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Groundbreaking Insights into the Role of Brain-Derived Neurotrophic Factor (BDNF) in Skin Senescence

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1. Introduction

The global aging population is rapidly increasing, prompting heightened attention towards the prevention of functional decline across various physiological systems. Brain-derived neurotrophic factor (BDNF) plays an essential role in preserving neural function by attenuating the progression of neurodegenerative diseases and responding to other age-related changes [1,2]. Its biological effects primarily mediated through the activation of tropomyosin receptor kinase B (TrkB) [3]. Despite extensive research into its neuroprotective effects, the involvement of BDNF and TrkB in skin aging process remains largely unexplored.

Recent evidence indicates that aged fibroblasts may promote an epithelial-to-mesenchymal transition (EMT)-like phenotype in keratinocytes through activation of the BDNF-TrkB signaling axis [4], with elevated levels of EMT-associated proteins observed in aged tissues [5]. Aged skin exhibits a marked accumulation of senescent cells. Although cellular senescence fulfills essential physiological roles during development and wound healing [6], the chronic persistence of senescent cells disrupts tissue homeostasis, drives inflammation, and facilitates extracellular matrix (ECM) degradation through the secretion of a diverse array of factors collectively termed the senescence-associated secretory phenotype (SASP) [7].

In recent years, the field of neurocosmetics has seen growing interest, as it offers new insights into how the brain interprets and responds to cosmetic applications. Additionally, conventional neurotrophic may play previously unrecognized roles in modulating skin cell function. In this study, we investigated, for the first time, the impact of aged dermal fibroblasts on BDNF-TrkB signaling and its potential association with SASP-related factors in epidermal cells, shedding light on its novel role in skin senescence.

2. Materials and Methods

Preparation for the Natural Skin Protectant

By employing advanced AI-driven screening technology, A Moroccan Flower Extract (MFE) was sourced from flowers grown on our own certified organic plantation in Morocco.

Traditionally used in perfumery, hair care, and food applications, MFE is valued for its natural benefits. *In vitro* tests were conducted using the pure active matter of MFE at a concentration of 0.02% to assess its effectiveness in skincare applications.

Human Fibroblast and Keratinocyte Co-Culture with BDNF-TrkB Measurement

Human fibroblasts were obtained from a 54-year-old donor and classified into two groups: young fibroblasts (low passage, 3rd passage) and aged fibroblasts (high passage, 15th passage). Both groups were incubated for 24 hours in the presence or absence of MFE at a concentration of 0.02 mg/ml. After incubation, the culture medium was collected to measure BDNF using a sensitive ELISA kit. Keratinocytes, obtained from a 49-year-old donor (low passage, 2nd passage), were incubated for 24 hours either with conditioned medium from young or aged fibroblasts, or without fibroblast medium (Control without fibroblast and Control with non-conditioned fibroblast medium). After incubation, cell monolayers were rinsed with PBS and stored at -20°C for TrkB measurement. Phosphorylated TrkB levels in keratinocytes were quantified using an ELISA kit specific to TrkB.

Human Skin Explant Culture and UVA Exposure

Skin explants were obtained from a 37-year-old female donor (phototype III), with informed consent. Upon excision, skin biopsies were placed on inert culture grids and cultured at 37°C in a humidified atmosphere with 5% CO_2. The explants were divided into three experimental groups: Control (non-stressed), Stress (UVA stressed), and MFE + Stress (UVA stressed with pre-treatment of 0.02% MFE). MFE was applied topically at a dose of 30 µL/cm², once daily for 24 hours, prior to UVA exposure. The explants were then exposed to a defined dose of UVA radiation (LED source, λ = 365 nm). After 24 hours, explants were collected, embedded in an optimal cutting temperature compound (OCT), snap-frozen, and stored at -80°C for subsequent analysis.

Immunofluorescence and Biomarker Quantification

Skin tissue slices (5 µm thickness) were prepared using a Leica cryostat and fixed in a 95% ethanol / 5% acetic acid solution. Non-specific binding sites were blocked with PBS containing 3% BSA. Sections were then incubated with primary antibodies against Collagen 17 (COL17) (ab186415), Ki67 (ab92742), and CXCL10 (ab318282), all sourced from Abcam. After washing, slides were incubated with Alexa Fluor 647-conjugated secondary antibodies (anti-rabbit, Invitrogen, 10123672), and nuclei were counterstained with DAPI (4',6-diamidino-2-phénylindole). Fluorescent images were captured using a ThermoFisher EVOS M5000/M7000 epifluorescence microscope, and the images were collected in 16-bit .TIFF format. For CXCL10 and COL 17, the fluorescence intensity was quantified, while for Ki67, the number of positively labeled cells was manually counted per section and normalized to the total number of epidermal nuclei. β-galactosidase was also evaluated as a specific senescence marker using the Abcam Senescence Detection Kit (ab65351), which involves X-gal hydrolysis to form a blue chromogenic precipitate. The number of blue-positive cells was counted per image, and the mean values per group were calculated as an indicator of cellular senescence.
Statistical analyses were performed using Student's t-test to compare the "Control" and "Control + UV" conditions. One-way ANOVA followed by the Holm-Sidak test was used to assess the significance between the "Control + UV" condition and the test compound-treated groups, with significance levels indicated as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

3. Results

Modulation of BDNF Release and TrkB Phosphorylation Under MFE Exposure

As shown in Figure 1, aged fibroblasts exhibited increased BDNF secretion, whereas treatment with 0.02% MFE significantly attenuated age-related BDNF release (Figure 1a). Co-culture with young fibroblasts did not result in a significant change in keratinocyte TrkB phosphorylation. In contrast, aged fibroblasts markedly enhanced TrkB phosphorylation in keratinocytes, an effect that was significantly reduced following 0.02% MFE treatment (Figure 1b).

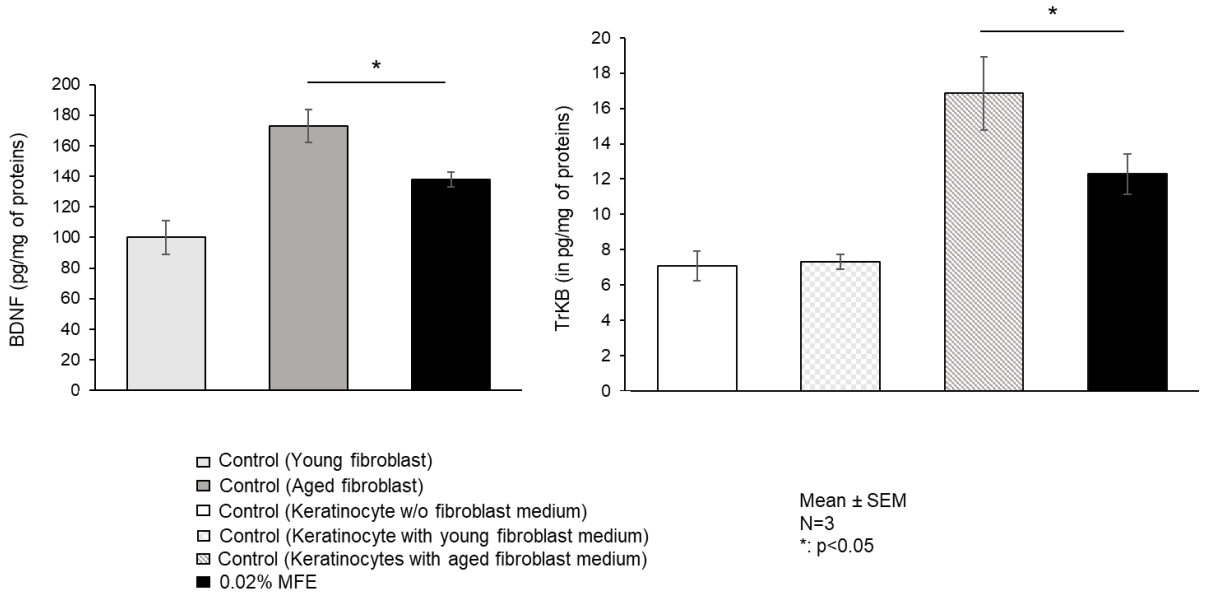

Figure 1. Modulation of the age-associated BDNF-TrkB signaling axis by 0.02% MFE treatment: (a) BDNF release by human normal fibroblasts in monolayer culture; (b) TrkB phosphorylation in human normal keratinocytes co-cultured with fibroblasts from panel (a). *Results are expressed as pg of BDNF or TrkB per mg of total proteins measured from cell monolayer lysate. Each experimental group are expressed as relative values (% vs Control). The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using GraphPad software with an unpaired t-test and Welch's correction (95% confidence interval).*

Visualization of Biomarker Expression

Immunofluorescence analysis of skin explants (Figure 2) revealed distinct modulation of biomarker expression across the different experimental conditions. UVA exposure led to a visible decrease in COL17 (Figure 2a) and Ki67 (Figure 2b) in the epidermis compared to the untreated controls, co-treatment with 02% MFE preserved the fluorescent intensity of both markers. Conversely, the pro-inflammatory cytokin CXCL10 (Figure 2c) strongly upregulated by UVA, was visibly reduced in MFE-treated samples. In addition, β-galactosidase staining (Figure 3) showed a lower number of β-galactosidase–positive cells in both epidermis and dermis following MFE treatment, supporting a reduction in senescence-associated β-galactosidase (SA-β-gal) accumulation.

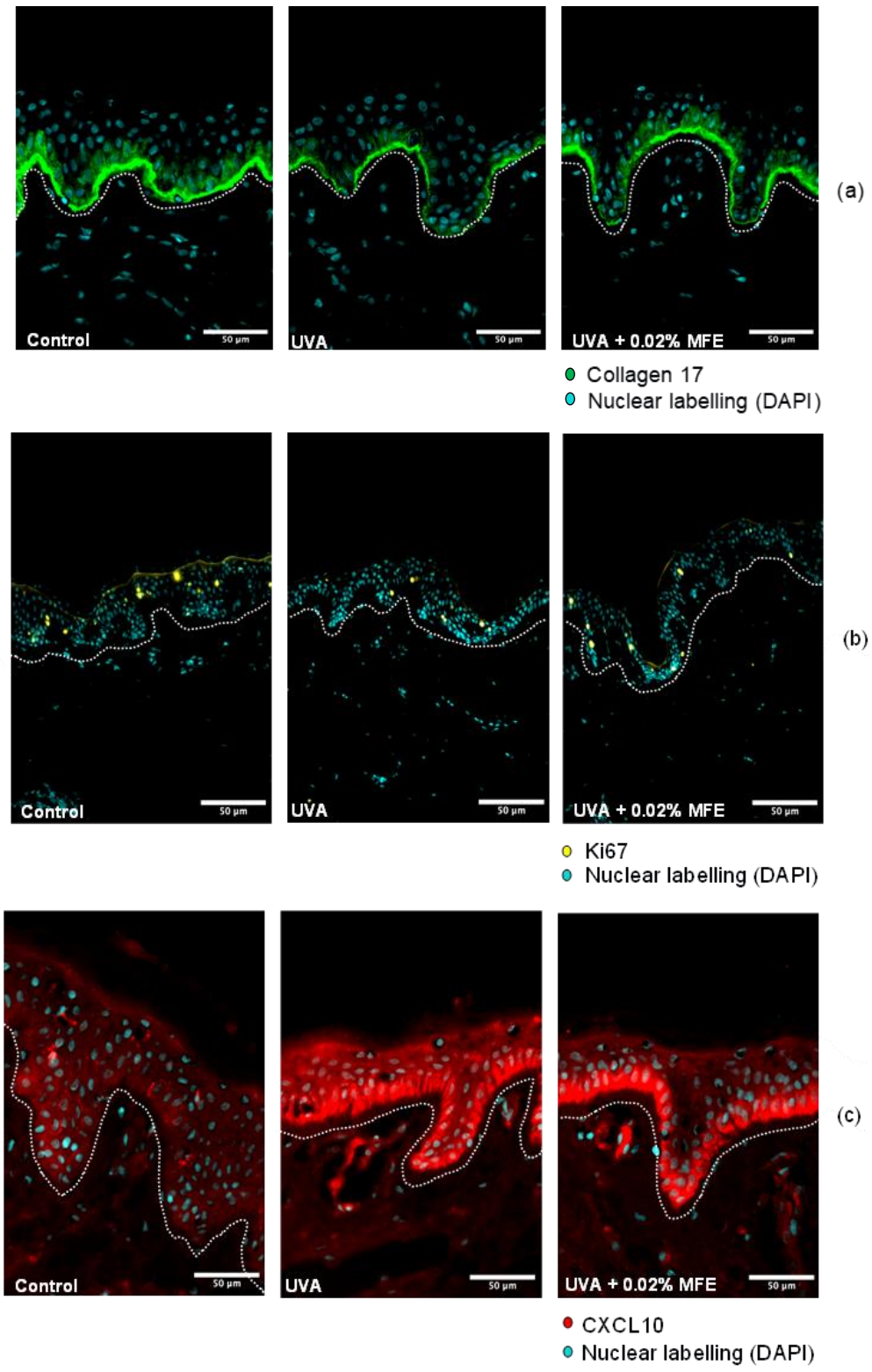

Figure 2. *In situ* visualization of biomarker expression in human skin explants following UVA exposure and treatment with 0.02% MFE: (a) COL17 (green); (b) Ki67 (yellow); and (c) CXCL10 (red) were visualized by immunofluorescence and superposed with nuclear staining using DAPI (cyan), epidermis and dermis were separated by white dotted line. *All images were acquired using epifluorescence microscopy with a 10× objective. Scale bar: 50 µm.*

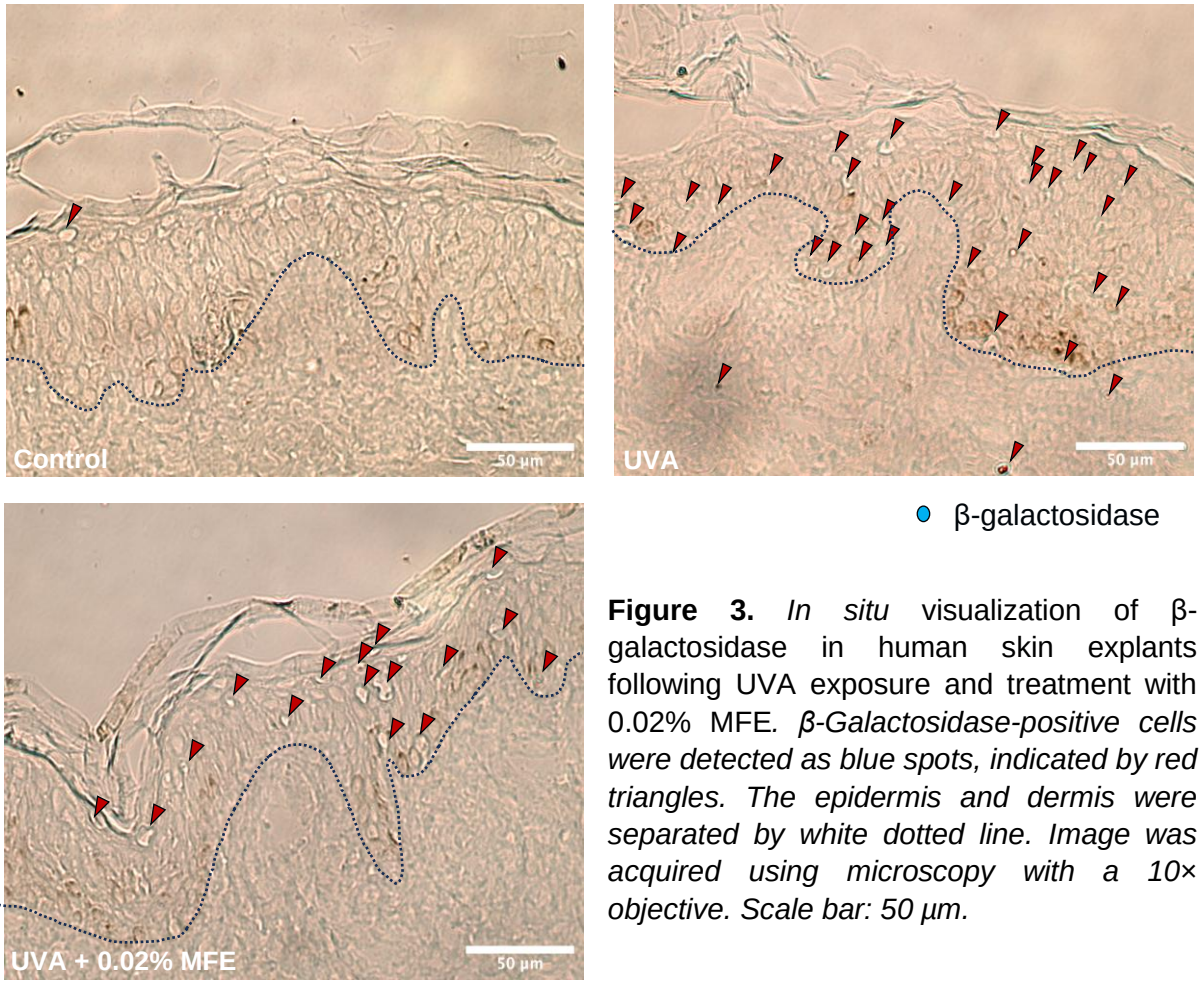

Figure 3. *In situ* visualization of β-galactosidase in human skin explants following UVA exposure and treatment with 0.02% MFE. *β-Galactosidase-positive cells were detected as blue spots, indicated by red triangles. The epidermis and dermis were separated by white dotted line. Image was acquired using microscopy with a 10× objective. Scale bar: 50 µm.*

Quantitative Analysis of Biomarker Expression in Skin Explants

Quantitative analysis (Figure 4) corroborated the differential modulation of biomarkers under MFE treatment compared to UVA exposure. Both COL17 and Ki67 signals, which were diminished upon UVA exposure, were significantly re-established following MFE treatment. indicating the preservation of epidermal structural integrity and the maintenance of proliferative capacity. In contrast, the fluorescence intensity of CXCL10, which was markedly induced by UVA, was significantly attenuated in MFE-treated samples, suggesting a reduction in pro-inflammatory signaling. Additionally, the number of β-galactosidase-positive cells, a well-established marker of cellular senescence, was significantly reduced following MFE treatment, supporting the attenuation of UVA-induced senescent-associated changes.

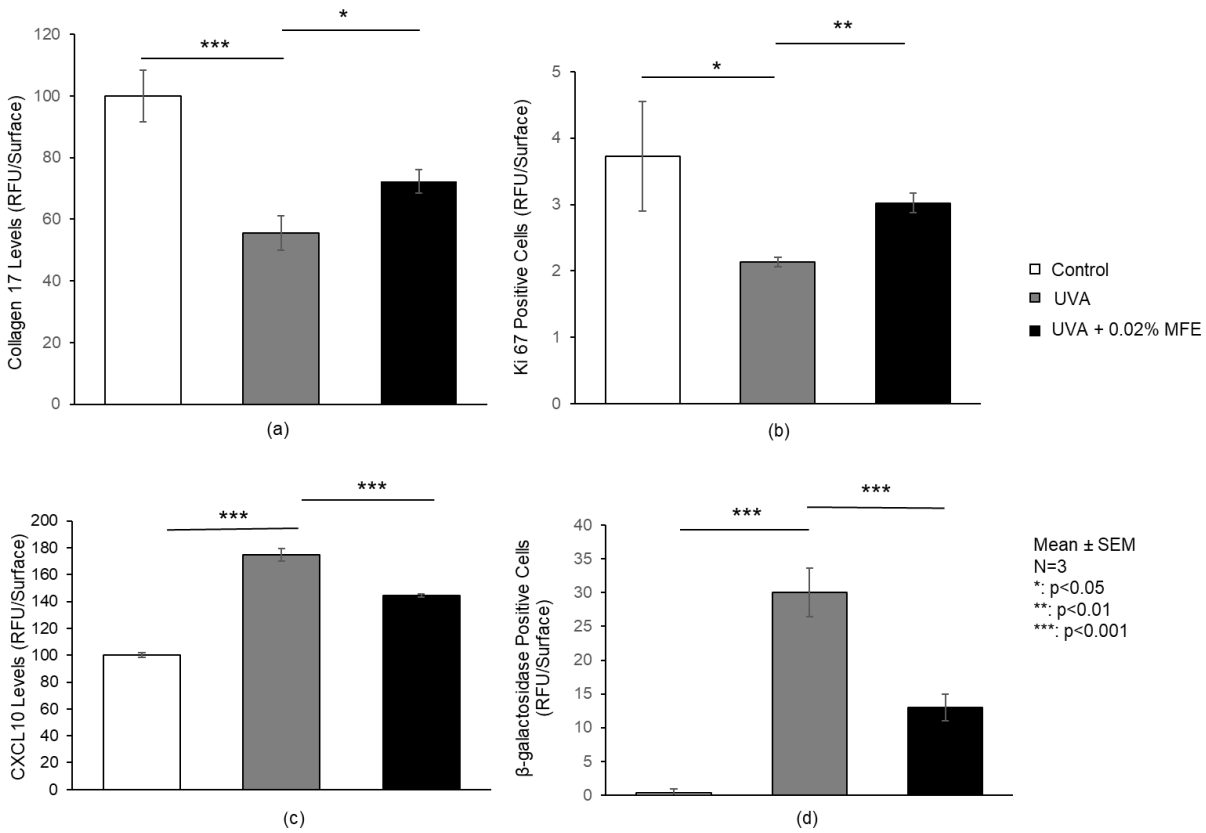

Figure 4. Quantification of biomarker expression in human skin explants following UVA exposure and treatment with 0.02% MFE: Histograms show the relative expression levels of: (a) COL 17, (b) Ki67-positive cells, (c) CXCL10, and (d) β-galactosidase-positive cells. *Results are expressed as relative fluorescence units per surface (RFU/surface) and presented as percentages versus* untreated *control. Error bars represent the standard error of the mean (SEM). Statistical analysis was performed using an unpaired t-test with Welch's correction (GraphPad Software; 95% confidence interval).*

4. Discussion

In recent years, skin is considered as a neurotrophic organ, with emerging evidence highlighting the significance of neurotrophic factors such as neurotrophins in the aging processes [8]. As previously mentioned, aged fibroblasts have been shown to promote an EMT-like phenotype in keratinocytes through activation of the BDNF-TrkB signaling pathway [4]. Our data revealed significantly elevated BDNF levels in aged fibroblasts compared to young fibroblasts, accompanied by upregulated TrkB expression in keratinocytes co-cultured with aged fibroblasts. In contrast, no significant changes in TrkB expression were observed in keratinocytes co-cultured with young fibroblasts. Therefore, it appears that only aged fibroblasts could stimulate the BDNF-TrkB signaling pathway through fibroblast-keratinocyte crosstalk.

EMT plays a pivotal role in tissue differentiation and regeneration. However, it is also implicated in the disruption of cell-cell adhesion and contributes to the pathogenesis of organ fibrosis [9]. Unlike the majority of collagens, COL17 is a transmembrane protein. Epidermal COL17 constitutes a critical component of hemidesmosomes [10], where it plays an essential role in maintaining keratinocyte adhesion [11]. Notably, COL17 expression has also been detected in

human neurons [12], suggesting potential involvement in neurotrophic signaling and functions beyond epithelial integrity. Our findings suggest that UVA-induced downregulation of COL17 may be attenuated by the natural skin protectant MFE, which appears to negatively correlate with modulation of BDNF-TrkB signaling in aged skin cells.

Recent data have demonstrated an association between COL17 overexpression and reduced Ki67 expression [13], implying a potential inverse relationship with cellular proliferation. Nevertheless, reports on age-related variations in Ki67 expression in human skin remain inconsistent across the literature [14–16]. Our findings indicate a positive correlation between UVA-induced alterations in COL17 and Ki67 expression levels, suggesting a coordinated response between structural integrity and proliferative activity under UVA exposure. Given that Ki67 represents a graded rather than binary marker distinguishing proliferation from quiescence [17], further investigation of additional SASP-related markers is warranted to comprehensively assess cellular senescence.

Evidence indicates that UVA exposure alone is insufficient to induce EMT [18], and that UVA radiation has been shown to downregulate BDNF expression, contributing to nerve cell damage within the central nervous system [19,20]. However, the BDNF-TrkB autocrine signaling loop has been shown to enhance the viability of senescent cells, thereby promoting their accumulation [21]. Notably, levels of BDNF and several SASP-associated cytokines, such as CXCL10, have been shown to exhibit similar upregulation in senescent cells. Consistent with these findings, our study showed the positive correlation between the age-related increases in BDNF levels and UVA-induced elevations in both CXCL10 expression and SA-β-gal activity. In this study, we demonstrate for the first time that the paracrine signaling network of BDNF-TrkB derived from aged fibroblasts may influence keratinocyte senescence, contributing to epidermal aging. This novel finding highlights the potential role of BDNF-TrkB axis in skin senescence and may offer new perspectives for developing strategies to prevent or mitigate skin aging.

5. Conclusion

In recent years, skin is considered as a neurotrophic organ, with emerging evidence highlighting the significance of neurotrophic factors in the aging processes. The BDNF-TrkB autocrine signaling loop has been shown to enhance the viability of senescent cells, thereby promoting their accumulation. In this study, we demonstrate for the first time that the paracrine signaling network of BDNF-TrkB derived from aged dermal fibroblasts may influence epidermal keratinocyte senescence and may be positively associated with the modulation of UV-induced SASP-related markers, contributing to epidermal aging. This novel finding suggests that the BDNF-TrkB axis may play a key role in skin senescence and may offer new perspectives for developing strategies to prevent or mitigate skin aging.

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7. Conflict of Interest Statement

The authors declare no competing interests.

8. References

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