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The ginsenoside Rg2 downregulates MMP-1 expression in keratinocyte (HaCaT)-conditioned medium-treated human fibroblasts (Hs68)

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Abstract

Keratinocytes exposed to UVB induce the production of cytokines, which activate fibroblasts and increase the expression of matrix metalloproteinases (MMPs). The increased expression of MMPs leads to connective tissue damage and wrinkle formation, resulting in skin aging. In this study, we used human dermal fibroblasts cultured in UVB-irradiated keratinocyte-conditioned medium (UV CM) to investigate the potential anti-aging effects of the ginsenoside Rg2 on skin. The inhibitory effect of Rg2 on the MMP-1 gene and protein was determined by real-time PCR and ELISA. We also examined the expression levels of proteins in the mitogen-activated protein kinase (MAPK) signaling pathway using western blotting, to elucidate the underlying mechanism of the inhibitory effect of Rg2. Rg2 inhibited MMP-1 mRNA and protein expression in a concentration-dependent manner. We found that Rg2 inhibited the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) but not that of p38. Therefore, our results suggest that Rg2 is a potential material for the prevention and treatment of photoaging.

Keywords Keratinocyte-conditioned medium, MMP-1, MAPKs, Ginsenoside Rg2

Introduction

The skin is an important physiological barrier, protecting the body from external stimuli [1]. Skin aging is a common phenomenon resulting from a combination of intrinsic and extrinsic aging. Intrinsic aging is natural aging that results from the passage of time, whereas extrinsic aging is caused by external factors including smoking, excessive alcohol consumption, malnutrition, and chronic exposure to UV light. Of these factors, exposure to UV light is known to be a major cause of photoaging, and is characterized by the formation of skin wrinkles [2, 3]. UV radiation is of three types: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–290 nm).

UVC is blocked by the ozone layer. Only UVA and UVB reach the skin. Among these, UVA reaches the dermis and can lead to photoaging. While UVB reaches the epidermis and can cause inflammation, intracellular ROS generation, and photoaging [4–6].

There are many cells in the epidermis through which UVB can penetrate, and keratinocytes and melanocytes are the main cell types [7]. Keratinocytes produce keratin and take up melanin produced by melanocytes. Thus, many cellular activities take place in the skin through interactions between cells [8].

Photoaging induced by UVB irradiation of the epidermis is associated with keratinocytes [9]. When exposed to UVB, keratinocytes produce and secrete cytokines and stimulate dermal fibroblasts [10, 11], which results in these fibroblasts being unable to synthesize collagen; this leads to the formation of wrinkles [12]. Thus, communication between skin cells plays

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an important regulatory role in cellular activities. To further investigate this phenomenon, UVB-irradiated keratinocyte-conditioned medium (UV CM) was used.

Photoaging refers to the formation of wrinkles that result from tissue and functional damage to the skin by exposure to the sun [13]. A previous study demonstrated that matrix metalloproteinases (MMPs) influence this process [14]. MMPs are zinc-containing peptide hydrolases that mediate the degradation of the ECM [15]. MMPs are classified into collagenases (MMP-1, MMP-8, MMP-13, and MMP-18), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), and elastase (MMP-12), according to their specificity, and 24 different types of MMPs are currently known to exist [16]. MMP expression is low in normal cells, and this low level of expression plays a role in maintaining connective tissue. However, MMP expression increases in response to external stimuli, resulting in connective tissue degradation and destruction [17]. Stimulation by UV light can increase the expression of MMPs, especially collagenase MMP-1, and is the major cause of skin aging, which is characterized by sagging skin or rough skin surfaces as the wrinkles deepen and skin elasticity decreases due to collagen degradation. Increased MMP-1 expression also induces breakdown of collagen fibers, and degraded collagen fragments are further broken down by MMP-3 and MMP-9 [18, 19].

MMP-1 expression is also increased by cytokines [20, 21]. Increased levels of cytokines are known to induce the activation of mitogen-activated protein kinases (MAPKs), which include extracellular-regulated protein kinase (ERK), Jun-N-terminal kinase (JNK), p38 kinase, and nuclear factor kappa B (NF-κB), in dermal fibroblasts [22]. MAPKs induce MMP-1 expression through the phosphorylation of activator protein-1 (AP-1), which drives the transcription of MMP-1, and NF-κB also increases MMP-1 expression through phosphorylation [23].

Ginseng, a well-known traditional therapeutic agent, is the dried root of *Panax ginseng*, and its major active ingredients are ginsenosides [24]. Ginsenosides include Rg1, Rg2, Rb2, and Rh1, and among these, Rg2, a triterpenoid saponin, has been reported to have antioxidant, antidiabetic, and anti-apoptotic activities [25, 26]. However, studies on the inhibitory effect of Rg2 on MMP-1 in human fibroblasts are limited. In this study, the ginsenoside Rg2 was used to determine the pathway responsible for the inhibitory effect of Rg2 on MMP-1 in human fibroblasts (Hs68) cultured in medium conditioned by keratinocytes damaged by UVB (UV CM).

Materials and methods

Materials

Ginsenoside Rg2 (purity ≥ 98%) was purchased from ChemFaces (Hubei, China) [27], and antibodies (Akt, p-Akt, Src, p-Src, MEK, p-MEK, SEK, p-SEK, ERK, p-ERK, JNK, p-JNK, p38, p-p38, p-c-Fos, p-c-Jun, NF-κB, p-NF-κB, and β-actin) were purchased from Cell Signaling Technology (Danvers, MA, USA). The primers used for real-time PCR [MMP-1 (Hs00899658_m1), GAPDH (Hs02786624_g1), IL-1β (Hs01555410_m1), IL-6 (Hs174131_m1), and TNF-α (Hs00174128_m1)] were purchased from Thermo Fisher Scientific(Thermo Fisher, USA). Hs68 cells, human skin fibroblasts, were obtained from the American Type Culture Collection (Manassas, USA), and HaCaT cells, human skin keratinocytes, were obtained from Cell Lines Service (CLS, Germany).

UVB irradiation and cell culture

HaCaT and Hs68 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37 °C in a 5% CO_2 incubator. For UVB irradiation, HaCaT cells were plated in 60 mm plates at 1×10^6 cells/well and incubated at 37 °C in a 5% CO_2 incubator for 24 h. After the HaCaT cells were grown to 80% confluence, the culture medium was removed.

The cells were washed twice with 1 mL of PBS, and 3 mL PBS was added again. After removing the covers, the plates were placed in a UV irradiation system (BIO-LINK BLX-312, France) and irradiated with 10 mJ/cm^2 UVB. Following irradiation, 3 mL of serum-free DMEM, containing no FBS or penicillin–streptomycin, was added, followed by incubation for 24 h. The suspension was then centrifuged, and the supernatant was collected and used as UV CM. To determine the effects of UV CM on Hs68 cells, HaCaT cell-conditioned medium not irradiated with UVB was also used as conditioned medium control (CM control).

Cell viability

To determine the cytotoxicity of Rg2 on Hs68 cells and the effect of Rg2 on Hs68 cell viability, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, Biobasic, USA) assays were performed. Hs68 cells were plated in 96-well plates at 5×10^4 cells/well and incubated at 37 °C in a 5% CO_2 incubator for 24 h. After 24 h, the cells were treated with different concentrations of Rg2 (12.5, 25, 50, and 100 μM) and/or UV CM. Cells were also treated with CM control. After 24 h of incubation, 20 μL of MTT solution (4 mg/mL) was added, and the plates were further incubated for 4 h. The supernatant was

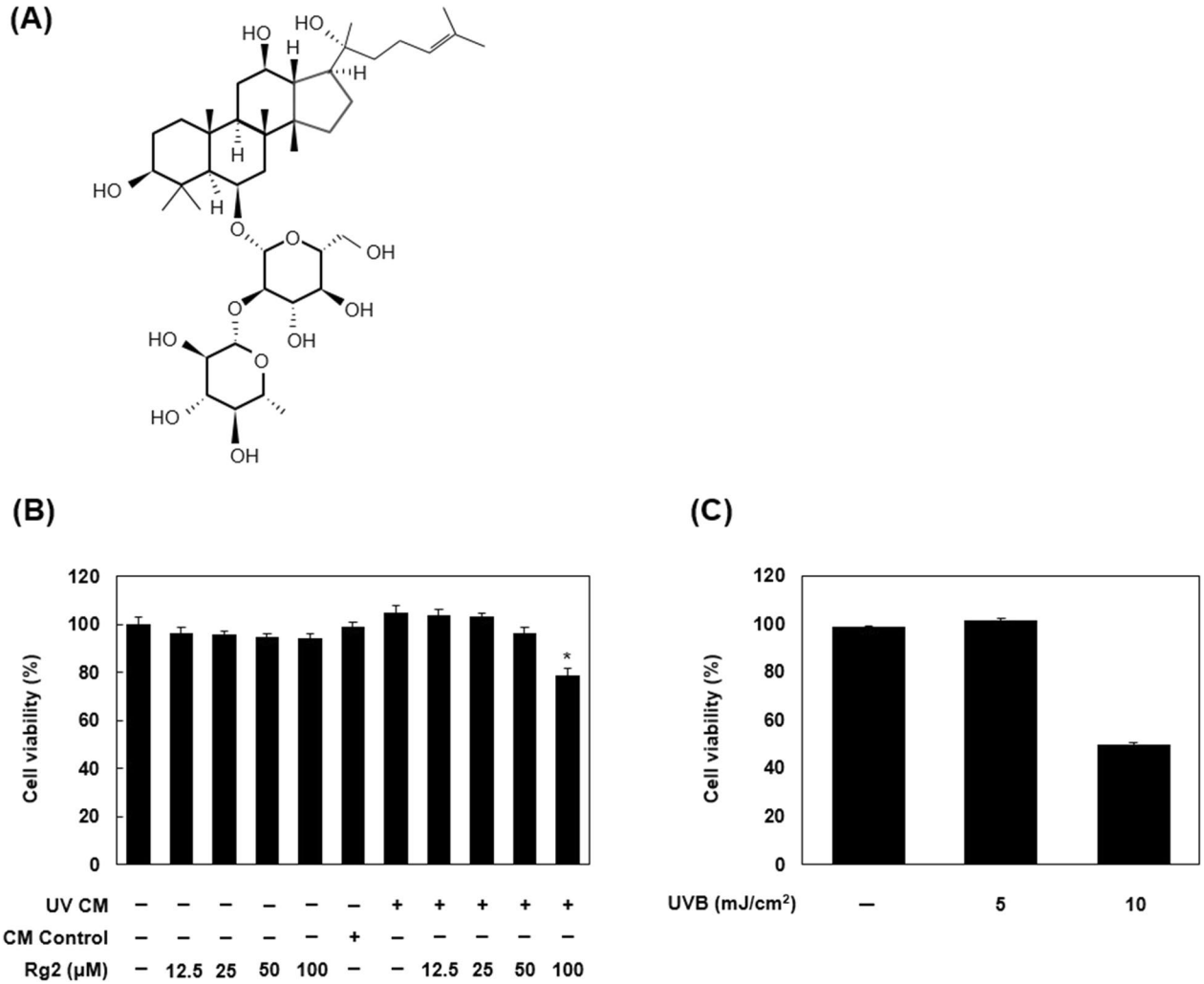

Fig. 1 Effect of Rg2 on the viability of Hs68 cells. Cells were treated with Rg2 (12.5–100 μM). **A** Chemical structure of ginsenoside Rg2. **B** Cytotoxicity of Rg2 in Hs68. Hs68 cells were incubated with 12.5, 25, 50 and 100 μM of Rg2 and CM for 24 h. **C** UVB irradiation of HaCaT cells by dose. Cell viability was measured using MTT assay

removed, and the cells were dissolved in 100 μL DMSO. Formazan formed from MTT reduction was quantified by measuring absorbance at 570 nm using a spectrophotometer (SpectraMax 190; Molecular Devices, Sunnyvale, CA, USA).

Measurement of MMP-1 and cytokines

To determine the inhibitory effect of Rg2 on MMP-1 and cytokine gene expression, Hs68 cells were plated in 60 mm plates at 5×10^5 cells/well and incubated at 37 °C in a 5% CO_2 incubator for 24 h. Next, the cells were co-treated with UV CM and Rg2 (12.5, 25, or 50 μM) and incubated for 24 h. After 24 h, RNA was extracted using TRIzol reagent (Ambion, USA). The RNA was dissolved in 20 μL of diethyl pyrocarbonate (DEPC)-treated water (Sigma, USA) and quantified using a NanoDrop 2000 (Thermo Fisher, USA). Total RNA was adjusted to 2 μg, and converted to cDNA using cDNA synthesis kits (ReverTra ACE-α-; Toyobo, Japan). The synthesized cDNA was then mixed with primer and TaqMan Master Mix (Thermo Fisher, USA), and real-time PCR was performed.

The amount of MMP-1 protein was measured using MMP-1 Human ELISA kits (GE Healthcare Life Sciences, Korea) in the supernatant from Hs68 cells that were co-treated with UV CM and 12.5, 25, or 50 μM Rg2 and incubated for 24 h.

Protein extraction and immunoblot analysis

To examine the effects of Rg2 on the MAPK signaling pathway, the phosphorylation levels of MAPKs were

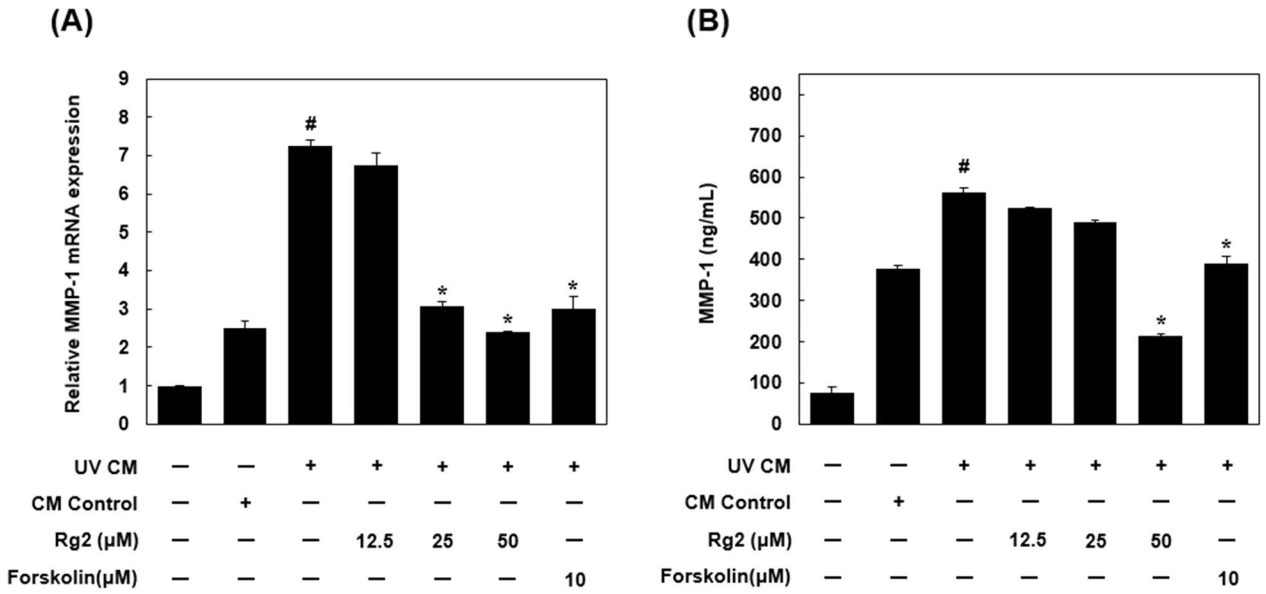

Fig. 2 UVB-induced keratinocyte-conditioned medium (UV CM) affected MMP-1 secretion and mRNA expression in human dermal fibroblasts (Hs68). **A** Rg2 inhibited MMP-1 mRNA expression. **B** Rg2 inhibited MMP-1 secretion. Hs68 cells were treated with UV CM with Rg2 (12.5–50 μM) for 24 h. Forskolin was the positive control. The CM control was cultivated in UVB non-irradiated HaCaT cell culture medium in Hs68 cells. $^{\#}p < 0.05$ compared with the vehicle control; $^{*}p < 0.05$ compared with the UVB

determined using western blotting. Hs68 cells were plated in 60 mm plates at 6×10^5 cells/well and incubated at 37 °C in a 5% CO_2 incubator for 24 h. After 24 h, the cells were co-treated with UV CM and 50 μM Rg2 and incubated for 15 min. Next, the supernatant was removed, and the cells were detached from the plates with 1 mL of trypsin-EDTA and transferred to E-tubes. The cells were dissolved in lysis buffer and the protein was quantified using a BCA assay (Thermo Fisher, USA). Loading dye was added to the quantified protein, and the proteins were subjected to heat treatment for 3 min to ensure they were uniformly charged. The proteins were then placed in a 10% Tris HCL gel, subjected to electrophoresis, and transferred to membranes. After protein transfer, the membranes were blocked with 5% skim milk and incubated with specific primary antibodies overnight with shaking, under refrigerated storage conditions. Next, the membranes were washed five times for 5 min each with Tris-buffered saline with Tween 20 (TBST), and incubated with secondary antibodies for 2 h with shaking. The membranes were again washed five times for 5 min with TBST, and phosphorylated proteins were detected.

Statistical analysis

The results were analyzed using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA). All data were expressed as mean ± standard deviation, and the effect of Rg2 was analyzed using Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

Results

Effects of Rg2 on the viability of Hs68 cells and HaCaT cells after UVB irradiation

MTT assays were performed to assess the toxicity of Rg2, UV CM, and CM control in Hs68 cells. The viability of Hs68 cells was measured after 24 h of treatment with Rg2 (12.5, 25, 50, and 100 μM) and UV CM or CM control, and the cell viabilities were found to be 96.5 ± 2.61%, 95.9 ± 1.25%, 94.5 ± 1.63%, and 94.4 ± 1.87%, respectively, for the Rg2 groups, 98.9 ± 2.08% for the UV CM group, and 105.1 ± 2.62% for the CM control group compared with that for the control group. Cells co-treated with UV CM and different concentrations of Rg2 showed cell viability of 103.9 ± 2.51%, 103.2 ± 1.46%, 96.5 ± 2.01%, and 78.9 ± 2.55%, respectively, compared with the control group. Therefore, the Rg2 (12.5–100 μM) and CM control treatments showed no cytotoxicity, whereas co-treatment with 100 μM Rg2 and UV CM showed cytotoxicity in Hs58 cells. Based on this result, Rg2 treatment concentrations of 12.5–50 μM were used. To determine the appropriate UVB radiation dose, HaCaT cells were irradiated with different doses of UVB, and their viability was determined. As shown in Fig. 1, HaCaT cells showed 50% cell viability at 10 mJ/cm^2 UVB, and therefore a UVB radiation dose of 10 mJ/cm^2 was used for the experiments.

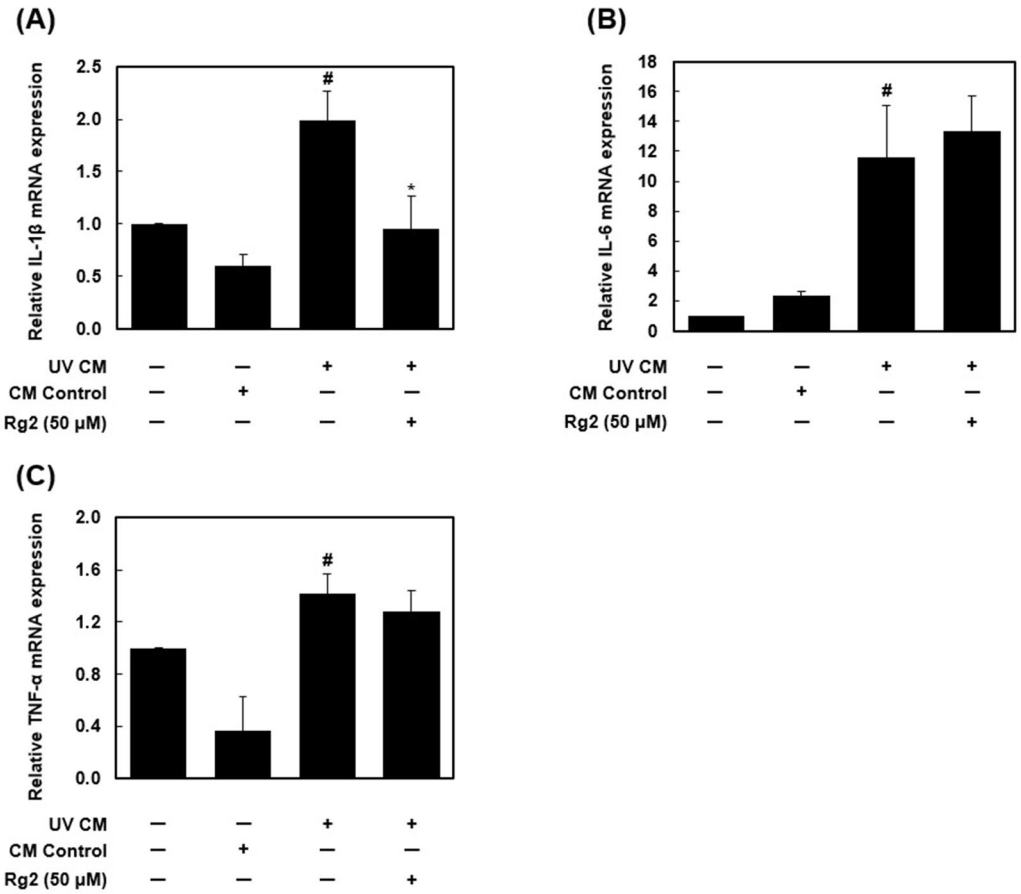

Fig. 3 Rg2 effects on cytokine mRNA expression induced by UV CM in human dermal fibroblasts (Hs68). **A** Rg2 inhibited IL-1β mRNA expression. **B** Rg2 had no effect on IL-6 mRNA expression. **C** Rg2 had no effect on TNF-α mRNA expression. mRNA levels were measured by real-time PCR. Hs68 cells were treated with UV CM with Rg2 (50 μM) for 24 h. CM control was cultivated in UVB non-irradiated HaCaT cell culture medium in Hs68 cells. UV CM was cultivated in UVB-irradiated HaCaT cell culture medium in Hs68 cells. $^{\#}p < 0.05$ compared with the vehicle control; $^{*}p < 0.05$ compared with the UV CM

Inhibitory effect of Rg2 on MMP-1 mRNA expression in Hs68 cells

The inhibitory effect of Rg2 on MMP-1 mRNA expression and protein secretion was examined. Hs68 cells were treated with UV CM and Rg2 (12.5–100 μM) for 24 h, and MMP-1 mRNA expression and protein secretion were measured by real-time PCR and ELISA kits. Rg2 inhibited MMP-1 mRNA expression and protein secretion, which was increased by UV CM (Fig. 2). MMP-1 ELISA results showed that Rg2 had a significant effect at 50 μM, and therefore subsequent experiments were performed using 50 μM Rg2.

Inhibitory effect of Rg2 on MMP-1 mRNA expression and protein secretion in Hs68 cells

The inhibitory effects of Rg2 on MMP-1 mRNA expression and protein secretion were investigated. Hs68 cells were treated with UV CM and Rg2 (12.5–100 μM) for 24 h. MMP-1 mRNA expression and protein secretion were measured with real-time PCR and ELISA kits respectively.

Both MMP-1 mRNA expression and protein secretion were increased by UV CM. Rg2 inhibited MMP-1 mRNA expression at 25, 50 μM (Fig. 2A). MMP-1 ELISA results show that Rg2 significantly inhibits its MMP-1 protein expression at 50 μM, and subsequent experiments were conducted using 50 μM Rg2 (Fig. 2B). In this experiment, Forskolin, a cAMP activator, was used as a positive control because it has been reported to inhibit MMP-1 by increasing cAMP levels that regulate various signaling pathways [28].

Inhibitory effect of Rg2 on expression of inflammatory cytokine mRNA in Hs68 cells

The mRNA expression levels of IL-1β, IL-6, and TNF-α were determined using real-time PCR, and the results showed that the levels of IL-1β, and IL-6 mRNA were

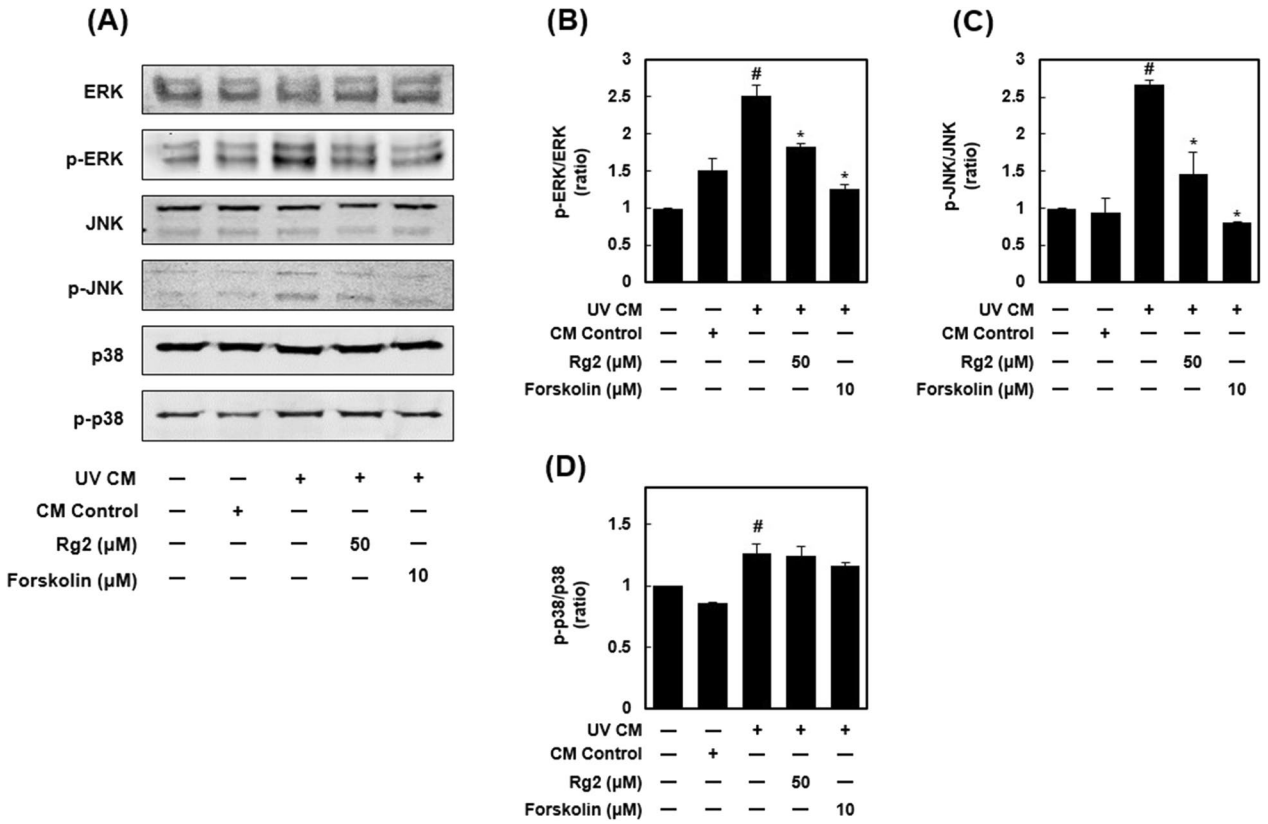

Fig. 4 Rg2 effects on the MAPK signaling pathway. **A** Representative western blots showing the effects of Rg2 on the phosphorylation of ERK, JNK and P38. Phosphorylation ratio analysis of ERK, JNK, and P38 respectively. **B**–**D** Hs68 cells were treated with UV CM with Rg2 (50 μM) for 15 min. Forskolin was the positive control. The CM control was cultivated in UVB non-irradiated HaCaT cell culture medium in Hs68 cells. UV CM was cultivated in UVB-irradiated HaCaT cell culture medium in Hs68 cells. $^{\#}p < 0.05$ compared with the vehicle control; $^{*}p < 0.05$ compared with the UV CM

significantly increased in the UV CM treated group. Of the cytokines with expression increased by UV CM, only the expression of IL-1β was inhibited by Rg2. UV CM treatment did not affect IL-6 and TNF-α mRNA expression (Fig. 3).

MMP-1 inhibition by Rg2 through the suppression of the ERK and JNK signaling pathways

MAPK plays an important role in transmitting signal from outside to inside of cell. In order to examine whether Rg2 suppressed MMP-1 expression via blocking the MAPK mediated signal pathway, We investigated the phosphorylated form of ERK, JNK and p38 protein using Western blotting. Elevated ratio of phosphorylated ERK and JNK in UV CM treated fibroblast was down-regulated by treatment with Rg2 (50 μM). However, Rg2 had no effect on the phosphorylation of p38 (Fig. 4). Binding of cytokines and growth factors to their receptors activates Src followed by phosphorylation of mitogen- activated protein kinase kinase (MEK), which leads to ERK-mediated pathway [29, 30]. In addition, JNK activation is regulated by stress-activated protein kinase/extracellular signal-regulated kinase kinase (SEK) phosphorylation [31–33]. Therefore, inhibitory effect of Rg2 on upstream pathway of ERK and JNK was determined. Data showed that Rg2 reduced the activation of Src, MEK and SEK induced by UV MC treatment (Fig. 5).

MMP-1 inhibition by Rg2 through the suppression of NF-κB and akt signaling pathway

The effect of Rg2 on NF-κB, a transcription factor involved in cytokine production, was examined, and the results showed that UV CM treatment led to phosphorylation of NF-κB, while Rg2 treatment (50 μM) significantly suppressed NF-κB (Fig. 6A, B). The effect of Rg2 on Akt, which is involved in the regulation of various cellular activities, was determined using western blotting. The results showed that UV CM treatment led to

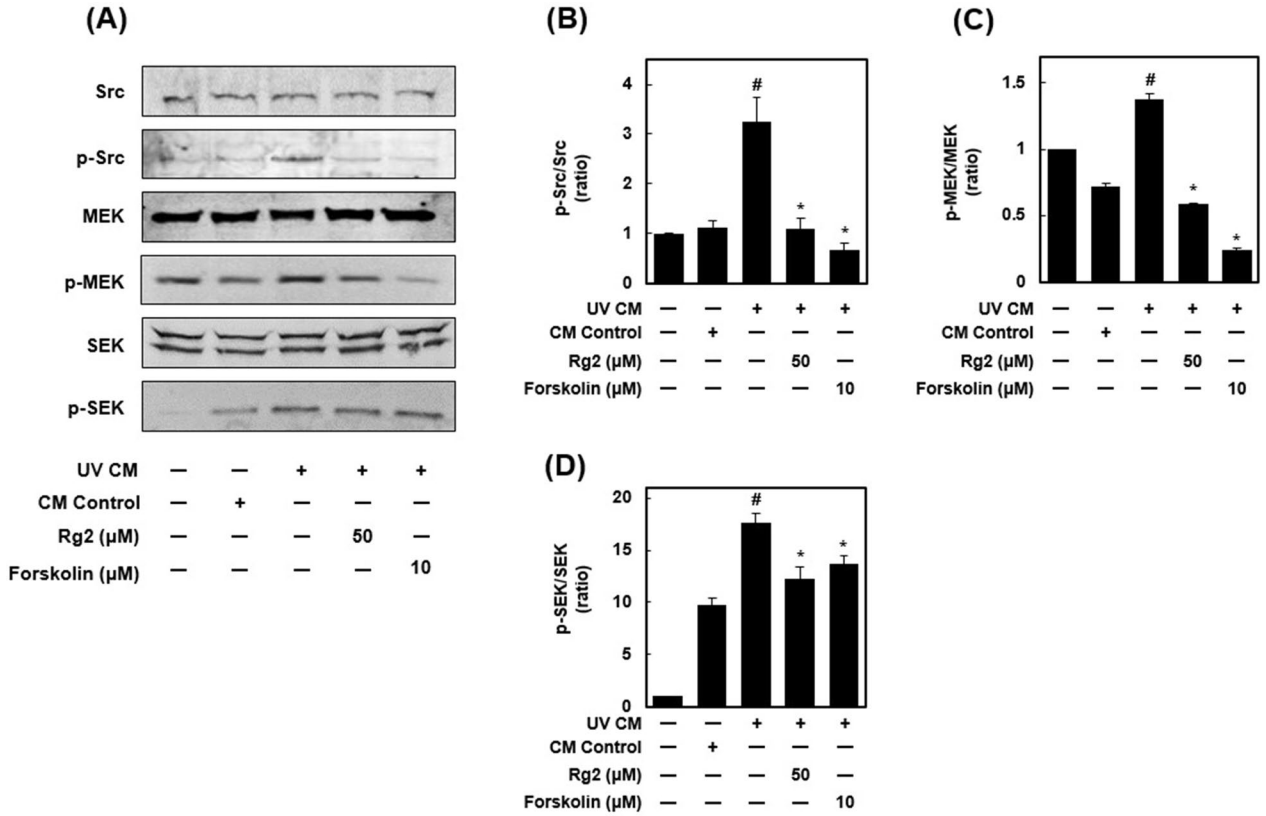

Fig. 5 Rg2 effects on the upstream signal molecules of MAPK signaling pathway. **A** Representative western blots showing the effects of Rg2 on the phosphorylation of Src, MEK and SEK. Phosphorylation ratio analysis of Src, MEK, and SEK respectively. **B**–**D** Hs68 cells were treated with UV CM with Rg2 (50 μM) for 15 min. Forskolin was the positive control. The CM control was cultivated in UVB non-irradiated HaCaT cell culture medium in Hs68 cells. UV CM was cultivated in UVB-irradiated HaCaT cell culture medium in Hs68 cells. $^{\#}p < 0.05$ compared with the vehicle control; $^{*}p < 0.05$ compared with the UV CM

phosphorylation of Akt, while Rg2 treatment (50 μM) significantly suppressed Akt expression (Fig. 6C, D).

Discussion

The skin, which is composed of the epidermis and dermis, ages over time. In particular, UV light stimulates the skin and induces epidermal keratinocytes to produce the cytokines IL-1β, IL-6, and TNF-α. The cytokines stimulate dermal fibroblasts, which in turn produce MMPs that can break down collagen and accelerate aging [2, 22]. In this study we investigated the inhibitory effect of the ginsenoside Rg2 on MMP-1 in Hs68 cells, in which MMP-1 expression was increased by treatment with UV CM obtained from HaCaT cells irradiated with UVB, to elucidate the signaling pathway responsible for the inhibitory effect exerted by Rg2.

UVB irradiation dose of 10 mJ/cm^2 was chosen after examining the IC50 viability of HaCaT cells irradiated with 10 mJ/cm^2 UVB. Rg2 showed no cytotoxicity to Hs68 cells at 100 μM, with a cell viability of 94.4%. However, cells co-treated with Rg2 and UV CM had a cell viability of 78.9% at an Rg2 concentration of 100 μM; therefore, Rg2 treatment concentrations of 12.5–50 μM were used (Fig. 1). Hs68 cells were treated with UV CM to examine the levels of MMP-1 and inflammatory cytokine mRNA expression and protein secretion. We found that UV CM increased MMP-1 mRNA expression and protein secretion in Hs68 cells, and increased the mRNA expression of the inflammatory cytokines IL-1β, IL-6, and TNF-α. Rg2 decreased the expression of MMP-1 and IL-1β mRNA (Figs. 2 and 3). One explanation may be that cytokines produced in UV-stimulated HaCaT cells were released into the extracellular space and included in the UV CM, and treatment with this UV CM led to increased MMP-1 expression in Hs68 cells [20]. Cultured keratinocytes release keratinocyte-specific proteins, such as stratifin,

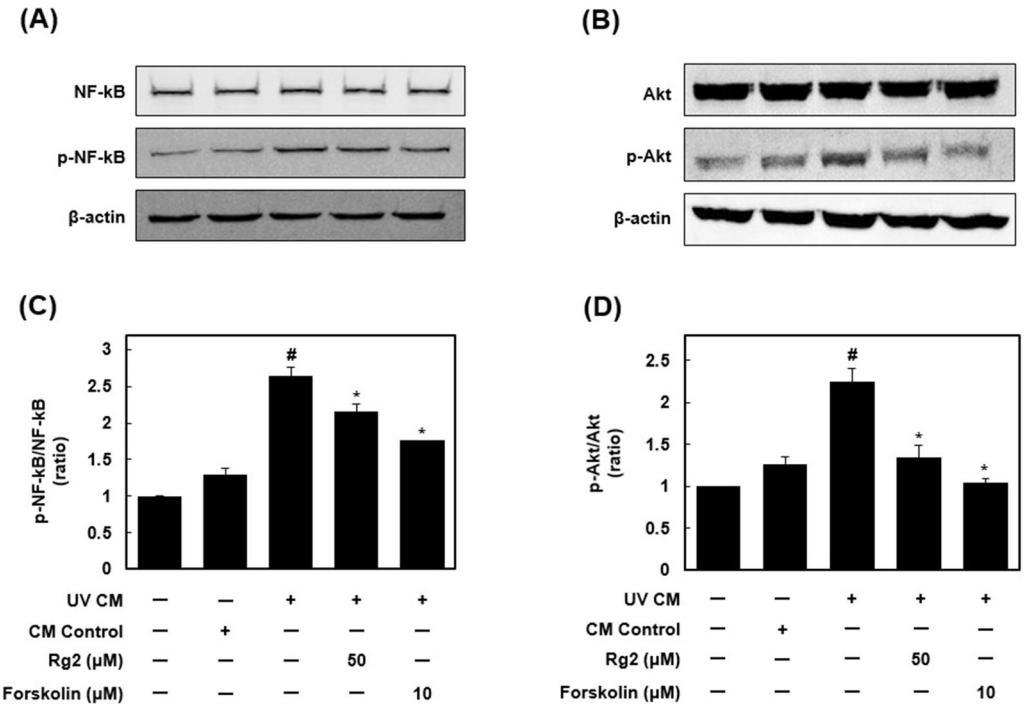

Fig. 6 The effect of Rg2 on the phosphorylation of NF-κB and Akt. **A**, **B** Analysis of the phosphorylation ratio of NF-κB and Akt. (**C**-**D**) Hs68 cells were treated with UV CM with Rg2 (50 μM) for 30 min or 24 hours. The CM control was cultivated in UVB non-irradiated HaCaT cell culture medium in Hs68 cells. UV CM was cultivated in UVB-irradiated HaCaT cell culture medium in Hs68 cells. $^{\#}p < 0.05$ compared with the vehicle control; $^{*}p < 0.05$ compared with the UV CM

into the extracellular space. These proteins are known to induce MMP-1 expression in fibroblasts [34]. Fibroblasts treated with keratinocyte conditioned medium (KCM) had enhanced MMP-1 expression through AP-1 activation via the MAPK signaling pathway [35]. Consistent with previous studies, the CM control group treated with keratinocyte-conditioned medium showed increased levels of MMP-1 mRNA expression and MMP-1 protein secretion compared with the no-treatment control group (Fig. 2).

Inside the cell, MMP expression is elicited when the transcription factor AP-1 is activated via the MAPK, Akt/PI3K, and NF-κB signaling pathways [36–38]. The MAPK signaling pathway transmits extracellular stimuli to the nucleus and plays an important role in the regulation of gene expression, cell proliferation, differentiation, apoptosis, and other cellular activities. ERK, JNK, and p38, which are MAPK pathways, transmit signals via phosphorylation [39]. In our study, UV CM activated all MAPK pathways, whereas Rg2 suppressed the ERK and JNK pathways, but not the p38 pathway (Fig. 4). The expression of the upstream regulators of MAPK, Src, MEK, and SEK were examined.

The Src pathway involves intracellular tyrosine kinases that are activated upon the binding of cytokines or growth factors to their receptors. The Src family consists of nine kinases, including the well-known Src, and participate in signal transduction [40]. Src signaling is transmitted to Ras and Raf, leading to activation of ERK1/2 [29]. Ras plays an important role in the signaling pathways associated with cell proliferation and differentiation, and can activate many signaling pathways, including the Raf, MEK, and NF-κB pathways [30]. In additioin, it has been known that activation of SEK mediates phosphorylation of JNK [31–33]. We found that UV CM activated Src, whereas Rg2 (50 μM) suppressed Src, and similar results were found for SEK and MEK expression (Fig. 5).

NF-κB is a transcription factor that affects the expression of inflammatory cytokines and MMP-1 [41]. As with the aforementioned signaling pathways, the NF-κB signaling pathway is activated by external stimuli, such as UV light and cytokines. In normal cells, NF-κB exists in a complex bound to IκB. Upon external stimulation of the cell, IκB kinase (IKK) is activated by phosphorylation. Activated IKK phosphorylates and phosphorylated

IκB are degraded and released from NF-κB [42]. NF-κB is activated and translocated to the nucleus, where it functions as a transcription factor [43]. In our study, NF-κB phosphorylation was increased by UV CM but suppressed by Rg2 (50 μM) (Fig. 6).

PI3K is activated by many cytokines and growth factors and transmits signals to Akt. The Akt/PI3K pathway controls a range of cellular processes [44]. This signaling activates AP-1, which consists of c-Fos and c-Jun, and activated AP-1 elicits MMP-1 expression. This activation has been attributed to the presence of an AP-1 binding site in the MMP-1 promoter region, to which activated AP-1 binds and promotes transcription [45]. Our study showed that UV CM activated Akt but it was suppressed by 50 μM Rg2 (Fig. 6).

Previous study showed that Rg2 protected the cell from UVB directly [25]. In this study, we found that Rg2 blocked the stimulatory signal pathway of MMP-1 expression in UV CM treated fibroblast, indicating that Rg2 has the potential to prevent photoaging through indirect as well as direct pathway. However, experimental model using in this study represents only the situation in which keratinocyte irradiated by UVB affected fibroblast in paracrine way. We will design the experimental models closer to real physiological and pathological condition to investigate the how Rg2 actually has effect on communication between keratinocyte and fibroblast in UVB-stimulated skin.

In order to use Rg2 as topical skin product ingredient, it should be confirmed that Rg2 is not only safe but also easy to apply to product. To our best knowledge, there is no clinical trial to show that Rg2 has no negative effect on skin health. However, Hwang et al. [46] reported that enzyme-modified *Panax ginseng* reduced the eye wrinkle formation without adverse-effect in clinical trial, which raises the expectation that product containing the Rg2 would be safe. In future, we hope that study on effect of Rg2 on skin should contribute to the skin health care industry.

Abbreviations

UVB	Ultraviolet B
MMP	Matrix metalloproteinase
CM	Conditioned medium
MAPK	Mitogen-activated protein kinase
ERK	Extracellular signal-regulated kinase
JNK	c-Jun N-terminal kinase
UV CM	UVB-irradiated keratinocyte-conditioned medium
NF-κB	Nuclear factor kappa B
AP-1	Activator protein-1
DMEM	Dulbecco's Modified Eagle's Medium
FBS	Fetal bovine serum
MTT	3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
IL	Interleukin
TNF	Tumor necrosis factor
SEK	Stress-activated protein kinase/extraceullar signal-regulated kinase kinase
MEK	Mitogen-activated protein kinase kinase
PI3K	Phosphoinositide 3-kinases

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Author contributions
Conceptualization: PL and YMK; methodology: YMK and SO; software: SP and YMK; validation: PL and SP; formal analysis: SO and SP; investigation: SO and SP; data curation: YMK and SO; writing—original draft preparation: YMK and SO; writing—review and editing: YMK and PL; visualization: SO and SP; supervision: YMK.

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Availability of data and materials
The data presented in this study are available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval and consent were not required as this study was based on publicly available data.

Competing interests
The authors declare that they have no competing interests.

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