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Recombinant human irisin regulated collagen II, matrix metalloproteinase-13 and the Wnt/β-catenin and NF-κB signaling pathways in interleukin-1β-induced human SW1353 cells

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Abstract. Osteoarthritis (OA) is a degenerative joint disease that seriously affects the quality of life of patients. Irisin has been reported to regulate bone metabolism via the cellular autocrine mechanism and play a protective role in rat OA. In the present study, a SW1353 chondrosarcoma cell line was treated with interleukin (IL)-1β and irisin. The present study evaluated cell viability, expression levels of collagen II (Col II) and matrix metalloproteinase-13 (MMP-13), and activity of the Wnt/β-catenin and NF-κB signaling pathways in treated SW1353 cells. The present results suggested that IL-1β could decrease Col II expression and increase MMP-13 expression at both the mRNA and protein levels, and also activate the Wnt/β-catenin and NF-κB signaling pathways in SW1353 cells. By contrast, irisin was identified to reverse the effects of IL-1β in IL-1β-induced SW1353 cells. The present results suggested that irisin treatment may have a cartilage-protective role in an IL-1β-induced SW1353 cell model.

Introduction

Osteoarthritis (OA), is the most prevalent joint disease characterized by loss of cartilage, subchondral bone sclerosis or cyst and osteophyte formation (1). The clinical manifestations of OA include joint stiffness, chronic pain and limited movement (2). Traditional treatment can only temporarily relieve clinical symptoms, and cannot effectively inhibit the pathological progress of OA (3). Therefore, it is important to understand the pathogenesis of OA and investigate novel safe and effective treatments. Degeneration of articular cartilage is one of the major pathological changes in OA (3). Many cytokines, growth factors and enzymes, such as interleukin (IL)-1β and collagenase, are involved in articular cartilage degeneration (4). IL-1β is secreted by synovial cells and OA inflammatory cells, and stimulates the production of proteolytic enzymes, such as matrix degrading enzymes and collagenase, causing synovial inflammation and bone resorption (4). Collagenase is upregulated in OA cartilage, and intra-articular injection of collagenase has successfully established an animal model of OA (4). In addition, matrix metalloproteinases (MMPs) have been shown to play an important role in the pathogenesis of OA (4). Inflammatory cytokines increase the secretion of MMP-13, and promote the degradation of collagen II (Col II) in chondrocytes, leading to the occurrence of OA (5).

Irisin, a myokine produced by skeletal muscle in response to physical exercise, promotes transdifferentiation of white adipose tissue into brown adipose tissue (6). Previous studies have suggested that irisin is also involved in the control of bone metabolism. Faienza *et al* (7) identified that irisin influences the treatment of pediatric patients with type 1 diabetes and promotes pediatric bone health. Previous studies have shown that irisin can directly enhance osteogenic differentiation of bone stromal cells and improve cortical quality (8). In addition, previous studies have suggested that irisin can activate the Wnt/β-catenin signaling pathway in MC3T3-E1 cells to promote osteoblast differentiation in OA mice (9,10). A previous study reported that irisin inhibits osteoclast differentiation by inhibiting the receptor of nuclear factor C1 of T cells activated by NF-κB ligand in RAW264.7 cells (9). Irisin can effectively enhance the osteogenesis process and reduce the occurrence of osteoporosis and fracture (9).

Many signaling pathways regulating joint formation and homeostasis are thought to be key factors in the pathogenesis of OA (10). The Wnt/β-catenin signaling pathway is considered to be one of the most important pathways associated with postnatal metabolism of articular cartilage matrix, differentiation and apoptosis of articular chondrocytes (10,11). β-catenin is a key factor in the Wnt/β-catenin signaling pathway and its expression level in the nucleus directly reflects the activation level of this signaling pathway (11). When the Wnt/β-catenin signaling pathway is activated, β-catenin can regulate the function of chondrocytes and change their physiological state, resulting in OA and other related diseases (10).

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The SW1353 cell line was initiated in 1977, and was later considered to be a valuable *in vitro* system for investigating catabolic gene regulation with IL-1β, tumor necrosis factor-α and fibroblast growth factors (12,13). At present, previous studies have focused on the bone and subchondral bone in OA joints. To the best of our knowledge, there are no studies investigating whether irisin directly acts on cartilage and plays a protective role in the process of OA. Furthermore, to the best of our knowledge, there are no data showing the close interaction between irisin and the Wnt/β-catenin and NF-κB signaling pathways in SW1353 cells. The present results suggested that irisin inhibited the Wnt/β-catenin and NF-κB signaling pathways in SW1353 cells.

Materials and methods

Materials. Recombinant human full-length irisin protein (112 amino acids, FNDC5 sequence 32-143) was purchased from Phoenix Pharmaceuticals, Inc. Recombinant human IL-1β was purchased from Bio-Techne, and lithium chloride (LiCl; molecular weight, 42.39400) was purchased from Shanghai Mintchem Development Co., Ltd.

Cell culture. The chondrosarcoma cell line SW1353, originating from a 72-year-old woman, was purchased from Procell Life Science & Technology, Co., Ltd. Cells were cultured with DMEM (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.) in a 5% CO_2 incubator at 37˚C.

Cell Counting Kit-8 (CCK-8) assay. A CCK-8 kit (Gibco; Thermo Fisher Scientific, Inc.) was used to evaluate the cytotoxicity of IL-1β and irisin. The experiment was performed according to the manufacturer's instructions. Cells (100 μl/well; ~5,000/well) were incubated for 4 h in 96-well plates in a humidified incubator (at 37˚C; 5% CO_2). Different concentrations of IL-1β (0, 5, 10, 20 or 50 ng/ml) or irisin (0, 10, 20, 50 or 100 mM) were added for 12, 24, 36 or 48 h. Then, 10 μl of CCK-8 solution was added to each well of the plate using a repeating pipettor and incubated at 37˚C for 4 h. The optical density was measured at a wavelength of 450 nm using a microplate reader (Bio-Rad Model 550; Bio-Rad Laboratories, Inc.).

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). SW1353 cells ($5x10^5$ in each dish) were seeded in a 6 cm dish and treated with 10 ng/ml IL-1β and/or 20 mM irisin at 37˚C for 24 h. The cells were harvested and then washed with PBS. Total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Then, 5 μl RNA was used for electrophoresis with a 1% agarose gel to detect the integrity of the RNA. The cDNA was synthesized from 1 μg total RNA using cDNA synthesis (Tiangen Biotech Co., Ltd.) according to the manufacturer's instructions. The expression levels of MMP-13, Col II and Wnt-1 were analyzed using the primer sequences and β-catenin as the reference gene as listed in Table I.

The experimental operation of the reaction system was carried out according to the manufacturer's instructions. The 20 μl reaction mixture consisted of 10 μl 2^*SuperReal PreMix Plus (SYBR Green; Tiangen Biotech Co., Ltd.) and each primer. The StepOne System (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used for qPCR using the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). The thermocycling conditions were as follows: Initial denaturation at 95˚C for 1 min; 40 cycles of 95˚C for 10 sec, 58˚C for 30 sec and 72˚C for 30 sec; and then the melt curve was analyzed. Target gene levels were analyzed using the $2^{-\Delta\Delta Cq}$ method (14).

Western blot analysis. SW1353 cells ($5x10^5$ in each dish) were seeded in a 6 cm dish and treated with 10 ng/ml IL-1β and/or 20 mM irisin at 37˚C for 24 h, then RIPA lysis buffer (Cell Signaling Technology, Inc.) was used to extract protein. Protein concentration was determined using a bicinchoninic acid kit (Sigma-Aldrich; Merck KGaA). A total of 20 μg of protein was loaded into each well of a 10% SDS-PAGE gel. After gel electrophoresis, the protein bands were separated from the gel, transferred to PVDF membranes after blocking with 5% non-fat milk at room temperature for 1 h by transfer electrophoresis and then incubated at 37˚C for 1 h with the following antibodies: MMP-13 (1:10,000; cat. no. DF6494; Affinity Biosciences), Col II (1:10,000 dilution; cat. no. AF5456; Affinity Biosciences), phospho-NFκB (1:10,000; cat. no. AF2006; Affinity Biosciences), inhibitor of NF-κB (IκB) α (1:10,000; cat. no. AF002; Affinity Biosciences), Wnt-1 (1:10,000; cat. no. DF514; Affinity Biosciences) and β-catenin (1:2,000; cat. no. ab1008; Abcam). β-actin (cat. no. ab051, Abcam) was used as the endogenous control. The membranes were then incubated with a goat anti-rabbit secondary antibody (1:10,000; cat. no. ab97051; Abcam) at 37˚C for 1 h. Protein bands were visualized with an ECL kit (Abcam). The data of study groups were quantitatively analyzed relative to the NC group using SPSS 19.0 (IBM Corp.).

Immunofluorescence analysis. SW1353 cells ($6x10^4$) were cultured in a 24-well plate and treated with 10 ng/ml IL-1β and/or 20 mM irisin at 37˚C for 24 h. After the culture solution was aspirated, cells were washed with PBS, and fixed with acetone at 4˚C for 10 min, and blocked with 5% BSA in PBS at 37˚C for 60 min. After the treatment with the primary antibody (rabbit LC3 antibody; 1:250; cat. no. DF674; Affinity Biosciences) and the secondary antibody (anti-rabbit IgG; 1:500; cat. no. AF313; Affinity Biosciences) at 37˚C for 1 h, the DAPI staining solution was added dropwise at 37˚C for 10 min, and the images were taken using a fluorescence microscope (magnification, 400x; Carl Zeiss AG).

Activation of the Wnt/β-catenin signaling pathway. LiCl is a specific activator of the Wnt/β-catenin signaling pathway. To investigate the effect of irisin on the activated Wnt/β-catenin signaling pathway in a non-inflammatory environment, the cells were pretreated with 10 mM LiCl at 37˚C for 24 h and treated with 20 nM irisin at 37˚C for a further 24 h. Phosphorylated-p65 (p-p65) primary antibody (1:500; cat. no. BC336) was purchased from Affinity Biosciences.

Statistical analysis. Data were analyzed using SPSS software version 12.0 (SPSS, Inc.) and are presented as the mean ± SD.

Table I. Primers used for reverse transcription-quantitative PCR.

Gene	Primer sequence (5'-3')	Length, bp
MMP-13	F: ACCCCAACCCTAAACATCC	155
	R: CGTTAAAAACAGCTCCGCA	
Collagen II	F: TGGTCTGAGGGGTCTTCC	172
	R: CTGGTCACCTGGTTTTCC	
β-catenin	F: CCAGTGGATTCTGTGTTGTT	170
	R: ATTTGAAGGCAGTCTGTCGT	
Wnt-1	F: CACAAACCGCCCTCCCCC	142
	R: GCAGCTCGCAGCCGTCCA	
β-actin	F: CCAAGGCCAACCGCGAGAA	187
	R: GCATGGGGGAGGGCATACC	

F, forward; R, reverse; MMP-13, matrix metalloproteinase-13.

Graphs were drawn using GraphPad Prism 7.00 (GraphPad Software, Inc.). One-way ANOVA with subsequent Tukey's test was used for multiple comparisons. The experiment was repeated three times in each group. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effects of irisin and IL-1β on cell viability of SW1353 cells. Effects of irisin and IL-1β on the cytotoxicity of SW1353 cells were evaluated by CCK-8 assay. Irisin at concentrations of 0, 10, 20, 50 and 100 mM did not have a significant effect on cell viability, after 24 h (Fig. 1A) or 48 h (Fig. 1B) of incubation. The concentration of 20 mM was chosen for the following experiments as previously described [12]. Furthermore, IL-1β at concentrations of 0, 5, 10, 20 and 50 ng/ml led to no difference in cell viability at 12 h (Fig. 1C) or 24 h (Fig. 1D). However, at 36 h (Fig. 1E) and 48 h (Fig. 1F) of incubation, IL-1β (10, 20 and 50 ng/ml) significantly decreased cell viability ($P<0.05$, $P<0.001$, $P<0.001$), and the effects of 50 ng/ml IL-1β were more significant ($P<0.001$). Therefore, 10 ng/ml IL-1β was used to stimulate cells.

Effect of irisin on IL-1β-induced expression of MMP-13 and Col II in SW1353 cells. The present study investigated the effect of irisin on IL-1β-induced MMP-13 and Col II expression levels using RT-qPCR, western blotting and immunofluorescence analysis. The present results indicated that the expression of Col II at the protein (Fig. 2A and C) and mRNA (Fig. 2D) levels was significantly decreased by IL-1β treatment, whereas irisin treatment reversed the IL-1β induced-decrease of Col II expression levels ($P<0.05$). Moreover, the expression of MMP-13 at the protein (Fig. 2B and C) and mRNA (Fig. 2E) levels in the SW1353 cells was upregulated by IL-1β treatment, and the effect of IL-1β was reversed by irisin treatment ($P<0.05$). Immunofluorescence analysis supported the present RT-qPCR results. The expression level of MMP-13 in SW1353 cells was downregulated after irisin treatment, whereas IL-1β treatment upregulated the expression level of MMP-13 in SW1353 cells compared with the control groups (Fig. 3A). However, the expression level of Col II in SW1353 cells was downregulated following IL-1β intervention, and subsequently upregulated by the irisin treatment compared with the control group (Fig. 3B).

Effect of irisin on IL-1β-induced activation of the Wnt/β-catenin signaling pathway in SW1353 cells. To study the anti-inflammatory mechanism of irisin, western blotting and RT-qPCR were used to investigated its effects on IL-1β-induced activation of the Wnt/β-catenin signaling pathway in SW1353 cells. The present results suggested that, compared with the negative control group, the protein and mRNA expression levels of β-catenin (Fig. 4A, C and D) and Wnt-1 (Fig. 4B, C and E) in SW1353 cells were upregulated by IL-1β treatment ($P<0.05$), indicating the activation of the Wnt/β-catenin signaling pathway. However, following irisin treatment, IL-1β-induced activation was suppressed ($P<0.05$). The present results suggested the IL-1β-induced activity of the Wnt/β-catenin signaling pathway was significantly decreased by irisin treatment.

Effect of irisin on the Wnt/β-catenin signaling pathway. LiCl significantly increased the mRNA expression levels of β-catenin (Fig. 5A) and Wnt-1 (Fig. 5B; $P<0.05$). Irisin treatment significantly decreased the expression levels of Wnt-1 and β-catenin that were induced by LiCl ($P<0.05$). SW1353 cells co-cultured with IL-1β and LiCl were also treated with irisin, and the present results indicated that treatment with irisin decreased the expression levels of β-catenin and Wnt-1 (Fig. 5C and D). The present results suggested that irisin may exerted its functions in SW1353 cells by inhibiting the Wnt/β-catenin signaling pathway (Fig. 5).

Effect of irisin on IL-1β-induced activation of NF-κB signaling in SW1353 cells. To investigate the effect of irisin on the NF-κB signaling pathway in IL-1β-induced SW1353 cells, the changes in IκBα and phosphorylated-p65 (p-p65) were detected by western blot analysis (Fig. 6C). Statistical analysis showed that the expression levels of p-p65 (Fig. 6A) and IκBα (Fig. 6B) were significantly increased by IL-1β, but were significantly decreased after irisin treatment compared with the IL-1β group. Therefore, the present results indicated that irisin could inhibit the level of cytoplasmic p-p65 and downregulate the activity of the NF-κB pathway.

Discussion

Different types of SW1353 cells have been applied in cell models of OA. Huang *et al* (14) utilized and treated SW1353 cells with IL-1β to imitate the microenvironment of OA for *in vitro* experiment. Feng *et al* (15) applied human SW1353 chondrocytes to evaluate the effect of salicin in OA. Lu *et al* (16) investigated the chondroprotective role of sesamol by downregulating MMP expression via retention of the NF-κB signaling pathway in activated SW1353 cells. In addition, Tetsunaga *et al* (17) analyzed the effect of runt-related transcription factor 2 on the mechanical stress-induced MMP-13 and A disintegrin and metalloproteinase with thrombospondin motifs 5 expression in SW1353 chondrocyte-like cells. Cheng *et al* (18) successfully established a cellular model

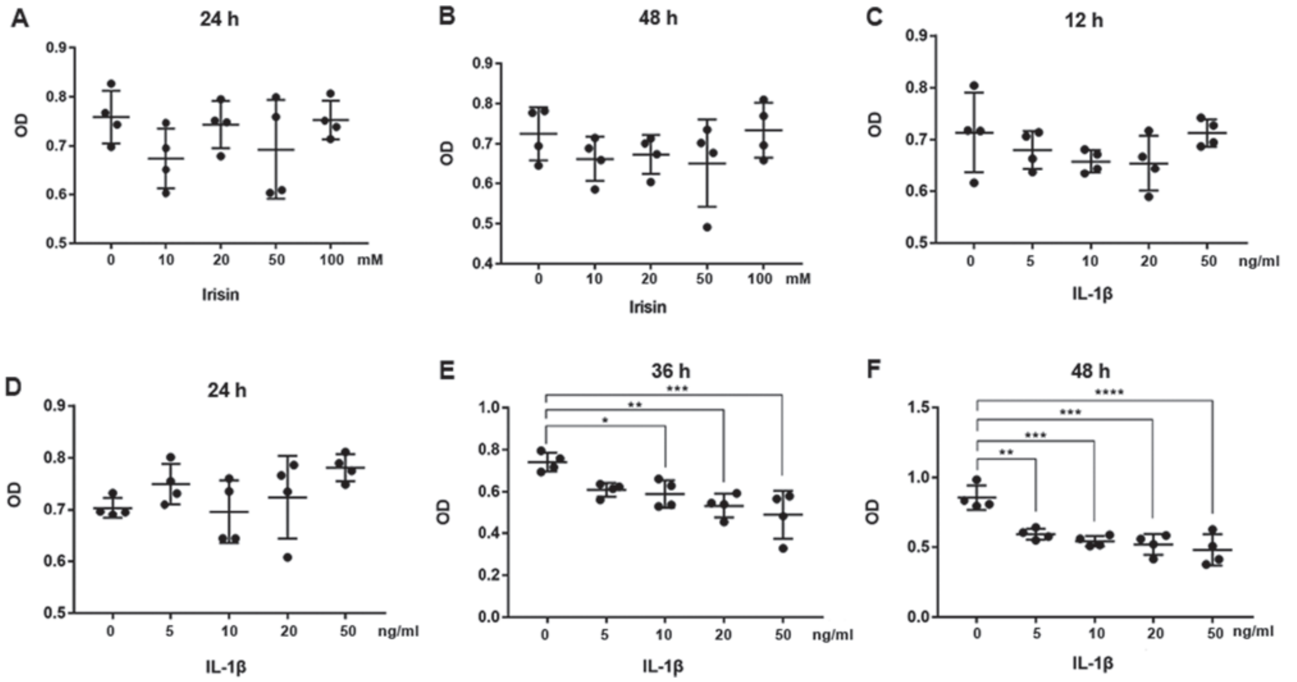

Figure 1. Effect of irisin on the viability of SW1353 cells. SW1353 cells were seeded (100 μl/well; ~5,000/well) in 96-well plates, treated with different concentrations of irisin, and assessed by CCK-8 assay at (A) 24 h and (B) 48 h. n=5/group. Cell viability in SW1353 cells treated with IL-1β was assessed by CCK-8 assay at (C) 12, (D) 24, (E) 36 and (F) 48 h. n=5/group. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. IL-1β, interleukin-1β; CCK-8, Cell Counting Kit-8; OD, optical density.

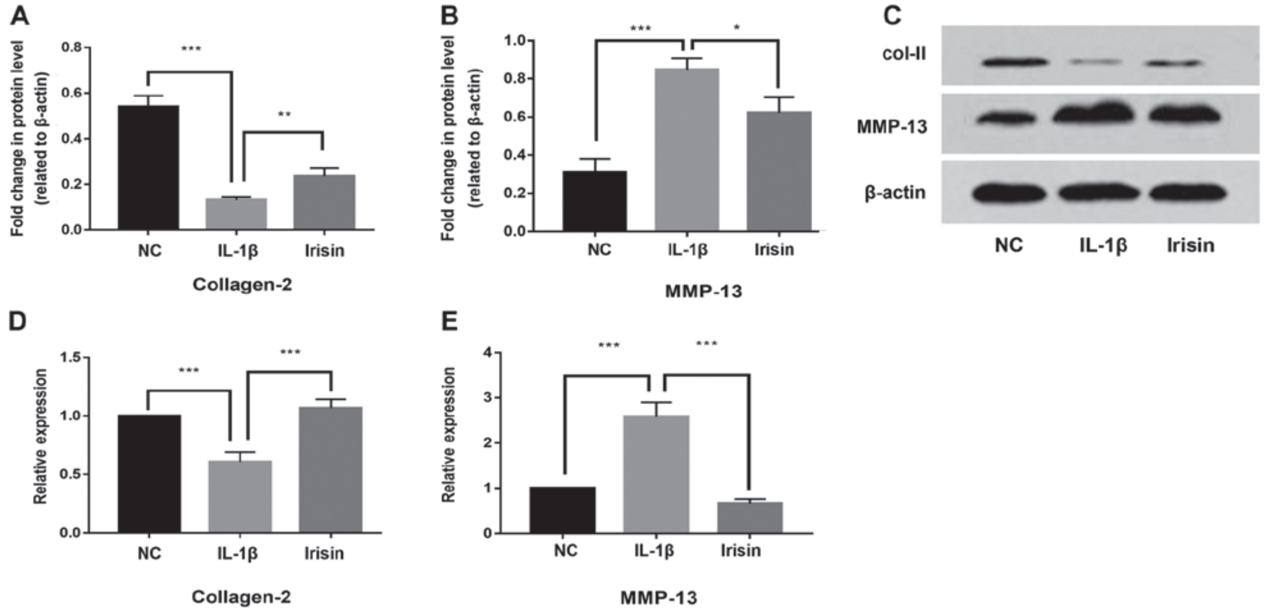

Figure 2. Effect of irisin on MMP-13 and Col-II in IL-1β-induced SW1353 cells. SW1353 cells were treated with 20 mM irisin for 24 h, with or without 10 ng/ml IL-1β. (A) Col II protein levels, (B) MMP-13 protein levels, (C) Col II and MMP-13 protein expression, (D) Col II mRNA levels and (E) MMP-13 mRNA levels were evaluated by reverse transcription-quantitative PCR and western blot analysis (n=5/group). *P<0.05, **P<0.01 and ***P<0.001. IL-1β, interleukin-1β; MMP-13, matrix metalloproteinase-13; Col-II, collagen II; NC, negative control.

of OA by stimulating SW1353 cells with IL-1β. All these previous studies have shown that SW1353 cells are a feasible cell line with which to establish OA models.

The present study used RT-qPCR, western blotting and immunofluorescence analysis to investigate whether irisin can protect against OA induced by IL-1β in SW1353 cells. Previous studies have confirmed that IL-1β plays an important role in the development of OA and can stimulate chondrocytes to produce OA (19,20). The present study performed CCK-8 analysis to identify the optimum concentration of IL-1β for modeling OA. Cartilage degradation in OA is mediated by the MMP family (21). MMP-13, as a member of the MMP family, can degrade collagen and reduce cartilage composition (22). High levels of MMPs are considered to be one of the

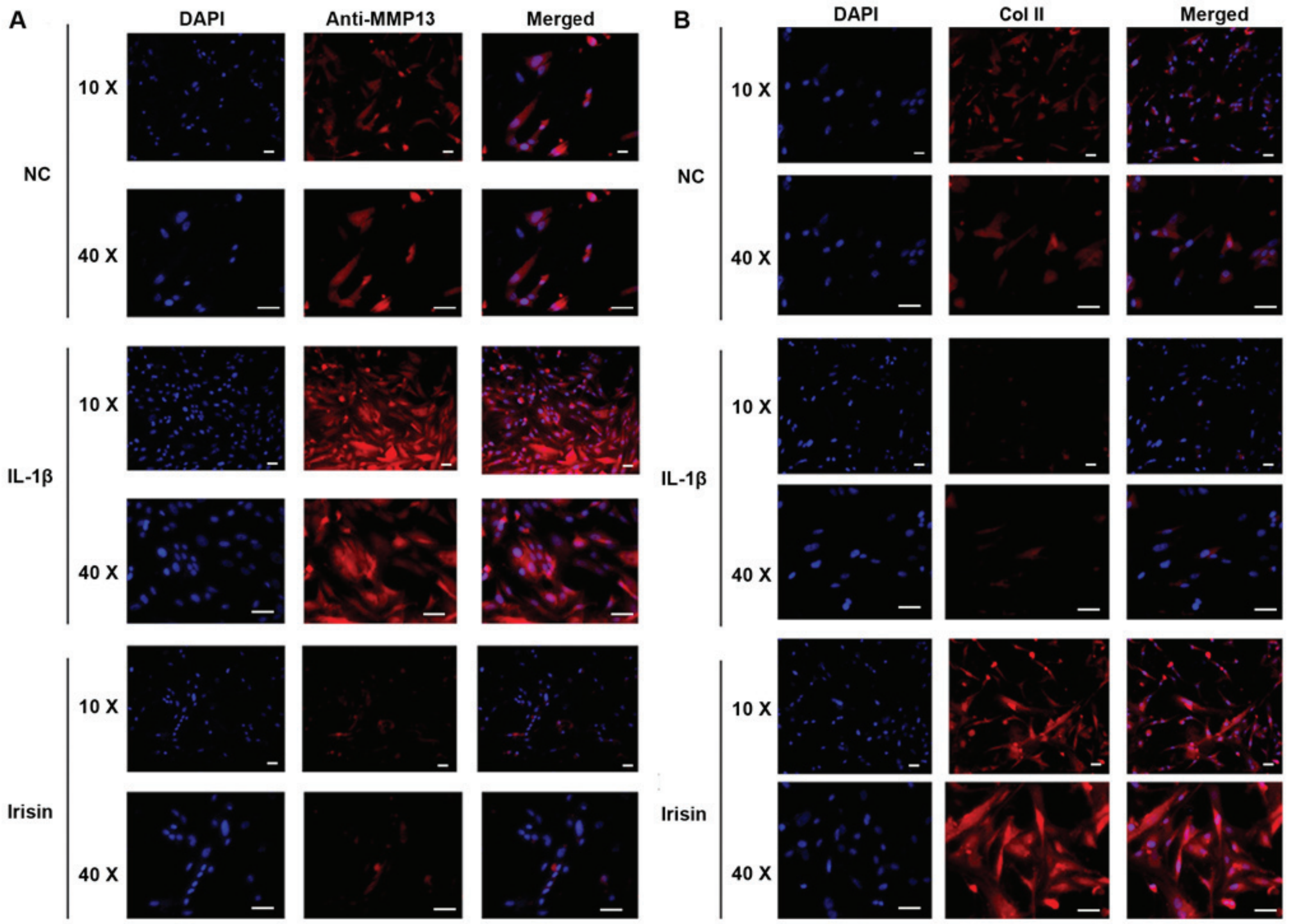

Figure 3. Immunofluorescence assay of the expression levels of (A) MMP-13 and (B) Col II. SW1353 cells were treated with different concentrations of 20 mM irisin for 6 h, with or without IL-β. The expression levels of MMP-13 and Col II were visualized using immunofluorescence. n=3/group. Scale bar, 10 μm. IL-1β, interleukin-1β; MMP-13, matrix metalloproteinase-13; Col-II, collagen II; NC, negative control.

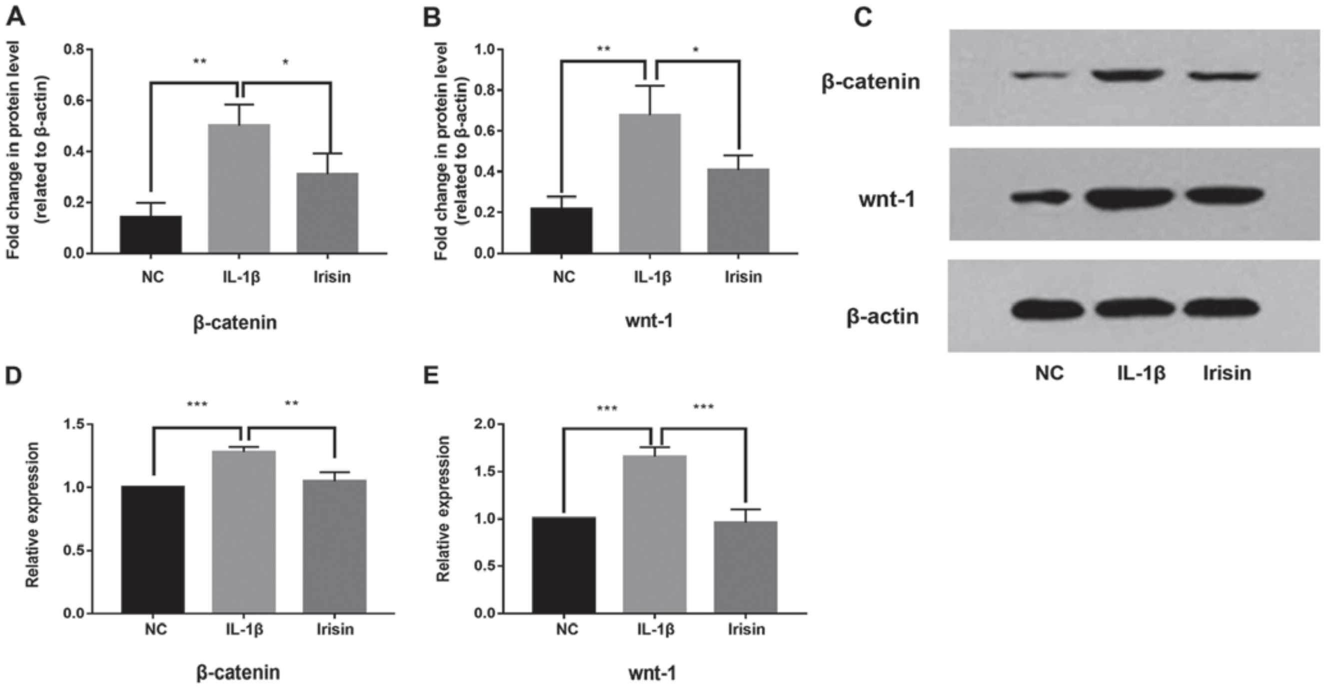

Figure 4. Effect of irisin on the activation of the Wnt/β-catenin signaling pathway in IL-1β-induced SW1353 cells. SW1353 cells were treated with 20 mM irisin for 24 h, with or without IL-1β, and (A) β-catenin protein levels, (B) Wnt-1 protein levels and (C) β-catenin and Wnt-1 protein expression,were evaluated by western blot analysis. n=5/group. mRNA expression levels of (D) β-catenin and (E) Wnt-1 were evaluated by reverse transcription-quantitative PCR. n=5/group. *P<0.05, **P<0.01 and ***P<0.001. IL-1β, interleukin-1β; NC, negative control.

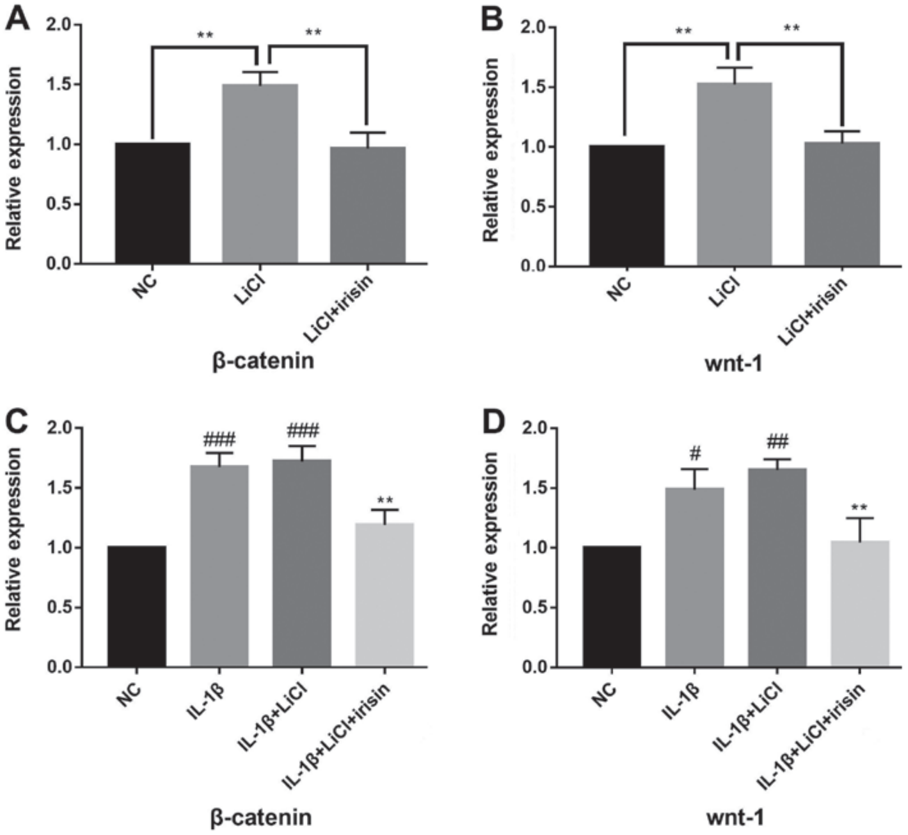

Figure 5. Effect of irisin on SW1353 cells after the addition of a Wnt/β-catenin signaling pathway activator. SW1353 cells were treated with 10 mM LiCl for 24 h, with or without IL-1β. mRNA expression levels of (A) β-catenin and (B) Wnt-1 were evaluated by RT-qPCR. n=5/group. SW1353 cells were treated separately with 10 ng/ml IL-1β, 10 ng/ml IL-1β + 10 mM LiCl or 10 ng/ml IL-1β + 10 mM LiCl + 20 mM irisin for 24 h. **P<0.01. mRNA expression levels of (C) β-catenin and (D) Wnt-1 were evaluated by RT-qPCR. n=5/group **P<0.01 vs. NC group; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. IL-1β+LiCl group. RT-qPCR, reverse transcription-quantitative PCR; IL-1β, interleukin-1β; NC, negative control; LiCl, lithium chloride.

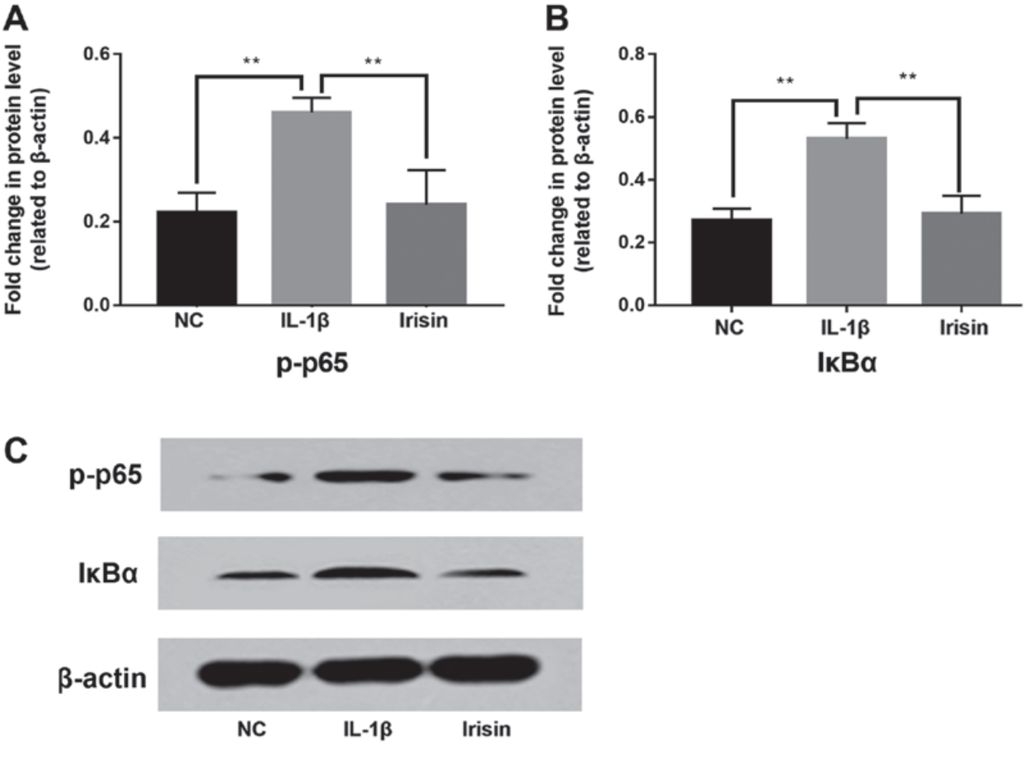

Figure 6. Effect of irisin on IL-1β-induced activation of NF-κB signaling in SW1353 cells. SW1353 cells were treated with 20 mM irisin for 24 h, with or without IL-1β. Protein expression levels of (A) p-p65 and (B) IκBα were evaluated by (C) western blot analysis. n=5/group. **P<0.01 vs. the NC group/IL-1β group. IκBα, inhibitor of NF-κB α; p-p65, phosphorylated p65; NC, negative control; IL-1β, interleukin-1β.

main characteristics of OA (22,23), which can combine with low levels of chondrocyte-specific proteins, such as Sox9 and collagen II (24), leading to cartilage degeneration. Therefore, it is important to regulate the expression levels of MMPs to facilitate the prevention and treatment of OA. The present results indicated that five different concentrations of irisin did not affect the viability of SW1353 cells. However, treatment with 20 mM irisin significantly reduced the expression levels of MMP-13 in SW1353 cells. In addition, irisin treatment increased the expression level of Col-II. The present results suggested that irisin exerted its protective effects by decreasing the expression level of MMP-13 and increasing the expression level of Col-II in IL-1β-induced SW1353 cells.

Previous study has shown that exercise causes production of skeletal muscle and release of irisin into the blood, which has beneficial effects in increasing bone mass, lowering body mass index, increasing insulin sensitivity and releasing total body energy (25). Colaianni *et al* (25) found that, compared with a restricted exercise group, the expression of irisin in mice after 3 weeks of treadmill exercise was increased significantly at both the mRNA and protein levels. A previous study showed that irisin can directly act on bone stromal cells and enhance osteogenic differentiation (25). A recent study also indicated that irisin activates the Wnt/β-catenin signaling pathway in pre-osteoblasts to promote osteoblast differentiation (9). A clinical investigation into OA showed that irisin expression levels in circulating and synovial fluids, and CRP expression levels in circulating fluid are closely related to the severity of OA (26), suggesting that irisin is associated with OA. Cartilage degeneration is an important part of the pathogenesis of OA (26). To the best of our knowledge, the present study is the first to investigate the direct effects of irisin on IL-1β-induced SW1353 cells.

Studies have shown that several signaling pathways regulating joint formation and homeostasis are key factors in the pathogenesis of OA (26). The NF-κB and Wnt/β-catenin signaling pathways are two major pathways involved in OA development (26). Wnt protein is a promoter of the Wnt/β-catenin signaling pathway (26). β-catenin is a key factor in the Wnt/β-catenin signaling pathway, which is encoded by the catenin β-1 gene (26). The expression level of β-catenin in the nucleus directly reflects the activation level of this signaling pathway (26). Under normal conditions, β-catenin remains stable in the cytoplasm. In the absence of Wnt protein promoter stimulation, β-catenin forms a complex with axis inhibitor, adenomatous polyposis coli and glycogen synthase kinase 3 (GSK3), among others. β-catenin is then phosphorylated by casein kinase 1 and GSK3, and the free β-catenin in the cytoplasm is reduced, and thereby the transcription of Wnt protein target genes in the nucleus is inhibited (27). Thus, the β-catenin expression level remains low due to continuous degradation. When the Wnt/β-catenin signaling pathway is activated, β-catenin becomes stable as proteasomal degradation is reduced, and stabilized β-catenin is then translocated into the nucleus to upregulate various inflammation-related genes, such as those encoding MMPs (27). Previous studies on OA showed the interaction of irisin and Wnt/β-catenin in osteogenesis (9); therefore, the present study investigated this interaction in IL-1β-induced SW1353 cells. The present RT-qPCR and western blotting results identified that irisin downregulated IL-1β-induced upregulation of Wnt-1 and β-catenin at both protein and mRNA expression levels in SW1353 cells. Therefore, the present results suggested that irisin could inhibit the Wnt/β-catenin signaling pathway. LiCl acts as a classical activator of the Wnt pathway, and can regulate GSK-3β (27). The primary role of GSK-3β is to phosphorylate free β-catenin (27). The present results suggested that irisin inhibited the Wnt pathway activated by LiCl, and this effect was also identified with irisin-treated cells co-treated with IL-1β and LiCl. Therefore, the present results suggested that irisin regulated the Wnt/β-catenin signaling pathway by inhibiting the phosphorylation of β-catenin.

The NF-κB pathway is another important pathway in the OA process, and could be a potential therapeutic target for OA (28). When the NF-κB pathway is activated by inflammatory mediators such as IL-1β, NF-κB-p65 in the cytoplasm, it is then translocated into the nucleus where it upregulates multiple inflammation-related genes, such as MMPs, cyclooxygenase-2 and prostaglandin E2 (28). Exposing chondrocytes to a variety of inflammatory cytokines leads to the degradation of IκB and further translocation of p65 into the nucleus (29). In the present study, western blot analysis indicated that IL-1β induced a significant increase in IκBα and p-p65 expression levels in SW1353 cells; however, this effect was reversed by irisin treatment. The present results suggested that irisin may reduce p-p65 in the cytoplasm to inhibit the NF-κB pathway, activated by inflammatory factors. Previous studies have shown that there is some crosstalk between the Wnt/β-catenin and NF-κB pathways (28,29). Activation of the Wnt/β-catenin pathway can enhance β-transducing repeat-containing protein-mediated degradation of IκB, and IκB kinase can also inhibit the degradation of β-catenin protein (29). However, the exact target of irisin in mitigating the IL-1β-induced inflammatory response is still unclear, and future studies will need investigate this in depth.

In conclusion, the present results suggested that irisin suppressed the activation of the Wnt/β-catenin and NF-κB signaling pathways, and may facilitate the treatment of OA.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XJL, YM and QHJ were responsible for the acquisition and interpretation of data. YL, QL and SW conducted the conception and design of the study, drafted the article. All authors read and approved final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Krasnokutsky S, Attur M, Palmer G, Samuels J and Abramson SB: Current concepts in the pathogenesis of osteoarthritis. Osteoarthritis Cartilage 16 (Suppl 3): S1-S3, 2008.
2. Buckland J: Osteoarthritis: Targeting cartilage erosion in OA. Nat Rev Rheumatol 6: 64, 2010.
3. Lahm A, Kasch R, Mrosek E, Spank H, Erggelet C, Esser J and Merk H: Semiquantitative analysis of ECM molecules in the different cartilage layers in early and advanced osteoarthritis of the knee joint. Histol Histopathol 27: 609-615, 2012.
4. Wang JH, Shih KS, Wu YW, Wang AW and Yang CR: Histone deacetylase inhibitors increase microRNA-146a expression and enhance negative regulation of interleukin-1β signaling in osteoarthritis fibroblast-like synoviocytes. Osteoarthritis Cartilage 21: 1987-1996, 2013.
5. Klatt AR, Klinger G, Paul-Klausch B, Kühn G, Renno JH, Wagener R, Paulsson M, Schmidt J, Malchau G and Wielckens K: Matrilin-3 activates the expression of osteoarthritis-associated genes in primary human chondrocytes. FEBS Lett 583: 3611-3617, 2009.
6. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ, *et al*: A PGC1-a-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481: 463-468, 2012.
7. Faienza MF, Brunetti G, Sanesi L, Colaianni G, Celi M, Piacente L, D'Amato G, Schipani E, Colucci S and Grano M: High irisin levels are associated with better glycemic control and bone health in children with type 1 diabetes. Diab Res Clin Pract 141: 10-17, 2018.
8. Colaianni G, Cuscito C, Mongelli T, Pignataro P, Buccoliero C, Liu P, Lu P, Sartini L, Di Comite M, Mori G, *et al*: The myokine irisin increases cortical bone mass. Proc Natl Acad Sci USA 112: 12157-12162, 2015.
9. Zhang J, Valverde P, Zhu X, Murray D, Wu Y, Yu L, Jiang H, Dard MM, Huang J, Xu Z, *et al*: Exercise-induced irisin in bone and systemic irisin administration reveal new regulatory mechanisms of bone metabolism. Bone Res 5: 16056, 2017.
10. Weng X, Lin P, Liu F, *et al*: Achyranthes bidentata polysaccharides activate the Wnt/β-catenin signaling pathway to promote chondrocyte proliferation. Int J Mol Med 3: 1045-1050, 2014.
11. Lotz MK and Carames B: Autophagy and cartilage homeostasis mechanisms in joint health, aging and OA. Nat Rev Rheumatol 7: 579-587, 2011.
12. Liacini A, Sylvester J, Li WQ, Huang W, Dehnade F, Ahmad M and Zafarullah M: Induction of matrix metalloproteinase-13 gene expression by TNF-alpha is mediated by MAP kinases, AP-1, and NF-kappaB transcription factors in articular chondrocytes. Exp Cell Res 288: 208-217, 2003.
13. Schaefer JF, Millham ML, de Crombrugghe B and Buckbinder L: FGF signaling antagonizes cytokine-mediated repression of Sox9 in SW1353 chondrosarcoma cells. Osteoarthritis Cartilage 11: 233-241, 2003.
14. Huang Y, Wan G and Tao J: C1q/TNF-related protein-3 exerts the chondroprotective effects in IL-1β-treated SW1353 cells by regulating the FGFR1 signaling. Biomed Pharmacother 85: 41-46, 2017.
15. Feng G and Zhang S: Salicin inhibits AGE-induced degradation of type II collagen and aggrecan in human SW1353 chondrocytes: Therapeutic potential in osteoarthritis. Artif Cells Nanomed Biotechnol 47: 1043-1049, 2019.
16. Lu YC, Jayakumar T, Duann YF, Chou YC, Hsieh CY, Yu SY, Sheu JR and Hsiao G: Chondroprotective role of sesamol by inhibiting MMPs expression via retaining NF-κB signaling in activated SW1353 cells. J Agric Food Chem 11: 4969-4978, 2011.
17. Tetsunaga T, Nishida K, Furumatsu T, Naruse K, Hirohata S, Yoshida A, Saito T and Ozaki T: Regulation of mechanical stress-induced MMP-13 and ADAMTS-5 expression by RUNX-2 transcriptional factor in SW1353 chondrocyte-like cells. Osteoarthritis Cartilage 19: 222-232, 2011.
18. Cheng NT, Meng H, Ma LF, Zhang L, Yu HM, Wang ZZ and Guo A: Role of autophagy in the progression of osteoarthritis: The autophagy inhibitor, 3-methyladenine, aggravates the severity of experimental osteoarthritis. Int J Mol Med 39: 1224-1232, 2017.
19. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
20. Chen WP, Hu ZN, Jin LB and Wu LD: Licochalcone A inhibits MMPs and ADAMTSs via the NF-κB and Wnt/β-catenin signaling pathways in rat chondrocytes. Cell Physiol Biochem 43: 937-944, 2017.
21. Troeberg L and Nagase H: Proteases involved in cartilage matrix degradation in osteoarthritis. Biochim Biophys Acta 1824: 133-145, 2012.
22. Tchetverikov I, Lohmander LS, Verzijl N, Huizinga TW, TeKoppele JM, Hanemaaijer R and DeGroot J: MMP protein and activity levels in synovial fluid from patients with joint injury, inflammatory arthritis, and osteoarthritis. Ann Rheum Dis 64: 694-698, 2005.
23. Tetlow LC, Adlam DJ and Woolley DE: Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: Associations with degenerative changes. Arthritis Rheum 44: 585-594, 2001.
24. Matyas JR, Huang D, Chung M and Adams ME: Regional quantification of cartilage type II collagen and aggrecan messenger RNA in joints with early experimental osteoarthritis. Arthritis Rheum 46: 1536-1543, 2002.
25. Colaianni G, Cuscito C, Mongelli T, Oranger A, Mori G, Brunetti G, Colucci S, Cinti S and Grano M: Irisin enhances osteoblast differentiation in vitro. Int J Endocrinol 2014: 902186, 2014.
26. Marcu KB, Otero M, Olivotto E, Borzi RM and Goldring MB: NF-κB signaling: Multiple angles to target OA. Curr Drug Targets 11: 599-613, 2010.
27. MacDonald BT, Tamai K and He X: Wnt/β-catenin signaling: Components, mechanisms, and diseases. Dev Cell 17: 9-26, 2009.
28. Roman-Blas JA and Jimenez SA: NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. Osteoarthritis Cartilage 14: 839-848, 2006.
29. Ma B and Hottiger MO: Crosstalk between Wnt/β-catenin and NF-κB signaling pathway during inflammation. Front Immunol 7: 378, 2016.