pEGF treatment mitigates UV-induced epidermal hyperplasia (**Fig. 2C**).

3) Collagen density (MT staining)

MT staining confirmed the UVB-induced reduction in collagen density, with the no-treatment ($0.73 \pm 0.02\%$) and vehicle-treated ($0.64 \pm 0.01\%$) groups showing significant collagen depletion, compared to the control group ($0.9 \pm 0.01\%$) ($p < 0.001$) (**Fig. 3A**).

Treatment with pEGF led to a significant increase in collagen density ($0.83 \pm 0.01\%$), suggesting partial restoration of dermal collagen and improved ECM structure following pEGF application (**Fig. 3B**).

4) ECM remodeling and collagen preservation (IHC staining)

IHC demonstrated that MMP-1 and MMP-3 were significantly

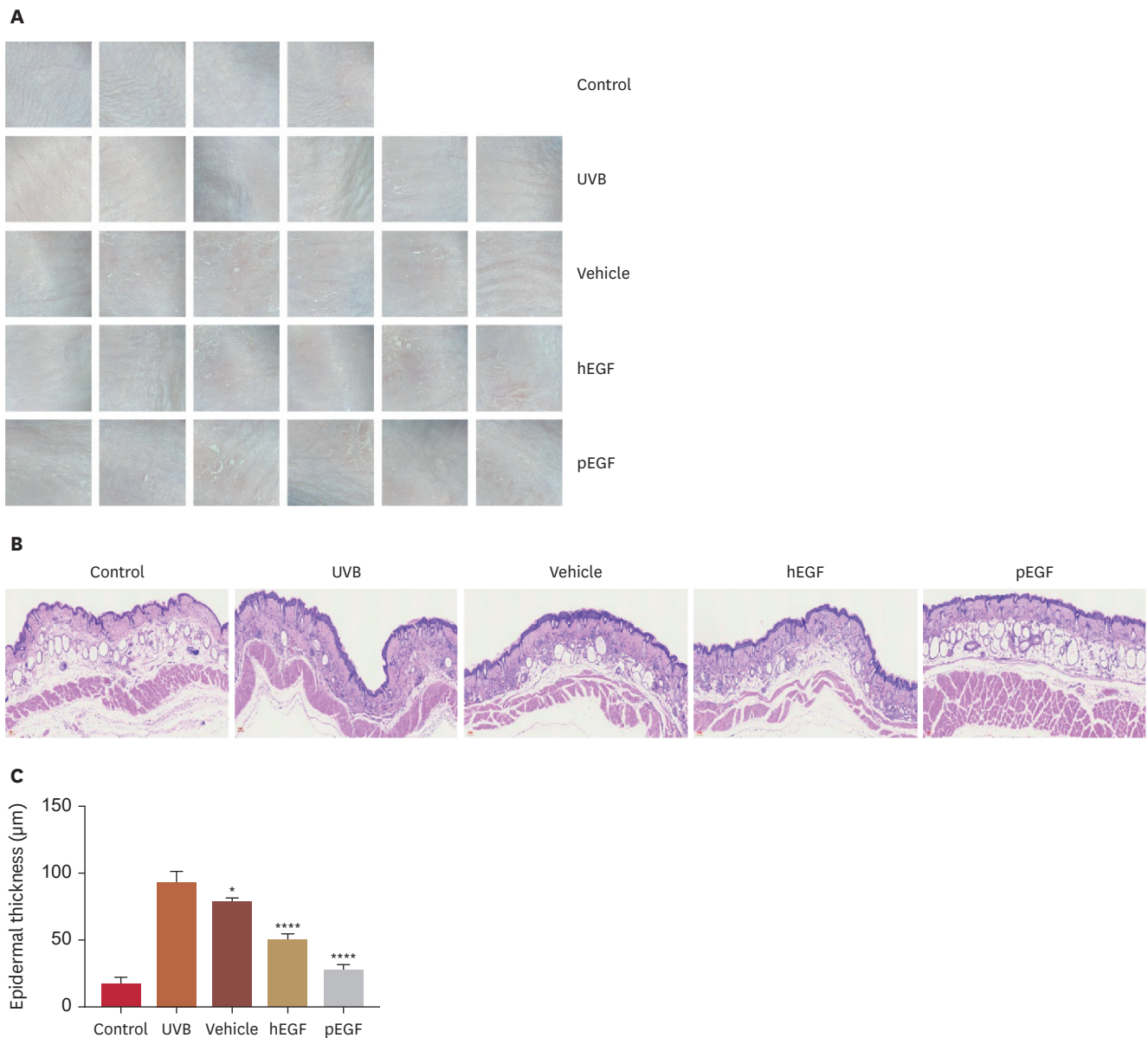


Fig. 2. Dermoscopic and histological assessment of UVB-induced photoaging and therapeutic effects of topical epidermal growth factor in SKH1 hairless mice. (A) Dermoscopic images of dorsal skin from each group (Control, UVB, Vehicle, hEGF, pEGF) at week 13. The pEGF-treated group showed improvement in skin texture, reduced scaling, and diminished fine wrinkles compared to UVB and vehicle groups. hEGF treatment also improved epidermal smoothness and elasticity, but pEGF produced the most pronounced effects. (B) Representative H&E-stained sections of dorsal skin from each experimental group (scale bar=100 μm). UVB exposure resulted in marked epidermal hyperplasia, which was attenuated by topical hEGF and further reduced by pEGF. (C) Quantitative analysis of epidermal thickness in each group based on H&E-stained sections. Topical pEGF treatment significantly reduced UVB-induced epidermal thickening. Data are expressed as mean ± standard deviation (n=6).

UVB: ultraviolet B, hEGF: human epidermal growth factor, pEGF: probiotic-derived epidermal growth factor, H&E: hematoxylin and eosin.

* $p < 0.05$, **** $p < 0.0001$ vs. UVB group.

upregulated in the no-treatment group. Conversely, the pEGF-treated group exhibited a significantly reduced expression of MMP-1 and MMP-3. Col1 and Col3 expressions were significantly reduced in the no-treatment and vehicle-treated groups, reflecting photoaging-associated collagen loss. Treatment with pEGF significantly increased Col1 and Col3 expression, enhancing collagen

synthesis and ECM restoration. TIMP-1 and TIMP-2, which are known MMP inhibitors, were significantly downregulated in UV-exposed skin. However, EGF treatment restored TIMP-1 and TIMP-2 expression, indicating that EGF treatment contributes to ECM stabilization by modulating the MMP/TIMP balance (**Fig. 3C**).

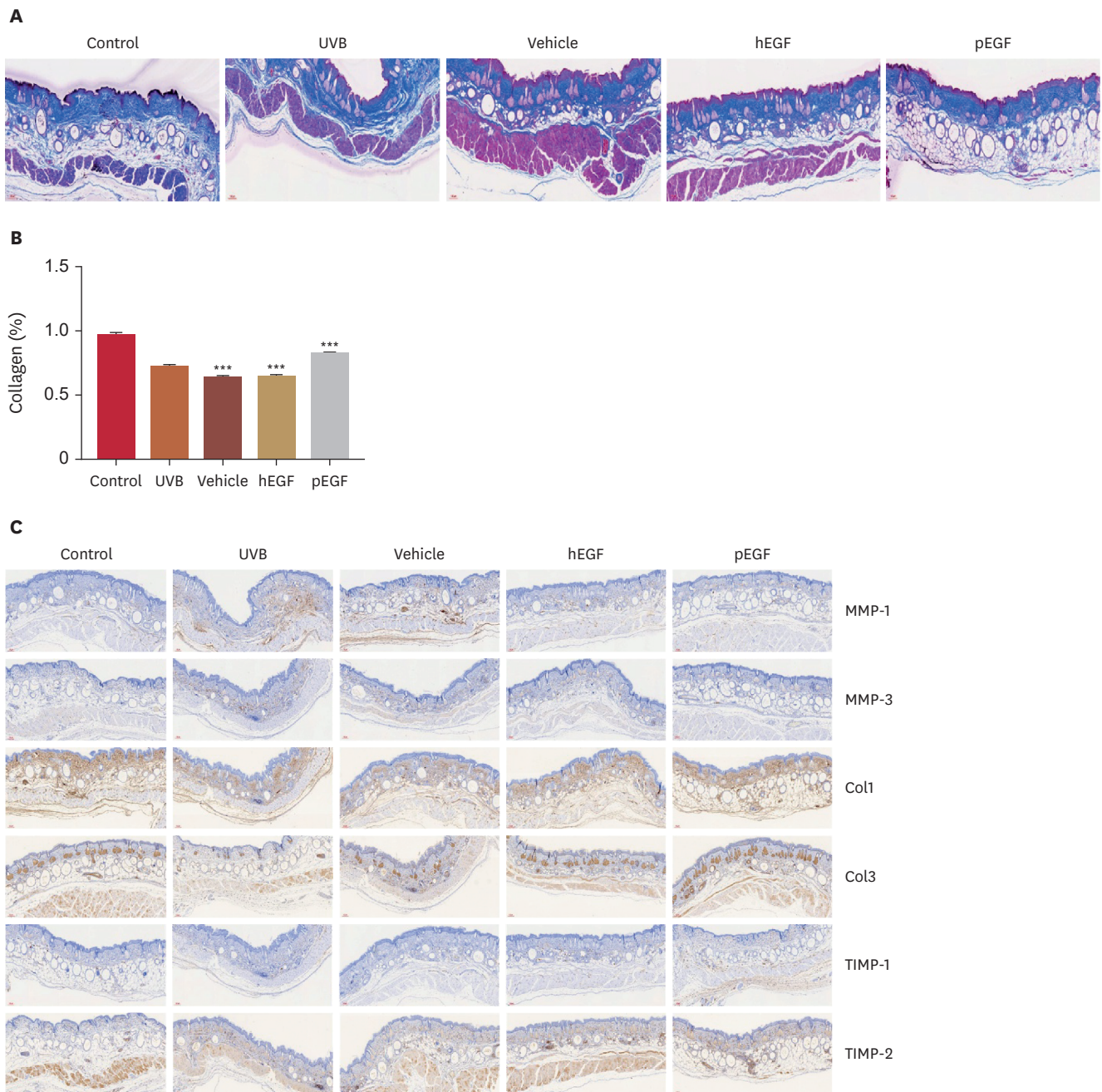


Fig. 3. Collagen preservation and ECM integrity after pEGF treatment. (A) Representative MT staining of dorsal skin sections from each group. Blue staining indicates collagen fibers. The UVB-exposed and vehicle-treated groups showed marked collagen degradation, while topical pEGF application restored collagen density. (scale bar=100 μ m). (B) Quantitative analysis of collagen density. Quantification of collagen density in each group expressed as the percentage of MT-stained area per field. UVB exposure significantly reduced collagen content compared to the control group, and pEGF treatment increased collagen density. Data are presented as mean $\pm$ standard deviation (n=6). (C) Immunohistochemical staining for representative ECM markers (MMP-1, MMP-3, Col1, Col3, TIMP-1, and TIMP-2) across experimental groups. UVB exposure resulted in increased MMP expression and reduced collagen and TIMP levels, whereas pEGF treatment reversed these effects. (scale bar=100 μ m).

ECM: extracellular matrix, pEGF: probiotic-derived epidermal growth factor, MT: Masson's trichrome, UVB: ultraviolet B, MMP: matrix metalloproteinase, Col1: type I collagen, Col3: type III collagen, TIMP: tissue inhibitors of metalloproteinase, hEGF: human epidermal growth factor.

*** $p < 0.001$ vs. UVB group.

Gene expression analysis (qPCR)

1) ECM remodeling and collagen synthesis

qPCR analysis revealed that Col1 and Col3 expression were significantly reduced in the no-treatment and vehicle-treated groups compared to those in the control group ($p < 0.001$). However, pEGF treatment, restored Col1 and Col3 expression, suggesting that pEGF promoted collagen synthesis and dermal repair.

Conversely, MMP-1 and MMP-3 expression were significantly upregulated in the no-treatment and vehicle-treated groups, indicating increased collagen degradation. EGF treatment led to reduction in MMP-1 and MMP-3 expression, with the pEGF group exhibiting the most significant inhibition ($p < 0.01$).

Furthermore, TIMP-1 and TIMP-2, which were significantly downregulated in the UVB-exposed skin, were restored following EGF treatment (Fig. 4).

2) Inflammatory gene expression

The no-treatment group showed a significant increase in tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) expression, indicating photoaging-associated inflammation. EGF treatment reduced TNF- α and IL-1 β expression, with the pEGF group exhibiting the most pronounced anti-inflammatory effect ($p < 0.001$) (Fig. 5).

Protein expression analysis (Western blot)

Western blot analysis further confirmed the significant alterations in ECM-related protein expression following EGF treatment. The expression of MMP-1 and MMP-3 was significantly upregulated in the no-treatment group, consistent with enhanced ECM degradation. In contrast, pEGF treatment significantly reduced MMP-1 and MMP-3 expression, indicating protection against UV-induced collagen breakdown. Col1 and Col3 protein levels were reduced considerably in the no-treatment group. In contrast, EGF treatment restored their expression with the 10% pEGF group showing the most significant upregulation. TIMP-1 and TIMP-2, which were downregulated in the no-treatment group, were significantly restored in the EGF-treated groups, indicating improved ECM homeostasis and collagen stabilization (Fig. 6A).

Protein array

Protein arrays were constructed to compare the protein expression patterns among the no-treatment, pEGF-treated, and control groups. Thirteen proteins were identified as differentially expressed in UVB-exposed skin compared to the control, including upregulated pro-inflammatory cytokines TNF- α and IL-1 β , as well as lysine demethylase 4B (KDM4B), MMP-1, MMP-3, and

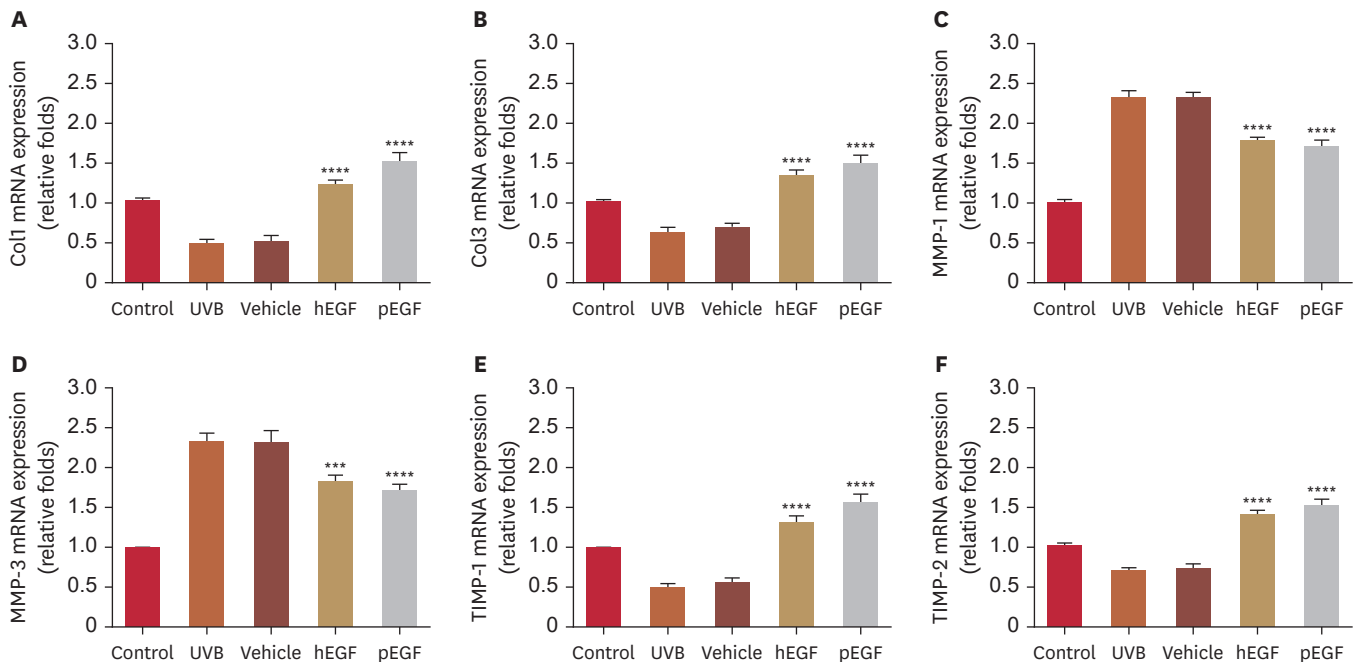


Fig. 4. Quantitative reverse transcription quantitative real-time polymerase chain reaction analysis of extracellular matrix remodeling-related gene expression in UVB-exposed and pEGF-treated mouse skin. Relative mRNA expression of (A) Col1, (B) Col3, (C) MMP-1, (D) MMP-3, (E) TIMP-1, and (F) TIMP-2 in dorsal skin tissues. UVB exposure significantly reduced Col1/3 and TIMP-1/2 while increasing MMP-1/3 expression. Both hEGF and pEGF treatments reversed these changes, with pEGF showing stronger restorative effects. Data are presented as mean $\pm$ standard deviation ($n=6$). Statistical significance was determined using one-way analysis of variance with Tukey's *post hoc* test.

UVB: ultraviolet B, pEGF: probiotic-derived epidermal growth factor, Col1: type I collagen, Col3: type III collagen, MMP: matrix metalloproteinase, TIMP: tissue inhibitors of metalloproteinase, hEGF: human epidermal growth factor.

*** $p < 0.001$, **** $p < 0.0001$ vs. UVB group.

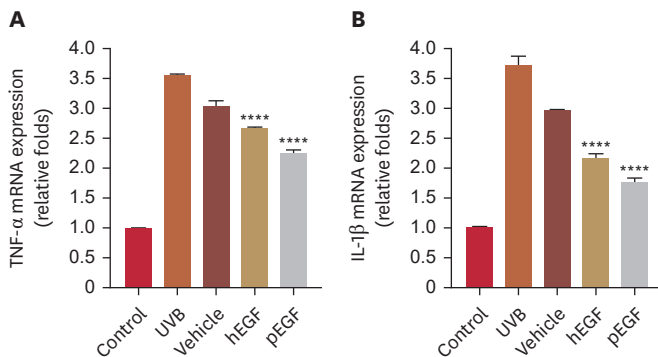


Fig. 5. Anti-inflammatory effects of pEGF assessed by mRNA expression of pro-inflammatory cytokines. (A) TNF- α and (B) IL-1 β mRNA expression levels were significantly increased in the UVB-exposed and vehicle-treated groups, indicating photoaging-associated inflammation. Both hEGF and pEGF treatments significantly suppressed the expression of these cytokines, with pEGF showing a more pronounced anti-inflammatory effect. Data are presented as mean $\pm$ standard deviation (n=6). Statistical analysis was performed using one-way analysis of variance followed by Tukey's *post hoc* test. pEGF: probiotic-derived epidermal growth factor, TNF- α : tumor necrosis factor- α , IL-1 β : interleukin-1 β , UVB: ultraviolet B, hEGF: human epidermal growth factor. ****p<0.0001 vs. UVB group.

CXCL1, among others. KDM4B showed the highest fold increase (1.85-fold) in the no-treatment group compared to the control group. In the pEGF treatment group, TNF- α , IL-1 β , MMP-1, MMP-3, and KDM4B levels were reduced compared to the no-treatment group. The most pronounced reduction was observed in KDM4B, showing a fold change of -0.65 (no-treatment vs. EGF group). Normalized log2 expression levels confirmed this trend: KDM4B expression was elevated in the no-treatment group (6.75) and decreased in the pEGF group (6.10), approaching the control levels (6.03) (**Fig. 6B**).

Hierarchical clustering and heatmap visualization showed that the no-treatment group had a distinct expression pattern compared with the control and EGF groups. The pEGF group clustered closer to the control group, indicating a partial normalization of UVB-induced changes. Pathway classification of the significantly regulated proteins revealed the enrichment of DNA replication, chromatin organization, oxidative stress response, and keratinization in the UVB group. In contrast, the EGF group showed an increased expression of proteins related to cell adhesion, wound

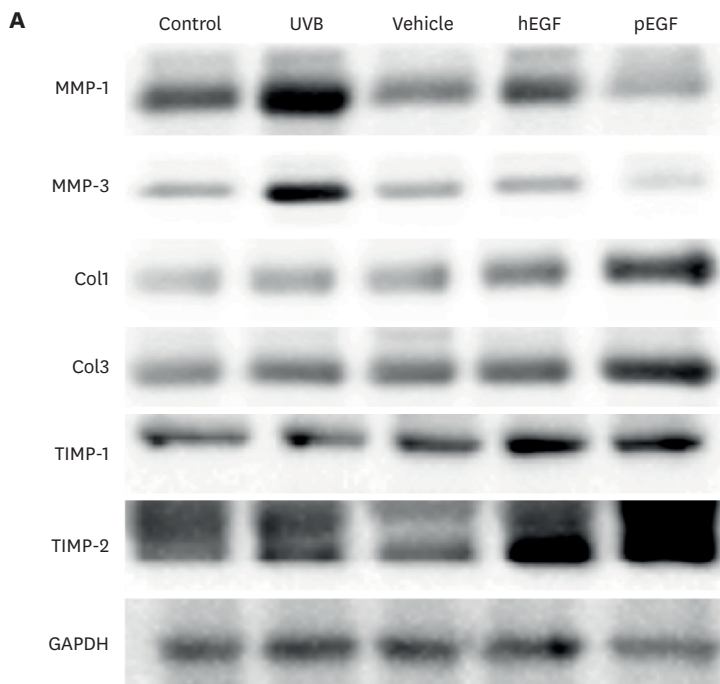


Fig. 6. Proteomic and protein-level validation of ECM remodeling after pEGF treatment. (A) Western blot analysis of ECM-related proteins, including MMP-1, MMP-3, Col1, Col3, TIMP-1, and TIMP-2, in dorsal skin lysates. UVB exposure markedly increased MMP expression and decreased collagen and TIMP expression, whereas hEGF and pEGF restored them. GAPDH served as a loading control. (B) Scatter plots comparing normalized log2 protein expression levels between the control group and UVB-exposed or 10% pEGF-treated groups. Red and green dots indicate upregulated and downregulated proteins, respectively, beyond the ± 1.5 -fold threshold. (C) Heatmap of selected differentially expressed proteins showing distinct expression patterns across UVB, hEGF, and pEGF groups. KDM4B was notably upregulated in UVB-exposed mice and downregulated by pEGF treatment. (D) Line plot showing the log2 fold changes of selected proteins relative to the control group. The 10% pEGF treatment reversed many UVB-induced protein expression changes and clustered more closely with the control group, indicating partial normalization of the UVB-altered proteomic profile. ECM: extracellular matrix, pEGF: probiotic-derived epidermal growth factor, MMP: matrix metalloproteinase, Col1: type I collagen, Col3: type III collagen, TIMP: tissue inhibitors of metalloproteinase, hEGF: human epidermal growth factor, GAPDH: glyceraldehyde 3-phosphate dehydrogenase, UVB: ultraviolet B, KDM4B: lysine demethylase 4B. (continued to the next page)

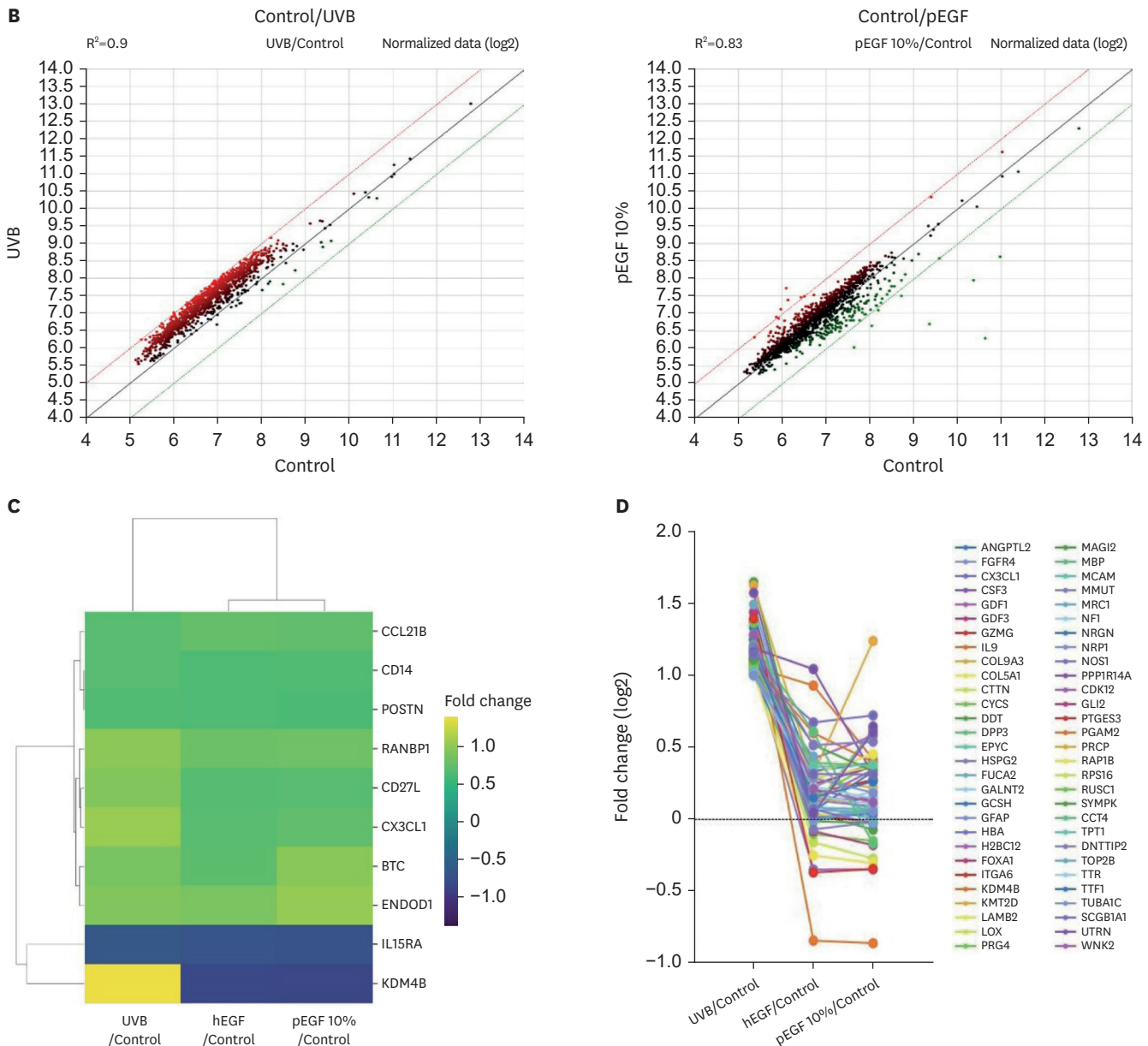


Fig. 6. (Continued) Proteomic and protein-level validation of ECM remodeling after pEGF treatment. (A) Western blot analysis of ECM-related proteins, including MMP-1, MMP-3, Col1, Col3, TIMP-1, and TIMP-2, in dorsal skin lysates. UVB exposure markedly increased MMP expression and decreased collagen and TIMP expression, whereas hEGF and pEGF restored them. GAPDH served as a loading control. (B) Scatter plots comparing normalized log₂ protein expression levels between the control group and UVB-exposed or 10% pEGF-treated groups. Red and green dots indicate upregulated and downregulated proteins, respectively, beyond the ± 1.5 -fold threshold. (C) Heatmap of selected differentially expressed proteins showing distinct expression patterns across UVB, hEGF, and pEGF groups. KDM4B was notably upregulated in UVB-exposed mice and downregulated by pEGF treatment. (D) Line plot showing the log₂ fold changes of selected proteins relative to the control group. The 10% pEGF treatment reversed many UVB-induced protein expression changes and clustered more closely with the control group, indicating partial normalization of the UVB-altered proteomic profile. ECM: extracellular matrix, pEGF: probiotic-derived epidermal growth factor, MMP: matrix metalloproteinase, Col1: type I collagen, Col3: type III collagen, TIMP: tissue inhibitors of metalloproteinase, hEGF: human epidermal growth factor, GAPDH: glyceraldehyde 3-phosphate dehydrogenase, UVB: ultraviolet B, KDM4B: lysine demethylase 4B.

healing, lipid metabolism, and epidermal development (**Fig. 6C**). Line plot further demonstrated a broader range of protein level changes in the no-treatment group compared to the relatively tighter distribution in the EGF-treated samples (**Fig. 6D**).

DISCUSSION

Chronic UVB exposure is the principal contributor to skin photoaging, leading to visible signs such as wrinkles, pigmentation,

and decreased elasticity. These manifestations are primarily due to the overproduction of ROS, activation of MMPs, and degradation of the ECM¹⁷. The UVB-induced photoaging model employed in this study is well-established and replicates many features of human skin aging, including ECM fragmentation and increased MMP activity. Our study confirmed that prolonged UVB exposure in SKH1 hairless mice led to characteristic molecular and histological changes, validating this model for evaluating regenerative interventions.

Histological assessments, including H&E and MT staining, revealed that the topical application of pEGF significantly reversed UVB-induced epidermal thickening and restored collagen fiber density. IHC further demonstrated that pEGF upregulated Col1 and Col3 while suppressing the expression of MMP-1 and MMP-3. These findings indicate that pEGF exerts dual actions, enhancing collagen synthesis while attenuating matrix degradation, likely through the stimulation of fibroblasts and suppression of proteolytic enzyme activity. At both the transcriptional and protein levels, real-time PCR and western blot analyses confirmed that PP-EGF downregulated MMP-1 and MMP-3 and upregulated TIMP-1 and TIMP-2. This modulation of the MMP/TIMP balance is consistent with the current understanding of ECM homeostasis during photoaging¹. Given the critical role of TIMPs in inhibiting collagenase activity, our findings support the potential of pEGF in preserving the dermal architecture.

In addition to collagen remodeling, pEGF significantly downregulated pro-inflammatory cytokines such as TNF- α and IL-1 β , indicating its capability to counteract UVB-induced inflammation. This is particularly relevant because chronic low-grade inflammation and oxidative stress are central drivers of extrinsic skin aging⁷. *P. pentosaceus*, a microbial source of pEGF, has multiple health-related functions. Studies have shown that this probiotic can improve antioxidant defense and suppress inflammation in gastrointestinal models while producing antimicrobial peptides that exhibit selective cytotoxicity against cancer cells. Notably, *P. pentosaceus*-fermented extracts have been reported to enhance collagen synthesis in human dermal fibroblasts by increasing the levels of active saponin metabolites⁸. Additionally, strains of *P. pentosaceus* isolated from fermented foods show resistance to gastric acid and bile salts and possess antimicrobial and cholesterol-lowering properties⁹. These findings highlight the broad potential of *P. pentosaceus* as a bioactive platform for both systemic and cutaneous applications, supporting its selection as an EGF delivery system.

Our proteomic analysis indicates that KDM4B may act as an epigenetic response to UVB induced stress and may be modulated by EGF treatment. KDM4B, a histone demethylase that targets H3K9me3 and H3K36me3, has been shown to regulate chromatin accessibility, cell proliferation, DNA damage repair, and stress

response signaling^{10,11}. In the present study, KDM4B expression was markedly upregulated in UVB-irradiated mouse skin, consistent with its role as a stress-responsive epigenetic biomarker. This finding aligns with the recent evidence showing that KDM4B is activated under genotoxic and oxidative stress conditions and is associated with cellular adaptation and survival. Interestingly, treatment with pEGF significantly reduced KDM4B expression, suggesting that EGF may attenuate UVB-induced epigenetic stress responses. Given that EGF activates the MAPK/ERK and PI3K/AKT pathways involved in tissue regeneration and cellular recovery, the observed downregulation of KDM4B following EGF treatment may reflect reduced cellular stress, decreased chromatin remodeling activity, or restoration of homeostatic gene expression rather than a direct regulatory mechanism¹². This novel finding suggests that KDM4B may serve as a potential epigenetic biomarker of photoaging and underscores the broader regulatory effects of PP-EGF, extending beyond cytokine modulation and ECM remodeling.

Although recombinant hEGF has been extensively studied in dermatology, challenges remain regarding its stability and production costs. pEGF offers a promising alternative that combines EGF bioactivity with enhanced stability and cost-effectiveness, made possible by microbial bioengineering^{5,6}. Interestingly, the 10% pEGF formulation exhibited substantial regenerative effects, confirming its efficacy at this concentration, without observable adverse responses. Although higher EGF concentrations are often associated with potent regenerative outcomes, our data suggest that pEGF exerts meaningful anti-aging effects even at this dose, highlighting the importance of dose optimization in preclinical and clinical investigations.

Despite these promising outcomes, this study has certain limitations. First, the investigation primarily focused on histological and molecular endpoints. Functional parameters such as skin hydration, elasticity, and transepidermal water loss were not assessed but are essential to fully determine clinical translatability. Second, this study did not directly compare pEGF with conventional recombinant EGF or other skin-rejuvenating agents, making it difficult to contextualize the relative efficacy of this new approach. Third, although murine skin shares similarities with human skin, differences in the epidermal structure, thickness, and immune responses may limit the extrapolation of these results to humans. Finally, the long-term safety and potential immunogenicity of topically applied pEGF have not yet been evaluated. Future research, including functional measurements, head-to-head comparisons, long-term safety analyses, and human clinical trials, will be crucial for validating and optimizing the therapeutic application of pEGF.

Topical application of 10% pEGF cream significantly improved UVB-induced photoaging *in vivo* by promoting collagen synthesis,

reducing ECM degradation, and modulating oxidative and inflammatory responses. These findings support the clinical potential of pEGF as a regenerative and antiaging topical agent. Further research should explore functional outcomes and optimal dosing strategies in human trials.

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CONFLICTS OF INTEREST

Byung Chull An (researcher), Yongku Ryu (principal researcher), and Myung Jun Chung (CEO) are employed at Cell Biotech, Co., Ltd. However, they did not influence the study design, data interpretation, or decision to publish the results.

DATA SHARING STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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