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Research article

Ginsenoside Rg3 attenuates skin disorders via down-regulation of MDM2/HIF1α signaling pathway

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ABSTRACT

Background: Thymic stromal lymphopoietin (TSLP) acts as a master switch for inflammatory responses. Ginsenoside Rg3 (Rg3) which is an active ingredient of Panax ginseng Meyer (Araliaceae) is known to possess various therapeutic effects. However, a modulatory effect of Rg3 on TSLP expression in the inflammatory responses remains poorly understood.

Methods: We investigated antiinflammatory effects of Rg3 on an in vitro model using HMC-1 cells stimulated by PMA plus calcium ionophore (PMACI), as well as an in vivo model using PMA-induced mouse ear edema. TSLP and vascular endothelial growth factor (VEGF) levels were detected using enzyme-linked immunosorbent assay or real-time PCR analysis. Murine double minute 2 (MDM2) and hypoxia-inducible factor 1α (HIF1α) expression levels were detected using Western blot analysis.

Results: Rg3 treatment restrained the production and mRNA expression levels of TSLP and VEGF in activated HMC-1 cells. Rg3 down-regulated the MDM2 expression level increased by PMACI stimulation. The HIF1α expression level was also reduced by Rg3 in activated HMC-1 cells. In addition, Rg3-administered mice showed the decreased redness and ear thickness in PMA-irritated ear edema. Rg3 inhibited the TSLP and VEGF levels in the serum and ear tissue homogenate. Moreover, the MDM2 and HIF1α expression levels in the ear tissue homogenate were suppressed by Rg3.

Conclusion: Taken together, the current study identifies new mechanistic evidence about MDM2/HIF1α pathway in the antiinflammatory effect of Rg3, providing a new effective therapeutic strategy for the treatment of skin inflammatory diseases.

1. Introduction

Inflammation is associated with numerous skin diseases [1–3]. Inflammatory reactions are characterized by swelling, heat, pain, and redness. Moreover, inflammation is regarded as an important factor of organ dysfunction which requires pharmacological intervention [4].

Various cells such as macrophages, keratinocytes, T cells, B cells, monocytes, eosinophils, neutrophils, and basophils are associated with the inflammatory reactions [5–7]. Mast cells also play roles in the inflammatory reactions [8]. Young et al. [9] reported that mast cells enhanced inflammation in chronic nonbacterial osteomyelitis and mast cell deficiency showed decreased inflammatory reactions in mice. Mast cell-depleted mice showed reduced joint inflammation in arthritis models [10]. Furthermore, mast cells ablation inhibited ear swelling response induced by 2,4-dinitrofluorobenzene (DNFB) in mice [11].

Thymic stromal lymphopoietin (TSLP) is crucial in the pathogenesis of skin inflammatory disorders such as contact dermatitis and atopic dermatitis. Recombinant TSLP treatment in the nape of the neck elevated scratching frequency in mice [12]. TSLP produced by mast cells plays a role in skin inflammatory responses [13].

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Wong and colleagues [14] reported that TSLP acts as a master switch for inflammatory reactions by means of mast cell activation.

TSLP was produced via murine double minute 2 (MDM2) signaling in macrophages [15]. Because MDM2 induces tissue inflammation, blockade of MDM2 would show potent antiinflammatory effects [16]. MDM2 induces activation of hypoxia-inducible factor 1α (HIF1α) [17]. Our previous study showed that treatment with MDM2 inhibitor (nutlin-3a) resulted in down-regulation of HIF1α, indicating that HIF1α is a downstream factor of MDM2 [15]. Jang et al. [18] reported that TSLP expression was mediated by HIF1α in keratinocytes.

Ginsenoside Rg3 (Rg3) is an active ingredient of Korean Red Ginseng (*Panax ginseng* Meyer) and has a variety of beneficial effects such as anticancer, antidiabetes, antivirus, antiinflammatory, antiosteoporotic, antiatherosclerosis, antiarthritic, antifatigue, antiadipogenic, and antioxidant [19–26]. In addition, Rg3 promoted beta-amyloid peptide degradation, suggesting a preventive effect on Alzheimer's disease [27]. However, the beneficial effect and precise mechanism of Rg3 on TSLP level in mast cells have not been clearly elucidated.

We hypothesized that Rg3 has a regulatory effect by inhibiting the TSLP production level via blockade of MDM2-HIF1α signaling pathway in mast cells during inflammatory responses. Thus, we assumed that inhibitions of MDM2 and HIF1α expression levels by Rg3 would reduce TSLP levels in serum and ear edema in mice.

2. Materials and methods

2.1. Enzyme-linked immunosorbent assay (ELISA)

The levels of TSLP and vascular endothelial growth factor (VEGF) in HMC-1 cell supernatants, sera and ear tissue homogenates were detected according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA), as previously described [28]. Each cytokine level was quantified by analyzing the absorbance with a microplate absorbance reader (Versa Max, Molecular Devices, Sunnyvale, CA, USA).

2.2. Quantitative RT-qPCR

Total RNA was extracted with an easy-BLUE™ RNA extraction kit (iNtRON Biotech Inc., Seongnam, Korea) and mixed with chloroform. After centrifugation, the collected upper aqueous phase was mixed with isopropanol. After centrifugation, RNA was treated with 75% and 100% ethanol specifically. The RNA was reverse-transcribed with a cDNA synthesis kit (Bioneer Corporation, Daejeon, Korea). The cDNAs were used for quantification of gene expression by quantitative real-time PCR (Applied Biosystems, Foster City, CA, USA) using Power SYBR® Green Master Mix (Thermo Fisher Scientific, Waltham, MS, USA), as previously described [29]. The primer sequences were shown in Supplementary Table 1. The mRNA expression levels of TSLP and VEGF were normalized to GAPDH for each sample. Samples were run in duplicate.

2.3. Western blot analysis

The harvested HMC-1 cells were lysed in cell lysis buffer (Invitrogen™, Carlsbad, CA, USA) for analyses of MDM2 and GAPDH or lysed in nuclear extraction reagent (Thermo Fisher Scientific) for analyses of HIF1α and Poly (ADP-ribose) polymerase (PARP) with minor modifications [30,31]. Lysates were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After proteins were transferred to nitrocellulose membranes (GE Healthcare, Chicago, IL, USA), the membranes were blocked with 5% bovine serum albumin diluted in phosphate buffered saline (PBS) with Tween 20 (Sigma Chemical Co.). The membranes were incubated overnight with primary antibodies against MDM2, GAPDH, HIF1α or PARP. GAPDH and PARP were used as loading controls. The washed membranes were incubated with horseradish peroxidase-conjugated secondary anti-mouse antibodies (all primary and secondary antibodies from Santa Cruz Biotechnology). An enhanced chemiluminescence solution (DoGenBio Co., Seoul, Korea) was used for development. Blots were quantified by Image J software (National Institute of Health, Bethesda, MD, USA). The density of each target band was normalized to GAPDH or PARP.

3. Results

3.1. Rg3 attenuates TSLP levels in HMC-1 cells stimulated with PMACI

To investigate whether Rg3 would regulate TSLP levels, we first examined the effect of Rg3 on cell viability via an MTT assay. HMC-1 cells were pre-incubated with Rg3 (0.1, 1 and 10 μg/ml) of in the presence of PMA plus calcium ionophore (PMACI) by referring to the study of [19]. As shown in Fig. 1A, Rg3 treatment up to 10 μg/ml did not decrease the cell viability. The final concentration of a vehicle control (DMSO) was < 0.025% and did not affect the assay (Fig. 1A). DEX (100 nM) which is a critical antiinflammatory agent [32], did not affect this assay and was used as a positive control (Fig. 1A). In addition, the treatment with Rg3 reduced β-galactosidase activity in activated HMC-1 cells ($p < 0.05$; Fig. 1B). Thus, the concentrations of 0.1, 1 and 10 μg/ml of Rg3 were used to assess the regulatory effect of Rg3 in HMC-1 cells. In our previous study [33], TSLP mRNA expression was reached its peak 5 h after PMACI stimulation. Thus, we activated HMC-1 cells for 7 h to measure TSLP production level. Rg3 inhibited the increase in TSLP production level in a concentration-dependent manner in activated HMC-1 cells ($p < 0.001$; Fig. 1C). A vehicle control did not have an effect on the TSLP production level in HMC-1 cells stimulated with PMACI (Fig. 1C). Thus, the following experiments were performed excluding the vehicle control group. Rg3 suppressed the mRNA expression level of TSLP increased by PMACI in a concentration-dependent manner ($p < 0.001$; Fig. 1D). The inhibitory effects of Rg3 on the production and mRNA expression levels of TSLP were similar to those of DEX ($p < 0.001$; Fig. 1C and D).

3.2. Rg3 attenuates VEGF levels in HMC-1 cells stimulated with PMACI

VEGF is associated not only with pathological angiogenesis, but also with various inflammatory diseases [34]. Thus, we evaluated a regulatory effect of Rg3 on VEGF production level in activated HMC-1 cells. In response to PMACI, VEGF production level increased in HMC-1 cells. Rg3 suppressed the increase in the VEGF production level in a concentration-dependent manner in HMC-1 cells stimulated with PMACI ($p < 0.001$; Fig. 1E). A vehicle control did not have an effect on the VEGF production in activated HMC-1 cells (Fig. 1E). Rg3 significantly reduced the mRNA expression level of VEGF increased by PMACI ($p < 0.001$; Fig. 1F). These inhibitory effects of Rg3 on the VEGF levels were similar to those of DEX ($p < 0.001$; Fig. 1E and F).

3.3. Rg3 down-regulates MDM2 signaling pathways in HMC-1 cells stimulated with PMACI

A previous study reported that Rg3 down-regulates NF-κB signaling pathway in activated HMC-1 cells [20]. MDM2 activates the NF-κB signaling pathway and acts as a co-transcription factor

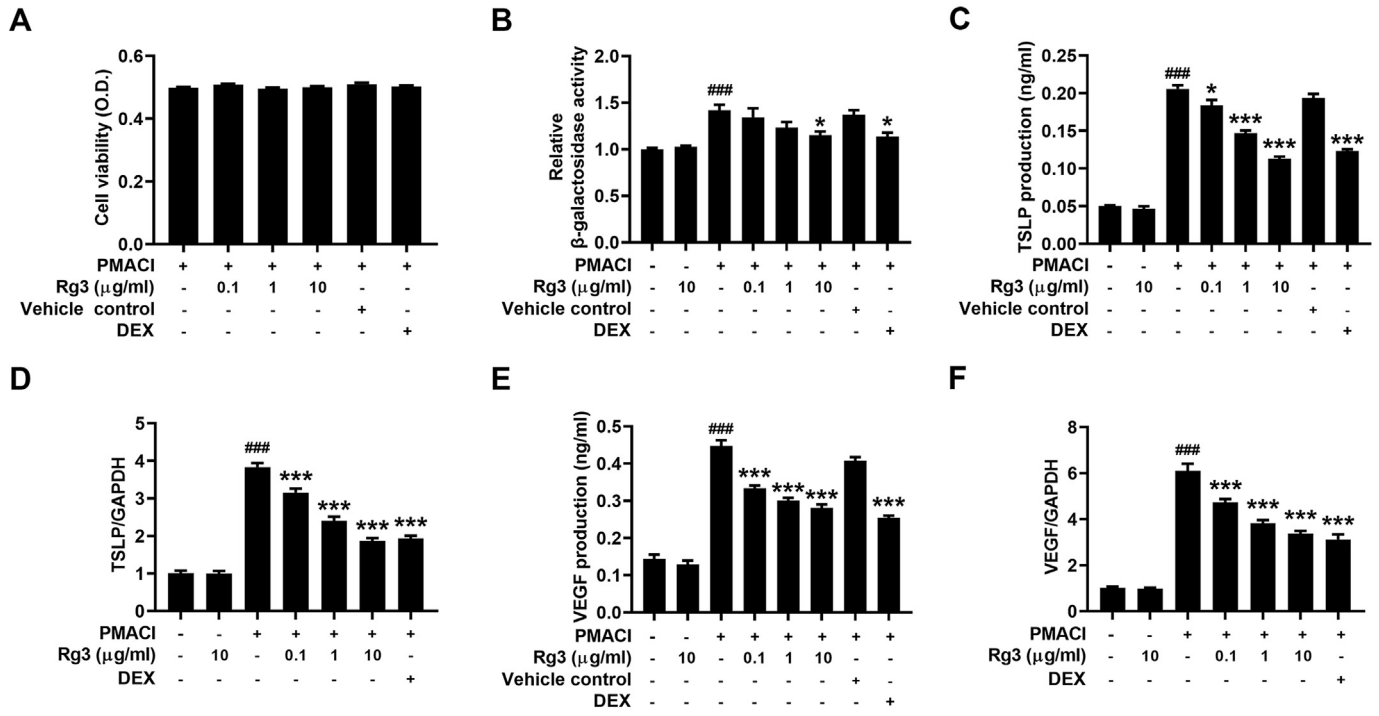

Fig. 1. Rg3 attenuates TSLP and VEGF levels in HMC-1 cells stimulated with PMACI. HMC-1 cells (4×10^5) were pre-incubated with Rg3, vehicle control (DMSO, 0.025%) and DEX (100 nM) for 1 h and then activated with PMACI for 7 h without media change. (A) Cell viability was measured by an MTT assay. (B) β-Galactosidase activity was analyzed by a β-galactosidase assay kit. (C,E) HMC-1 cells (4×10^5) were pre-incubated with Rg3, vehicle control (DMSO, 0.025%) and DEX (100 nM) for 1 h and then activated with PMACI for 7 h without media change. The TSLP and VEGF production levels in cell supernatants were determined by ELISA. (D,F) HMC-1 cells were pre-incubated with Rg3 and DEX (100 nM) for 1 h and then activated with PMACI for 5 h without media change. The TSLP and VEGF mRNA expression levels in harvested cells were determined by real time-PCR. ###$p < 0.001$ considered significant compared to PMACI-inactivated group; *$p < 0.05$ and ***$p < 0.001$ considered significant compared to PMACI-activated group.

for NF-κB target genes [35]. Thus, we attempted to further investigate whether Rg3 would regulate the TSLP and VEGF levels via MDM2 signaling pathways. First, we pre-treated an MDM2 inhibitor, nutlin-3a, and activated HMC-1 cells with PMACI. The TSLP ($p < 0.001$) and VEGF ($p < 0.001$) production levels were significantly reduced by nutlin-3a treatment (Fig. 2A and B). The inhibitory effects of nutlin-3a on the production levels were similar to those of Rg3 (Fig. 2A and B). Next, we investigated whether MDM2 expression level would up-regulated by PMACI stimulation. As shown in Fig. 2C and D, MDM2 expression began to be significantly induced 1 h after PMACI stimulation ($p = 0.007$). Elevation of MDM2 expression was the most at 3 h time point ($p = 0.011$) following PMACI stimulation (Fig. 2C and D). Thus, we selected 3 h time point to analyze the regulatory effect of Rg3 on the MDM2 expression. Expectedly, Rg3 treatment significantly reduced the MDM2 expression level increased by PMACI stimulation ($p < 0.001$, Fig. 2E and F). DEX also significantly decreased the MDM2 expression level ($p < 0.001$, Fig. 2E and F).

3.4. Rg3 down-regulates HIF1α signaling pathways in HMC-1 cells stimulated with PMACI

MDM2 is a positive activator of HIF1α and VEGF [17]. Both of MDM2 and HIF1α are key modulators of VEGF pathways [36]. Thus, we treated nutlin-3a in HMC-1 cells to study whether HIF1α expression would be regulated through MDM2 signaling pathways. To analyze HIF1α expression level, HMC-1 cells were activated with PMACI for 4 h, referring to the report of [37]. The PMACI stimulation significantly induced a marked increase in the HIF1α expression level (Fig. 3A and B, $p < 0.001$). The increased HIF1α expression level was significantly suppressed by nutlin-3a ($p = 0.006$, Fig. 3A and B). Next, we investigated whether Rg3 would down-regulate HIF1α expression in activated HMC-1 cells. Rg3 significantly restrained the HIF1α expression level in activated HMC-1 cells ($p = 0.004$, Fig. 3C and D). DEX also significantly restrained the HIF1α expression level ($p = 0.003$, Fig. 3). Moreover, an HIF1α inhibitor, YC-1 significantly diminished the production levels of TSLP ($p < 0.001$) and VEGF ($p < 0.001$) in activated HMC-1 cells (Fig. 2A and B). The inhibitory effects of YC-1 on the production levels were similar to those of Rg3 (Fig. 2A and B).

3.5. Rg3 attenuates PMA-irritated ear edema

Inflammatory reactions can occur when vascular tissues are subjected to harmful irritation [38]. Based on the above findings, we used an in vivo model of PMA-irritated ear edema and further investigated the regulatory effect of Rg3 on inflammatory responses. An increase in ear thickness indicates the degree of inflammation responses [39]. The PMA irritation induced significant redness and increases in ear thickness ($p < 0.05$, Fig. 4A and Table 1). However, Rg3-administered mice showed the decreased redness and ear thickness when compared to PMA control mice ($p < 0.05$, Fig. 4A and Table 1). In addition, Rg3 significantly decreased the serum TSLP ($p = 0.003$) and VEGF ($p = 0.044$) levels increased by PMA irritation (Fig. 4B and C). The TSLP ($p < 0.001$) and VEGF ($p = 0.004$) levels in the ear tissue homogenate were also markedly reduced by Rg3 administration (Fig. 4D and E). DEX also significantly inhibited these responses (Fig. 4 and Table 1). Finally, to study the regulatory effect of Rg3 on MDM2-HIF1α pathways in the in vivo model of PMA-irritated ear edema, we analyzed the MDM2 and HIF1α expression levels in the ear tissue homogenate. As shown in Fig. 5, Rg3 significantly suppressed the MDM2

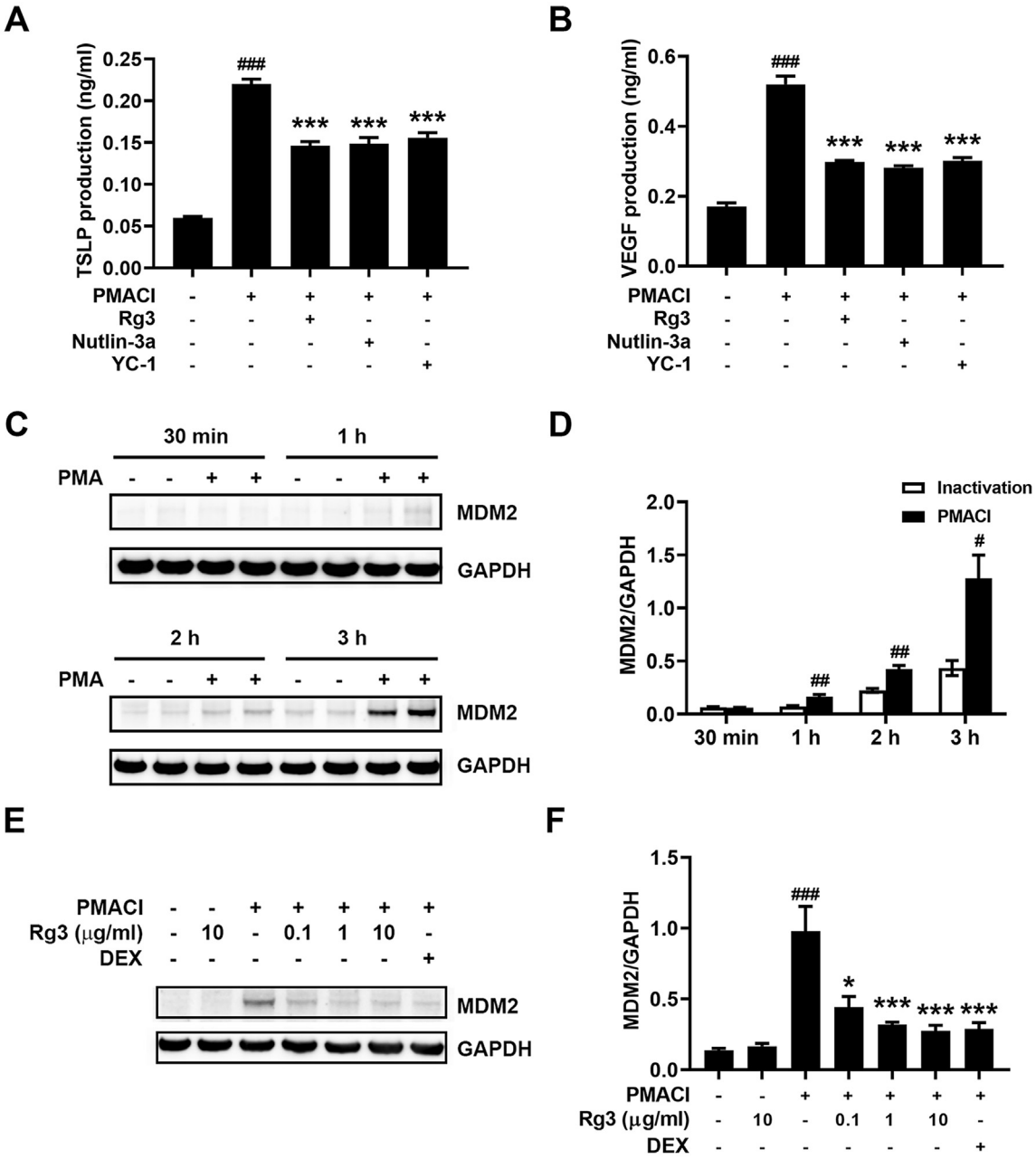

Fig. 2. Rg3 down-regulates MDM2 signaling pathways in HMC-1 cells stimulated with PMACI. HMC-1 cells (4×10^5) were pre-incubated with Rg3 (10 μg/ml), nutlin-3a (1 μM) or YC-1 (10 μM) for 1 h and then activated with PMACI for 7 h without media change. (A) TSLP and (B) VEGF production levels in cell supernatants were assessed by ELISA. (C) HMC-1 cells (5×10^6) were activated with PMACI for the indicated period. The MDM2 expression level was detected by immunoblot assay. (D) Quantification of relative levels was expressed as MDM2/GAPDH. (E) HMC-1 cells (5×10^6) were pre-incubated with Rg3 and DEX (100 nM) for 1 h and then activated with PMACI for 3 h without media change. The MDM2 expression level was detected by immunoblot assay. (F) Quantification of relative levels was expressed as MDM2/GAPDH. #$p < 0.05$, ##$p < 0.01$ and ###$p < 0.001$ considered significant compared to PMACI-inactivated group; *$p < 0.05$ and ***$p < 0.001$ considered significant compared to PMACI-activated group.

($p < 0.001$) and HIF1α ($p = 0.002$) expression levels increased by PMA irritation in the ear tissue homogenate. DEX also significantly decreased the MDM2 ($p < 0.001$) and HIF1α ($p = 0.011$) expression levels (Fig. 5).

4. Discussion

Newly synthesized cytokines are secreted to the extracellular space for leading to inflammation after mast cell activation [40]. This mast cell activation can be reproduced by means of chemical stimuli. Stimulation with protein kinase C activator PMA plus calcium ionophore A23187 led to release of various inflammatory cytokines from HMC-1 cells [41]. Critical inflammatory cytokines, TSLP and VEGF were also produced by PMACI [33,37,41]. Serum and skin lesions of patients with atopic dermatitis contained high levels of TSLP [42]. TSLP receptor deficiency led to decreased skin inflammation in mice [43]. TSLP deficiency resulted in reduced skin inflammation in mice [13]. Transgenic overexpression of VEGF in the skin resulted in features of skin inflammation [44]. Scaldaferri et al. [45] suggested that agents that block VEGF signaling could suppress inflammatory responses in patients with inflammatory disease. Our findings presented that Rg3 reduced the production

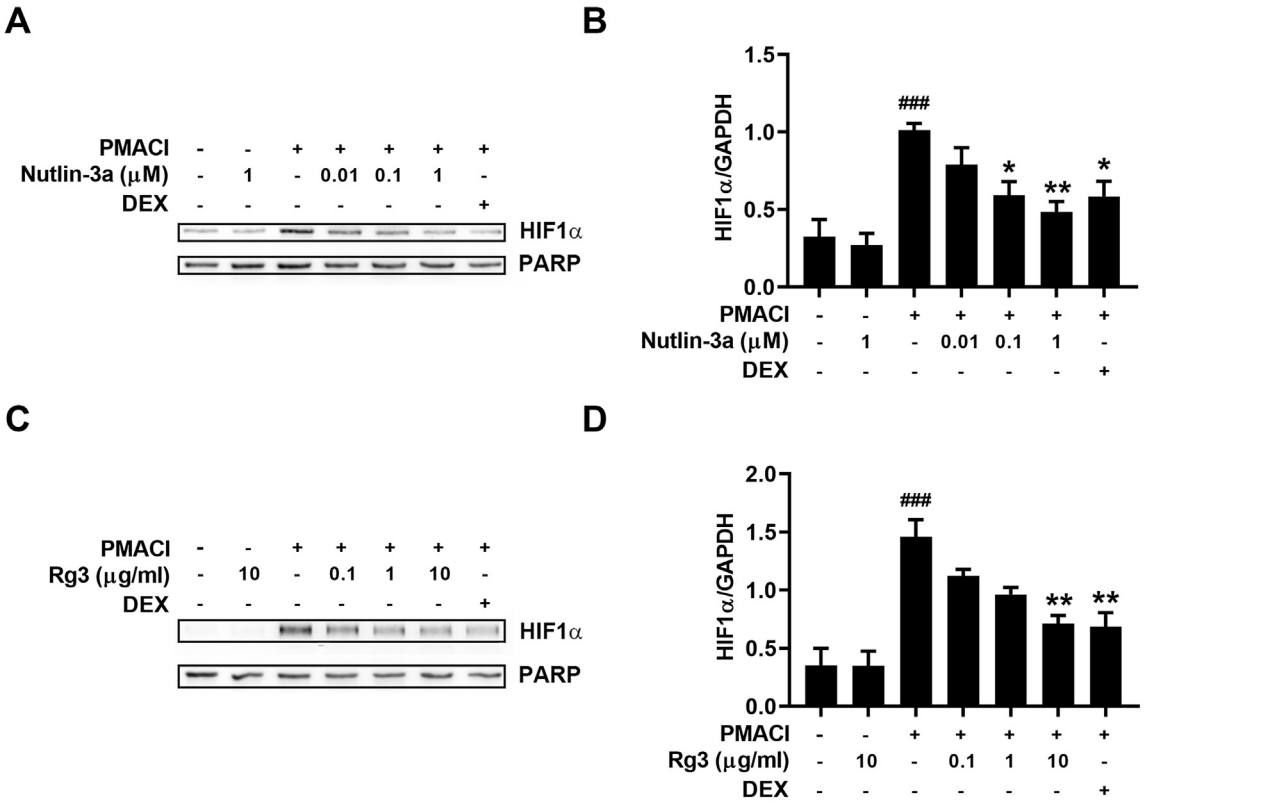

Fig. 3. Rg3 down-regulates HIF1α signaling pathways in HMC-1 cells stimulated with PMACI. HMC-1 cells (5×10^6) were pre-incubated with (A) nutlin-3a, (C) Rg3 or DEX (100 nM) for 1 h and then activated with PMACI for 4 h without media change. The expression levels of HIF1α and PARP in nuclear extraction were detected by immunoblot assay. (B,D) Quantification of relative levels was expressed as HIF1α/PARP. ###p < 0.001 considered significant compared to PMACI-inactivated group; *p < 0.05 and **p < 0.01 considered significant compared to PMACI-activated group.

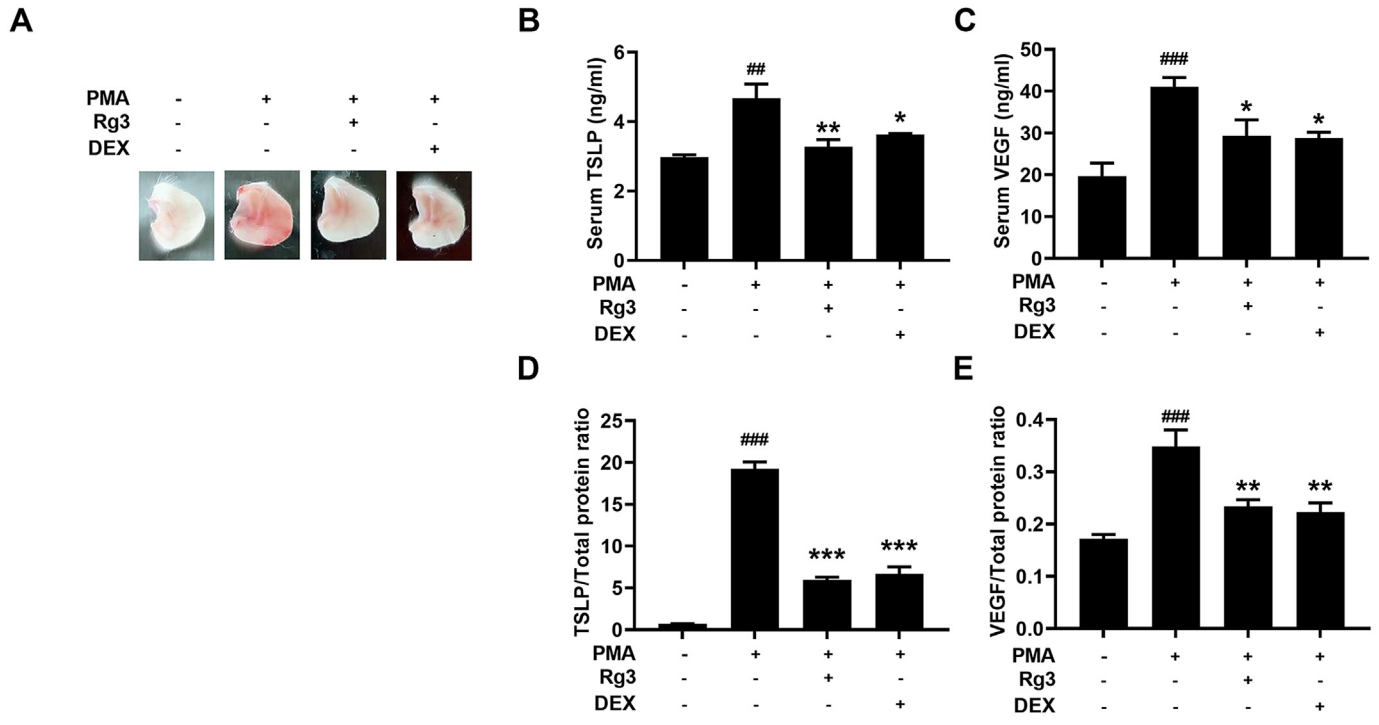

Fig. 4. Rg3 attenuates PMA-irritated ear edema. (A) Ears were photographed after analyzing the ear thickness. (B) TSLP and (C) VEGF levels in serum were measured by ELISA. (D) TSLP and (E) VEGF levels in the ear tissue homogenate were assessed by ELISA. The total protein levels were assayed with a bicinchoninic acid protein assay kit. ##p < 0.01 and ###p < 0.001 considered significant compared to blank group; *p < 0.05, **p < 0.01, and ***p < 0.001 considered significant compared to PMA control group.

Table 1
Quantification of the ear thickness before/after Rg3 treatment (n = 5/Group)

Treatment	Dose	Pre-thickness (mm)	Post-thickness (mm)	Increase (mm)
Blank	-	0.3410 ± 0.0034	0.3454 ± 0.0024	0.0044 ± 0.0015
PMA control	-	0.3414 ± 0.0015	0.4396 ± 0.0064	0.0982 ± 0.0064#
PMA + Rg3	10 mg/kg	0.3412 ± 0.0019	0.3910 ± 0.0054	0.0498 ± 0.0042 *
PMA + DEX	100 nM	0.3414 ± 0.0030	0.3932 ± 0.0051	0.0518 ± 0.0051 *

Blank, acetone-applied and PBS-administered mice; PMA control, PMA-irritated and PBS-administered mice; PMA + Rg3, PMA-irritated and Rg3-administered mice; PMA + DEX, PMA-irritated and DEX-administered mice. #p < 0.05 considered significant compared to blank group; *p < 0.05 considered significant compared to PMA control group.

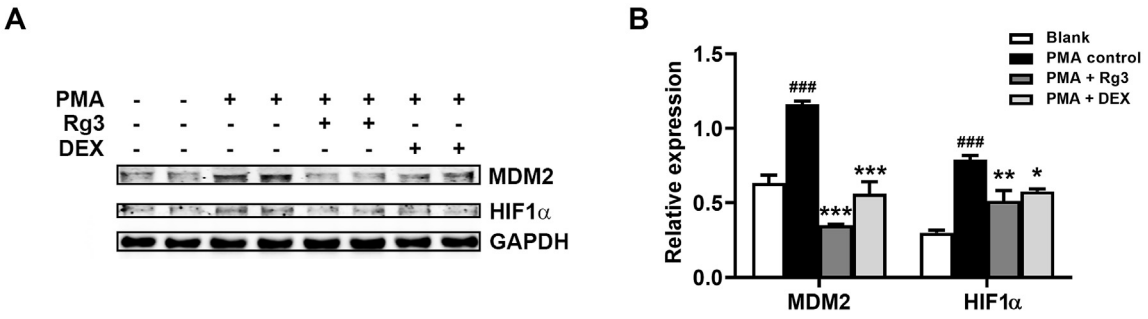

Fig. 5. Rg3 down-regulates MDM2 and HIF1α expression levels in PMA-irritated ear edema. (A) MDM2 and (C) HIF1α expression levels in the ear tissue homogenate were detected by immunoblot assay. (B,D) Quantification of relative levels was expressed as MDM2/GAPDH or HIF1α/GAPDH. ###p < 0.001 considered significant compared to blank group; *p < 0.05, **p < 0.01, and ***p < 0.001 considered significant compared to PMA control group.

and mRNA expression of TSLP and VEGF in HMC-1 cells (Fig. 1). Thus, we presume that Rg3 may be useful for us to prevent and/or treat skin inflammatory disorders.

In general, it has been known that NF-κB is a regulator of inflammation-related TSLP expression at transcription level [33,46]. MDM2 is an upstream factor of NF-κB. MDM2 transcriptionally up-regulates NF-κB expression and activates NF-κB-mediated gene expression [47]. Intradermal injection of MDM2 siRNA significantly suppressed TSLP mRNA and protein levels in the skin of DNFB-applied mice [48]. Jang and colleagues [18] suggested that HIF1α also is an important transcription factor of TSLP. MDM2 is an upstream factor of HIF1α [15]. In the present study, Rg3 inhibited activation of MDM2 and HIF1α in HMC-1 cells (Figs. 2 and 3). Thus, we postulate that Rg3 might regulate skin inflammatory reactions through MDM2/HIF1α signal pathway. Inflammatory stimuli (i.e., PMACI stimulation) increased the activation of MDM2 as well as HIF1α. Furthermore, an MDM2 inhibitor (nutlin-3a) dose-dependently inhibited HIF1α activation levels. To our knowledge, this is the first study showing new signal cascade (MDM2/HIF1α) for TSLP expression in mast cells.

PMA-induced ear edema model is a well-established *in vivo* model to confirm *in vitro* results in many studies [1,49–51]. Our findings presented that treatment with Rg3 ameliorated the ear swelling responses resulted from PMA irritation (Table 1) and reduced the levels of TSLP and VEGF as well as the activation of MDM2 and HIF1α in mice (Figs. 4 and 5). Thus, we assume that the antiinflammatory effects of Rg3 would be easily translated to the human skin inflammatory disease.

A recent study suggested that the no-observed-adverse-effect level (NOAEL) for Rg3 in dogs is 20 mg/kg [52]. Additionally, Li and colleagues [53] reported that the NOAEL value for Rg3 in rats is 180 mg/kg. Highest concentration of Rg3 in the present study is 10 mg/kg. Hence, we presume that 10 mg/kg of Rg3 would not be toxic to humans.

Conclusionally, we elucidated the beneficial effect and precise mechanism of Rg3 on TSLP production in activated mast cells and found that Rg3 suppressed the TSLP production through down-regulation of MDM2/HIF1α signaling pathway in an in vitro model of PMACI-stimulated HMC-1 cells and a PMA-induced mouse ear edema model of inflammation. Our findings provided the new mechanistic evidence about MDM2/HIF1α pathway in the antiinflammatory effect of Rg3, suggesting a new effective therapeutic strategy for the treatment of skin inflammatory diseases.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2021.06.008.

References

[1] Wu BC, Lee AH, Hancock REW. Mechanisms of the innate defense regulator peptide-1002 anti-inflammatory activity in a sterile inflammation mouse model. J Immunol 2017;199:3592–603.
[2] Lorz LR, Kim MY, Cho JY. Medicinal potential of Panax ginseng and its ginsenosides in atopic dermatitis treatment. J Ginseng Res 2020;44:8–13.
[3] Lorz LR, Kim D, Kim MY, Cho JY. Panax ginseng-derived fraction BIOGF1K reduces atopic dermatitis responses via suppression of mitogen-activated protein kinase signaling pathway. J Ginseng Res 2020;44:453–60.
[4] Mehrzadi S, Khalili H, Fatemi I, Malayeri A, Siahpoosh A, Goudarzi M. Zingerone mitigates carrageenan-induced inflammation through antioxidant and anti-inflammatory activities. Inflammation 2021;44:186–93.
[5] Nakashima C, Ishida Y, Kitoh A, Otsuka A, Kabashima K. Interaction of peripheral nerves and mast cells, eosinophils, and basophils in the development of pruritus. Exp Dermatol 2019;28:1405–11.

[6] Yu CH, Suh B, Shin I, Kim EH, Kim D, Shin YJ, Chang SY, Baek SH, Kim H, Bae ON. Inhibitory effects of a novel chrysin-derivative, CPD 6, on acute and chronic skin inflammation. Int J Mol Sci 2019;20:2607.
[7] Nakashima C, Ishida Y, Kitoh A, Otsuka A, Kabashima K. Interaction of peripheral nerves and mast cells, eosinophils, and basophils in the development of pruritus. Exp Dermatol 2019;28:1405–11.
[8] Dong J, Chen L, Zhang Y, Jayaswal N, Mezghani I, Zhang W, Veves A. Mast cells in diabetes and diabetic wound healing. Adv Ther 2020;37:4519–37.
[9] Young S, Sharma N, Lee JH, Chitu V, Neumeister V, Sohr E, Stanley ER, Hedrich CM, Craig AWB. Mast cells enhance sterile inflammation in chronic nonbacterial osteomyelitis. Dis Model Mech 2019;12:dmm040097.
[10] Schubert N, Dudeck J, Liu P, Karutz A, Speier S, Maurer M, Tuckermann J, Dudeck A. Mast cell promotion of T cell-driven antigen-induced arthritis despite being dispensable for antibody-induced arthritis in which T cells are bypassed. Arthritis Rheumatol 2015;67:903–13.
[11] Dudeck A, Dudeck J, Scholten J, Petzold A, Surianarayanan S, Köhler A, Peschke K, Vöhringer D, Waskow C, Krieg T, et al. Mast cells are key promoters of contact allergy that mediate the adjuvant effects of haptens. Immunity 2011;34:973–84.
[12] Jang H, Matsuda A, Jung K, Karasawa K, Matsuda K, Oida K, Ishizaka S, Ahn G, Amagai Y, Moon C, et al. Skin pH is the master switch of kallikrein 5-mediated skin barrier destruction in a murine atopic dermatitis model. J Invest Dermatol 2016;136:127–35.
[13] Moon PD, Han NR, Kim HM, Jeong HJ. High-fat diet exacerbates dermatitis through up-regulation of TSLP. J Invest Dermatol 2019;139:1198–201.
[14] Wong CK, Hu S, Cheung PF, Lam CW. Thymic stromal lymphopoietin induces chemotactic and prosurvival effects in eosinophils: implications in allergic inflammation. Am J Respir Cell Mol Biol 2010;43:305–15.
[15] Han NR, Moon PD, Kim HM, Jeong HJ. TSLP exacerbates septic inflammation via murine double minute 2 (MDM2) signaling pathway. J Clin Med 2019;8:1350.
[16] Ebrahim M, Mulay SR, Anders HJ, Thomasova D. MDM2 beyond cancer: podoptosis, development, inflammation, and tissue regeneration. Histol Histopathol 2015;30:1271–82.
[17] Patterson DM, Gao D, Trahan DN, Johnson BA, Ludwig A, Barbieri E, Chen Z, Diaz-Miron J, Vassilev L, Shohet JM, et al. Effect of MDM2 and vascular endothelial growth factor inhibition on tumor angiogenesis and metastasis in neuroblastoma. Angiogenesis 2011;14:255–66.
[18] Jang Y, Jeong SH, Park YH, Bae HC, Lee H, Ryu WI, Park GH, Son SW. UVB induces HIF-1α-dependent TSLP expression via the JNK and ERK pathways. J Invest Dermatol 2013;133:2601–8.
[19] Lee A, Yun E, Chang W, Kim J. Ginsenoside Rg3 protects against iE-DAP-induced endothelial-to-mesenchymal transition by regulating the miR-139-5p-NF-κB axis. J Ginseng Res 2020;44:300–7.
[20] Kee JY, Hong SH. Ginsenoside Rg3 suppresses mast cell-mediated allergic inflammation via mitogen-activated protein kinase signaling pathway. J Ginseng Res 2019;43:282–90.
[21] Siddiqi MH, Siddiqi MZ, Kang S, Noh HY, Ahn S, Simu SY, Aziz MA, Sathishkumar N, Jiménez Pérez ZE, Yang DC. Inhibition of osteoclast differentiation by ginsenoside Rg3 in RAW264.7 cells via RANKL, JNK and p38 MAPK pathways through a modulation of cathepsin K: an in silico and in vitro study. Phytother Res 2015;29:1286–94.
[22] Guo M, Xiao J, Sheng X, Zhang X, Tie Y, Wang L, Zhao L, Ji X. Ginsenoside Rg3 mitigates atherosclerosis progression in diabetic apoE-/- mice by skewing macrophages to the M2 phenotype. Front Pharmacol 2018;9:464.
[23] Zhang Y, Wang S, Song S, Yang X, Jin G. Ginsenoside Rg3 alleviates complete freund's adjuvant-induced rheumatoid arthritis in mice by regulating CD4+CD25+Foxp3+Treg cells. J Agric Food Chem 2020;68:4893–902.
[24] Zhang W, Wang X, Zhang M, Xu M, Tang W, Zhang Y, Lu L, Gao J, Gao S. Intranasal delivery of microspheres loaded with 20 (R)-ginsenoside Rg3 enhances anti-fatigue effect in mice. Curr Drug Deliv 2017;14:867–74.
[25] Zhang L, Zhang L, Wang X, Si H. Anti-adipogenic effects and mechanisms of ginsenoside Rg3 in pre-adipocytes and obese mice. Front Pharmacol 2017;8:113.
[26] Wei X, Su F, Su X, Hu T, Hu S. Stereospecific antioxidant effects of ginsenoside Rg3 on oxidative stress induced by cyclophosphamide in mice. Fitoterapia 2012;83:636–42.
[27] Yang L, Hao J, Zhang J, Xia W, Dong X, Hu X, Kong F, Cui X. Ginsenoside Rg3 promotes beta-amyloid peptide degradation by enhancing gene expression of neprilysin. J Pharm Pharmacol 2009;61:375–80.
[28] Moon PD, Lee JS, Kim HY, Han NR, Kang I, Kim HM, Jeong HJ. Heat-treated Lactobacillus plantarum increases the immune responses through activation of natural killer cells and macrophages on in vivo and in vitro models. J Med Microbiol 2019;68:467–74.
[29] Moon PD, Han NR, Lee JS, Hong S, Yoo MS, Kim HJ, Kim JH, Kang S, Jee HW, Kim HM, et al. Use of physcion to improve atopic dermatitis-like skin lesions through blocking of thymic stromal lymphopoietin. Molecules 2019;24:1484.
[30] Moon PD, Han NR, Lee JS, Kim HM, Jeong HJ. Ursolic acid downregulates thymic stromal lymphopoietin through the blockade of intracellular calcium/caspase-1/NF-κB signaling cascade in HMC-1 cells. Int J Mol Med 2019;43:2252–8.
[31] Han NR, Kim HY, Kang S, Kim MH, Yoon KW, Moon PD, Kim HM, Jeong HJ. Chrysophanol, an anthraquinone from AST2017-01, possesses the anti-proliferative effect through increasing p53 protein levels in human mast cells. Inflamm Res 2019;68:569–79.
[32] Achuthan A, Aslam ASM, Nguyen Q, Lam PY, Fleetwood AJ, Frye AT, Louis C, Lee MC, Smith JE, Cook AD, et al. Glucocorticoids promote apoptosis of proinflammatory monocytes by inhibiting ERK activity. Cell Death Dis 2018;9:267.
[33] Moon PD, Kim HM. Thymic stromal lymphopoietin is expressed and produced by caspase-1/NF-κB pathway in mast cells. Cytokine 2011;54:239–43.
[34] Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669–76.
[35] Thomasova D, Mulay SR, Bruns H, Anders HJ. p53-independent roles of MDM2 in NF-κB signaling: implications for cancer therapy, wound healing, and autoimmune diseases. Neoplasia 2012;14:1097–101.
[36] Farhang Ghahremani M, Goossens S, Nittner D, Bisteau X, Bartunkova S, Zwolinska A, Hulpiau P, Haigh K, Haenebalcke L, Drogat B, et al. p53 promotes VEGF expression and angiogenesis in the absence of an intact p21-Rb pathway. Cell Death Differ 2013;20:888–97.
[37] Choi IY, Kim SJ, Jeong HJ, Park SH, Song YS, Lee JH, Kang TH, Park JH, Hwang GS, Lee EJ, et al. Hesperidin inhibits expression of hypoxia inducible factor-1 alpha and inflammatory cytokine production from mast cells. Mol Cell Biochem 2007;305:153–61.
[38] Dellinger A, Zhou Z, Lenk R, MacFarland D, Kepley CL. Fullerene nanomaterials inhibit phorbol myristate acetate-induced inflammation. Exp Dermatol 2009;18:1079–81.
[39] Chen L, Deshpande M, Grisotto M, Smaldini P, Garcia R, He Z, Gulko PS, Lira SA, Furtado GC. Skin expression of IL-23 drives the development of psoriasis and psoriatic arthritis in mice. Sci Rep 2020;10:8259.
[40] Chelombitko MA, Chernyak BV, Fedorov AV, Zinovkin RA, Razin E, Paruchuru LB. The role played by mitochondria in FcεRI-dependent mast cell activation. Front Immunol 2020;11:584210.
[41] Moon PD, Kim HM. Anti-inflammatory effect of phenethyl isothiocyanate, an active ingredient of Raphanus sativus Linne. Food Chem 2012;131:1332–9.
[42] Hammad H, Lambrecht BN. Barrier epithelial cells and the control of type 2 immunity. Immunity 2015;43:29–40.
[43] Oyoshi MK, Venturelli N, Geha RS. Thymic stromal lymphopoietin and IL-33 promote skin inflammation and vaccinia virus replication in a mouse model of atopic dermatitis. J Allergy Clin Immunol 2016;138:283–6.
[44] Detmar M, Brown LF, Schön MP, Elicker BM, Velasco P, Richard L, Fukumura D, Monsky W, Claffey KP, Jain RK. Increased microvascular density and enhanced leukocyte rolling and adhesion in the skin of VEGF transgenic mice. J Invest Dermatol 1998;111:1–6.
[45] Scaldaferri F, Vetrano S, Sans M, Arena V, Straface G, Stigliano E, Repici A, Sturm A, Malesci A, Panes J, et al. VEGF-A links angiogenesis and inflammation in inflammatory bowel disease pathogenesis. Gastroenterology 2009;136.585-595.e5.
[46] Vu AT, Chen X, Xie Y, Kamijo S, Ushio H, Kawasaki J, Hara M, Ikeda S, Okumura K, Ogawa H, et al. Extracellular double-stranded RNA induces TSLP via an endosomal acidification- and NF-κB-dependent pathway in human keratinocytes. J Invest Dermatol 2011;131:2205–12.
[47] Gu L, Findley HW, Zhou M. MDM2 induces NF-kappaB/p65 expression transcriptionally through Sp1-binding sites: a novel, p53-independent role of MDM2 in doxorubicin resistance in acute lymphoblastic leukemia. Blood 2002;99:3367–75.
[48] Han NR, Oh HA, Nam SY, Moon PD, Kim DW, Kim HM, Jeong HJ. TSLP induces mast cell development and aggravates allergic reactions through the activation of MDM2 and STAT6. J Invest Dermatol 2014;134:2521–30.
[49] Moon PD, Han NR, Lee JS, Kim HM, Jeong HJ. Effects of linalyl acetate on thymic stromal lymphopoietin production in mast cells. Molecules 2018;23:1711.
[50] Moon PD, Kim HM. Suppression of thymic stromal lymphopoietin production by rutin in mast cells. Food Chem 2012;133:76–81.
[51] Li M, Zhang L, Cai RL, Gao Y, Qi Y. Lipid-soluble extracts from Salvia miltiorrhiza inhibit production of LPS-induced inflammatory mediators via NF-κB modulation in RAW 264.7 cells and perform antiinflammatory effects in vivo. Phytother Res 2012;26:1195–204.
[52] Gao Y, Wang G, Wang T, Li G, Lin J, Sun L, Wu X, Sun X, Wang H, Li C, et al. A 26-week 20(S)-ginsenoside Rg3 oral toxicity study in Beagle dogs. Regul Toxicol Pharmacol 2020;110:104522.
[53] Li C, Wang Z, Li G, Wang Z, Yang J, Li Y, Wang H, Jin H, Qiao J, Wang H, et al. Acute and repeated dose 26-week oral toxicity study of 20(S)-ginsenoside Rg3 in Kunming mice and Sprague-Dawley rats. J Ginseng Res 2020;44:222–8.