J Ginseng Res 44 (2020) 274–281

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research Article

Inhibitory mechanism of ginsenoside Rh3 on granulocyte–macrophage colony-stimulating factor expression in UV-B–irradiated murine SP-1 keratinocytes

Young Sun Park [1,☆], Ji Eun Lee [1,☆], Jong Il Park [1], Cheol hwan Myung [1], Young-Ho Lim [2], Chae Kyu Park [2], Jae Sung Hwang [1,*]

[1] Department of Genetic Engineering & Graduate School of Biotechnology, Kyung Hee University, Yongin, Republic of Korea
[2] KGC R&D Headquarters, Daejeon, Republic of Korea

ARTICLE INFO

Article history:
Received 26 July 2018
Received in Revised form
20 November 2018
Accepted 18 December 2018
Available online 24 December 2018

Keywords:
Ginsenoside Rh3
GM-CSF
Keratinocytes
PKC
Ultraviolet radiation

ABSTRACT

Background: Ultraviolet (UV) goes through the epidermis and promotes release of inflammatory cytokines in keratinocytes. Granulocyte–macrophage colony-stimulating factor (GM-CSF), one of the keratinocyte-derived cytokines, regulates proliferation and differentiation of melanocytes. Extracellular signal–regulated kinase (ERK1/2) and protein kinase C (PKC) signaling pathways regulate expression of GM-CSF. Based on these results, we found that ginsenoside Rh3 prevented GM-CSF production and release in UV-B–exposed SP-1 keratinocytes and that this inhibitory effect resulted from the reduction of PKCδ and ERK phosphorylation.

Methods: We investigated the mechanism by which ginsenoside Rh3 from *Panax ginseng* inhibited GM-CSF release from UV-B–irradiated keratinocytes.

Results: Treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA) or UV-B induced release of GM-CSF in the SP-1 keratinocytes. To elucidate whether the change in GM-CSF expression could be related to PKC signaling, the cells were pretreated with H7, an inhibitor of PKC, and irradiated with UV-B. GM-CSF was decreased by H7 in a dose-dependent manner. When we analyzed which ginsenosides repressed GM-CSF expression among 15 ginsenosides, ginsenoside Rh3 showed the largest decline to 40% of GM-CSF expression in enzyme-linked immunosorbent assay. Western blot analysis showed that TPA enhanced the phosphorylation of PKCδ and ERK in the keratinocytes. When we examined the effect of ginsenoside Rh3, we identified that ginsenoside Rh3 inhibited the TPA-induced phosphorylation levels of PKCδ and ERK.

Conclusion: In summary, we found that ginsenoside Rh3 impeded UV-B–induced GM-CSF production through repression of PKCδ and ERK phosphorylation in SP-1 keratinocytes.

© 2019 The Korean Society of Ginseng, Published by Elsevier Korea LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The skin is the organ that covers all of the body and is mainly composed of the epidermis and dermis. The epidermis consists of keratinocytes (approximately 90%) and melanocytes (5–10%) [1]. Melanocytes originate from neural crest cells and synthesize melanin pigments [2]. Skin pigmentation plays many beneficial roles that include determination of skin color of humans and ultraviolet (UV) radiation protection [3]. Excessive exposure to UV-B (280–320nm) accelerates skin aging such as wrinkle formation and hyperpigmentation [1]. Production of proinflammatory cytokines and growth factors mediates skin damage [4]. There is evidence showing that granulocyte–macrophage colony-stimulating factor (GM-CSF) is an important cytokine in the control of the proliferation and differentiation of melanocytes in pigmented spots induced by UV [5]. Previous studies have shown that protein kinase C (PKC) and the following activation of the extracellular signal–regulated kinase (ERK) pathway regulate GM-CSF expression [6].

* Corresponding author. Department of Genetic Engineering & Graduate School of Biotechnology, Kyung Hee University, Yongin-si, Gyeonggi-do, 17104, Republic of Korea
E-mail address: jshwang@khu.ac.kr (J.S. Hwang).
☆ These authors made equal contributions to the paper.

https://doi.org/10.1016/j.jgr.2018.12.006
p1226-8453 e2093-4947/$ – see front matter © 2019 The Korean Society of Ginseng, Published by Elsevier Korea LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

PKC is composed of a large family of 11 members of kinases, which are categorized into conventional (cPKCs; α, βI, βII, γ), novel (nPKCs; δ, e, η, θ, μ), and atypical (aPKCs; τ, ζ) protein kinases [7,8]. The cPKCs and nPKCs can be stimulated using phorbol esters and diacylglycerol; on the other hand, the aPKCs have been reported to show no response to phorbol esters and diacylglycerol either *in vivo* or *in vitro* [9]. It has been reported that UV-B markedly induces the activation of PKCδ and PKCε but has no effect on PKCα [10,11]. The Korean Red Ginseng (KRG) saponin has been known to exert anticancer, antiaging, antioxidant, and antiinflammatory effects, with a low rate of adverse effects [12,13].

Previous studies have shown that UV-B irradiation stimulated production of GM-CSF in SP-1 keratinocytes and that this induction of GM-CSF promoted mouse melanocyte proliferation. In addition, KRG saponin inhibited secretion of GM-CSF from mouse keratinocytes induced by UV-B [12].

Saponin consists of various ginsenosides that are divided into two major groups, the panaxadiols and panaxatriols, based on the chemical structure [13]. Ginsenosides are the main compounds responsible for most of the pharmacological and immunological effects of saponin [14]. However, the inhibitory mechanisms of ginsenoside activity on UV-induced skin pigmentation still remain unclear. In this research, we investigated the inhibitory mechanism of ginsenoside Rh3 in cellular protection against expression and release of GM-CSF induced by UV-B in SP-1 keratinocytes.

2. Materials and methods

2.1. Cell line and cell culture

SP-1 keratinocytes from Sencar mice were kindly gifted from Dr. Stuart H. Yuspa (Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, NIH, USA). Eagle's minimum essential medium (EMEM) complemented with 8% Chelex-treated heat-inactivated newborn calf serum (Carlsbad, CA, USA), 1% penicillin–streptomycin, and 0.05 mM Ca^{2+} was used for the *in vitro* culture of the SP-1 keratinocytes.

2.2. Materials

Ginsenosides compound K, F1, F2, Rb1, Rb2, Rb3, Rc, Rd, Re, Rh1, Rh3, Rg1, Rg2, Rf, and Ro were provided by Korea Ginseng Corporation (Daejeon, Korea).

2.3. Irradiation

For irradiation, the medium was changed to Dulbecco's phosphate-buffered saline, and the keratinocytes were treated with UV-B (30 mJ/cm^2). The cells were then maintained in EMEM containing 2% newborn calf serum for the indicated times.

2.4. Cell viability assay

The SP-1 keratinocytes were cultured and placed in 96-well plates (1.0×10^4 cells/well). After 24 h, the cells were treated with ginsenoside Rh3 (1, 10, 100, and 1000 μM) and incubated for 24 h. Then, cell viabilities were determined using an EZ-Cytox assay kit (Daeil Lab Service, Seoul, Korea).

2.5. Reverse transcription polymerase chain reaction analysis

TRIzol reagent (Takara Bio, Inc., Tokyo, Japan) was used to extract total RNAs from the SP-1 keratinocytes. Complementary DNAs were amplified by reverse transcription polymerase chain reaction (RT-PCR) using the following primers: GM-CSF, forward:

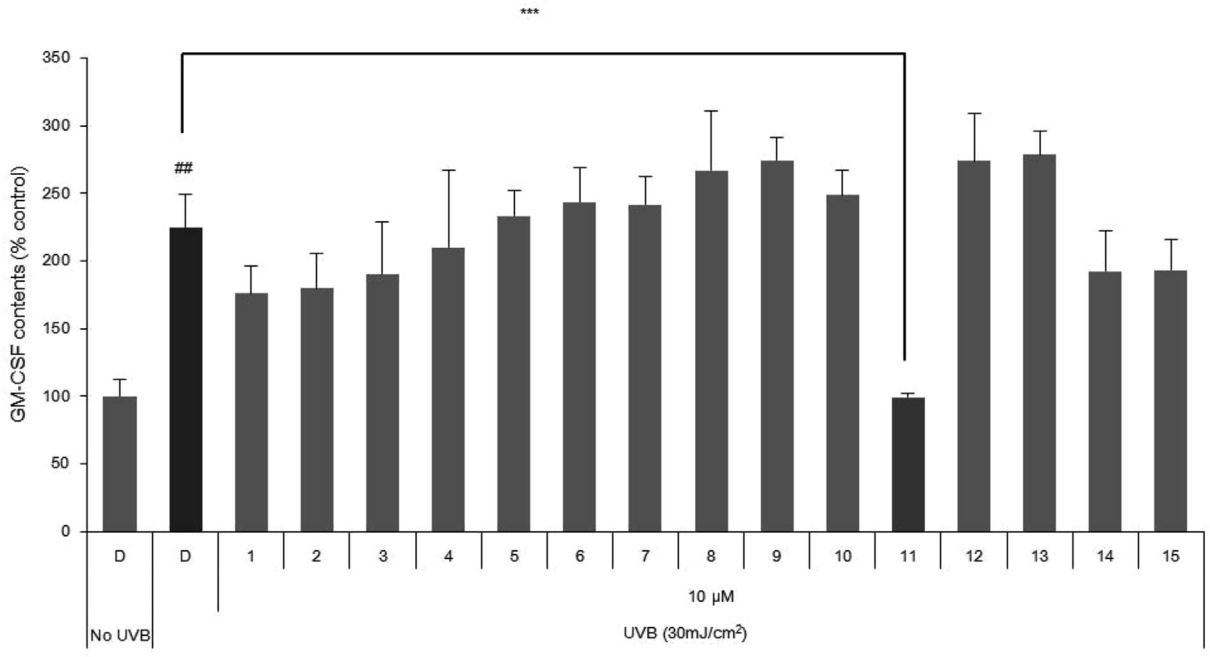

Fig. 1. Ginsenoside Rh3 inhibited production of GM-CSF in the UV-B–exposed SP-1 keratinocytes. SP-1 cells were treated with 15 types of ginsenosides: (1) ginsenoside compound K, (2) ginsenoside F1, (3) ginsenoside F2, (4) ginsenoside Rb1, (5) ginsenoside Rb2, (6) ginsenoside Rb3, (7) ginsenoside Rc, (8) ginsenoside Rd, (9) ginsenoside Re, (10) ginsenoside Rh1, (11) ginsenoside Rh3, (12) ginsenoside Rg1, (13) ginsenoside Rg2, (14) ginsenoside Rf, and (15) ginsenoside Ro (10 μM). After 24 h, the cells were exposed to UV-B (30 mJ/cm^2) and were treated with 15 types of ginsenosides. GM-CSF levels in the supernatant were measured by ELISA. ***$p < 0.001$, compared with the UV-B–treated control group. ##$p < 0.01$ compared with dimethyl sulfoxide (D) 0.1% control. ***$p < 0.001$ compared with the UV-B control, n = 3.
ELISA, enzyme-linked immunosorbent assay; GM-CSF, granulocyte–macrophage colony-stimulating factor.

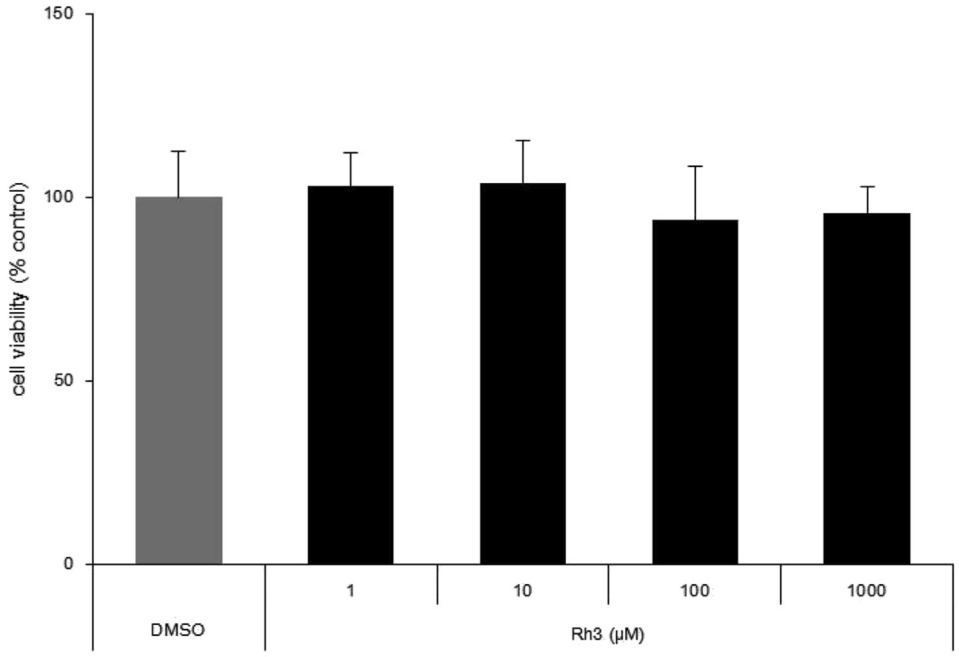

Fig. 2. SP-1 keratinocyte viability assay. Cell viability of the SP-1 keratinocytes under treatment with ginsenoside Rh3 (1, 10, 100, and 1000 μM) was determined by EZ-Cytox assay. These experiments were performed in triplicate.
DMSO, dimethyl sulfoxide.

5′ - GCCATC AAAGAAGCCCTGAA - 3′ and reverse: 5′ - GCGGGTCTGCACACATGT TA - 3′. The mouse β-actin gene served as the normalization control. PCR amplifications were performed for 28 cycles under the optimal condition of denaturation at 95°C, annealing at 59°C, and extension at 70°C. The product of PCR amplification was separated on 2% agarose gels and detected by using RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology, Seongnam, Korea).

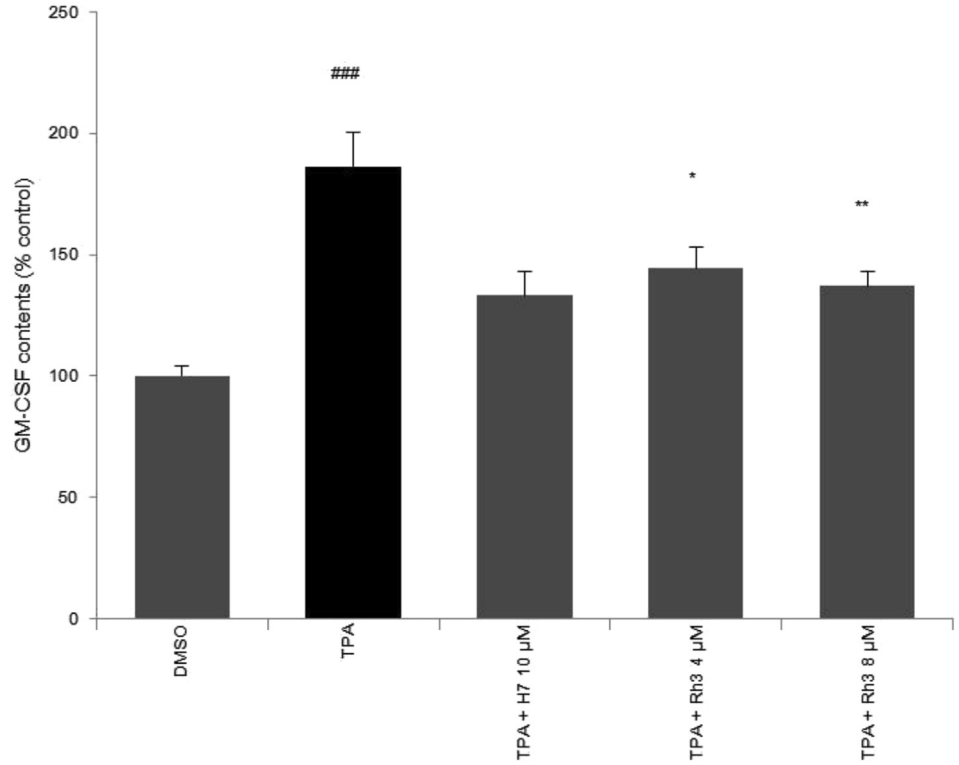

Fig. 3. Ginsenoside Rh3 reduced TPA-induced GM-CSF production. SP-1 cells were treated with 50 nM TPA after 24 h of treatment with ginsenoside Rh3. Six hours later, the conditioned medium was collected to measure the concentration of GM-CSF. Expression of GM-CSF was detected by ELISA. $^{###}p < 0.001$ compared with dimethyl sulfoxide (D, "DMSO") 0.1% control, $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the TPA-treated group, n = 3.
ELISA, enzyme-linked immunosorbent assay; GM-CSF, granulocyte–macrophage colony-stimulating factor; TPA, 12-O-tetradecanoylphorbol-13-acetate.

2.6. Western blot analysis

Total extracts were prepared with radioimmunoprecipitation assay buffer (RIPA buffer) (Noble Bio, Hwaseong, Korea) containing 1 mM phenylmethanesulfonyl fluoride and protease inhibitor cocktail (cat#P8340; Sigma, St. Louis, MO, USA). The proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using NuPAGE 10% Bis–Tris gels and transferred to a polyvinylidene fluoride transfer membrane (Pall Corporation, Port Washington, NY, USA) using NuPAGE 3-(N-morpholino) propane sulphonic acid (MOPS) SDS running buffer (cat# NP0001; Invitrogen) and NuPAGE transfer buffer 20× (cat# NP0006; Invitrogen™).

Western blot was performed using antibodies against anti–GM-CSF antibody (Abcam, Cambridge, UK), anti–phospho-p42/44 mitogen-activated protein kinase (MAPK) (ERK1/2) antibody, anti–p42/44 MAPK (ERK1/2) antibody (Cell Signaling Technology, Beverly, MA, USA), anti–PKCα antibody (Santa Cruz Biotechnology, Dallas, TX, USA), anti–PKCδ antibody (Santa Cruz Biotechnology, Dallas, TX, USA), anti–phospho-PKCα antibody (Abcam, Cambridge, MA), anti–phospho-PKCδ antibody (Abcam, Cambridge, MA), anti–β-actin antibody (Sigma, St. Louis, MO, USA), goat anti–mouse IgG antibody (Bio-Rad Laboratories Inc, Hercules, CA, USA), and rabbit IgG heavy- and light-chain antibody (Bethyl Laboratories, Montgomery, Texas, USA). The membranes were incubated in WEST-ZOL plus (iNtRON Biotechnology, Seongnam, Korea), and ChemiDoc XRS densitometry systems (Bio-Rad) were used for visualization.

2.7. Measurement of GM-CSF by enzyme-linked immunosorbent assay

Culture supernatants were collected 24 h after UV-B irradiation. The medium was centrifuged at 1000 rpm for 5 min and stored at −80°C. The GM-CSF in the culture supernatants was determined using a mouse GM-CSF enzyme-linked immunosorbent assay kit (eBioscience, San Diego, CA, USA) as per the manufacturer's instructions.

2.8. Statistical analysis

All values are expressed as mean ± standard error of mean. Significant differences between the results were evaluated using

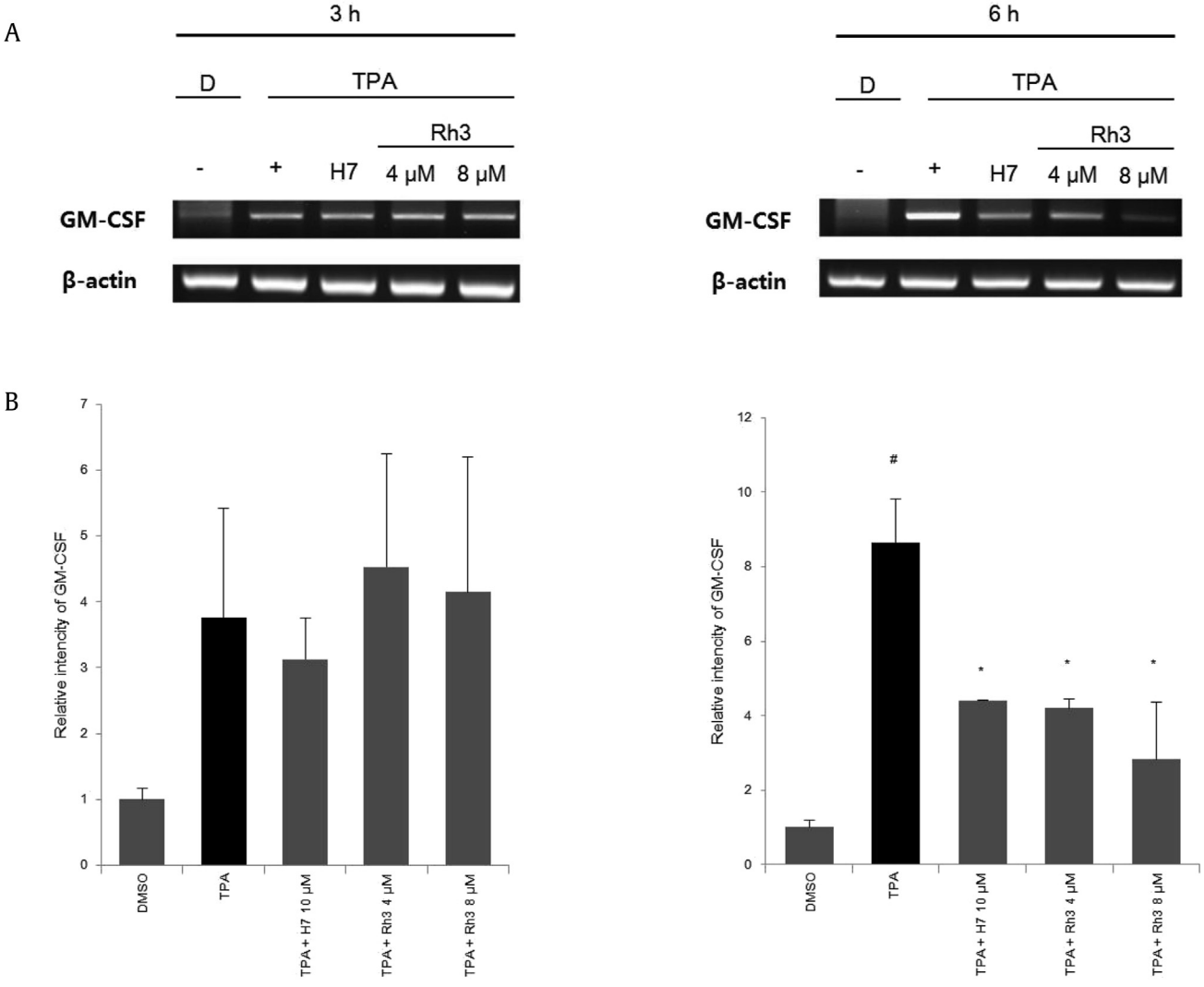

Fig. 4. Ginsenoside Rh3 reduced TPA-induced GM-CSF mRNA expression. The SP-1 keratinocytes were treated with ginsenoside Rh3 (4 μM and 8 μM). After 24 h, the cells were treated with 50 nM TPA. After UV-B irradiation, the cells were treated with 2% NBCS EMEM containing ginsenoside Rh3. (A) After 3 h and 6 h, the RNA level of GM-CSF was detected by RT-PCR. (B) The RNA level of GM-CSF was quantified using Image J software. The level of β-actin was used for loading control. $^{\#}p < 0.05$ compared with DMSO (D) 0.1% control, $^{*}p < 0.05$ compared with the TPA control, n = 3.

DMSO, dimethyl sulfoxide; EMEM, Eagle's minimum essential medium; GM-CSF, granulocyte–macrophage colony-stimulating factor; NBCS, newborn calf serum; RT-PCR, reverse transcription polymerase chain reaction; TPA, 12-O-tetradecanoylphorbol-13-acetate.

the Student *t* test. The *p*-values < 0.05 were regarded as statistically significant.

3. Results

3.1. Ginsenoside Rh3 inhibited production of GM-CSF in the UV-B–exposed SP-1 keratinocytes

We identified 15 types of ginsenosides to determine which ginsenoside reduced GM-CSF expression in SP-1 keratinocytes. Enzyme-linked immunosorbent assaywas used to quantify the GM-CSF levels from the SP-1 keratinocyte–cultured media. GM-CSF was increased in the culture media after UV-B exposure compared with the nonirradiated control cell media. Among the compounds tested, ginsenoside Rh3 resulted in an approximate 40% decrease in the GM-CSF level. Based on these results, we selected ginsenoside Rh3 for further study (Fig. 1).

3.2. Effect of ginsenoside Rh3 on cell viability

The cytotoxic effect of ginsenoside Rh3 was investigated on the SP-1 keratinocytes. We found that ginsenoside Rh3 did not affect cell viability at concentrations of 1–1000 μM (Fig. 2).

3.3. Ginsenoside Rh3 reduced 12-O-tetradecanoylphorbol-13-acetate–induced GM-CSF production

We investigated whether ginsenoside Rh3 could inhibit 12-O-tetradecanoylphorbol-13-acetate (TPA)–induced GM-CSF production in SP-1 cells. H7 (PKC inhibitor) was used as a positive control for the reduction in GM-CSF induced by TPA. We found that ginsenoside Rh3 reduced production of GM-CSF induced by 50 nM TPA in a dose-dependent manner (Fig. 3).

3.4. Ginsenoside Rh3 reduced TPA-induced GM-CSF mRNA expression

The SP-1 cells were treated with 50 nM TPA and ginsenoside Rh3 for 0, 3, and 6 h. To detect the GM-CSF mRNA level, we used RT-PCR. We observed that GM-CSF mRNA levels significantly increased at 3 and 6 h after TPA treatment and that ginsenoside Rh3 and H7 decreased the GM-CSF mRNA level in the TPA-treated SP-1 cells at 6 h (Fig. 4).

3.5. Differential activity of PKC isoforms in the UV-B radiation–induced SP-1 keratinocytes

We then examined which PKC isoforms are activated by UV-B radiation. By Western blot analysis, we confirmed that the PKCδ isoform was activated 5 min after UV-B exposure. However, the PKCα isoform was not activated after UV-B irradiation (Fig. 5). These results demonstrated that PKCδ is involved in UV-B–induced GM-CSF expression.

3.6. Effect of ginsenoside Rh3 on phosphorylation of PKCδ induced by TPA in the SP-1 keratinocytes

We identified whether ginsenoside Rh3 could suppress the TPA-induced phosphorylation of PKCδ and ERK. We observed that the phosphorylation levels of PKCδ and ERK increased rapidly after 3 min of 100 nM TPA treatment and that ginsenoside Rh3 reduced the phosphorylation (Fig. 6A). The results of the Western blot analysis of phospho-PKCδ and phospho-ERK were quantified using an image analyzer (Fig. 6B and C).

4. Discussion

Exposure to continuous and excessive UV-B radiation is a major cause of skin damage including sunburn, skin aging, erythema, and skin cancer [15]. UV irradiation promotes the secretion of various inflammatory cytokines and chemokines from keratinocytes [4,16]. It is known that GM-CSF is produced by UV irradiation in keratinocytes and increases skin pigmentation by controlling the proliferation and differentiation of melanocytes [17]. In our previous study, treatment with KRG saponin and ginsenoside Rh3 decreased GM-CSF release and expression in UV radiation–exposed keratinocytes [18]. Saponin consists of various ginsenosides, which have numerous functions [12]. We tested 15 types of ginsenosides to identify which ginsenosides repress GM-CSF expression. We found that ginsenoside Rh3 inhibited GM-CSF production in UV-B–

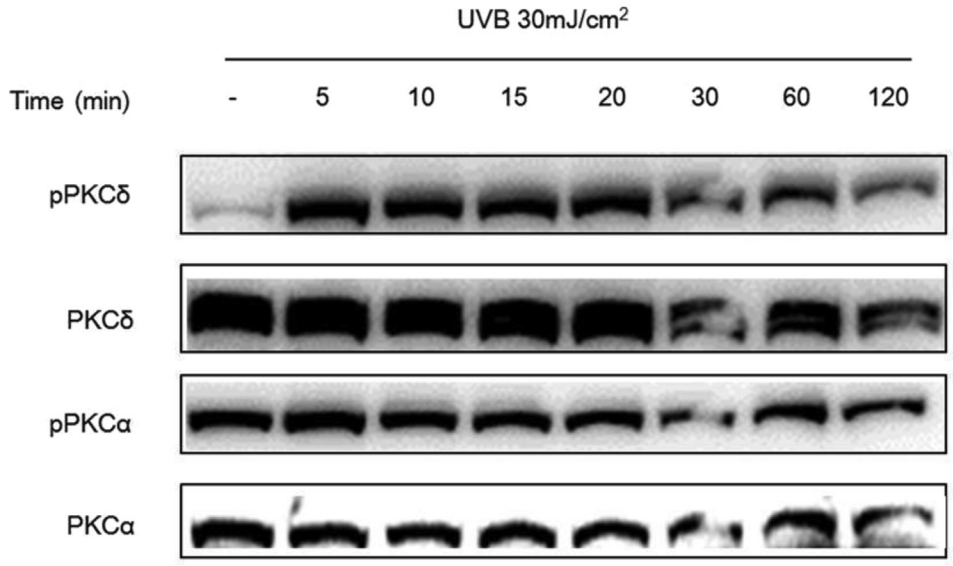

Fig. 5. Differential activity of PKC isoforms in the UV-B radiation–induced SP-1 keratinocytes. SP-1 cells were exposed to UV-B (30 mJ/cm^2) for different time points. The level of phospho-PKCδ (pPKCδ), total PKCδ (PKCδ), phospho-PKCα (pPKCα), and total PKCα (PKCα) was determined by Western blot at the indicated times.
PKC, protein kinase C.

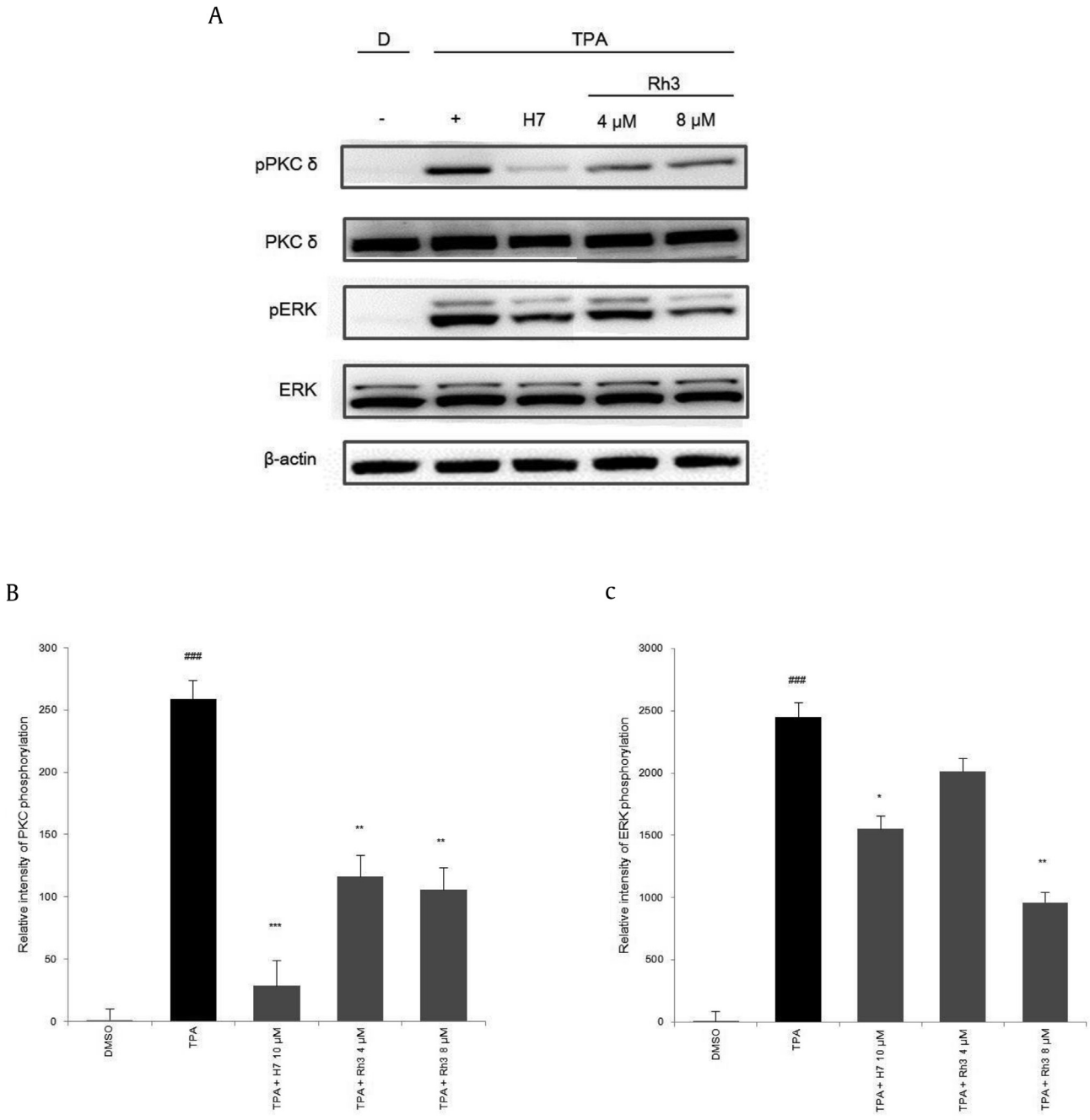

Fig. 6. Effect of ginsenoside Rh3 on phosphorylation of PKCδ and ERK induced by TPA in the SP-1 keratinocytes. The SP-1 keratinocytes were treated with ginsenoside Rh3 (4 and 8 μM). After 24 h, a mixture of ginsenoside Rh3 and 100 nM TPA was applied to the keratinocytes for 2 min. (A) The phosphorylated forms of PKCδ and ERK were analyzed by Western blot analysis. (B) The relative intensity of phospho-PKCδ and ERK was quantified using Image J software. Band densities of phospho-PKCδ were normalized to those of total PKCδ. Band densities of phospho-ERK were normalized to those of total ERK. $^{###}p < 0.001$ compared with DMSO (D) 0.1% control, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with TPA control, n = 3.
DMSO, dimethyl sulfoxide; ERK, extracellular signal−regulated kinase; PKC, protein kinase C; TPA, 12-O-tetradecanoylphorbol-13-acetate.

exposed SP-1 keratinocytes (Fig. 1). Ginsenoside Rh3 is a metabolite of ginsenoside Rg5 in humans [19]. Pharmacological studies found that ginsenoside Rg5 and Rh3 have anticancer and antiinflammatory activities [19,20]. In addition, Rh3 was reported to inhibit lipopolysaccharides (LPS-)induced cytokines such as inducible nitric oxide synthase, tumor necrosis factor-α, and interleukin-1β (IL-1β) [21]. In our study, ginsenoside Rh3 suppressed TPA-induced GM-CSF protein expression (Fig. 3) and reduced GM-CSF mRNA level in the TPA-treated SP-1 cells (Fig. 4). The cPKC and nPKC can be directly activated by TPA and inhibited by H7 [22,23]. It has been reported that PKC signaling is activated in response to UV irradiation [24]. However, the PKC isoforms have different functions and reactions. UV irradiation activates phosphorylation of PKCδ, but not PKCα [25]. We investigated whether phosphorylation of PKC isoforms is activated by UV-B irradiation in SP-1 cells. After UV-B irradiation (30 mJ/cm^2), PKCδ was phosphorylated after 5 min, and this phosphorylation was maintained for 1 hr. However, PKCα was not phosphorylated by UV-B irradiation (Fig. 5). These results demonstrate that PKCδ is involved in UV-B−induced GM-CSF production. The PKC-ERK pathway regulates

proliferation and differentiation and is an antiapoptotic pathway in keratinocytes [26]. It has been reported that PKC activates MAPK/ERK Kinase (MEK)-ERK pathway [27]. Furthermore, phosphorylation of ERK contributes to the generation of GM-CSF through the translation and stabilization of GM-CSF [28]. Similar to UV-B irradiation, treatment of cells with TPA increased phosphorylation of ERK and PKCδ. We found that ginsenoside Rh3 inhibited TPA-induced phosphorylation of PKCδ and ERK (Fig. 6). Taken together, we identified that UV-B increased GM-CSF expression through PKCδ activation. Our results showed the inhibitory effect of ginsenoside Rh3 on UV-B–induced GM-CSF expression and the inhibitory mechanism of GM-CSF through the repression of PKCδ phosphorylation in SP-1 cells. Thus, our finding shows that ginsenoside Rh3 can suppress UV-B–induced PKCδ activation. GM-CSF is also induced by UV light and has autocrine or paracrine function in human skin [29,30]. It is therefore possible that ginsenoside Rh3 has an effect on the production of GM-CSF in cultured human keratinocytes.

PKCδ has been known to implicate in various cellular processes such as apoptosis, migration, and differentiation [9,31,32]. In addition, PKCδ plays an important role in regulation of inflammatory cytokines, including IL-6, IL-8, and interferon-γ. Immunohistochemistry and PCR analysis showed decreased expression of IL-6 and monocyte chemoattractant protein-1 in the aortic tissues of PKCδ knockout mice [33,34]. Despite the fact that PKCδ is involved in diverse biological activities such as glucose metabolism, neuropathogenesis, and tonic tension, few PKCδ inhibitors have been reported [35–38]. Recently, we found that ginsenoside Rh3 decreased the proliferation of mouse melanocytes through down-regulation of microphthalmia-associated transcription factor, a key regulator of melanocytes [39]. In this regard, we suggest that ginsenoside Rh3 may be a bioactive compound targeting PKCδ and a potential agent to suppress UV-B–induced skin pigmentation.

An earlier report has shown that ginsenoside Re has a protective role in methamphetamine-induced apoptosis via PKCδ inhibition in SH-SY5Y neuroblastoma cells [40]. In contrast, other groups suggested that ginsenoside Rh2 induced PKCδ activity and apoptosis in SK-HEP-1 hepatoma cells [41]. Our findings that ginsenoside Rh3 may be a potential PKCδ inhibitor can be applied to the aforementioned studies. Further studies on the effect of ginsenoside Rh3 on reactive oxygen species produced by UV-B, based on the reported result that the PKCs are regulated by UV-induced reactive oxygen species, will need to be performed [42,43].

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was supported by the 2017 grant from the Korean Society of Ginseng and by a grant (D171754) from Gyeonggi Technology Development Program funded by Gyeonggi Province.

References

[1] Li M, Chiba H, Warot X, Messaddeq N, Gérard C, Chambon P, Metzger D. RXR-alpha ablation in skin keratinocytes results in alopecia and epidermal alterations. Development 2001;128:675–88.
[2] Hirobe T. Role of keratinocyte-derived factors involved in regulating the proliferation and differentiation of mammalian epidermal melanocytes. Pigm Cell Res 2005;18:2–12.
[3] Prota G. Pigment cell research: what directions? Pigm Cell Res 1997;10:5–11.
[4] Lee EJ, Jeon MS, Kim BD, Kim JH, Kwon YG, Lee H, Lee YS, Yang JH, Kim TY. Capsiate inhibits ultraviolet B-induced skin inflammation by inhibiting Src family kinases and epidermal growth factor receptor signaling. Free Radic Biol Med 2010;48:1133–43.
[5] Hirobe T, Furuya R, Hara E, Horii I, Tsunenaga M, Ifuku O. Granulocyte-macrophage colony-stimulating factor (GM-CSF) controls the proliferation and differentiation of mouse epidermal melanocytes from pigmented spots induced by ultraviolet radiation B. Pigm Cell Res 2004;17:230–40.
[6] Reibman J, Talbot AT, Hsu Y, Ou G, Jover J, Nilsen D, Pillinger MH. Regulation of expression of granulocyte-macrophage colony-stimulating factor in human bronchial epithelial cells: roles of protein kinase C and mitogen-activated protein kinases. J Immunol 2000;165:1618–25.
[7] Gilchrest BA, Park HY, Eller MS, Yaar M. Mechanisms of ultraviolet light-induced pigmentation. Photochem Photobiol 1996;63:1–10.
[8] Liu JF, Crépin M, Liu JM, Barritault D, Ledoux D. FGF-2 and TPA induce matrix metalloproteinase-9 secretion in MCF-7 cells through PKC activation of the Ras/ERK pathway. Biochem Biophys Res Commun 2002;293:1174–82.
[9] Brodie C, Blumberg P. Regulation of cell apoptosis by protein kinase c δ. Apoptosis 2003;8:19–27.
[10] Chen N, Ma W-y, Huang C, Dong Z. Translocation of protein kinase Cε and protein kinase Cδ to membrane is required for ultraviolet B-induced activation of mitogen-activated protein kinases and apoptosis. J Biol Chem 1999;274:15389–94.
[11] Aziz MH, Manoharan HT, Verma AK. Protein kinase Cε, which sensitizes skin to sun's UV radiation–induced cutaneous damage and development of squamous cell carcinomas, associates with Stat3. Cancer Res 2007;67:1385–94.
[12] Oh CT, Park JI, Jung YR, Joo YA, Shin DH, Cho HJ, Ahn SM, Lim Y-H, Park CK, Hwang JS. Inhibitory effect of Korean Red Ginseng on melanocyte proliferation and its possible implication in GM-CSF mediated signaling. J Ginseng Res 2013;37:389.
[13] Attele AS, Wu JA, Yuan C-S. Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol 1999;58:1685–93.
[14] Yayeh T, Jung K-H, Jeong HY, Park J-H, Song Y-B, Kwak Y-S, Kang H-S, Cho JY, Oh J-W, Kim S-K. Korean red ginseng saponin fraction downregulates proinflammatory mediators in LPS stimulated RAW264. 7 cells and protects mice against endotoxic shock. J Ginseng Res 2012;36:263.
[15] Sies H, Stahl W. Nutritional protection against skin damage from sunlight. Annu Rev Nutr 2004;24:173–200.
[16] Köck A, Schwarz T, Kirnbauer R, Urbanski A, Perry P, Ansel J, Luger T. Human keratinocytes are a source for tumor necrosis factor alpha: evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. J Exp Med 1990;172:1609–14.
[17] Yamaguchi Y, Hearing VJ. Physiological factors that regulate skin pigmentation. Biofactors 2009;35:193–9.
[18] Chung I, Lee J, Park YS, Lim Y, Chang DH, Park J, Hwang JS. Inhibitory mechanism of Korean red ginseng on GM-CSF expression in UVB-irradiated keratinocytes. J Ginseng Res 2015;39:322–30.
[19] Shin Y, Bae E, Kim D. Inhibitory effect of ginsenoside Rg5 and its metabolite ginsenoside Rh3 in an oxazolone-induced mouse chronic dermatitis model. Arch Pharm Res 2006;29:685–90.
[20] Lee YY, Park J, Lee E, Lee S, Kim D, Kang JL, Kim H. Anti-inflammatory mechanism of ginseng saponin metabolite Rh3 in lipopolysaccharide-stimulated microglia: critical role of 5′-adenosine monophosphate-activated protein kinase signaling pathway. J Agric Food Chem 2015;63:3472–80.
[21] Park J, Park E, Kim D, Jung K, Jung J, Lee E, Hyun J, Kang JL, Kim H. Anti-inflammatory mechanism of ginseng saponins in activated microglia. J Neuroimmunol 2009;209:40–9.
[22] Rivedal E, Opsahl H. Role of PKC and MAP kinase in EGF-and TPA-induced connexin43 phosphorylation and inhibition of gap junction intercellular communication in rat liver epithelial cells. Carcinogenesis 2001;22:1543–50.
[23] Siflinger-Birnboim A, Goligorsky M, Del Vecchio P, Malik A. Activation of protein kinase C pathway contributes to hydrogen peroxide-induced increase in endothelial permeability. Lab Invest: J Tech Methods Pathol 1992;67:24–30.
[24] Denning MF, Wang Y, Nickoloff BJ, Wrone-Smith T. Protein kinase Cδ is activated by caspase-dependent proteolysis during ultraviolet radiation-induced apoptosis of human keratinocytes. J Biol Chem 1998;273:29995–30002.
[25] Bode AM, Dong Z. Mitogen-activated protein kinase activation in UV-induced signal transduction. Sci Signal 2003;2003:re2.
[26] Jirousek MR, Goekjian PG. Protein kinase C inhibitors as novel anticancer drugs. Expert Opin Investig Drugs 2001;10:2117–40.
[27] Ueda Y, Hirai S-i, Osada S-i, Suzuki A, Mizuno K, Ohno S. Protein kinase C δ activates the MEK-ERK pathway in a manner independent of Ras and dependent on Raf. J Biol Chem 1996;271:23512–9.
[28] Esnault S, Malter JS. Hyaluronic acid or TNF-α plus fibronectin triggers granulocyte macrophage-colony-stimulating factor mRNA stabilization in eosinophils yet engages differential intracellular pathways and mRNA binding proteins. J Immunol 2003;171:6780–7.
[29] Kawada A, Hiruma M, Noguchi H, Ishibashi A, Motoyoshi K, Kawada I. Granulocyte and macrophage colony-stimulating factors stimulate proliferation of human keratinocytes. Arch Dermatol Res 1997;289:600–2.
[30] Braunstein S, Kaplan G, Gottlieb AB, Schwartz M, Walsh G, Abalos RM, Fajardo TT, Guido LS, Krueger JG. GM-CSF activates regenerative epidermal growth and stimulates keratinocyte proliferation in human skin in vivo. J Invest Dermatol 1994;103(4).
[31] Mondrinos MJ, Zhang T, Sun S, Kennedy PA, King DJ, Wolfson MR, Knight LC, Scalia R, Kilpatrick LE. Pulmonary endothelial protein kinase C-delta (PKCδ)

regulates neutrophil migration in acute lung inflammation. Am J Pathol 2014;184:200–13.
[32] Hamdorf M, Berger A, Schüle S, Reinhardt J, Flory E. PKCδ-Induced PU. 1 phosphorylation promotes hematopoietic stem cell differentiation to dendritic cells. Stem Cells 2011;29:297–306.
[33] Page K, Li J, Zhou L, Iasvoyskaia S, Corbit KC, Soh J, Weinstein IB, Brasier AR, Lin A, Hershenson MB. Regulation of airway epithelial cell NF-κB-dependent gene expression by protein kinase cδ. J Immunol 2003;170:5681–9.
[34] Morgan S, Yamanouchi D, Harberg C, Wang Q, Keller M, Si Y, Burlingham W, Seedial S, Lengfeld J, Liu B. Elevated protein kinase C-δ contributes to aneurysm pathogenesis through stimulation of apoptosis and inflammatory signaling. Arterioscler Thromb Vasc Biol 2012;32:2493–502.
[35] Mayr M, Chung Y, Mayr U, McGregor E, Troy H, Baier G, Leitges M, Dunn MJ, Griffiths JR, Xu Q. Loss of PKC-δ alters cardiac metabolism. Am J Physiol Heart Circ Physiol 2004;287:H945.
[36] Kokorovic A, Cheung GW, Breen DM, Chari M, Lam CK, Lam TK. Duodenal mucosal protein kinase C-δ regulates glucose production in rats. Gastroenterology 2011;141:1720–7.
[37] Jin H, Kanthasamy A, Harischandra DS, Kondru N, Ghosh A, Panicker N, Anantharam V, Rana A, Kanthasamy AG. Histone hyperacetylation upregulates PKCδ in dopaminergic neurons to induce cell death: relevance to epigenetic mechanisms of neurodegeneration in Parkinson′ s disease. J Biol Chem 2014. jbc. M114. 576702.
[38] Lim K, Kwon J, Kim K, Noh J, Kang S, Park J, Lee M, Bae O, Chung J. Emodin inhibits tonic tension through suppressing PKCδ-mediated inhibition of myosin phosphatase in rat isolated thoracic aorta. Br J Pharmacol 2014;171:4300–10.
[39] Lee JE, Park JI, Myung CH, Hwang JS. Inhibitory effects of ginsenosides on basic fibroblast growth factor-induced melanocyte proliferation. J Ginseng Res 2017;41:268–76.
[40] Oh J, Chun K, Joo S, Oh Y, Lee S. Caspase-3-dependent protein kinase C delta activity is required for the progression of ginsenoside-Rh2-induced apoptosis in SK-HEP-1 cells. Cancer Lett 2005;230:228–38.
[41] Nam Y, Wie MB, Shin E, Nguyen TL, Nah S, Ko SK, Jeong JH, Jang C, Kim H. Ginsenoside re protects methamphetamine-induced mitochondrial burdens and proapoptosis via genetic inhibition of protein kinase C δ in human neuroblastoma dopaminergic SH-SY5Y cell lines. J Appl Toxicol 2015;35:927–44.
[42] Lee H-G, Yang J-H. PKC-δ mediates TCDD-induced apoptosis of chondrocyte in ROS-dependent manner. Chemosphere 2010;81:1039–44.
[43] Makino J, Kamiya T, Hara H, Adachi T. TPA induces the expression of EC-SOD in human monocytic THP-1 cells: involvement of PKC, MEK/ERK and NOX-derived ROS. Free Radical Res 2012;46:637–44.