Original Paper

Down-Regulation of MiR-150 Alleviates Inflammatory Injury Induced by Interleukin 1 via Targeting Kruppel-Like Factor 2 in Human Chondrogenic Cells

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Key Words

Mir-150 • KLF2 • Osteoarthritis • IL-1 • Inflammation

Abstract

Background/Aims: Interleukin-1 (IL-1) is known to be involved in cartilage degeneration following joint injury or due to osteoarthritis. In the present study, we explored the effects of miR-150 on IL-1-stimulated human chondrogenic cells ATDC5. **Methods:** ATDC5 cells were transfected with the mimic, inhibitor or negative controls specific for miR-150, and subsequently treated by IL-1. CCK8 assay, PI and FITC-conjugated Annexin V double-staining, Western blot, qRT-PCR and ELISA assay were performed to determine the changes of cell viability, apoptosis, and the release of pro-inflammatory cytokines. Targeting relationship between miR-150 and KLF2 was detected by dual luciferase activity assay. **Results:** IL-1 reduced cell viability, induced apoptosis, and enhanced the expression and release of pro-inflammatory cytokines (IL-6, IL-8 and TNF- α) in ATDC5 cells. IL-1 also increased the expression of miR-150. Suppression of miR-150 alleviated IL-1-induced cell damage in ATDC5 cells, while overexpression of miR-150 resulted in an opposite impact. KLF2 was negatively regulated by miR-150, and it was proved as a target gene of miR-150. KLF2 overexpression exhibited protective actions in IL-1-injured ATDC5 cells, even if miR-150 was suppressed in cell. Moreover, IL-1-induced activation of NF- κ B and Notch pathways was alleviated by KLF2 overexpression. **Conclusions:** Suppression of miR-150 led to up-regulation of KLF2, which in turn protected ATDC5 cells against IL-1-induced injury.

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Introduction

Osteoarthritis (OA) is a common form of degenerative joint disease which is accompanied by pain and joint dysfunction. Currently, available treatments for OA are restricted to relieve symptoms which cannot achieve the goal of preventing or curing OA. Patients with OA take X. Yang and Q. Zhang contributed equally to this work.

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paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors or injection of glucocorticoid and hyaluronic acid, but there are several side-effects. For example, naproxen, one kind of NSAIDs, is associated with an elevated risk of gastrointestinal tract complications [1]. Celecoxib, a selective inhibitor of COX-2, has no significantly adverse gastrointestinal effects, but higher risk of myocardial infarction [2]. Thus, novel, safely, and effectively therapeutic strategies are required to be developed for the treatment of OA.

OA has been identified as a non-inflammatory arthritis. However, the dichotomy between inflammatory and degenerative arthritis is still not clear with the recognition of a plethora of ongoing immune processes within the OA joint and synovium. Anti-anabolic and pro-catabolic activities of inflammatory cytokines, particularly interleukin-1 (IL-1) are known to be involved in cartilage degeneration following joint injury or due to OA. IL-1 production is triggered in injured and degenerated joints, which in turn is known to induce pro-inflammatory pathways. Nowadays, IL-1 has been widely used for establishing the *in vitro* model of OA [3, 4].

microRNAs (miRNAs) are single-stranded RNA molecules that are widespread presented in eukaryotes, containing about 17 to 25 nucleotides. During OA, many miRNAs are expressed abnormally, and thus miRNAs present in human plasma and synovial fluid have been considered as promising biomarkers for OA [5]. For instance, the expressions of miR-483, miR-22, and miR-377 were elevated, and miR-140, miR-29a and miR-25 were down-regulated in OA chondrocytes [6]. Besides, a growing number of miRNAs have been reported to be implicated in the development of OA. Hwang and his colleagues have demonstrated that miR-365 prevented IL-1-induced extracellular matrix loss by targeting HIF-2 α [7]. Wu et al., reported that suppression of miR-1246 significantly reduced LPS-induced inflammatory injury in chondrogenic cells [8].

miR-150 is one of the most popular and widely studied miRNAs. It has been reported to be low expressed in osteosarcoma tissues and cell lines, and acted as an anti-cancer gene [9]. Besides, it is a key modulator involved in orchestrating inflammation [10]. However, the role of miR-150 in OA has not been revealed yet. This study was designed to investigate the effect of miR-150 dysregulation on IL-1-induced chondrocyte injury *in vitro*. In addition, the relationship between miR-150 and KLF2 was explored to reveal the possibly underlying mechanisms of miR-150's action.

Materials and Methods

Cell culture and stimulation

The human chondrogenic ATDC5 cells (ECACC, Porton Down, Wiltshire, UK) were cultured in DMEM/F12 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 5% FBS (Gibco, Grand Island, NY), 100 U/mL penicillin (Sigma-Aldrich), and 100 μ g/mL streptomycin (Sigma-Aldrich) in a humidified incubator with 95% air and 5% CO₂ at 37°C. The cells were routinely cultured in 6-well plates (Corning, New York, NY) with a density of 6×10^4 /well.

The recombinant IL-1 β with purity greater than 98% (HPLC) was purchased from Sigma-Aldrich. Cells were treated by 1 ng/mL IL-1 β for 12 h, as previously described [11].

Cell transfection

The miR-150 mimic, miR-150 inhibitor and their correspondingly controls (Scramble and anti-NC) were synthesized by GenePharma Co (Shanghai, China).

The full-length wide-type KLF2 sequences were inserted into the pEX-2 plasmid (GenePharma). An empty pEX-2 plasmid was transfected as a negative control. siRNA specific for KLF2 was constructed into U6/Neo plasmid (GenePharma). An empty U6/Neo plasmid with non-targeting sequences was transfected as a negative control. Cell transfections were conducted using Lipofectamine 3000 reagent (Invitrogen) under non-serum condition.

CCK-8 assay

The transfected ATDC5 cells (5×10^3) in 96-well plate were treated by IL-1 for 12 h. After another 24 h of incubation at fresh culture medium, cell viability was assessed by using CCK-8 (Dojindo Molecular Technologies, Kyushu, Japan). Briefly, the culture medium was removed, and the cells were washed twice with phosphate buffer saline (PBS). 10 μ L CCK-8 solution was added, and the plates were incubated for 1 h at 37°C. The absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA).

Apoptosis assay

The ATDC5 cells (5×10^5) in 6-well plates were undergone transfection and IL-1 treatment, after which cell apoptosis was analyzed using Annexin V-FITC/PI apoptosis detection kit (Invitrogen, Carlsbad, CA). Briefly, 1×10^5 cells from each sample were washed twice with PBS, and stained with Annexin V-FITC and PI according to the manufacturer's protocol in the presence of 50 μ g/mL RNase A (Sigma-Aldrich). Apoptotic cells were distinguished by the FACS can (Beckman Coulter, Fullerton, CA). Data were quantified by using FlowJo software (Tree Star Inc. Ashland, OR).

qRT-PCR

qRT-PCR was performed to detect the mRNA levels of IL-6, IL-8, TNF- α , KLF2, and the RNA levels of miR-150 in ATDC5 cells after transfection and IL-1 treatment. Total RNAs in ATDC5 cells were extracted using RNA pure Rapid Extraction Kit (Biotek Corporation, Beijing, China). cDNA was transcribed using SuperScript™ IV First-Strand Synthesis System (Invitrogen). SYBRTM Green PCR Master Mix (Applied Biosystems, Foster City, CA) and mirVana™ qRT-PCR miRNA Detection kit (Invitrogen) were respectively used for detection of mRNA and miRNA expressions. GAPDH and U6 were used for internal controls. Data were quantified using the $2^{-\Delta\Delta CT}$ method.

ELISA

The ATDC5 cells (5×10^4) in 24-well plates were undergone transfection and IL-1 treatment, after which culture supernatant was collected. The concentrations of IL-6, IL-8 and TNF- α in the culture supernatant were measured by ELISA kits (R&D Systems, Abingdon, UK) according to the manufacturers' protocol.

Dual luciferase activity assay

The 3'UTR target site was generated by PCR and the luciferase reporter constructs with the KLF2 3'UTR carrying a putative miR-150-binding site into pMiR-report vector were amplified by PCR. Cells were co-transfected with the reporter construct, control vector and miR-150 or scramble using Lipofectamine 3000 (Life Technologies, USA). Reporter assays were done using the dual-luciferase assay system (Promega) following to the manufacturer's information.

Western blot

Total protein in ATDC5 cells after relevant transfection and treatment were extracted using RIPA lysis buffer (Beyotime, Shanghai, China). The purity of proteins was quantified by the BCA™ Protein Assay Kit (Pierce, Appleton, WI). Western blot system was established using a Bio-Rad Bis-Tris Gel system (Bio-Rad Laboratories, Hercules, CA). Proteins in equal concentration were electrophoresed in PAGE-SDS and transferred onto PDVF membranes (Millipore, Bedford, MA). After blocking in the 5% blocking buffer (Beyotime), the membranes were incubated with primary antibodies which were prepared in 5% blocking buffer at a dilution of 1:1,000. After incubation with primary antibodies at 4°C overnight, the PDVF membranes were incubated with secondary antibody for 1 h at room temperature. Signals of the bands were captured using Bio-Rad ChemiDoc™ XRS system (Bio-Rad). The intensity of the bands was quantified using Image Lab™ Software (Bio-Rad).

Statistical analysis

Unless part of the data from Western blot analysis, all other data is presented as the mean $\pm$ SD. Statistics were analyzed by the one-way analysis of variance (ANOVA) in SPSS 19.0 statistical software (SPSS Inc., Chicago, IL). A p-value of lower than 0.05 was considered as a significant result.

Results

IL-1 induces cell injury and release of pro-inflammatory cytokines in chondrocytes

A significant reduction of cell viability measured by CCK-8 assay was observed in ATDC5 cells treated with IL-1 as compared to the control cells ($p < 0.05$) (Fig. 1A). Apoptosis measured by PI and FITC double-staining was significantly increased in cells treated with IL-1 compared to control cells ($p < 0.001$) (Fig. 1B). The induced apoptosis by IL-1 was also confirmed by performing Western blot analysis. As shown in Fig. 1C, Bcl-2 was down-regulated, Bax was up-regulated and caspase-3 and -9 were cleaved remarkably in cells treated with IL-1 as compared to the control cells. Moreover, cells treated with IL-1 showed increased mRNA expressions (Fig. 1D) and concentrations of IL-6, IL-8 and TNF- α (Fig. 1E, F and G).

IL-1 induces the expression of miR-150

Enhanced expression of miR-150 quantified by RT-PCR was observed in cells treated with IL-1 as compared to control cells ($p < 0.01$) (Fig. 2).

Overexpression and suppression of miR-150

Significantly enhanced expression of miR-150 was observed in cells transfected with miR-150-mimic as compared to scramble cells ($p < 0.01$). Expression of miR-150 was suppressed in cells transfected with anti-miR-150 as compared to anti-NC ($p < 0.01$) (Fig. 3).

Fig. 1. Effects of IL-1 on chondrogenic ATDC5 cells.

A: IL-1 inhibited cell viability as assessed by CCK8 assay; B: IL-1 promoted cell apoptosis as assessed by iodide (PI) and fluorescein isothiocyanate (FITC)-conjugated Annexin V double-staining; C: IL-1 promoted cell apoptosis by regulating apoptosis-related proteins, as detected by Western blotting analysis; D: IL-1 promoted the mRNA expressions of inflammatory cytokines (IL-6, IL-8 and TNF- α), as evaluated by qRT-PCR; E-G: IL-1 promoted the releases of IL-6, IL-8, and TNF- α , as evaluated by ELISA assay. * indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$.

Fig. 2. Effects of IL-1 on miR-150 expression in chondrogenic ATDC5 cells. IL-1 induced the expression of miR-150 as evaluated by qRT-PCR analysis. ** indicates $p < 0.01$.

Fig. 3. Effects of miRNA transfection on miR-150 expression in chondrogenic ATDC5 cells. miR-150 mimic overexpressed miR-150 expression, and anti-miR-150 suppressed miR-150 expression, as detected by qRT-PCR. ** indicates $p < 0.01$.

Fig. 4. Effects of miR-150 on IL-1-injured chondrogenic ATDC5 cells. A: Overexpression of miR-150 decreased cell viability; B: enhanced cell apoptosis; C: promoted the dysregulations of apoptosis-related proteins; D: increased the expressions of pro-inflammatory cytokines; and E-G: increased the releases of pro-inflammatory cytokines, in ATDC5 cells treated by IL-1. * indicates $p < 0.05$.

Overexpression of miR-150 aggravates IL-1-induced cell injury

A decreased viability was observed in cells transfected with miR-150-mimic compared to scramble cells ($p < 0.05$) (Fig. 4A). Of contrast, an increased cell-viability was observed in cells transfected with anti-miR-150 compared to anti-NC ($p < 0.05$) (Fig. 4A). Apoptosis was significantly increased in cells transfected with miR-150-mimic compared to scramble cells ($p < 0.05$) (Fig. 4B and C). A significant decrease in apoptosis was observed in cells transfected with anti-miR-150 compared to anti-NC ($p < 0.05$) (Fig. 4B and C). Also, enhanced expression and release of IL-6, IL-8 and TNF- α were observed in cells transfected with miR-150-mimic compared to scramble cells ($p < 0.05$), as observed by qRT-PCR and ELISA assay (Fig. 4D, E, F and G). As expected, anti-miR-150 impacted the expression and release of IL-6, IL-8 and TNF- α resulted in completely apposite results.

MiR-150 negatively regulates KLF2, and KLF2 is a target of miR-150

A significant decrease of KLF2 expression was observed in cells transfected with miR-150-mimic compared to scramble cells ($p < 0.05$). On the contrary, an increased expression of KLF2 was observed in cells transfected with anti-miR-150 compared to anti-NC ($p < 0.01$) (Fig. 5A and B). The dual luciferase assay demonstrated that KLF2 was a target of miR-150, as observed by significantly reduced luminescence in cells transfected with miR-150-mimic compared to scramble control cells ($p < 0.05$) (Fig. 5C).

Fig. 5. Effects of miR-150 on the expression of KLF2 in chondrogenic ATDC5 cells. A: miR-150 negatively regulated KLF2 mRNA expression, as assessed by qRT-PCR; B: miR-150 negatively regulated KLF2 protein expression, as assessed by Western blotting analysis; C: KLF2 was a target of miR-150, as assessed by dual luciferase activity assay. * indicates $p < 0.05$, and ** indicates $p < 0.01$.

Fig. 6. A: Effects of co-transfection with anti-miR-150 and sh-KLF2 on IL-1-injured chondrogenic ATDC5 cells. A-B: pEX-KLF2 overexpressed KLF2 expression, and sh-KLF2 suppressed KLF2 expression, as detected by qRT-PCR and Western blot. C: Suppression of miR-150 attenuated IL-1-impaired cell viability by overexpression of KLF2; D-E: Suppression of miR-150 attenuated IL-1-induced cell apoptosis by overexpression of KLF2; F: Suppression of miR-150 attenuated IL-1-induced the expressions of pro-inflammatory cytokines by overexpression of KLF2; G-I: Suppression of miR-150 attenuated IL-1-induced the releases of pro-inflammatory cytokines by overexpression of KLF2. * indicates $p < 0.05$, and ** indicates $p < 0.01$.

Suppression of miR-150 reduces cell injury by overexpression of KLF2

A significant increase of KLF2 expression was observed in pEX-KLF2 transfected cells compared to pEX transfected cells ($p < 0.01$) (Fig. 6A and B). Meanwhile, KLF2 expression was significantly suppressed by sh-KLF2 compared to sh-NC ($p < 0.01$) (Fig. 6A and B). Of note, compared to the IL-1+anti-miR-150+shNC group, cell viability was reduced ($p < 0.05$, Fig. 6C), apoptosis was enhanced ($p < 0.01$, Fig. 6D and E), and the expression and release of pro-inflammatory cytokines were increased ($p < 0.05$, Fig. 6F, G, H and I) significantly in IL-1+anti-miR-150+sh-KLF2 group.

Overexpression of KLF2 reduces IL-1-induced cell injury

Cell viability was significantly increased in cells transfected with pEX-KLF2 compared to cells transfected with pEX ($p < 0.05$); meanwhile, a significant decrease of viability was observed in sh-KLF2 compared to sh-NC ($p < 0.05$) (Fig. 7A). Similarly, a decrease in

Fig. 7. Effects of KLF2 on IL-1-injured chondrogenic ATDC5 cells. A: Overexpression of KLF2 attenuated IL-1-induced viability inhibition; B-C: attenuated IL-1-induced cell apoptosis; D: attenuated IL-1-induced the expressions of pro-inflammatory cytokines; E-G: attenuated IL-1-induced the releases of pro-inflammatory cytokines. * indicates $p < 0.05$.

Fig. 8. Effects of KLF2 on NF- κ B and Notch pathways. Overexpression of KLF2 repressed the activation of NF- κ B and Notch pathways, as detected by Western blot analysis.

apoptosis was observed in cells transfected with pEX-KLF2 compared to cells transfected with pEX ($p < 0.05$), and apoptosis was significantly induced by sh-KLF2 compared to sh-NC ($p < 0.05$) (Fig. 7B and 7C). Significantly decreased expression and release of IL-6, IL-8 and TNF- α were observed in cells transfected pEX-KLF2 ($p < 0.05$) when compared to pEX (Fig. 7D, E, F and G). sh-KLF2 affected on the expression and release of IL-6, IL-8 and TNF- α resulted in apposite results.

Overexpression of KLF2 represses the activation of NF- κ B and Notch pathways

IL-1 remarkably phosphorylated p65, and IkB α , and up-regulated Notch-1, Notch-2 and Notch-3 (Fig. 8). The phosphorylation and up-regulation induced by IL-1 were attenuated by pEX-KLF2, while were accelerated by sh-KLF2 (Fig. 8).

Discussion

OA is often associated with multiple risk factors, most notably age, joint trauma, altered biomechanics, and obesity [12, 13]. There are scientific reports suggest that pathologic changes related to OA share a common final pathway and subsequently operate to perpetuate joint destruction and eventual failure. It was reported that IL-1 was involved in the pathogenesis of OA, that it could significantly reduce chondrocytes proliferation, induced cell death, and accelerate inflammation [14, 15]. This was also confirmed in the current study, that IL-1 reduced ATDC5 cells viability, induced apoptosis and the increased the release of pro-inflammatory cytokines, like IL-6, IL-8 and TNF- α . These data indicated that apoptotic and inflammatory injury was significantly induced by IL-1 in ATDC5 cells.

We also showed that miR-150 was highly expressed in response to IL-1 treatment. More importantly, miR-150 suppression attenuated IL-1-induced injury in ATDC5 cells, as evidenced by the increased viability, the reduced apoptosis and the repressed release of pro-inflammatory cytokines. The functions of miR-150 in cell survival have been widely studied previously, but the exact role of miR-150 was confusing. Several studies suggested that miR-

150 acted as anti-apoptotic and anti-inflammatory modulator in response to stimulations. For example, Xue and Li demonstrated that, miR-150 could protect lung and airway epithelial cells against smoke-induced inflammation and apoptosis [16]. Zhu et al., indicated that miR-150 could attenuate hypoxia-induced apoptosis and immune inflammatory response in dendritic cells and cardiomyocytes [17]. Of contrast, Qin et al., demonstrated that ectopic expression of miR-150 accelerated oxidized low-density lipoprotein (ox-LDL)-induced apoptosis in endothelial cells [18]. Our findings were consistence with the study of Qin, suggested the protective effects of miR-150 suppression on IL-1-injured ATDC5 cells. This contradiction may be caused by different cell types and stimulations.

KLFs are a member of a multigene family of transcriptional factors having a three-Cys2-His2-zinc-finger domain [19, 20]. Studies demonstrate a key role for the KLFs in various processes of cell growth and differentiation. KLF2 is a zinc finger-containing transcription factor, and has been identified as a modulator of the pro-inflammatory response [21]. KLF2 has been shown to mediate anti-inflammatory effects of atheroprotective flow by inhibition of pro-inflammatory transcription factor ATF2 [22]. Das et al. reported that THP-1 cells overexpressing KLF2 showed reduced LPS-mediated induction of pro-inflammatory cytokines and chemokines [23]. In the present study, we found that KLF2 was a target gene of miR-150. Besides, the protective actions of miR-150 suppression on IL-1-induced cell injury were partially eliminated by KLF2 silencing. Our data indicated that miR-150 suppression alleviated inflammatory injury induced by IL-1 possibly via targeting KLF2 in ATDC5 cells.

The pathogenesis of OA involves multiple signaling pathways, among which NF- κ B and Notch signaling pathways play significant roles in cartilage damage [24]. Following IL-1 treatment, transcriptional activity of NF- κ B is enhanced [25], which in turn regulates genes involved in immune response, inflammation and cell survival [26, 27]. Notch signaling determines cell fate via regulation of differentiation and apoptosis [28]. In a murine surgical model, inhibition of Notch signaling in chondrocytes suppresses OA development [29]. Thus, core factors in NF- κ B and Notch signaling pathways have been considered as potential therapeutic OA targets [30]. Herein, we showed that IL-1-induced activation of NF- κ B and Notch pathways was alleviated by KLF2 overexpression, indicating KLF2 protected ATDC5 via modulation of these two pathways.

To conclude, our findings in this study demonstrated a protective function of miR-150 suppression on IL-1-injured ATDC5 cells. The protective actions might be released by targeting KLF2 and modulation of NF- κ B and Notch pathways. This study provides a novel insight into the role of miR-150 in IL-1 induced injury in OA.

Abbreviations

KLF2 (Kruppel-like factor 2); shRNA (Short hairpin RNA); siRNA (small interfering RNA); qRT-PCR (Quantitative real-time polymerase chain reaction); siNC (small interfering negative control).

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Disclosure Statement

These authors declare to have no conflict of interests..

References

- 1 Flood J: The role of acetaminophen in the treatment of osteoarthritis. *Am J Manag Care* 2010;16 Suppl Management:S48-54.
- 2 Chen YF, Jobanputra P, Barton P, Bryan S, Fry-Smith A, Harris G, Taylor RS: Cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs (etodolac, meloxicam, celecoxib, rofecoxib, etoricoxib, valdecoxib and lumiracoxib) for osteoarthritis and rheumatoid arthritis: a systematic review and economic evaluation. *Health Technol Assess* 2008;12:1-278, iii.
- 3 Zhou Z, Du D, Chen A, Zhu L: Circular RNA expression profile of articular chondrocytes in an IL-1beta-induced mouse model of osteoarthritis. *Gene* 2018;644:20-26.
- 4 Lou Y, Wang C, Zheng W, Tang Q, Chen Y, Zhang X, Guo X, Wang J: Salvianolic acid B inhibits IL-1beta-induced inflammatory cytokine production in human osteoarthritis chondrocytes and has a protective effect in a mouse osteoarthritis model. *Int Immunopharmacol* 2017;46:31-37.
- 5 Trachana V, Ntounou E, Anastasopoulou L, Tsezou A: Studying microRNAs in osteoarthritis: critical overview of different analytical approaches. *Mech Ageing Dev* 2018;10.1016/j.mad.2018.02.006
- 6 Iliopoulos D, Malizos KN, Oikonomou P, Tsezou A: Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One* 2008;3:e3740.
- 7 Hwang HS, Park SJ, Lee MH, Kim HA: MicroRNA-365 regulates IL-1beta-induced catabolic factor expression by targeting HIF-2alpha in primary chondrocytes. *Sci Rep* 2017;7:17889.
- 8 Wu DP, Zhang JL, Wang JY, Cui MX, Jia JL, Liu XH, Liang QD: MiR-1246 Promotes LPS-Induced Inflammatory Injury in Chondrogenic Cells ATDC5 by Targeting HNF4gamma. *Cell Physiol Biochem* 2017;43:2010-2021.
- 9 Xu J, Wang Z, Liao Z, Dai D, Ma X: MicroRNA-150 functions as an antioncogenic regulator in osteosarcoma. *Oncol Lett* 2017;14:2483-2490.
- 10 Sang W, Wang Y, Zhang C, Zhang D, Sun C, Niu M, Zhang Z, Wei X, Pan B, Chen W, Yan D, Zeng L, Loughran TP, Jr., Xu K: MiR-150 impairs inflammatory cytokine production by targeting ARRB-2 after blocking CD28/B7 costimulatory pathway. *Immunol Lett* 2016;172:1-10.
- 11 Yasuhara R, Miyamoto Y, Akaike T, Akuta T, Nakamura M, Takami M, Morimura N, Yasu K, Kamijo R: Interleukin-1beta induces death in chondrocyte-like ATDC5 cells through mitochondrial dysfunction and energy depletion in a reactive nitrogen and oxygen species-dependent manner. *Biochem J* 2005;389:315-323.
- 12 Felson DT: Clinical practice. Osteoarthritis of the knee. *N Engl J Med* 2006;354:841-848.
- 13 Das M, Lu J, Joseph M, Aggarwal R, Kanji S, McMichael BK, Lee BS, Agarwal S, Ray-Chaudhury A, Iwenofu OH, Kuppusamy P, Pompili VJ, Jain MK, Das H: Kruppel-like factor 2 (KLF2) regulates monocyte differentiation and functions in mBSA and IL-1beta-induced arthritis. *Curr Mol Med* 2012;12:113-125.
- 14 Sandell LJ, Aigner T: Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. *Arthritis Res* 2001;3:107-113.
- 15 Ji B, Guo W, Ma H, Xu B, Mu W, Zhang Z, Amat A, Cao L: Isoliquiritigenin suppresses IL-1beta induced apoptosis and inflammation in chondrocyte-like ATDC5 cells by inhibiting NF-kappaB and exerts chondroprotective effects on a mouse model of anterior cruciate ligament transection. *Int J Mol Med* 2017;40:1709-1718.
- 16 Xue H, Li MX: MicroRNA-150 protects against cigarette smoke-induced lung inflammation and airway epithelial cell apoptosis through repressing p53: MicroRNA-150 in CS-induced lung inflammation. *Hum Exp Toxicol* 2017;10.1177/0960327117741749960327117741749.
- 17 Zhu J, Yao K, Guo J, Shi H, Ma L, Wang Q, Liu H, Gao W, Sun A, Zou Y, Ge J: miR-181a and miR-150 regulate dendritic cell immune inflammatory responses and cardiomyocyte apoptosis via targeting JAK1-STAT1/c-Fos pathway. *J Cell Mol Med* 2017;21:2884-2895.
- 18 Qin B, Shu Y, Xiao L, Lu T, Lin Y, Yang H, Lu Z: MicroRNA-150 targets ELK1 and modulates the apoptosis induced by ox-LDL in endothelial cells. *Mol Cell Biochem* 2017;429:45-58.
- 19 Bieker JJ: Kruppel-like factors: three fingers in many pies. *J Biol Chem* 2001;276:34355-34358.
- 20 Feinberg MW, Lin Z, Fisch S, Jain MK: An emerging role for Kruppel-like factors in vascular biology. *Trends Cardiovasc Med* 2004;14:241-246.
- 21 Liu Y, Wang J, Yi Y, Zhang H, Liu J, Liu M, Yuan C, Tang D, Benjamin IJ, Xiao X: Induction of KLF4 in response to heat stress. *Cell Stress Chaperones* 2006;11:379-389.

- 22 Fledderus JO, van Thienen JV, Boon RA, Dekker RJ, Rohlena J, Volger OL, Bijnens AP, Daemen MJ, Kuiper J, van Berkel TJ, Pannekoek H, Horrevoets AJ: Prolonged shear stress and KLF2 suppress constitutive proinflammatory transcription through inhibition of ATF2. *Blood* 2007;109:4249-4257.
- 23 Das H, Kumar A, Lin Z, Patino WD, Hwang PM, Feinberg MW, Majumder PK, Jain MK: Kruppel-like factor 2 (KLF2) regulates proinflammatory activation of monocytes. *Proc Natl Acad Sci U S A* 2006;103:6653-6658.
- 24 Saito T, Tanaka S: Molecular mechanisms underlying osteoarthritis development: Notch and NF-kappaB. *Arthritis Res Ther* 2017;19:94.
- 25 Toegel S, Wu SQ, Otero M, Goldring MB, Leelapornpisid P, Chiari C, Kolb A, Unger FM, Windhager R, Viernstein H: Caesalpinia sappan extract inhibits IL1beta-mediated overexpression of matrix metalloproteinases in human chondrocytes. *Genes Nutr* 2012;7:307-318.
- 26 Bonizzi G, Karin M: The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004;25:280-288.
- 27 Hacker H, Karin M: Regulation and function of IKK and IKK-related kinases. *Sci STKE* 2006;2006:re13.
- 28 Yoon K, Gaiano N: Notch signaling in the mammalian central nervous system: insights from mouse mutants. *Nat Neurosci* 2005;8:709-715.
- 29 Hosaka Y, Saito T, Sugita S, Hikata T, Kobayashi H, Fukai A, Taniguchi Y, Hirata M, Akiyama H, Chung UI, Kawaguchi H: Notch signaling in chondrocytes modulates endochondral ossification and osteoarthritis development. *Proc Natl Acad Sci U S A* 2013;110:1875-1880.
- 30 Marcu KB, Otero M, Olivetto E, Borzi RM, Goldring MB: NF-kappaB signaling: multiple angles to target OA. *Curr Drug Targets* 2010;11:599-613.