RETRACTED ARTICLE: Lycium barbarum polysaccharide alleviates IL-1β-evoked chondrogenic ATDC5 cell inflammatory injury through mediation of microRNA-124

Huawei Ni⁎, Guoxiu Wang⁎⁎, Yu Xu⁎, Xiaomin Gu⁎, Chengzhang Sun⁎ and Haibo Li⁎

⁎Department of Orthopedics, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, China; ⁎Department of Orthopedics, Shenzhen University General Hospital, Shenzhen, China; ⁎⁎Department of Orthopedics, Shaoxing People’s Hospital (Shaoxing Hospital of Zhejiang University), Shaoxing, China

ABSTRACT

Background: Lycium barbarum polysaccharide (LBP) is a promising therapeutic drug in inflammation-related injuries, nevertheless the mechanism of LBP’s action is still elusive. The study is to explore the effect of LBP on IL-1β-evoked ATDC5 cell inflammatory injury.

Methods: ATDC5 cells were administrated with 10 ng/mL interleukin (IL)-1β to establish an in vitro model of cartilage damage. After management, cell viability was tested through CCK8 assay. The pro-inflammatory cytokines and cyclooxygenase (Cox)-2 were assessed through ELISA, western blot and qRT-PCR. MiR-124 expression in ATDC5 cells was silenced by transfecting with miR-124 inhibitor, and the pro-inflammatory cytokines and Cox-2 were re-assessed. NF-κB and JNK pathways were measured through western blot.

Results: IL-1β stimulation accelerated the release of IL-1β, IL-6 and TNF-α; elevated Cox-2 expression. LBP significantly eased IL-1β-induced inflammation. MiR-124 expression was observed to enhance by LBP, and the impacts of LBP on ATDC5 cells were lightened via transfection with miR-124 inhibitor. NF-κB and JNK pathways were activated after IL-1β stimulation, nevertheless were inactivated by LBP administration. Besides, LBP-evoked the repression of NF-κB and JNK pathways were overturned by miR-124 inhibitor.

Conclusions: Our study suggests that LBP protects ATDC5 cells from IL-1β-evoked injury through up-regulating miR-124 via blocking NF-κB and JNK pathways.

ARTICLE HISTORY

Received 28 June 2019
Revised 12 August 2019
Accepted 16 August 2019

KEYWORDS

Lycium barbarum polysaccharide; osteoarthritis; inflammatory injury; microRNA-124

Introduction

Osteoarthritis (OA) is a chronic sickness of movable articular cartilage degeneration caused by various factors and mechanical injuries, main characteristics of which are progressive destruction of articular cartilage. It causes a series of pathophysiological changes, damages the cartilage structure, further causing biomechanical disorders and leading to more obvious clinical manifestations of osteoarthritides [1]. Recently, the prevalence of OA is increasing accompanied by the gradually increasing of aging population and changes in living habits. Previous study showed that about 120 million people in China are suffering from OA and more than half of them are over 60 years old. Besides, the incidence of OA in female is higher than that in male [2]. OA is a kind of aseptic inflammatory disease, and its pathogenesis is considered as a specific immune response. Inflammatory factors, such as interleukin (IL)-1β, IL-6 and cyclooxygenase (Cox-2) are highly correlated with immune response and pain in OA, among which interleukin can be used as a promoter of immune response, and Cox-2 is considered to be the key factor of pain and inflammatory response [3]. However, there are no effective therapies for OA due to limited understanding of OA pathogenesis.

Lycium barbarum L. is a traditional and commonly used Chinese medicine and Lycium barbarum polysaccharide (LBP) is the dominating active element of Lycium barbarum L. It has been proved that LBP has many functions such as protecting liver, lowering blood lipid, lowering blood sugar, anti-fatigue, anti-aging, enhancing immunity and anti-tumor [4–6]. Besides that, LBP is widely used in inflammation-related injuries. For example, LBP has protective effects on cerebral ischemia reperfusion injury in mice by inhibiting NF-κB and inflammatory reactions [7]. Besides, LBP was reported to effectively attenuate CIA-evoked bone damage and bone loss, simultaneously reduce CIA-stimulated inflammation and MMPs expression in type II collagen-induced arthritis in mice [8].

Micro RNA (miRNA) is a class of small endogenous RNA with chemical stability and length of about 21–23 nucleotides. Its conventional action is to specifically bind to the 3'UTR of the target gene by the principle of base
complementary pairing to inhibit the expression of the target gene [9]. In the current research of osteoarthritis, researchers have found that more and more miRNAs have a close correlation with the pathogenesis of arthritis. Miyaki S et al. reported, miR-140 regulated chondrogenesis and homeostasis, as well as the deletion of miR-140 contributed to the age-related OA-like changes [10]. In addition, Wu et al. testified that miR-1246 inhibition mitigated LPS-evoked inflammatory injury via up-regulating HNF4α and activation of PI3K/AKT and JAK/STAT pathways [11]. In addition, miR-124 has been observed to show neglectable impacts in mediating inflammation. For example, in vitro and in vivo experiments corroborated that PPARγ-stimulated miR-124 was involved in the repression of pro-inflammatory cytokine [12]. Yu et al. also revealed that miR-124 ameliorated ICH-induced inflammatory injury through C/EBP-α pathway in intracerebral hemorrhage [13]. Above researches imply that miR-124 may be a latent therapeutic target for inflammatory sickness.

In our present study, we aimed to probe the impact of LBP in IL-1β-evoked inflammatory injury in ATDC5 cells. Additionally, the involvements of miR-124 as well as NF-κB and JNK pathways were also delved in this research to further disclose the associated mechanism. The research might unveil a neoteric therapeutic strategy for OA.

Materials and methods

Cell culture and treatment

ATDC5 cells acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA) were cultivated in a 5% CO2 incubator at 37°C utilizing RPMI-1640 medium supplied with 10% FBS. After thawing, cells between the fifth and tenth passages were pre-cultured in a 75 cm2 flask. Until confluence achieved, ATDC5 cells were administrated by IL-1β 10 ng/mL for 6 h. Lycium barbarum purchased from Ning Xia was utilized to extract the polysaccharide content on the basis of phenolsulfuric method. The extract powder of LBP was utilized to extract the polysaccharide content on the basis of phenolsulfuric method. The extract powder of LBP was diluted with DMEM and adjusted the dosages to 0–500 μg/mL. ATDC5 cells were disposed above LBP for 12 h in the next experiments.

Cell counting kit-8 (CCK-8) assay

According to the specifications of CCK-8 kit (Dojindo, Gaithersburg, MD), cell viability was probed into ATDC5 cells. Briefly, ATDC5 cells were cultivated into 96-well plate and stimulated with the diverse dosages of LBP for 12 h. Afterward, 10 μL CCK-8 solution was mixed into the culture plate, and was co-cultivated with ATDC5 cells for extra 1 h at 37°C. The absorbance at 450 nm wavelength was executed utilizing a Microplate Reader (Bio-Rad, Hercules, CA).

Western blot

After administration, the protein of ATDC5 cells was prepared exploiting RIA lysis buffer (Beyotime Biotechnology, Shanghai, China), and the total proteins concentrations were quantified by utilizing the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). The proteins were resolved by SDS-PAGE, and then transferred to the Polyvinylidene Difluoride (PVDF) membrane. The extraordinary primary antibodies were sealed in 5% BAS at a dilution of 1:1,000, and next co-incubated with the PVDF membrane at 4°C overnight. The secondary antibody was then utilized to co-culture with above membrane for another 1 h. The common used ECL reagent was exploited to visualize the specific bindings. The protein bands were analyzed via Image Lab™ Software (Bio-Rad).

Enzyme-linked immunosorbent assay (ELISA)

After administration with LBP and IL-1β the culture supernatant was gathered from 24-well culture plates and the productions of IL-6, IL-8 and TNF-α were checked via exploiting the related ELISA kits (R&D Systems, Abingdon, UK) on the basis of these kit specifications.

Transfection

MiR-124 inhibitor and its related control (GenePharma Co., Shanghai, China) were synthesized. Lipofectamine 3000 reagent (Invitrogen) were mixed with above plasmids, meanwhile transfected into ATDC5 cells on the basis of the manufacturer’s protocol. After 48 h, the transfected cells were collected and employed in the subsequent researches.

Real time-polymerase chain reaction (qRT-PCR)

After administration and transfection, total RNA was isolated from these cells utilizing TRizol reagent (Invitrogen). The 1 μL total RNA was exploited to configure the reverse transcription system according the instruction of MultiscribeRTkit (Applied Biosystems). Relative IL-6, IL-8, TNF-α, Cox-2 and miR-124 expression levels were analyzed by SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). β-actin and U6 were considered to be the endogenous controls, as well as these data were gauged by 2−ΔΔCt method.

Statistical analysis

The results from above researches are shown as the mean ± SD. SPSS 19.0 statistical software was utilized to figure out the results in different group. The significant differences were gauged via ANOVA, and p values of <.05 was regarded as a statistically significant result.

Results

LBP alleviates IL-1β-evoked inflammatory factor levels

Firstly, cytotoxicity of LBP on ATDC5 cells was detected through CCK-8 assay. The results showed that 0–300 μg/mL LBP had no obvious influences in the proliferation of ATDC5 cells but 400–500 μg/mL LBP promoted the proliferation of ATDC5 cells (p < .05, Figure 1(A)). Thus, 300 μg/mL LBP was selected in our following experiments. Then, ELISA, western
blot and qRT-PCR were all conducted to detect the impacts of LBP on IL-1β-induced inflammation. The results of above experiments all showed that elevated levels of IL-6, IL-8, and TNF-α evoked by IL-1β were then suppressed by LBP ($p < .05$ or $p < .01$, Figure 1(B)–1(D)). Thus, the above consequence reveals that LBP alleviates IL-1β-induced inflammation.

**LBP alleviates IL-1β-evoked elevated Cox-2 expression**

The level of Cox-2 which played a momentous role in the pathological process of inflammation was examined. The result analyzed by western blot showed that Cox-2 expression was heightened by IL-1β stimulation and elevated level of Cox-2 was obviously suppressed by LBP treatment (Figure 2(A)). Similarly results were shown in Figure 2(B), elevated Cox-2 mRNA in IL-1β-induced group was suppressed by the adding of LBP ($p < .05$). The above results indicate that LBP mitigates IL-1β-evoked Cox-2 expression.

**LBP suppressed IL-1β-induced elevated expression of inflammatory factors and Cox-2 through up-regulating miR-124**

Relevant regulatory mechanisms were further explored. We found that miR-124 was abated in IL-1β-induced group and the treatment of LBP successfully rescued the deletion of miR-124 ($p < .1$, Figure 3(A)). Specific miR-124 inhibitor was used in the next experiments to specifically knock down miR-124 expression as shown in Figure 3(B) ($p < .01$). Then, interesting results were shown by detecting IL-6, IL-8, TNF-α and Cox-2 levels through ELISA, western blot and qRT-PCR, respectively. The results in Figure 3(C)–3(E) showed that inhibiting effect of LBP was partly counteracted by the combination of miR-124 inhibitor ($p < .01$). As expect, LBP suppressed the increased Cox-2 level induced by IL-1β ($p < .001$), but Cox-2

![Figure 1](image1.png)  
**Figure 1.** LBP alleviates IL-1β-evoked inflammatory factor levels. A. Cell viability was detected through CCK-8 assay. B. The IL-6, IL-8 and TNF-α productions in cell supernatant were tested through ELISA. C. The IL-6, IL-8 and TNF-α protein levels in ATDC5 cells were checked through western blot. D. The IL-6, IL-8 and TNF-α mRNA levels in ATDC5 cells were measured through qRT-PCR. The bars showed means ± SD accompanied by three independent experiments. *$p < .05$; **$p < .01$; ***$p < .001$; ns, no significance.

![Figure 2](image2.png)  
**Figure 2.** LBP alleviates IL-1β-induced the elevation of Cox-2. A. Cox-2 expression in ATDC5 cells was admeasured through western blot. B. Cox-2 mRNA level in ATDC5 cells was gauged through qRT-PCR. The bars showed means ± SD accompanied by three independent experiments. *$p < .05$; **$p < .01$. 


The level was raised in LBP + miR-124 inhibitor group as relative to the miR-124 inhibitor group (p < .01, Figure 3(F)). The above results show that LBP suppresses IL-1β-evoked Cox-2 elevation through up-regulating miR-124.

LBP restrained IL-1β-triggered activation of NF-κB and JNK pathways via up-regulating miR-124

Associated signal pathways were also investigated in our following research. In Figure 4(A) and 4(B), p-p65 and p-IκBα levels were significantly elevated in IL-1β-induced group (p < .01), identifying that IL-1β-evoked the activation of NF-κB pathway through promoting the phosphorylation of p65 and IκBα. Then, LBP treatment restrained NF-κB signal pathway (p < .05). Nevertheless, the combination of miR-124 inhibitor with LBP weakened the inhibiting effect of LBP on NF-κB signal pathway activation (p < .05). Similar results were shown in the detection of JNK pathway. We observed that suppressed level of p-JNK was elevated by the combination of miR-124 inhibitor with LBP (p < .05, Figure 4(C) and 4(D)). The above results hinted that LBP restrained IL-1β-provoked activation of NF-κB and JNK pathways via up-regulating miR-124.

Discussion

OA is a chronic degenerative joint sickness, which brings great burden to patients worldwide [14]. The pathophysiology of OA has not been explored thoroughly, but amount of evidences have authenticated that pro-inflammatory cytokines produced from cartilage are closely associated with the progression of OA [15]. At present, clinical treatment of OA mainly focuses on relieving symptoms, such as injecting hyaluronic acid into the joint cavity of patients, or using antipyrétic analgesics, which alleviate the clinical symptoms of OA but also bring different degrees of side effects on patients [16]. Therefore, it is necessary to further clarify its pathogenesis and drug action mechanism on the basis of therapeutic research.

Scholars have found that the pathological process of OA is mediated by some pro-inflammatory factors. IL-1 is one of the most important one, which is closely related to the pathological process of cartilage matrix degradation in OA. The content of IL-1 in normal synovial fluid is very low, most of which are in the form of IL-1β [17]. Fell et al. for the first time reported that IL-1β could induce chondrocytes to produce proteolytic enzymes, which in turn mediate a series of
related roles of chondrocytes in cartilage degeneration [18]. At present, IL-1β has been widely used as an inducer of OA in vitro [19]. In our present study, ATDC5 cells were subjected to IL-1β (10 ng/mL for 6 h) to set up an in vitro model of cartilage damage. As an important constituent of traditional Chinese medicine, LBP has been extensively adopted in inflammatory-related diseases due to its remarkable anti-oxidant and anti-inflammation function [20]. In our present study, we observed that IL-6, IL-8 and TNF-α levels were all elevated by IL-1β stimulation. Then, the treatment of LBP effectively suppressed the elevated IL-6, IL-8 and TNF-α levels. Cox-2 mainly exists in the nuclear membrane and is not expressed in resting state only when chondrocytes were stimulated by IL-1β and other substances. Previous studies have found that controlling the expression of Cox-2 could suppress the inflammation in the early stage of OA, so as to alleviate symptoms and relieve pain [21]. We also found that IL-1β induced overexpression of Cox-2 and the combination with LBP suppressed the level of Cox-2. The above studies demonstrated that LBP was able to suppress IL-1β-induced chondrogenic ATDC5 cells inflammatory injury.

LBP is the dominating ingredients extracting from *lycium barbarum* L., which anti-inflammatory function has been certified in diverse illnesses [22,23]. Currently, several studies testified that the active ingredients extracted from traditional Chinese medicines exhibit the anti-inflammatory activity through mediating miRNAs. A fascinating research disclosed that LBP lightened H2O2-induced the repression of cell viability, and the promotion of apoptosis and autophagy through down-regulating miR-194 in PC-12 and SH-SY5Y cells [24]. It has been proved that miRNAs are involved in the regulation of chondrocyte differentiation and metabolism. Thus, we then set to explore whether LBP was associated with miRNA regulation in OA. Jones et al. found 16 differentially expressed miRNAs in cartilage tissues of 365 patients with OA [25]. Because of different types and different targeting gene, the miRNAs play a role in inhibiting [26] or promoting [27] the pathogenesis of OA. For example, Zhang et al. found that miR-146a aggravated pro-inflammatory cytokines, meanwhile the articular cartilage degeneration of miR-146a knockout mice was relieved as relative to that of the wild-type mice [28]. However, miRNA-145 was able to attenuate TNF-α-driven cartilage matrix degradation in osteoarthritis via targeting mitogen-activated protein kinase kinase 4 (MKK4) [29]. The anti-inflammation effect of miR-124 has been revealed in previous studies. Wang et al. has reported that PPARγ-evoked the elevation of miR-124 to inhibit pro-inflammatory cytokines productions in patients with sepsis [12]. On the basis of these researches, we speculate that miR-124 might join in mediating the anti-inflammatory activity of LBP in ATDC5 cells. Just as we imagined, the research revealed that LBP treatment successfully rescued the decreased level
of miR-124 triggered by IL-1β, prompting us to speculate that LBP exerted anti-inflammation effect through up-regulating miR-124 in IL-1β-managed ATDC5 cells. The research firstly disclosed the impacts of LBP-miR-124 axis in ATDC5 cells, which might provide a neoteric view for OA treatment.

NF-κB pathway is identified as a classical pro-inflammatory pathway due to the association of NF-κB with pro-inflammatory cytokines, chemokines, and adhesion molecules [30]. Zuo et al. demonstrated that iodoids restrained LPS-evoked inflammation by restraining the NF-κB pathway in polyycistic ovary syndrome [31]. JNK is another signal pathway which is closely associated with inflammation regulation. Sadeghi et al. reported that resveratrol ameliorated palmitate-evoked inflammation in skeletal muscle cells through assauging JNK/NF-κB pathway [32]. In our study, we also discovered that the NF-κB and JNK pathways were both activated by IL-1β stimulation. LBP administration impeded the NF-κB and JNK pathways via suppressing the rate of p/t-p65, p/t-IκBα and p/t-JNK. Then, the adding of miR-124 inhibitor offset the effect of LBP by promoting the phosphorylation of p65, IκBα and JNK, hinting that LBP suppressed IL-1β-triggered the activation of NF-κB and JNK pathways via enhancement of miR-124.

In conclusion, the findings from the current researches supply the important evidences that LBP may be a promising therapeutic target for OA due to its protecting effect on ATDC5 cells from IL-1β-evoked inflammatory injury. In addition, we for the first time revealed that LBP exerted anti-inflammation effect through up-regulating the expression of miR-124 via hindering NF-κB and JNK pathways. Nevertheless, because of the practical constraints, the current research did not provide the related in vivo experiments, which will be a vital point for the future research.

Disclosure statement

Authors declare that there is no conflict of interests.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Author contribution

Conceives and designed the experiments: Haibo Li, Huawei Ni and Guoxiu Wang. Performed the experiments: Huawei Ni, Guoxiu Wang and Yu Xu. Analyzed the data: Huawei Ni, Guoxiu Wang and Xiaomin Gu. Contributed reagents/materials/analysis tools: Chengzhang Sun. Wrote the paper: Haibo Li.

References

[1] Hashimoto M, Nakasa T, Hikata T. Molecular network of cartilage homeostasis and osteoarthritis. Med Res Rev. 2008;28(3):464–481.
[2] Tang X, Wang S, Zhan S, et al. The prevalence of symptomatic knee osteoarthritis in China: results from the China health and retirement longitudinal study. Arthritis Rheumatol (Hoboken, NJ). 2016;68(3):648–653.
[3] Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthr Cartil. 2013;21(1):16–21.
[4] Cai H, Yang X, Cai Q, et al. Lycium barbarum L. polysaccharide (LBP) reduces glucose uptake via down-regulation of SGLT-1 in Caco2 cell. Molecules (Basel, Switzerland). 2017;22(2):341.
[5] Mao Y, Xiao B, Jiang Z, et al. Growth inhibition and cell-cycle arrest of human gastric cancer cells by Lycium barbarum polysaccharide. Med Oncol. 2010;27(3):785–790.
[6] Yi R, Liu X-M, Dong Q. A study of Lycium barbarum polysaccharides (LBP) extraction technology and its anti-aging effect. AJTCAM. 2013;10(4):171–174.
[7] Ge JB, Lu HJ, Song XJ. [Protective effects of LBP on cerebral ischemia reperfusion injury in mice and mechanism of inhibiting NF-kappaB, TNF-alpha, IL-6 and IL-1beta]. Zhongguo Zhong Yao za zhi – Zhongguo zhongyao za zhi – CJCM. 2017;42(2):326–331.
[8] Liu Y, Lv J, Yang B, et al. Lycium barbarum polysaccharide attenuates type II collagen-induced arthritis in mice. Int J Biol Macromol. 2015;78:318–323.
[9] Elbashir SM, Harborth J, Lendeckel W, et al. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001;411(6836):494–499.
[10] Miyaki S, Sato T, Inoue H, et al. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev. 2010;24(11):1173–1185.
[11] Wu DP, Zhang JL, Wang YJ, et al. MiR-1246 promotes LPS-induced inflammatory injury in chondrogenic cells ATDC5 by targeting HNF4-gamma. Cell Physiol Biochem. 2017;43(5):2010–2021.
[12] Wang D, Shi L, Xin W, et al. Activation of PPAR-gamma inhibits pro-inflammatory cytokines production by upregulation of miR-124 in vitro and in vivo. Biochem Biophys Res Comm. 2017;486(3):726–731.
[13] Yu A, Zhang T, Duan H, et al. MiR-124 contributes to M2 polarization of microglia and confers brain inflammatory protection via the C/EBP-alpha pathway in intracerebral hemorrhage. Immunol Lett. 2017;182:1–11.
[14] Chu CR, Millis MB, Olson SA. Osteoarthritis: from palliation to prevention: AOA critical issues. J Bone Joint Surg Am Vol. 2014;96(15):e130.
[15] Novakofski KD, Torre CJ, Fortier LA. Interleukin-1alpha, -6, and -8 levels and their associations with knee osteoarthritis in China: results from the China health and retirement longitudinal study. Arthrit Rheumatol (Hoboken, NJ). 2012;68(3):653–663.
[16] Daheshia M, Yao JQ. The interleukin 1beta pathway in the pathogenesis of osteoarthritis. J Rheumatol. 2008;35(12):2306–2312.
[17] Yu A, Zhang T, Duan H, et al. MiR-124 promotes LPS-induced inflammatory injury in chondrogenic cells ATDC5 by targeting HNF4-gamma. Cell Physiol Biochem. 2017;43(5):2010–2021.
[18] Taruc-Uy RL, Lynch SA. Diagnosis and treatment of osteoarthritis. Primary Care. 2013;40(4):821–836.
[19] Daheshia M, Yao JQ. Interleukin 1beta pathway in the pathogenesis of osteoarthritis. J Rheumatol. 2008;35(12):2306–2312.
[20] Corbett PR, Honey GD, Fletcher PC. From prediction error to psychosis: ketamine as a pharmacological model of delusions. J Psychopharmacol. 2007;21(3):238–252.
[21] Shakibaei M, John T, Seifarth C, et al. Resveratrol inhibits IL-1beta-induced stimulation of caspase-3 and cleavage of PARP in human articular chondrocytes in vitro. Ann New York Acad Sci. 2013;1271(1):251–257.
[22] Novakofski KD, Torre CJ, Fortier LA. Interleukin-1alpha, -6, and -8 levels and their associations with knee osteoarthritis in China: results from the China health and retirement longitudinal study. Arthrit Rheumatol (Hoboken, NJ). 2012;68(3):653–663.
microRNA-194 in PC-12 and SH-SY5Y cells. Cell Physiol Biochem. 2018;50(2):460–472.

[25] Jones SW, Watkins G, Le Good N, et al. The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-alpha and MMP13. Osteoarthr Cartil. 2009; 17(4):464–472.

[26] Stanczyk J, Pedrioli DM, Brentano F, et al. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis Rheum. 2008;58(4):1001–1009.

[27] Sugatani T, Hruska KA. Impaired micro-RNA pathways diminish osteoclast differentiation and function. J Biol Chem. 2009;284(7):4667–4678.

[28] Zhang X, Wang C, Zhao J, et al. miR-146a facilitates osteoarthritis by regulating cartilage homeostasis via targeting Camk2d and Ppp3r2. Cell Death Dis. 2017;8(4):e2734.

[29] Hu G, Zhao X, Wang C, et al. MicroRNA-145 attenuates TNF-alpha-driven cartilage matrix degradation in osteoarthritis via direct suppression of MKK4. Cell Death Dis. 2017;8(10):e3140.

[30] Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harbor Persp Biol. 2009;16(6):a001651.

[31] Zuo T, Zhu M, Xu W, et al. Iridoids with genipin stem nucleus inhibit lipopolysaccharide-induced inflammation and oxidative stress by blocking the NF-kappaB pathway in polycystic ovary syndrome. Cell Physiol Biochem. 2017;43(5):1855–1865.

[32] Sadeghi A, Seyyed Ebrahimi SS, Golestani A, et al. Resveratrol ameliorates palmitate-induced inflammation in skeletal muscle cells by attenuating oxidative stress and JNK/NF-kappaB pathway in a SIRT1-independent mechanism. J Cell Biochem. 2017;118(9):2654–2663.