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Protection of ginsenoside Rg1 on chondrocyte from IL-1β-induced mitochondria-activated apoptosis through PI3K/Akt signaling

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Abstract Chondrocyte apoptosis is closely related to the development and progression of osteoarthritis. Ginsenoside Rg1 protects cells by antagonizing apoptosis. This study aimed to investigate the protective effect of Rg1 on interleukin 1β (IL-1β)-induced chondrocyte apoptosis and the underlying molecular mechanisms. Chondrocytes were harvested from the joints of 1-week-old Sprague–Dawley rats. After treated with 10 μg/mL Rg1 for 2 h, the chondrocytes were cultured with 10 ng/mL IL-1β to induce cytotoxicity. Cell viability was assessed with MTT assays. Annexin V/propidium iodide staining and terminal deoxynucleotidyl transferase dUTP nick-end labeling were used to detect chondrocyte apoptosis. The contents of total Akt, phosphorylated Akt (p-Akt), Bcl-2, Bax, and cytochrome *C* (Cyt *c*) were determined by Western blotting assay. A quantitative colorimetric assay was used to determine caspase-3 activity. Our present findings have shown that pre-treatment of chondrocytes with Rg1 reduces IL-1β induced cytotoxicity/apoptosis. Rg1 pretreatment also decreases the activity of IL-1β that reduces expression of Bcl-2 and level of p-Akt, and increases Bax activity, Cyt *c* release, and caspase-3 activation. It also reverses the activity of IL-1β that reduces the expression of tissue inhibitor of metalloproteinase-1 expression and increased the synthesis of matrix metalloproteinase-13, with the net effect of inhibiting extracellular matrix degradation. These results indicate that Rg1 may protect chondrocytes from IL-1β-induced apoptosis via the phosphatidylinositol 3-kinase/protein kinase B signaling pathway, through preventing caspase-3 release.

Keywords Ginsenoside Rg1 · Osteoarthritis · Chondrocytes · Apoptosis · Akt

Introduction

Osteoarthritis (OA) is a degenerative disease characterized by the progressive loss of articular cartilage and destruction of cartilage matrix. Pro-inflammatory cytokines such as interleukin-1β (IL-1β) play an important role in promoting OA lesions by inducing chondrocytes to secrete matrix metalloproteinases (MMPs), which degrade the extracellular matrix [1] and facilitate chondrocyte apoptosis [2]. Reported studies have shown that chondrocyte loss and cartilage tissue degeneration induced by excessive chondrocyte apoptosis are among the main causes of OA [3].

Apoptosis is an autonomous programmed cell death process regulated by multiple signaling pathways. One of the important pathways during the apoptotic process involves in the activation of caspase-3, and then induces hydrolysis of nucleic acids and cytoskeletal proteins [4]. In vitro studies have confirmed that caspase-3 activity is significantly increased in IL-1β-induced chondrocyte apoptosis [5]. Importantly, caspase-3 is activated by cytochrome *c* (Cyt *c*) that is induced by apoptotic signals [6, 7]. These signaling events are set in motion by the pro-apoptotic protein Bax, a member of the Bcl-2 family, which migrates to the mitochondrial membrane and induces Cyt

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c release. Therefore, inhibiting the mitochondrial pathway that can activate caspase-3 is of major importance for cell apoptosis inhibition. This can be achieved by Bcl-2 via binding to and inhibiting Bax [8].

The phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway is considered important for inhibiting chondrocyte apoptosis [9]. Phosphorylated PI3K/Akt promotes Bcl-2 release in chondrocytes, thus inhibiting Bax activity and preventing cell apoptosis [10, 11]. Therefore, PI3K/Akt signaling may effectively reduce mitochondrial pathway-induced cell apoptosis.

Ginsenoside is a medicinal ingredient extracted from ginseng, and Rg1 is among the most important and active ingredients in various ginsenosides. It was recently demonstrated that Rg1 protects against neuronal apoptosis induced by 6-hydroxydopamine (6-OHDA), hydrogen peroxide, and β-amyloid [12–14]. Further studies revealed that PI3K/Akt signaling is an important neuroprotective mechanism of Rg1 [12, 15]. Others have reported that Rg3, Bb3, and Rd, all of which have a similar structure to that of Rg1, suppress MMP secretion and inhibit cartilaginous matrix degeneration [16, 17]. However, it remains unclear whether Rg1 can inhibit chondrocyte apoptosis, or reduce cartilaginous matrix degeneration through activating PI3K/Akt signaling.

In this study, we investigated whether Rg1 could inhibit chondrocyte apoptosis and suppress the secretion of matrix-degrading enzymes by in an in vitro model of rat chondrocyte apoptosis. We also explored the mechanism of the protective role of Rg1 on chondrocytes by studying changes in PI3K/Akt and mitochondrial signaling pathways.

Materials and methods

Collection, isolation, and culture of rat articular chondrocytes

The cartilage of 1-week-old Sprague–Dawley rats was harvested and minced before being digested with 0.25 % trypsin (Gibco, Grand Island, NY, USA). The trypsin was then removed, and the cartilage was washed with phosphate-buffered saline (PBS) three times, after which 0.2 % collagenase II (Gibco) was added for digestion at 37 °C for 4–5 h. A 200-μm mesh strainer was used to filter the above solution, and the cells were collected by centrifugation. The cells were then cultured in Dulbecco's modified Eagle's medium (DMEM)-F12 medium (Gibco) supplemented with 10 % fetal bovine serum (Gibco) and 1–2 % penicillin/streptomycin (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd, Hangzhou, China) and incubated with 5 % CO_2 at 37 °C. The F1 generation of chondrocytes was used for the experiments in this report.

Experimental grouping

The cells were divided into seven groups and treated with Rg1 in following concentrations: 0, 0.001, 0.01, 0.1, 1, 10, or 100 μg/mL. MTT [3-(4,5-dimethylthiazol -2-yl)-2,5-diphenytetrazolium bromide] assays were performed 72 h after incubation.

To investigate the protective effect of Rg1 on IL-1β-induced cytotoxicity, cells were incubated with Rg1 for 2 h, after which 10 ng/mL IL-1β was added. Cells in the control group were cultured without any treatment. The MTT assay was performed to detect cell viability after 72 h.

For flow cytometry and TUNEL experiments, the cells were divided into five groups: control, cells were cultured without any treatment; IL-1β, cells cultured for 96 h with 10 ng/mL IL-1β; Rg1 alone, cells cultured for 96 h with 10 μg/mL Rg1 without IL-1β; Rg1, cells cultured for 2 h with 10 μg/mL Rg1 followed by 96 h with 10 ng/mL IL-1β; and inhibition, in which the cells were incubated with 25 μmol/mL LY294002 and 10 μg/mL Rg1 for 2 h followed by 96 h with 10 ng/mL IL-1β. For protein expression level and caspase-3 activity assay by Western blotting, the cells were treated by IL-1β in five groups for 24 h.

MTT assay

Cell viability was assessed with MTT assays. Briefly, chondrocytes were plated in a 96-well plate and cultured for 12 h in serum-free DMEM. After appropriate treatment according to the experimental grouping, cells were incubated with MTT (Sigma, St. Louis, MO, USA) at a final concentration of 0.5 mg/mL at 37 °C for 4 h. The absorbance of each well was determined at a wavelength of 490 nm with a reference wavelength of 630 nm using a Dynatech MR5000 plate reader (Dynatech, Chantilly, VA, USA).

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining

TUNEL assays were performed to assess chondrocyte apoptosis. Chondrocytes were plated in a 24-well plate and cultured for 12 h in serum-free DMEM. After appropriate treatment according to the experimental grouping, cell samples were fixed with 4 % paraformaldehyde and incubated with 3 % H_2O_2 in methanol for 10 min. Then, the samples were incubated with 0.1 % Triton X-100 on ice for 2 min. After two washes in PBS, 50 μL mixed TUNEL (Roche, Indianapolis, IN, USA) was added to each well for incubation at 37 °C for 1 h. The cells were then washed with PBS three times, after which 4′,6-diamidino-2-phenylindole (DAPI; Beyotime, Haimen, China) was added.

Cells were observed under an inverted fluorescence microscope (Olympus, Tokyo, Japan) at 200× magnification. Randomly selected fields were photographed, and representative pictures were chosen to count apoptotic cells and total cells. The rate of TUNEL-positive cells in each field was calculated.

Protein extraction

Total protein was extracted as follows: treated samples were collected and washed twice with pre-cooled PBS. After the PBS was removed, protein extraction reagents containing protease inhibitors (Beyotime) were added. The lysate was collected and centrifuged at 14,000×g for 15 min at 4 °C. The protein-containing supernatant was then divided and stored at −20 °C.

Mitochondrial and cytosolic proteins were extracted according to the mitochondria isolation kit instructions (Pierce, Rockford, IL, USA). Briefly, cells were incubated with 800 μL mitochondria isolation reagent A and then homogenized on ice in a Dounce homogenizer. Unlysed cells and large debris were pelleted by centrifugation at 700×g for 10 min at 4 °C. The supernatant was further centrifuged at 12,000×g for 15 min at 4 °C. The supernatant (cytosolic fraction) was collected, and the pellet (mitochondrial fraction) was lysed in 2 % CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate) in Tris-buffered saline (TBS) (25 mM Tris, 0.15 M NaCl, pH 7.2).

Western blotting

Protein mixtures were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were incubated with appropriate antibodies (Cell Signaling Technology, Danvers, MA, USA) overnight at 4 °C. The blots were then incubated with secondary antibody (Cell Signaling Technology) and detected using enhanced chemiluminescence. The results were scanned using a gel imaging system (UVP Company, Upland, CA, USA), and densitometry measurements were performed with Image lab software (Bio-Rad Laboratories, Hercules, CA, USA).

Flow cytometry

Apoptotic cell analysis was also performed after annexin V and propidium iodide (PI) staining of the cells by flow cytometry according to the manufacturer's protocol (BD PharMingen, San Diego, CA, USA). After treatment, 1×10^6 cells were harvested and washed three times with PBS, then resuspended in binding buffer followed by Annexin V/PI labeling at room temperature for 15 min in the dark. The samples were analyzed using a FACScan flow cytometer (BD Biosciences, San Jose, CA, USA).

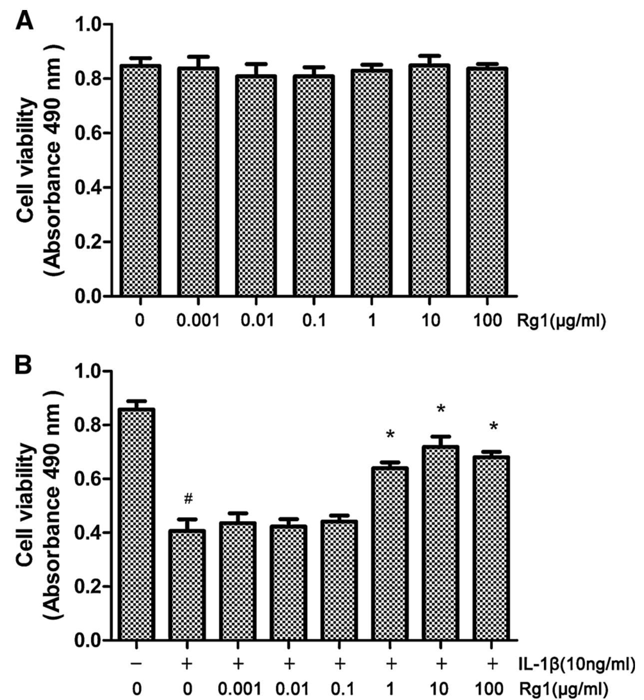

Fig. 1 Effect of Rg1 on chondrocyte viability and IL-1β-induced damage. The viabilities of cells in different treatment groups were assessed using MTT assay. **a** Cells were treated with 0.001, 0.01, 0.1, 1, 10, or 100 μg/mL of Rg1 for 72 h, and cells in the control group were cultured without any treatment. **b** Rg1 (0, 0.001, 0.01, 0.1, 1, 10, or 100 μg/mL) was added for 2 h prior to 72-h treatment with 10 ng/mL IL-1β. All data are shown as mean ± SEM obtained from five separate experiments performed in triplicate. *Statistically significant difference ($P \leq 0.05$) versus the IL-1β group. #Statistically significant results ($P \leq 0.05$) versus control

Caspase-3 activity assay

Caspase-3 activity was measured using a caspase-3 colorimetric assay kit (Kaiji Biological Engineering Materials Co., Ltd, Nanjing, China) according to the manufacturer's instructions. Briefly, cells were collected and lysed using the lysis buffer provided. The caspase-3 activity colorimetric assay is based on hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp-p-nitroaniline by caspase-3, resulting in the release of *p*-nitroaniline moiety, which has a high absorbance at 405 nm that was detected by a plate reader. Caspase-3 activity in the experimental groups was normalized to the control group.

Statistical analysis

Data were analyzed using SPSS 18.0 statistical software (SPSS Inc., Chicago, IL, USA). Results are shown as mean ± SEM unless stated otherwise. One-way analysis of

variance followed by Dunnett's multiple comparisons and Student's unpaired t tests were used for the statistical analyses. $P < 0.05$ was considered statistically significant.

Results

Effect of Rg1 on chondrocyte viability and IL-1β-induced damage

To study whether Rg1 has any potential cytotoxic effect on chondrocytes, we used MTT assays to examine the cell viability upon Rg1 treatment. Cells were cultured with Rg1 in the following series of concentration: 0, 0.001, 0.01, 0.1, 1, 10, or 100 μg/mL. The cytotoxic effect of Rg1 was detected at 72 h after incubation. There were no significant differences in absorbance among the treated groups (Fig. 1a), indicating that Rg1 had no detectable cytotoxic effects on chondrocytes at the above concentrations ($P > 0.05$).

We then studied the protective effect of Rg1 on IL-1β-induced cytotoxicity. Cells were pretreated with Rg1 (0, 0.001, 0.01, 0.1, 1, 10, or 100 μg/mL) for 2 h, followed by incubation with 10 ng/mL IL-1β. MTT assays were performed 72 h later. Cell viability was significantly decreased by IL-1β (Fig. 1b), and IL-1β-induced cell damage was visibly reduced by the addition of Rg1 with its concentration in the range of 1, 10, or 100 μg/mL ($P < 0.05$). The protective effect in the three concentrations of Rg1 has no significant difference, although the group with 10 μg/mL of Rg1 has shown relatively stronger effect (Fig. 1b). Thus, 10 μg/ml of Rg1 was used in the following experiments.

Antagonistic effect of Rg1 on chondrocyte apoptosis

To study whether IL-1β induced cytotoxicity was mediated by apoptotic process, we used TUNEL and flow cytometry assays to assess chondrocyte apoptosis. Chondrocytes were pre-treated with Rg1 (10 μg/mL) with or without LY294002 for 2 h before adding IL-1β (10 ng/mL) to treat the cells for 96 h. For a negative control group without Rg1 pretreatment, the cells were treated with IL-1β alone and cultured for 96 h. As shown in Fig. 2a, bright green apoptotic cells with condensed chromatin were observed using DAPI and TUNEL staining. The results showed that IL-1β significantly increased the percentage of TUNEL-positive cells compared to that in the control group ($P < 0.05$). Figure 2b showed that pretreatment with 10 μg/mL Rg1 significantly reduced the percentage of IL-1β induced apoptotic chondrocytes. Flow cytometry after annexin V/PI staining was also used to detect chondrocyte apoptosis. The trend of the results was consistent with that of TUNEL staining. The percentage of apoptotic chondrocytes in the Rg1 pretreated groups was significantly decreased compared to that in the IL-1β alone group ($P < 0.05$) (Fig. 2a).

Rg1 reduced IL-1β-induced chondrocyte apoptosis via PI3K/Akt signaling

To further investigate the molecular mechanism through which Rg1 protects chondrocytes from IL-1β-induced apoptosis, Western blot analyses were performed to study changes in PI3K/Akt signaling. As shown in Fig. 3, p-Akt levels were significantly decreased after 24 h with IL-1β stimulation compared to the control group ($P < 0.05$). Notably, 2 h pretreatment with 10 μg/mL Rg1 significantly increased p-Akt expression in IL-1β-stimulated chondrocytes ($P < 0.05$). Total Akt levels were unchanged.

To further explore the effect of PI3K/Akt signaling in Rg1-mediated protection of IL-1β-induced chondrocyte apoptosis, the PI3K-specific inhibitor LY294002 was added together with Rg1. LY294002 blocked the Rg1 mediated increase of p-Akt (Fig. 3), and also reduced the Rg1 suppression on chondrocyte apoptosis induced by IL-1β (Fig. 2a, b). No direct effect of Rg1 on Akt phosphorylation was detected. Collectively, these results support the hypothesis that the protective effects of Rg1 may be achieved via enhanced PI3K/Akt signaling.

Rg1 protects against IL-1β-induced chondrocyte apoptosis by inhibiting the mitochondrial apoptosis pathway and reducing caspase-3 activation

To explore the role of the mitochondrial apoptosis pathway on Rg1-mediated protection against IL-1β-induced chondrocyte apoptosis, expression levels of the Bcl-2 family proteins Bcl-2 and Bax were detected with Western blotting analysis. As shown in Fig. 4a, compared to control cells, expression of the anti-apoptotic protein Bcl-2 was significantly reduced while that of Bax was significantly increased in the IL-1β group ($P < 0.05$). Similar results were obtained in cells pretreated with LY294002 and Rg1 before IL-1β ($P < 0.05$) (Fig. 4a). When 10 μg/mL Rg1 was added prior to IL-1β, Bcl-2 expression was significantly higher than that in the IL-1β group and Bax expression was significantly lower ($P < 0.05$) (Fig. 4a). In addition, we investigated Cyt c release from mitochondria into the cytosol. Figure 4a shows that after IL-β stimulation, significantly increased Cyt c was released compared to the control group ($P < 0.05$), and this release was obviously reduced by the addition of Rg1 ($P < 0.05$). To a certain extent, LY294002 blocked the inhibitory effect of Rg1 on IL-1β-mediated Cyt c release. Cells treated with Rg1 alone did not show any change in these signals compared to control.

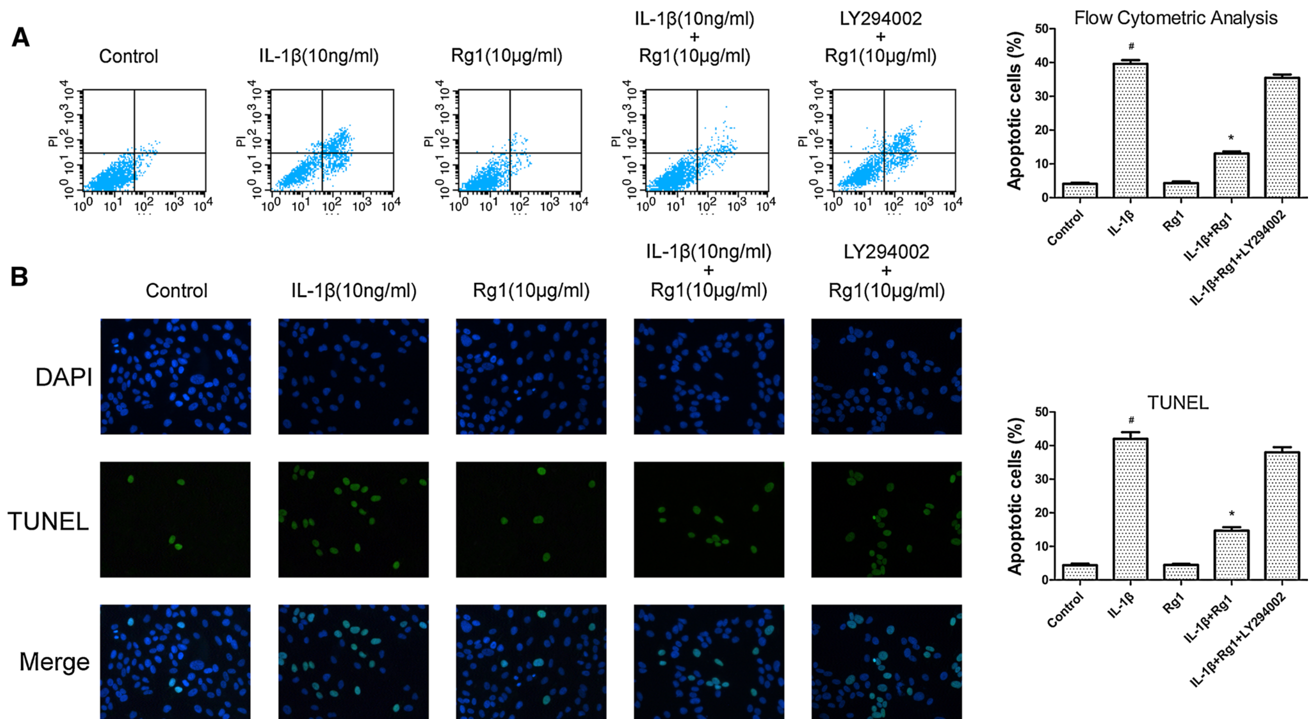

Fig. 2 Antagonistic effect of Rg1 on chondrocyte apoptosis. Chondrocytes were treated with 10 μg/mL of Rg1 with or without LY294002 for 2 h prior to 96-h treatment with 10 ng/mL IL-1β. **a** Annexin V/PI staining and flow cytometry assays, and **b** TUNEL labeling and DAPI staining were employed to assess chondrocyte apoptosis. All data are mean ± SEM obtained from five separate experiments performed in triplicate. *Statistically significant difference ($P \leq 0.05$) versus cells subjected to IL-1β treatment alone. #Statistically significant difference ($P \leq 0.05$) versus control

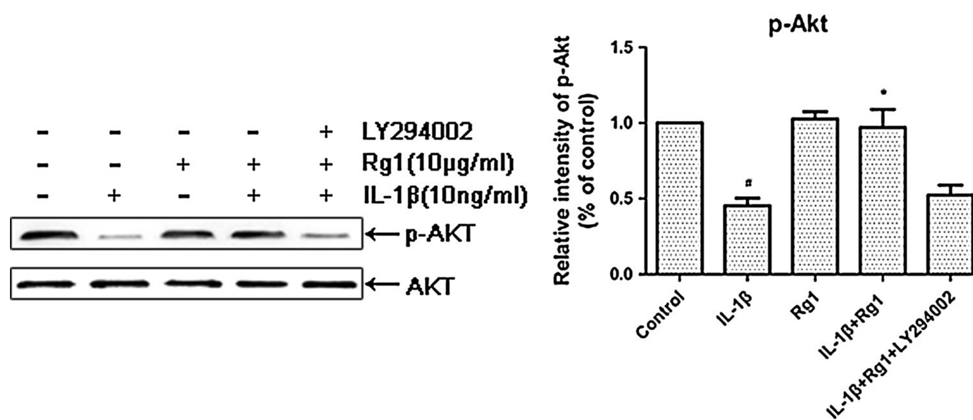

Fig. 3 Rg1 reduced IL-1β-induced chondrocyte apoptosis via PI3K/AKT signaling. Chondrocytes were treated with 10 μg/mL Rg1 or 10 μg/mL Rg1 + LY294002 for 2 h before 24-h IL-1β (10 ng/mL) treatment. Total Akt and p-Akt were detected by western blot. p-Akt levels were normalized to Akt levels. The histograms show the fold change compared with control. All data are shown as mean ± SEM obtained from five separate experiments performed in triplicate. *Statistically significant difference ($P \leq 0.05$) versus cells treated with IL-1β alone. #Statistically significant difference ($P \leq 0.05$) versus control

A caspase-3 fluorometric assay kit was used to detect changes in caspase-3 activity. Rg1 treatment alone did not affect the caspase-3 activity. However, as shown in Fig. 4b, addition of Rg1 significantly inhibited IL-1β induced caspase-3 activation, suggesting that it may protect chondrocyte apoptosis. This effect was partly attenuated by LY294002, indicating that Rg1 exerts its effects via increased PI3K/Akt signaling.

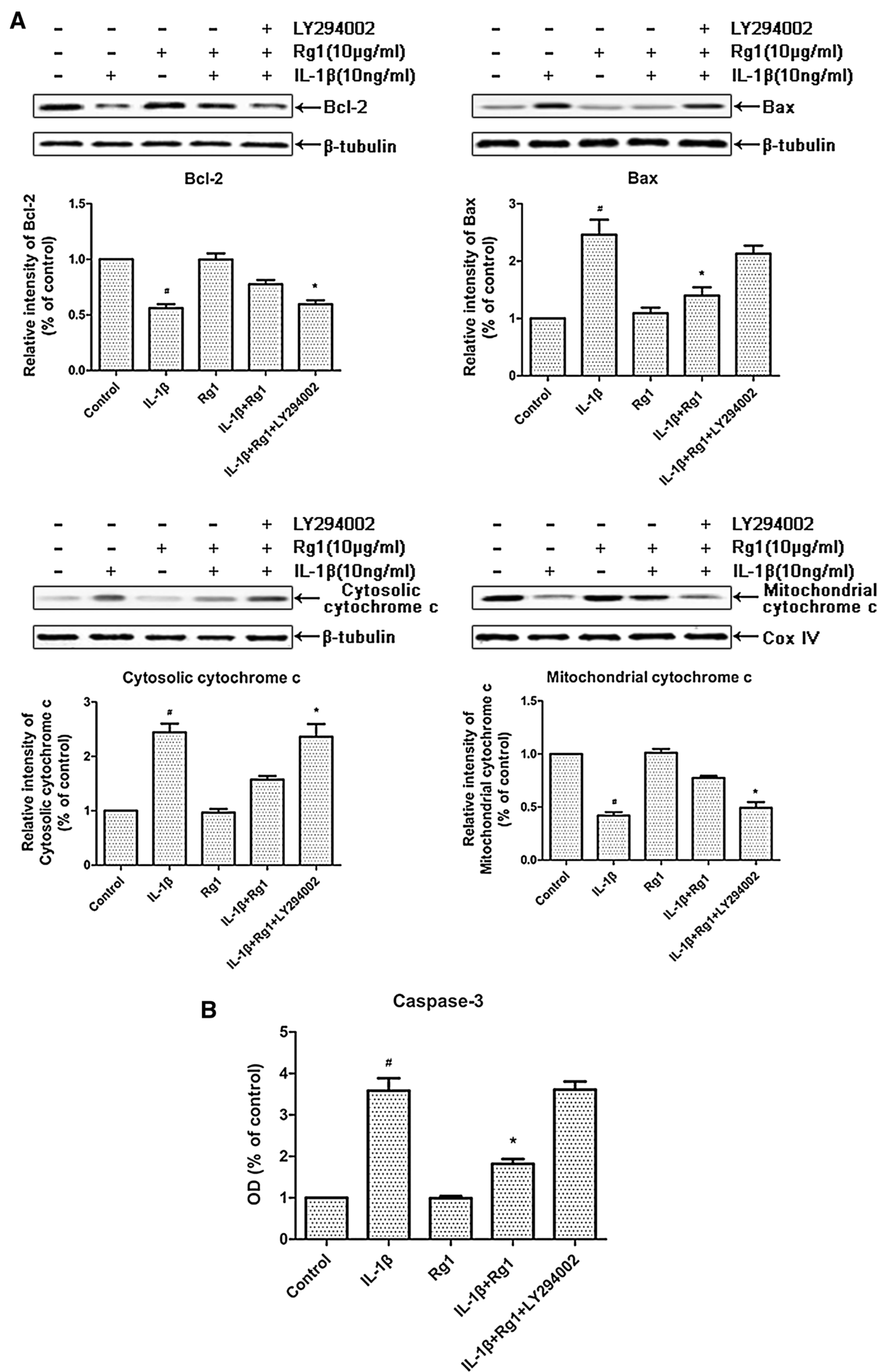

Fig. 4 Protective effect of Rg1 against IL-1β-induced chondrocyte apoptosis via mitochondrial apoptosis pathway inhibition and caspase-3 activation reduction. Chondrocytes were treated with 10 μg/mL Rg1 or 10 μg/mL Rg1 + LY294002 for 2 h prior to 24-h treatment with 10 ng/mL IL-1β. **a** Bcl-2, Bax, and Cyt *c* were detected with western blots. β-tubulin was used as internal reference for Bcl-2, Bax, and cytoplasmic Cyt c, and COX IV was used as the internal reference for mitochondrial Cyt c. Bcl-2, Bax, and Cyt *c* contents were normalized to β-tubulin and COX IV. Histograms show the fold change of each group compared with control. **b** Changes in caspase-3 activity were measured by spectrophotometry. The data are shown as mean ± SEM obtained from five separate experiments performed in triplicate. *Statistically significant difference ($P \leq 0.05$) versus cells treated with IL-1β alone. #Statistically significant difference ($P \leq 0.05$) versus control

Rg1 promoted tissue inhibitor of metalloproteinase-1 (TIMP-1) expression and inhibited matrix metalloproteinase-13 (MMP-13) synthesis

The balance between TIMP and 1–MMP-13 is vital for appropriate synthesis and degradation of the extracellular matrix of chondrocyte and hence their viability. To examine whether the protective effect of Rg1 pretreatment was due to the regulation of the balance of two proteins, we examined their expression level with above experimental system. As shown in Fig. 5, Western blotting assay revealed that, compared to the IL-β group, Rg1 pretreatment significantly promoted TIMP-1 expression and inhibited IL-1β-induced MMP-13 secretion. Again, LY294002 partially blocked the effects of Rg1 (Fig. 5), indicating that PI3K/Akt signaling was involved in the Rg1 regulated protein expression. Consistently, no direct effect of Rg1 on TIMP-1 and MMP-13 expression was detected.

Discussion

Ginsenoside Rg1 has shown therapeutic effect on nervous system diseases and cardiovascular diseases [14, 18]. However, the effects of Rg1 in OA have not yet been reported. Our present findings indicate that pre-treatment with Rg1 in IL-1β induced apoptosis of chondrocyte promotes Bcl-2 expression, inhibits Bax activity, and reduces both Cyt *c* release and caspase-3 activation. It also enhanced TIMP-1 expression and inhibited MMP-13 synthesis, with the net effect of inhibiting extracellular matrix degradation. These effects by Rg1 are partially mediated via enhanced PI3K/Akt signaling.

Pro-inflammatory cytokines such as IL-1β can induce chondrocyte apoptosis in rats and humans, and this is closely related to the occurrence and development of OA [19]. Previous studies have demonstrated that Rg1 effectively reduces the pain response caused by IL-1β and regulates IL-1β-induced changes in feeding behaviors, indicating that Rg1 may inhibit IL-1β-induced biological effects [20, 21]. Gong et al. [22] and Yan et al. [23] reported that Rg1 antagonized β-amyloid induced apoptosis of hippocampal neurons and endothelial cells. The present study provides first hand evidence that Rg1 (10 μg/mL) significantly reduced IL-1β-induced chondrocyte apoptosis and thus Rg1 may play a protective role against IL-1β related occurrence and development of OA.

Earlier studies have indicated that the PI3K/Akt pathway promoted chondrocyte survival and extracellular matrix synthesis [9, 24]. Further studies also confirmed that inhibited Akt phosphorylation was an important mechanism of 4-hydroxynonenal-induced chondrocyte apoptosis and that LY294002 blocked the effect of insulin-like growth factor-1 and caused a significant increase in

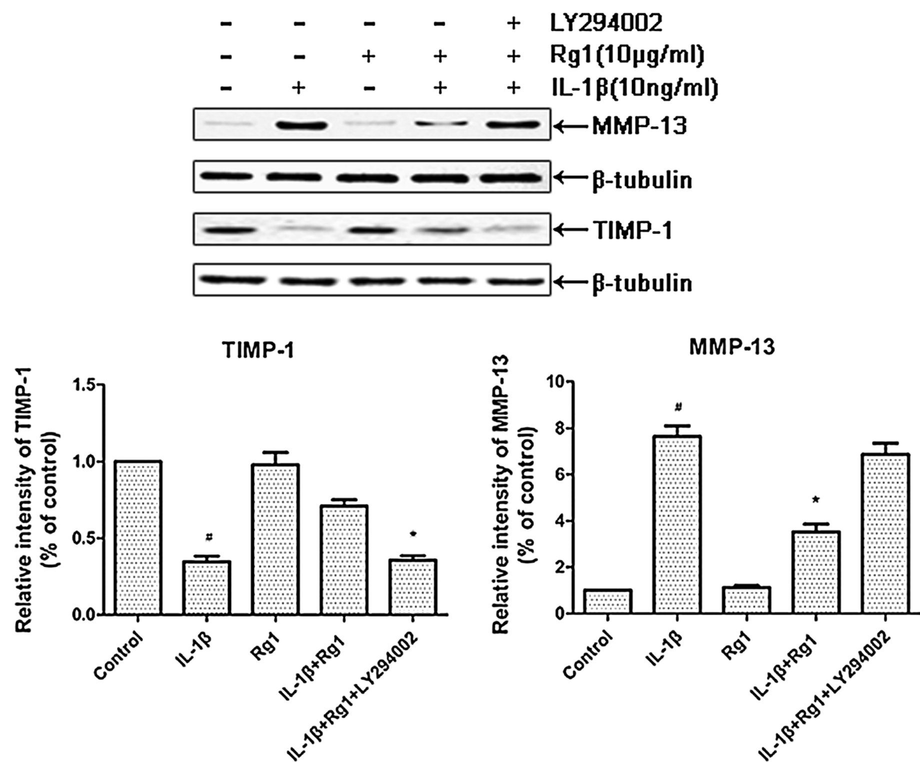

Fig. 5 Rg1 promoted TIMP-1 expression and inhibited MMP-13 synthesis. Chondrocytes were treated with 10 μg/mL Rg1 and 10 μg/mL Rg1+LY294002 for 2 h prior to 24-h treatment with 10 ng/mL IL-1β. TIMP-1 and MMP-13 expressions were detected by western blot, and β-tubulin was used as the internal reference. TIMP-1 and MMP-13 contents were normalized to β-tubulin. Histograms show the fold change compared with control. All data are shown as mean ± SEM obtained from five separate experiments performed in triplicate. *Statistically significant difference ($P \leq 0.05$) compared with cells treated with IL-1β alone. #Statistically significant difference ($P \leq 0.05$) compared with control

chondrocyte apoptosis [11, 25]. In this study, we found that pretreatment of Rg1 reverse the effects of IL-1β on Akt phosphorylation inhibition and chondrocyte apoptosis. Furthermore, the anti-apoptotic effect of Rg1 was significantly decreased by the PI3K-specific inhibitor LY294002. Both findings were consistent with the published work. Therefore, the collective evidence indicates that PI3K/Akt signaling is an important molecular target of OA and that Rg1 may have a potential therapeutic role via this signaling pathway.

The release of Cyt *c* due to decreased mitochondrial function can lead to a series of signaling cascades culminating in chondrocyte apoptosis in OA. An appropriate Bcl-2/Bax balance is a key to mitochondrial function [26]. P-Akt can activate one of its downstream substrates, Bad, which depolymerizes with Bcl-2, resulting in the release of Bcl-2 and subsequent inhibition of apoptosis [27]. Lee et al. [10] found that *Clematis mandshurica* promoted Akt phosphorylation and inhibited Bad activity, which increased the Bcl-2/Bax ratio and inhibited chondrocyte apoptosis. Our present results demonstrated that Rg1 pretreatment on IL-1β induced apoptosis increased the Bcl-2/Bax ratio and inhibited Cyt *c* release from mitochondria to the cytosol, indicating that Rg1 may decrease mitochondrial permeability in chondrocytes to inhibit Cyt *c* release and block IL-1β induced apoptosis. Interestingly, a previous work in vascular endothelial cells revealed that the ginseng extract Rg3 inhibited cell apoptosis by activating the Akt/Bad pathway with decreased Bcl-2/Bax ratio [28], suggesting that different ginsenoside components exert different anti-apoptotic mechanisms.

Caspase-3 is a member of the downstream Cyt *c* signaling pathway and the most important executor of cell apoptosis. One previous study reported that 17β-estradiol activated the PI3K/Akt pathway and inhibited the mitochondrial pathway, ultimately inhibiting caspase-3 activation and chondrocyte apoptosis [29, 30]. Therefore, inhibition of caspase-3 activation may be the key to decrease chondrocyte apoptosis. Our work here indicated that Rg1 can maintain mitochondrial function and inhibit caspase-3 activation induced by IL-1β. Supported by a similar inhibitory role of Rg1 with another ginseng extract Rb1 on caspase-3 activation by activating Akt [31], this may be one of the most important anti-apoptotic mechanisms for Rg1.

In addition to inhibiting chondrocyte apoptosis, drugs used to treat OA are anticipated for promoting chondrocyte matrix synthesis. Previous study has shown that TIMP-1 blocked MMP-13 activity and inhibited extracellular matrix degradation [32]. Recent studies indicate that chondrocyte expression of TIMP-1 and MMP-13 is regulated by PI3K/Akt signaling [33]. The current study revealed that Rg1 pretreatment in IL-1β induced cytotoxicity promoted TIMP-1 expression but inhibited MMP-13 expression. Addition of the PI3K-specific inhibitor LY294002 further confirmed that PI3K/Akt signaling plays an important role during this process, a finding that is consistent with the results reported by Zheng et al. [33]. Thus, we speculate that as a target of Rg1, Akt can both inhibit chondrocyte apoptosis and reduce extracellular matrix degradation.

There are several limitations to our study. Notably, all of our experiments were performed in a cell-based in vitro system. It remains to be determined whether equivalent effects would be observed consistently in the in vivo model and the patients. Furthermore, all our data were acquired with Rg1 pretreatment experiments, which seems essential for permitting the protective role of the compound in both our own and reported studies [13, 34]. It would be helpful in the future work to examine whether the pre-treated cells have altered signaling pathway in IL-1β and other biological process in order to better prepare the cell against apoptotic treatment.

In conclusion, our findings demonstrate that Rg1 inhibited IL-1β-induced chondrocyte apoptosis and promoted TIMP-1 expression and inhibited MMP-13 expression via its effects on PI3K/Akt/mitochondrial signaling pathways. Collectively, these results indicate that Rg1 has a potential therapeutic role for OA treatment.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Aida Y, Maeno M, Suzuki N, Shiratsuchi H, Motohashi M, Matsumura H (2005) The effect of IL-1beta on the expression of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in human chondrocytes. Life Sci 77:3210–3221. doi:10.1016/j.lfs.2005.05.052
2. Zhou PH, Liu SQ, Peng H (2008) The effect of hyaluronic acid on IL-1beta-induced chondrocyte apoptosis in a rat model of osteoarthritis. J Orthop Res 26:1643–1648. doi:10.1002/jor.20683
3. Kim HA, Blanco FJ (2007) Cell death and apoptosis in osteoarthritic cartilage. Curr Drug Targets 8:333–345
4. Li J, Yuan J (2008) Caspases in apoptosis and beyond. Oncogene 27:6194–6206. doi:10.1038/onc.2008.297
5. Ju XD, Deng M, Ao YF, Yu CL, Wang JQ, Yu JK, Cui GQ, Hu YL (2010) The protective effect of tetramethylpyrazine on cartilage explants and chondrocytes. J Ethnopharmacol 132:414–420. doi:10.1016/j.jep.2010.08.020
6. Antonsson B (2001) Bax and other pro-apoptotic Bcl-2 family "killer-proteins" and their victim the mitochondrion. Cell Tissue Res 306:347–361. doi:10.1007/s00441-001-0472-0
7. Fan TJ, Han LH, Cong RS, Liang J (2005) Caspase family proteases and apoptosis. Acta Biochim Biophys Sin (Shanghai) 37:719–727

8. Suen DF, Norris KL, Youle RJ (2008) Mitochondrial dynamics and apoptosis. Genes Dev 22:1577–1590. doi:10.1101/gad.1658508
9. Montaseri A, Busch F, Mobasheri A, Buhrmann C, Aldinger C, Rad JS, Shakibaei M (2011) IGF-1 and PDGF-bb suppress IL-1beta-induced cartilage degradation through down-regulation of NF-kappaB signaling: involvement of Src/PI-3 K/AKT pathway. PLoS One 6:e28663. doi:10.1371/journal.pone.0028663
10. Lee SW, Lee HJ, Moon JB, Choi SM, Kim DK, Kim IR, Choi WC, Park BS (2008) Purified extract from Clematis mandshurica prevents adenoviral-TRAIL induced apoptosis on rat articular chondrocytes. Am J Chin Med 36:399–410. doi:10.1142/s0192415x08005850
11. Vaillancourt F, Fahmi H, Shi Q, Lavigne P, Ranger P, Fernandes JC, Benderdour M (2008) 4-Hydroxynonenal induces apoptosis in human osteoarthritic chondrocytes: the protective role of glutathione-S-transferase. Arthritis Res Ther 10:R107. doi:10.1186/ar2503
12. Ge KL, Chen WF, Xie JX, Wong MS (2010) Ginsenoside Rg1 protects against 6-OHDA-induced toxicity in MES23.5 cells via Akt and ERK signaling pathways. J Ethnopharmacol 127:118–123. doi:10.1016/j.jep.2009.09.038
13. Liu Q, Kou JP, Yu BY (2011) Ginsenoside Rg1 protects against hydrogen peroxide-induced cell death in PC12 cells via inhibiting NF-kappaB activation. Neurochem Int 58:119–125. doi:10.1016/j.neuint.2010.11.004
14. Wu J, Pan Z, Wang Z, Zhu W, Shen Y, Cui R, Lin J, Yu H, Wang Q, Qian J, Yu Y, Zhu D, Lou Y (2012) Ginsenoside Rg1 protection against beta-amyloid peptide-induced neuronal apoptosis via estrogen receptor alpha and glucocorticoid receptor-dependent anti-protein nitration pathway. Neuropharmacology 63:349–361. doi:10.1016/j.neuropharm.2012.04.005
15. Leung KW, Yung KK, Mak NK, Chan YS, Fan TP, Wong RN (2007) Neuroprotective effects of ginsenoside-Rg1 in primary nigral neurons against rotenone toxicity. Neuropharmacology 52:827–835. doi:10.1016/j.neuropharm.2006.10.001
16. Shin JS, Park N, Ra J, Kim Y, Shin M, Hong M, Kim SH, Kwon HJ, Hong SP, Kim J, Bae H (2009) Panax ginseng C.A. Meyer modulates the levels of MMP3 in S12 murine articular cartilage cell line. J Ethnopharmacol 124:397–403. doi:10.1016/j.jep.2009.05.036
17. So MW, Lee EJ, Lee HS, Koo BS, Kim YG, Lee CK, Yoo B (2013) Protective effects of ginsenoside Rg3 on human osteoarthritic chondrocytes. Mod Rheumatol 23:104–111. doi:10.1007/s10165-012-0635-8
18. Chen H, Yin J, Deng Y, Yang M, Xu L, Teng F, Li D, Cheng Y, Liu S, Wang D, Zhang T, Wu W, Liu X, Guan S, Jiang B, Guo D (2012) The protective effects of ginsenoside Rg1 against hypertension target-organ damage in spontaneously hypertensive rats. BMC Complement Altern Med 12:53. doi:10.1186/1472-6882-12-53
19. Csaki C, Mobasheri A, Shakibaei M (2009) Synergistic chondroprotective effects of curcumin and resveratrol in human articular chondrocytes: inhibition of IL-1beta-induced NF-kappaB-mediated inflammation and apoptosis. Arthritis Res Ther 11:R165. doi:10.1186/ar2850
20. Kang M, Yoshimatsu H, Oohara A, Kurokawa M, Ogawa R, Sakata T (1995) Ginsenoside Rg1 modulates ingestive behavior and thermal response induced by interleukin-1 beta in rats. Physiol Behav 57:393–396
21. Seo YJ, Kwon MS, Choi HW, Jang JE, Lee JK, Sun Y, Jung JS, Park SH, Suh HW (2008) Intracerebroventricular ginsenosides are antinociceptive in proinflammatory cytokine-induced pain behaviors of mice. Arch Pharm Res 31:364–369. doi:10.1007/s12272-001-1165-x
22. Gong L, Li SL, Li H, Zhang L (2011) Ginsenoside Rg1 protects primary cultured rat hippocampal neurons from cell apoptosis induced by beta-amyloid protein. Pharm Biol 49:501–507. doi:10.3109/13880209.2010.521514
23. Yan J, Liu Q, Dou Y, Hsieh Y, Liu Y, Tao R, Zhu D, Lou Y (2013) Activating glucocorticoid receptor-ERK signaling pathway contributes to ginsenoside Rg1 protection against beta-amyloid peptide-induced human endothelial cells apoptosis. J Ethnopharmacol 147:456–466. doi:10.1016/j.jep.2013.03.039
24. Beier F, Loeser RF (2010) Biology and pathology of Rho GTPase, PI-3 kinase-Akt, and MAP kinase signaling pathways in chondrocytes. J Cell Biochem 110:573–580. doi:10.1002/jcb.22604
25. Oh CD, Chun JS (2003) Signaling mechanisms leading to the regulation of differentiation and apoptosis of articular chondrocytes by insulin-like growth factor-1. J Biol Chem 278:36563–36571. doi:10.1074/jbc.M304857200
26. Burlacu A (2003) Regulation of apoptosis by Bcl-2 family proteins. J Cell Mol Med 7:249–257
27. Stiles BL (2009) PI-3-K and AKT: onto the mitochondria. Adv Drug Deliv Rev 61:1276–1282. doi:10.1016/j.addr.2009.07.017
28. Min JK, Kim JH, Cho YL, Maeng YS, Lee SJ, Pyun BJ, Kim YM, Park JH, Kwon YG (2006) 20(S)-Ginsenoside Rg3 prevents endothelial cell apoptosis via inhibition of a mitochondrial caspase pathway. Biochem Biophys Res Commun 349:987–994. doi:10.1016/j.bbrc.2006.08.129
29. Kumagai K, Imai S, Toyoda F, Okumura N, Isoya E, Matsuura H, Matsusue Y (2012) 17beta-Oestradiol inhibits doxorubicin-induced apoptosis via block of the volume-sensitive Cl(−) current in rabbit articular chondrocytes. Br J Pharmacol 166:702–720. doi:10.1111/j.1476-5381.2011.01802.x
30. Zhong M, Carney DH, Boyan BD, Schwartz Z (2011) 17beta-Estradiol regulates rat growth plate chondrocyte apoptosis through a mitochondrial pathway not involving nitric oxide or MAPKs. Endocrinology 152:82–92. doi:10.1210/en.2010-0509
31. Hashimoto R, Yu J, Koizumi H, Ouchi Y, Okabe T (2012) Ginsenoside Rb1 Prevents MPP(+)-Induced Apoptosis in PC12 Cells by Stimulating Estrogen Receptors with Consequent Activation of ERK1/2, Akt and Inhibition of SAPK/JNK, p38 MAPK. Evid Based Complement Alternat Med 2012:693717. doi:10.1155/2012/693717
32. Goldring MB, Otero M, Plumb DA, Dragomir C, Favero M, El Hachem K, Hashimoto K, Roach HI, Olivotto E, Borzi RM, Marcu KB (2011) Roles of inflammatory and anabolic cytokines in cartilage metabolism: signals and multiple effectors converge upon MMP-13 regulation in osteoarthritis. Eur Cell Mater 21:202–220
33. Zheng X, Xia C, Chen Z, Huang J, Gao F, Li G, Zhang B (2012) Requirement of the phosphatidylinositol 3-kinase/Akt signaling pathway for the effect of nicotine on interleukin-1beta-induced chondrocyte apoptosis in a rat model of osteoarthritis. Biochem Biophys Res Commun 423:606–612. doi:10.1016/j.bbrc.2012.06.045
34. Zhu D, Wu L, Li CR, Wang XW, Ma YJ, Zhong ZY, Zhao HB, Cui J, Xun SF, Huang XL, Zhou Z, Wang SQ (2009) Ginsenoside Rg1 protects rat cardiomyocyte from hypoxia/reoxygenation oxidative injury via antioxidant and intracellular calcium homeostasis. J Cell Biochem 108:117–124. doi:10.1002/jcb.22233