



Research Article

A new role for the ginsenoside RG3 in antiaging via mitochondria function in ultraviolet-irradiated human dermal fibroblasts[☆]

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ABSTRACT

Background: The efficacy of ginseng, the representative product of Korea, and its chemical effects have been well investigated. The ginsenoside RG3 has been reported to exhibit apoptotic, anticancer, and antidepressant-like effects.

Methods: In this report, the putative effect of RG3 on several cellular function including cell survival, differentiation, development and aging process were evaluated by monitoring each specific marker. Also, mitochondrial morphology and function were investigated in ultraviolet (UV)-irradiated normal human dermal fibroblast cells.

Results: RG3 treatment increased the expression of extracellular matrix proteins, growth-associated immediate-early genes, and cell proliferation genes in UV-irradiated normal human dermal fibroblast cells. And, RG3 also resulted in enhanced expression of antioxidant proteins such as nuclear factor erythroid 2-related factor-2 and heme oxygenase-1. In addition, RG3 affects the morphology of UV-induced mitochondria and plays a role in protecting mitochondrial dysfunction.

Conclusion: RG3 restores mitochondrial adenosine triphosphate (ATP) and membrane potential via its antioxidant effects in skin cells damaged by UV irradiation, leading to an increase in proteins linked with the extracellular matrix, cell proliferation, and antioxidant activity.

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1. Introduction

Ultraviolet (UV) irradiation is well known as a main factor of skin aging [1–3]. The primary features of skin aging in humans include a decline in fibrous tissue, slow cellular regeneration, and a reduction of the vascular and glandular network [4]. The extracellular matrix (ECM) components of the skin must be well balanced to provide structurally functional support [5].

Collagen is the basic protein of structure in the extracellular space of various types of animal connective tissues and constitutes approximately 25–35% of all protein content in the body [6]. Matrix metalloproteinases (MMPs) degrade ECM connective tissue [7].

In addition to UV irradiation, other reactive oxygen species (ROS) and hydrogen peroxide (H₂O₂) are involved in the pathogenesis of several skin conditions, including aging, wrinkles, photosensitivity, and malignancy [8].

[☆] The English of this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: <http://www.textcheck.com/certificate/krdwNk>.

Abbreviations: NHDF, normal human dermal fibroblast; ECM, extracellular matrix.

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Commonly used in the pharmaceutical and cosmetic industries, plant extracts with antioxidant properties decrease the production of ROS. Various traditional plant extracts have well-known properties, including medicinal functions, pharmacological activities, and skin-protective effects [9,10]. The efficacy of ginseng, the representative product of Korea, and its chemical effects have been well investigated. The ginsenoside RG3 has been reported to exhibit apoptotic [11,12], anticancer [13,14], antiinflammatory [Cheng, 2016 #17], and antidepressant-like effects [15].

2. Materials and methods

2.1. RG3 material

The ginsenoside RG3 (>95% purity) was purchased from Korean Tobacco and Ginseng, Ltd. (Deajon, Korea).

2.2. Cell culture and cell viability

Cell culture and the cell viability assay were performed as previously described [16].

2.3. Wound healing assay

For the cell migration assay, monolayers were carefully scratched using a 10- μ L pipette tip. After 24 hr of UV irradiation (40 J/m²) and treatment with UV crosslinker (Vilber Lourmat, Collégien, France) for 3 min, cells were untreated or treated with 1, 5, or 10 μ M RG3 extract for 24 h. Wounded areas were then photographed.

2.4. Western blot analysis and antibodies

Western blotting was performed as previously described [16,17]. Anticollagen (Abcam, Cambridge, UK), anti-dynamin-related protein 1 (DRP1), and anti-GTPase optic atrophy 1 (OPA1) antibodies were purchased from BD Biosciences (San Jose, CA, USA). Anti-actin antibody was obtained from Sigma-Aldrich (St. Louis, MO, USA), anti-phosphorylated DRP1 [pDRP1; phosphorylated serine 616 (pS616) or pS637] was purchased from Cell Signaling Technology (Boston, MA, USA), and anti-elastin, anti-MMP-3, anti-extracellular signal-regulated kinase (ERK), anti-translocase of mitochondrial outer-membrane 40 (TOM40), anti-mitofusin (MFN), and anti-mitochondrial fission protein 1 (FIS1) antibodies were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG secondary antibodies were obtained from Koma Biotech (Seoul, Korea).

2.5. Quantitative polymerase chain reaction

Quantitative polymerase chain reaction was performed as previously described [16].

2.6. ROS assay

Normal human dermal fibroblast (NHDF) cells were plated and incubated at a density of 1.0×10^6 cells/well in six-well culture plates for 24 h. The following day, cells were UV irradiated with 40 J/m² followed by treatment with UV crosslinker (Vilber Lourmat) for 3 min and either untreated or treated with RG3 extract for 24 h. Cells were washed, stained with 5 μ M 2',7'-dichlorodihydrofluorescein diacetate (CM-H₂DCFDA; Life Technologies, Carlsbad, CA, USA) and 20 mM dihydroethidium (DHE) in Hank's balanced salt solution and incubated at 37°C for 30 min. FACSCalibur flow cytometer (BD Biosciences) was used to measure H₂O₂ activity

using an emission filter at 532 nm. At least 10,000 cells were analyzed in three independent experiments.

2.7. Mitochondrial fraction

NHDF cells were washed with phosphate buffered saline (PBS) and resuspended in mitochondrial fraction buffer (20 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) [HEPES], pH 8.0, 10 mM KCl, 1.5 mM MgCl₂, 1 mM ethylenedinitrilo)tetraacetic acid (EDTA), 250 mM sucrose, 1 mM phenylmethane sulfonyl fluoride, 10 g/mL leupeptin, 10 g/mL aprotinin, and 0.2 mM sodium orthovanadate) for 30 min on ice, and homogenized. Unbroken cells and nuclei were pelleted by centrifugation at 1500 \times g for 10 min. The supernatant was centrifuged at 10,000 \times g for 30 min at 4°C and transferred to a new tube as the postmitochondrial fraction. Then, the supernatant was centrifuged at 10,000 \times g for 1 h at 4°C, and the resulting supernatant was used as the cytosolic fraction. The pellet containing the post-mitochondrial fraction was washed with 500 μ L of mitochondrial fraction buffer and used to isolate the mitochondrial fraction. Isolated mitochondria were treated with 2M sodium chloride, 100 mM sodium carbonate (pH 11.2), or 1% Triton X-100 for 30 min. The samples were ultracentrifuged to separate the supernatant (S) and precipitate (P) fractions, which were used to analyze tricarboxylic acid activity.

2.8. Confocal imaging analysis

NHDF cells were grown on glass coverslips to 50–70% confluency and transfected with pDsRed2-Mito using jetPEI reagents (polyplus transfection, NY, USA). After 48 h, cells were fixed in 4% paraformaldehyde at room temperature for 10 min and permeabilized with 0.2% Triton X-100 for 5 min at room temperature. Then, cells were incubated in blocking buffer containing 5% bovine serum albumin (Sigma-Aldrich) in 1 \times Tris-buffered saline for 1 h at 37°C. The rabbit polyclonal anti-cytochrome-c antibody was diluted 200-fold for use as a primary antibody and incubated overnight. Fluorescein isothiocyanate-conjugated anti-mouse antibody (BD Biosciences, Franklin Lakes, NJ, USA) was used as the secondary antibody. After appropriate rinsing, coverslips were mounted with Vectashield (Vector Laboratories, Burlingame, CA, USA) and visualized using a Leica confocal microscope (Leica Microsystems, Wetzlar, Germany).

2.9. Measurement of ATP levels in subcellular compartments

Mitochondrial adenosine triphosphate (ATP) was measured using a firefly luciferase construct conjugated with a COX8 mitochondrial targeting sequence (pcDNA3.1/Zeo-CHA-COX8-Luc) as previously described [18]. Briefly, light emission was measured in a luminometer at 5-s intervals until the maximum value of luminescence was reached. To normalize for the variability of luciferase expression in transfected cells, the relative luminescence values in each cellular compartment were expressed as a ratio to the total potential luminescence measured in equal amounts of the same lysed cells with a luciferase assay kit (Promega, Madison, WI, USA) in the presence of excess ATP (final concentration, 0.5 mmol/L).

2.10. Measurement of mitochondrial membrane potential ($\Delta\Psi_m$)

NHDF cells were irradiated with 40 J/m² UV followed by treatment with UV crosslinker (Vilber Lourmat) for 3 min and either untreated or treated with 10 μ M of RG3 extract for 24 h or 30 μ M carbonyl cyanide m-chlorophenyl hydrazone (CCCP) for 8 h as a control. Cells were stained with 1 μ M rhodamine-123 for 60 min to measure the mitochondrial transmembrane potential using flow cytometry. A minimum of 10,000 cells per sample were analyzed.

2.11. Statistical analysis

Statistical analysis was performed as previously described [16].

3. Results

3.1. Cell viability and wound healing of NHDF cells treated with RG3 extract

NHDF cells were treated with 1, 5, and 10 μM of RG3 extract for 24 hr. Compared with untreated cells, treatment with the RG3 extract did not exhibit cytotoxicity (Fig. 1A). To assess the anti-apoptotic effect of RG3, NHDF cells were induced to undergo apoptosis by exposure to 60 J/m^2 of UVC irradiation. The apoptosis marker caspase 3 was cleaved in a time-dependent manner (Fig. 1B). When confluent monolayers of cells were treated with RG3 for 24 hr after UV irradiation in a wound healing assay, cells closed readily when treated with doses from 1 to 10 μM in a dose-dependent manner (Fig. 1C).

3.2. Cell differentiation, development, and protective effects of RG3 after UV irradiation in NHDF cells

Protein kinase B (PKB)/AKT plays a fundamental role in signaling pathways that regulate a variety of cellular functions, such as cell survival, growth, nutrient metabolism, proliferation, and apoptosis.

Phosphorylation of PKB/AKT at Ser473 and Thr308 fully activates PKB/AKT [19]. Exposure of mouse epidermal JB6 Cl 41 cells to UV irradiation led to phosphorylation of PKB/AKT at Ser473 and Thr308 in a time-dependent manner. UV irradiation induced PKB/AKT phosphorylation of epidermal cells, indicating a correlation between the response to UV and PKB/AKT [20]. Activated ERKs translocate to the nucleus and promote changes in gene expression, growth, differentiation, or mitosis [21]. Mammalian cells respond quickly when exposed to UV, stimulating various factors, which ultimately results in the activation of transcription factors and proteins such as mitogen-activated protein kinases (MAPKs) and ERKs. MAPKs are activated by phosphorylation of specific tyrosine and threonine residues [22,23].

We also investigated the effect of UV irradiation on the phosphorylation and activity of PKB/AKT (S473) and ERK (T202 and Y204). Total protein levels of ERK were upregulated in RG3-treated cells after UV irradiation (Figs. 2A, 2B).

Protein extracts of NHDF cells either untreated or treated with RG3 extract for 24 h after UV irradiation were analyzed by Western blot. It has previously been reported that in human fibroblasts, type I collagen gene expression was regulated by the ERK signaling pathway [24]. Our data showed that the ERK protein level was dramatically increased in a dose-dependent manner (Fig. 2A). Expression of phosphorylated ERK (T44/T42) and PKB (S473) was significantly increased in a dose-dependent manner in UV-induced NHDF cells (Fig. 2A). UV irradiation is known to affect immune-

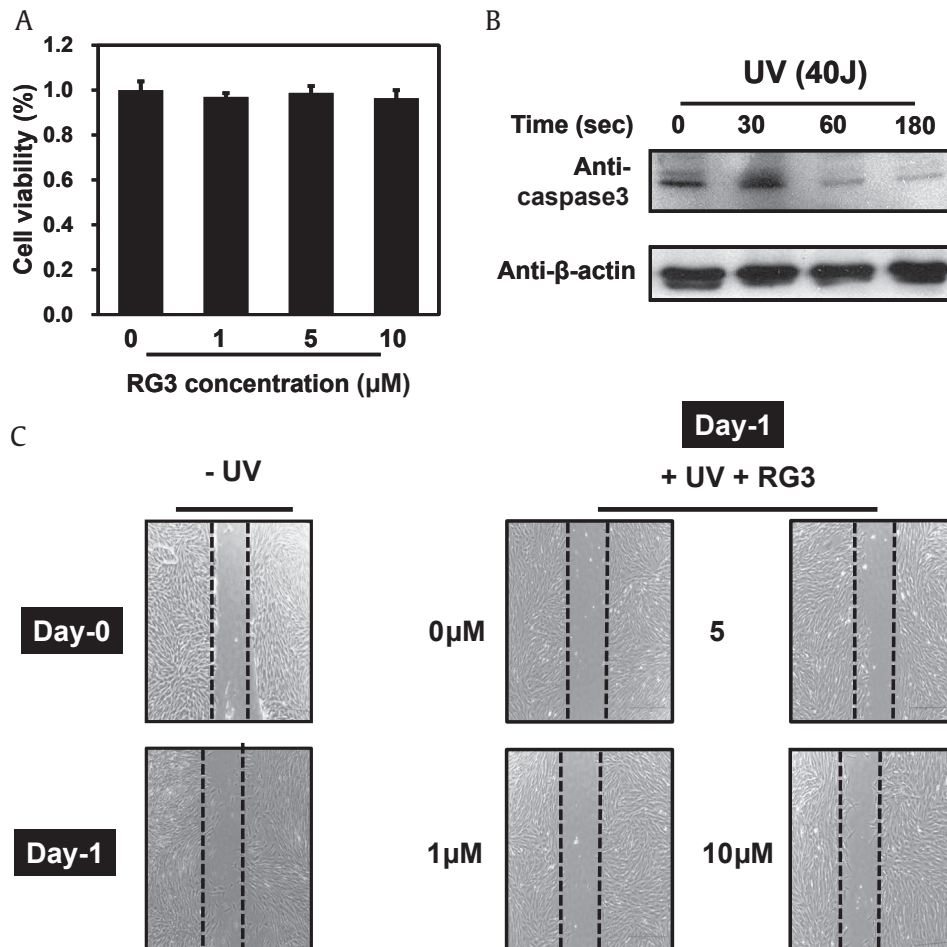


Fig. 1. Cell viability assay and cell wound healing assay. (A) RG3 extract did not result in cytotoxicity at 1, 5, and 10 $\mu\text{g}/\text{mL}$ by EZ-Cyto assay with NHDF cells. (B) Induction of apoptotic cell death was measured by Western blot analysis with anti-caspase 3 antibodies after UV irradiation of 40 J/m^2 in NHDF cells. (C) Cell wound healing analysis was applied to UV-irradiated cells for 24 hours after scratching. The UV-irradiated NHDF cells were further treated with RG3 extract in a dose-dependent manner. NHDF, normal human dermal fibroblast; UV, ultraviolet.

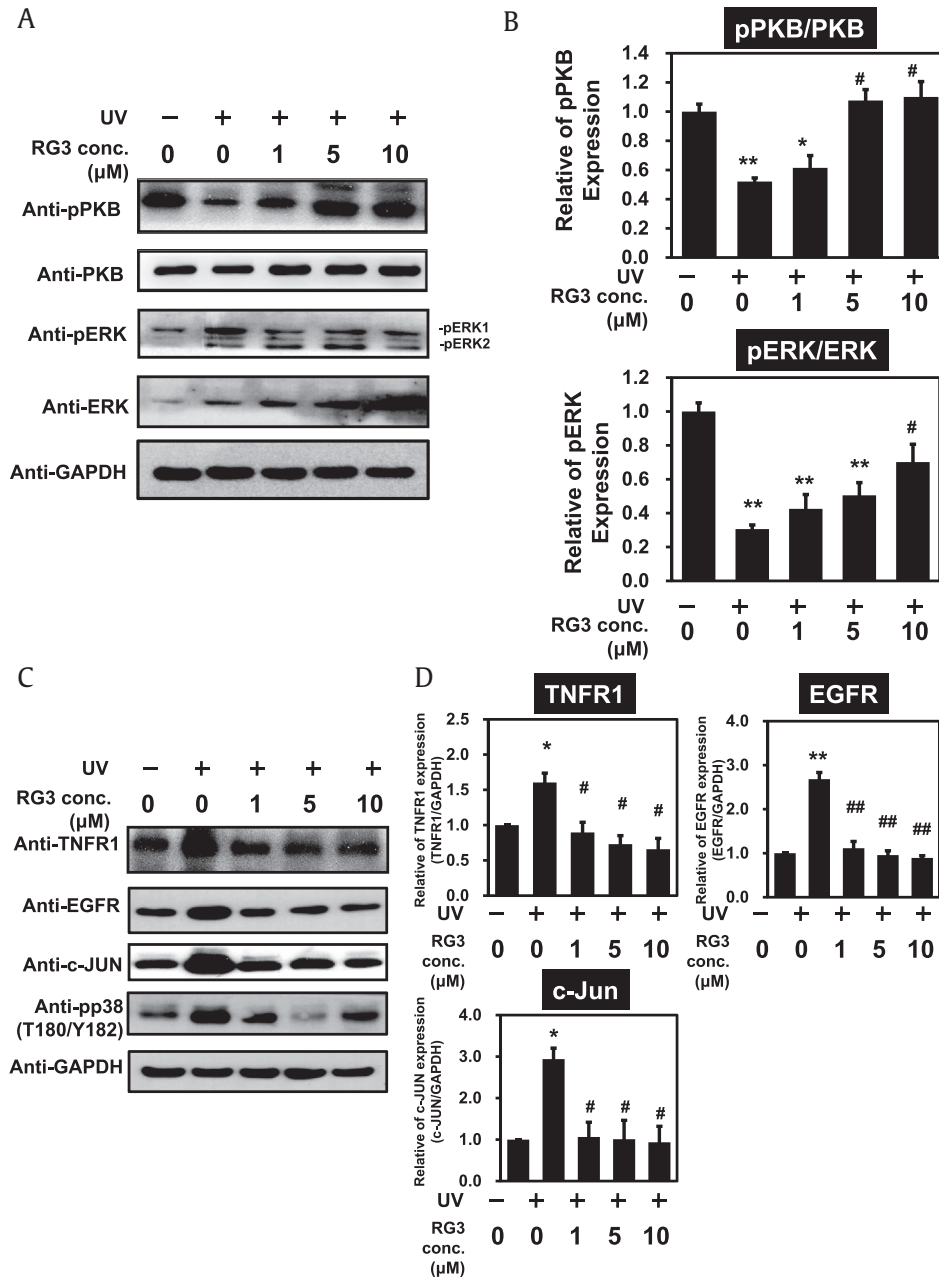


Fig. 2. Cell differentiation, development, and proliferation protein expression by Western blot. (A, C) Total protein extracted from untreated or RG3 extract-treated cells was analyzed by Western blot with anti-extracellular signal-regulated kinase (ERK), anti-phosphorylated ERK2, anti-protein kinase B (PKB), anti-phosphorylated PKB, anti-tumor necrosis factor receptor 1 (TNFR1), anti-epidermal growth factor receptor (EGFR), anti-c-JUN, and anti-phosphorylated-p38 antibodies. (B, D) Anti-ERK, anti-pERK, anti-PKB, anti-pPKB, anti-TNFR1, anti-EGFR, anti-c-JUN, and anti-pp38 were quantified by Western blotting, as described in the [Materials and methods](#) section. The values are means \pm SD of three independent experiments. Significant difference from negative control (Non-UV irradiated): *, $p < 0.05$ and **, $p < 0.01$. Significant difference from positive control (UV irradiated): #, $p < 0.05$ and ##, $p < 0.01$. pERK, phosphorylated ERK; SD, standard deviation; UV, ultraviolet.

regulatory factors and signal transduction in the skin. More specifically, UV irradiation increases the expression of several growth-associated immediate-early genes such as c-Jun [25,26]. Tumor necrosis factor (TNF)- α , which was the first cytokine linked to immunosuppression and skin carcinogenesis after UV irradiation, increases the production of TNF- α in keratinocytes during UV irradiation and contributes to the induction of skin cancer [27–29]. Growth factor receptor, Src, and Ras-Raf-MEK-MAPK signaling pathways are also important after UV exposure [30–34]. Rapid nuclear translocation of epidermal growth factor receptor (EGFR) was observed in UV-irradiated human primary and HaCaT keratinocytes. UV irradiation induces nuclear translocation of the EGFR in

human keratinocytes [35]. It has also been reported that structural changes were induced by UVB light in the epidermal growth factor-binding site [36]. Our data indicate that the expression of TNF receptor 1, EGFR, c-Jun, and pp38 was decreased in a dose-dependent manner in UV-induced NHDF cells (Figs. 2C, 2D).

3.3. RG3 enhanced the expression of ECM proteins in UV-irradiated NHDF cells

The general composition of the ECM varies between multicellular structures due to cell adhesion, intercellular communication, and differentiation [37,38]. Development, tissue repair, morphogenesis,

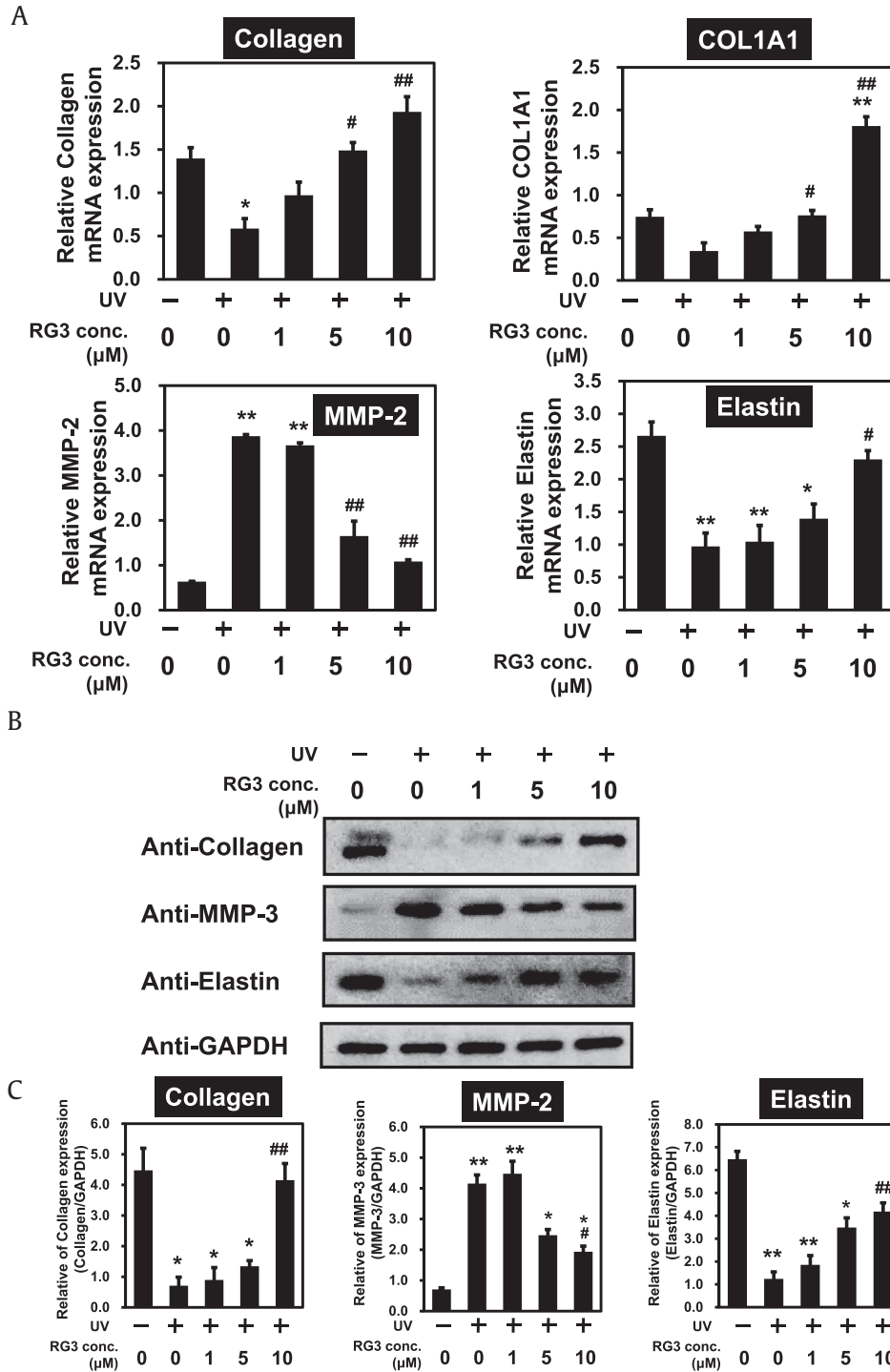


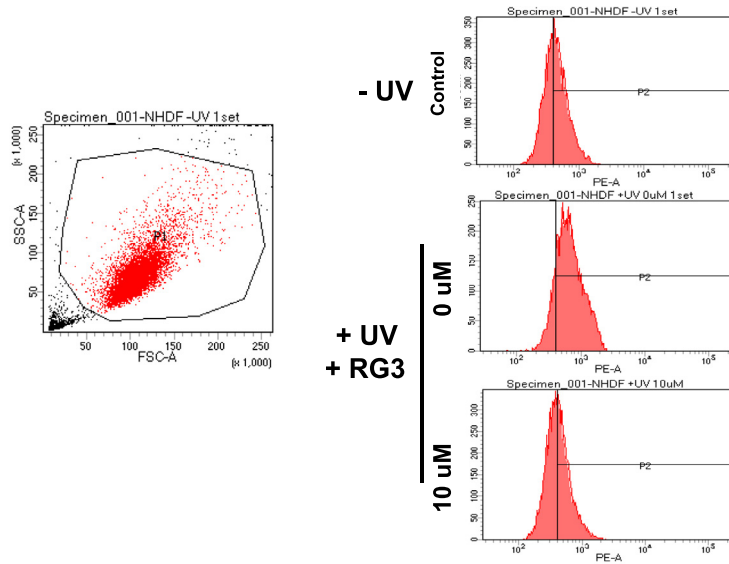
Fig. 3. Extracellular matrix mRNA and protein expression. (A) mRNA level of ECM genes in RG3-treated cell on UV irradiation. (B) Protein level of ECM in RG3-treated cell on UV irradiation. (C) Collagen, elastin, MMP-3, and GAPDH in the immunoprecipitation were quantified by Western blot analyses, as described in the [Materials and methods section](#). Bar heights are means ± SD of three independent experiments. Significant difference from negative control (Non-UV irradiated): *, $p < 0.05$ and **, $p < 0.01$. Significant difference from positive control (UV irradiated): #, $p < 0.05$ and ##, $p < 0.01$. COL1A1, alpha-1 type I collagen; ECM, extracellular matrix; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MMP, matrix metalloproteinase; SD, standard deviation; UV, ultraviolet.

and remodeling are characterized by degradation of the ECM. Normal physiological conditions regulate decomposition of the ECM; however, disability of this process can result in disease such as chronic inflammation and cancer [39].

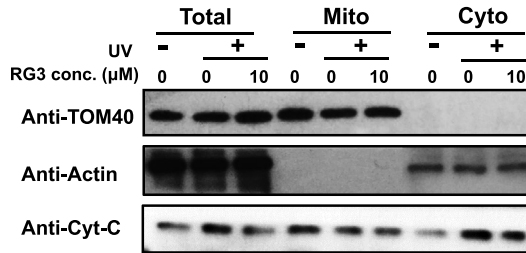
Collagen is the most abundant fibrous protein within the interstitial ECM and as the main structural elements of ECM

provide tensile strength, control cell adhesion, support chemotaxis and migration, and induce tissue development [40,41]. Collagen associates with elastin, and stretching is limited by close association with collagen fibers [42]. Elastin is a highly elastic ECM protein in connective tissue that helps maintain tissue integrity after stretching and contraction, especially when the skin is pinched or

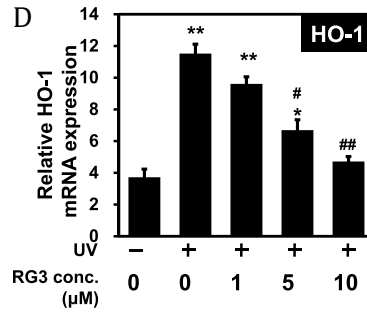
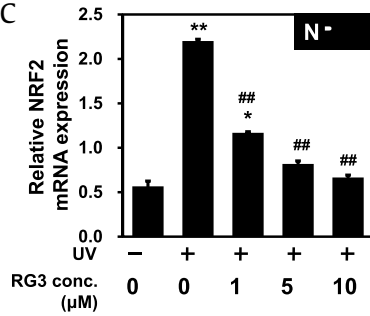
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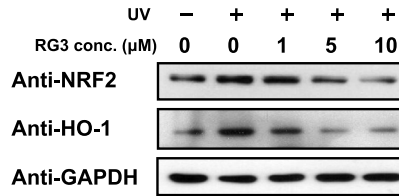
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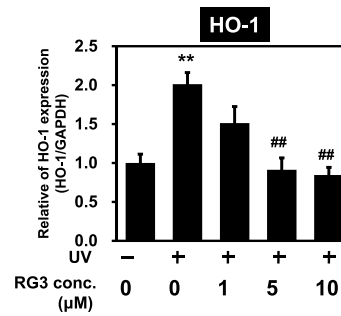
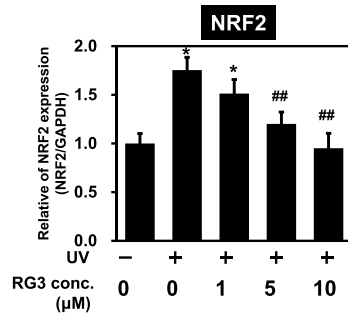
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F



pressed. The elastin of the tissue is combined with the fine fibers to form elastic fibers and in adult tissues constitutes the main component of elastic fibers [43].

In humans, alpha-1 type I collagen (COL1A1) is encoded by the COL1A1 gene, which encodes a major component of type I collagen, and includes fibrin collagen found in connective tissues, including cartilage [44]. After UV irradiation, skin is damaged. Infiltrating macrophages move to the site of damage and release interferon- γ , which binds to receptors in sun-damaged melanocytes to avoid detection by the immune system [45]. It is well known that interferon- γ inhibits COL1A1 expression in fibroblasts, principally at the transcriptional level [46].

We evaluated the mRNA levels of ECM proteins in RG3-treated cells after UV irradiation. Our data showed that the mRNA levels of collagen, elastin, and COL1A1 were increased in a dose-dependent manner, whereas that of MMP-2 was not (Fig. 3A). Protein extracts of untreated or RG3-treated cells after UV irradiation were analyzed by Western blotting. After UV exposure, the basal expression level of collagen and elastin was decreased dramatically, but RG3-treated cells showed increased collagen and elastin levels compared with untreated controls. The protein content of MMP-3 was increased compared to that of the normal state during UV irradiation, but the expression of MMP-3 decreased in proportion to the concentration of RG3 after UV irradiation (Figs. 3B, 3C).

It has been reported that the ERK signaling pathway regulates type I collagen gene expression in human fibroblasts [47]. Our data showed that the ERK protein level was significantly increased and that expression of the interstitial collagenase enzyme MMP-3 was significantly downregulated in a dose-dependent manner (Figs. 2A and 3A, 3B). These results suggested that the antiaging effects of RG3 treatment in UV-irradiated cells are associated with the transcriptional and translational regulation of molecules involved in the ECM.

3.4. Treatment with RG3 extract inhibited apoptosis after UV exposure in NHDF cells

UVA/UVB light stimulates the production of ROS such as O $_2^{\bullet-}$, H $_2$ O $_2$, and 1 O $_2$ in the skin [48–50]. The production of ROS occurs during physiological processes such as aging, metabolism, and apoptosis, as well as in a number of pathological conditions. Environmental stresses can lead to harmful oxidative reactions, and ROS are abolished via enzymatic as well as nonenzymatic antioxidative mechanisms [33]. Antioxidants are well known as a systemic light protectant against UVA- and UVB-induced skin damage [51]. For the removal of ROS, every organism has a complex defense system consisting of both antioxidants and antioxidant enzymes [52].

Exposure to UV or H $_2$ O $_2$ increases cell toxicity. In particular, H $_2$ O $_2$ exposure in muscle cells may increase cytotoxic inflammation and increase H $_2$ O $_2$ in atherosclerosis or chronic inflammatory disease [53]. ROS production has been shown to be increased in NHDF cells after UV irradiation [16]. In this study, fluorescence-activated cell sorting revealed that RG3-treated cells exhibit reduced production of ROS compared with untreated cells. The levels of H $_2$ O $_2$ were measured using the cell-permeable compound CM-H $_2$ DCFDA (Fig. 4A). UV irradiation increased the levels of H $_2$ O $_2$, whereas ROS levels were reduced after treatment with RG3. These results

indicated that the RG3 protects dermal fibroblasts from UV irradiation by reducing the production of ROS.

It has previously been reported that RG3 inhibits the release of cytochrome-c from depleted serum into the cytoplasm, indicating that mitochondrial-dependent caspase activity is inhibited by RG3 [54]. Cytochrome-c in total cell lysate and the release of cytochrome-c from mitochondria into the cytosol were increased after UV irradiation. We also showed that the release of cytochrome-c into the cytosol was decreased after treatment with RG3 (Fig. 4B), suggesting that apoptosis of UV-exposed skin cells is inhibited by RG3 treatment.

Nuclear factor erythroid 2-related factor-2 (NRF2) increases detoxification pathways and antioxidant potential and protects cardiac fibroblasts and cardiomyocytes against oxidative stress [55,56]. NRF2 also activates the antioxidant response against oxidative or electrophilic stress [57]. Thus, NRF2 is a major player in antioxidant and antiinflammatory response signaling pathways [58]. UVA can cause more damage to cells deficient in antioxidant defense, and low levels of UVA lead to the antioxidant defense regulated by NRF2 [59]. NRF2 regulates cellular resistance to oxidants, and NRF2 knockout mice have been shown to exhibit increased susceptibility to diseases related to chemical toxicity and oxidative pathology [60]. In UVA irradiation in human corneal endothelial cells, antioxidant protection is controlled by increased transcription and translation of NRF2 [59]. NRF2 has also been shown to increase heme oxygenase (HO)-1 after UVA (320–380 nm) irradiation of human skin fibroblasts to control antioxidant activity [61].

Examination of HO-1 and NRF2 mRNA and protein levels in the presence or absence of RG3 treatment after UV exposure revealed that mRNA and protein expression increased in a dose-dependent manner (Figs. 4C–4F). Our results suggest that treatment with RG3 increases the expression of NRF2, which upregulates the expression of HO-1 after inflammation and oxidative stress (Figs. 4C–4F).

3.5. RG3-induced changes in mitochondrial activity and morphology after UV exposure

Mitochondrial quality is maintained by performing qualitative control through a dynamic interconnected network that is continuously dividing and fusing over short periods of time. The proteins that mediate mitochondrial division are DRP1, mitochondrial fission factor (MFF), and FIS1, which are large GTPases, and mitochondrial fusion proteins (MFN1, MFN2), and OPA1, which are GTPases involved in mitochondrial fusion [62–64]. Phosphorylation of DRP1 on Ser616 promotes mitochondrial fission, whereas Ser637 phosphorylation of DRP1 inhibits mitochondrial fission [65]. Mitochondria have been shown to execute a variety of roles in processes such as the transduction of metabolic and stress signals [66] and the production of free radicals such as ROS [67]. When UV light is applied to keratinocytes [68], H $_2$ O $_2$ accumulates in mitochondria, causing mutations in mitochondrial DNA, which results in skin aging and skin cancer [69]. Mitochondrial DNA is also known as a biomarker that can present genetic damage by ultraviolet light in the skin of animals and humans [70]. Mitochondria are extremely dynamic organelles whose morphology is regulated by fusion and fission [71,72]. Our data have shown that mitochondrial proteins related to fusion, such as OPA1, MFN2, and

Fig. 4. RG3 inhibits ROS through antioxidant effect. (A) ROS measurement of H $_2$ O $_2$ using 2', 7'-dichlorodihydrofluorescein diacetate (CM-H $_2$ DCFDA). (B) The expression level of cytochrome-c after the isolation of mitochondria and cytosols in RG3-treated cell line after UV irradiation. (C, D) qPCR analysis was performed using total RNA from UV-exposed RG3-treated samples. NRF2, HO-1, and β -actin genes were amplified by qPCR using corresponding primers. (E) Protein level of NRF2 and HO-1 from UV-exposed RG3-treated samples by Western blot. (F) Graphical representation. Significant difference from negative control (Non-UV irradiated): *, $p < 0.05$ and **, $p < 0.01$. Significant difference from positive control (UV irradiated): #, $p < 0.05$ and ##, $p < 0.01$. HO-1, heme oxygenase-1; NRF2, nuclear factor erythroid 2-related factor-2; qPCR, quantitative polymerase chain reaction; ROS, reactive oxygen species; SD, standard deviation; UV, ultraviolet.

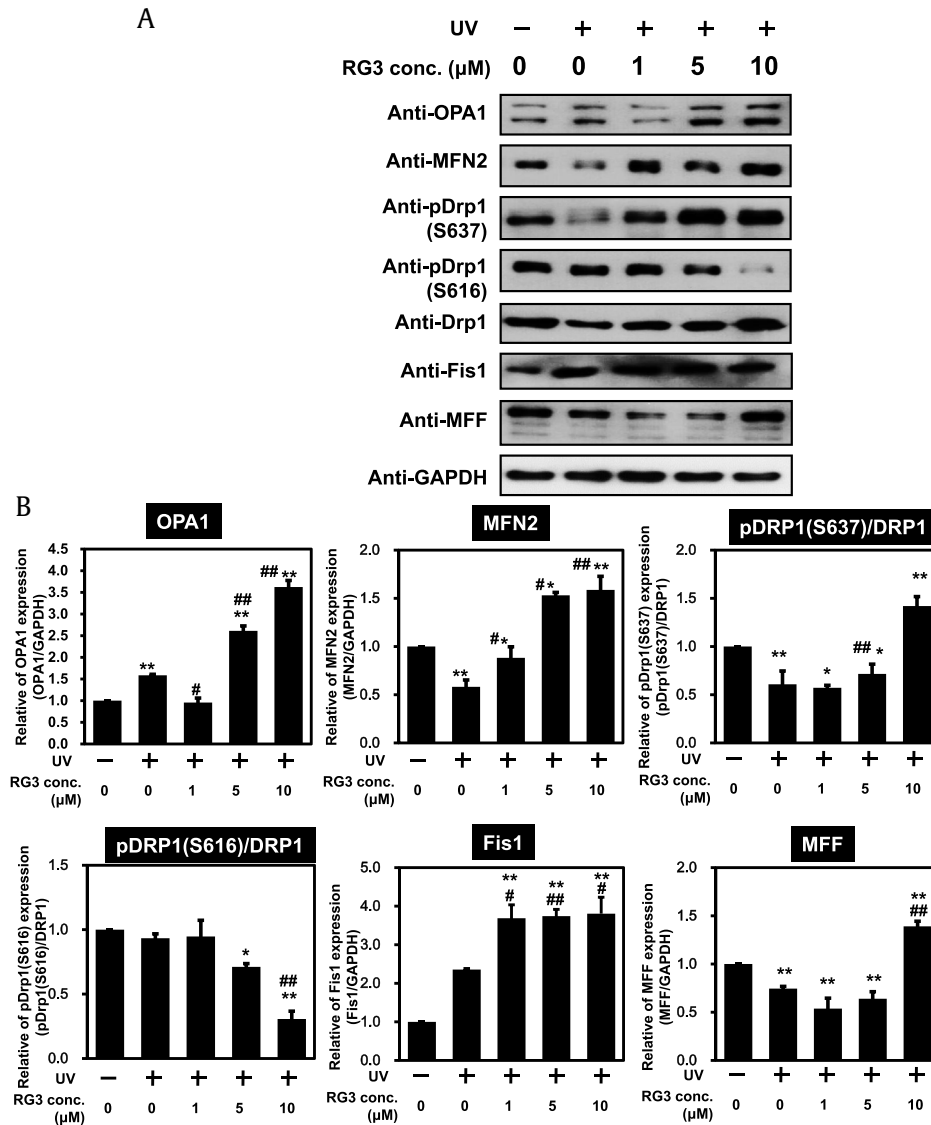


Fig. 5. Changes in mitochondrial activity and morphology by RG3 treatment after UV exposure. (A) The expression level of fusion and fission proteins in RG3-treated cell line after UV irradiation. (B) Graphical representation. (C) Image of MitoRed staining from UV-exposed RG3-treated samples. (D) Mitochondrial membrane potential ($\Delta\Psi_m$; mitochondrial respiratory capacity) from UV-exposed RG3-treated samples. (E) ATP of mitochondria from UV-exposed RG3-treated samples. Significant difference from negative control (Non-UV irradiated): *, $p < 0.05$ and **, $p < 0.01$. Significant difference from positive control (UV irradiated): #, $p < 0.05$ and ##, $p < 0.01$. DRP1, dynamin-related protein 1; FIS1, mitochondrial fission protein 1; MFN2, mitofusin-2; UV, ultraviolet.

pDRP1 (S637), were increased and that one of the mitochondrial proteins related to fission pDRP1 (S616) was decreased, but other fission proteins, such as FIS1 and MFF, were unchanged (Figs. 5A, 5B). Confocal imaging showed that after UV exposure, mitochondria fragment into even smaller units compared with the absence of UV exposure. Treatment with RG3 (10 μM) rescued mitochondrial morphology in UV-exposed NHDF cells (Fig. 5C). Similar to the reduction in mitochondrial membrane potential induced by UV irradiation in keratinocytes [73], the UV irradiation-induced decrease in mitochondrial membrane potential in NHDF cells was increased after RG3 treatment (Fig. 5D). Following UV exposure, mitochondrial membrane potential and mitochondrial ATP were significantly elevated in RG3-treated cells compared with control cells (Figs. 5D, 5E).

4. Discussion

RG3 plants have been investigated for different pharmacological properties; however, no study of the mitochondria of human

fibroblast cells after UV damage has been performed. Here, we analyzed several beneficial activities, including antiaging, antioxidant, and recovered mitochondrial functional properties of UV-exposed NHDF cells treated with RG3 extract. Skin is constantly exposed to oxidative stress induced by the production of ROS, and research has focused on antioxidants to combat the pathological conditions caused by oxidative stress [32,74].

Pretreatment of UV-exposed NHFB cells with RG3 extract inhibited the detrimental effects of oxidative stress, as demonstrated by the decrease in ROS generation and increase in cell growth, survival, and proliferation and signaling pathways, including ERK- and AKT/PKB-dependent pathways. Treatment of UV-exposed NHFB cells with RG3 extract also resulted in increased levels of ECM proteins such as collagen and elastin but decreased the function of inhibitory collagenases, such as MMP-2 and MMP-3 (Fig. 3).

Cytochrome-c, a proapoptotic factor released from mitochondria after UV irradiation, was reduced after treatment with RG3. This suggests that UV irradiation induces apoptosis and that the

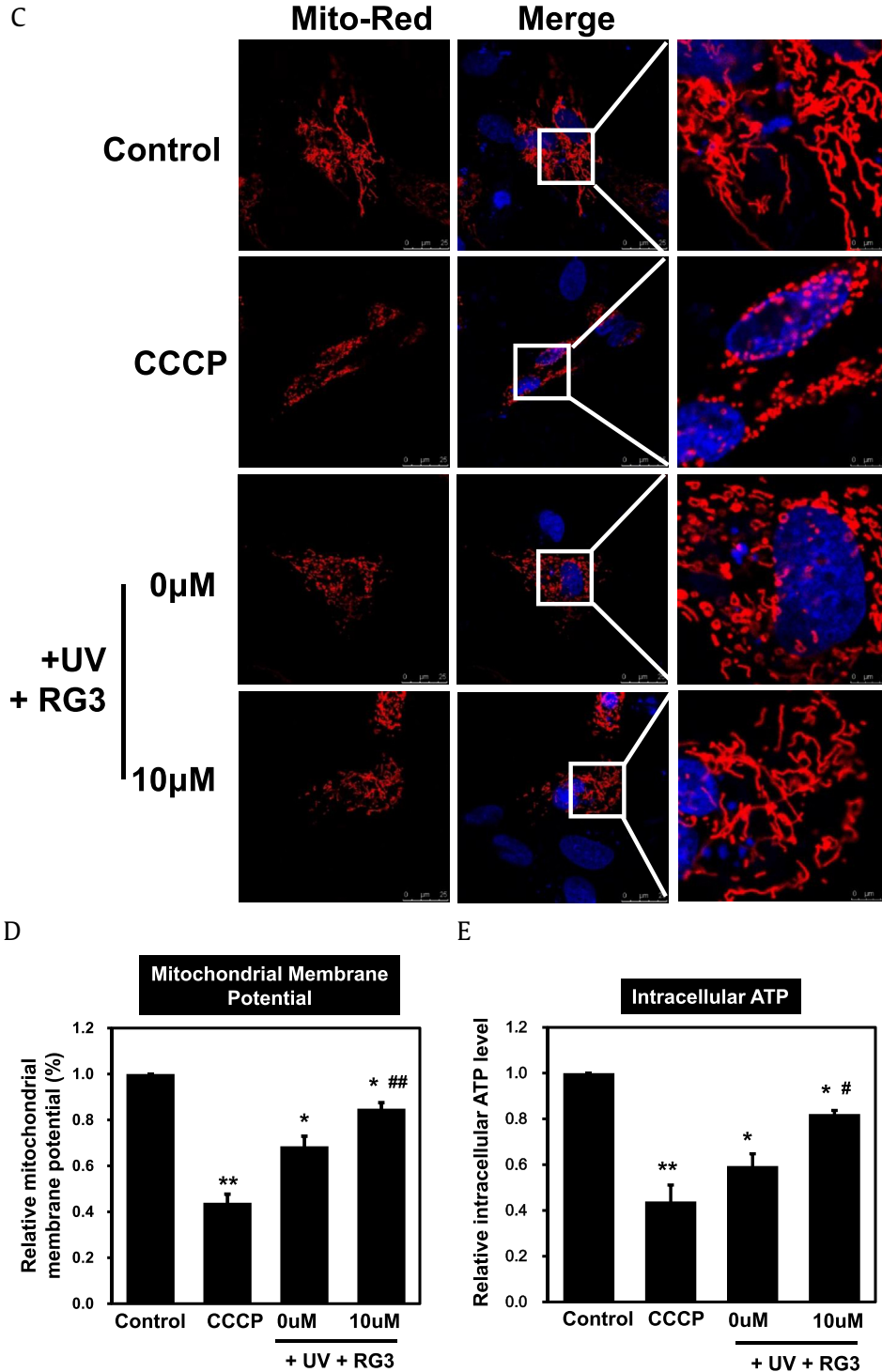


Fig. 5. (continued).

antioxidant activity of RG3 suppresses the release of cytochrome-c from mitochondria and inhibits apoptosis. The antioxidant activity of RG3 in mitochondria transformed mitochondria fragmented by UV exposure into the tubular form and increased the expression of fusion proteins (Figs. 4B, 4C and 5A, 5B).

RG3 restores mitochondrial ATP and membrane potential via its antioxidant effects in skin cells damaged by UV irradiation, leading to an increase in proteins linked with the ECM, cell proliferation, and antioxidant activity.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgments

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