

MiR-379-5p Promotes Chondrocyte Proliferation via Inhibition of PI3K/Akt Pathway by Targeting YBX1 in Osteoarthritis

Hongjun Zhang¹, Wendi Zheng¹, Du Li¹, and Jia Zheng¹ 

Abstract

Objective. We evaluated the ability of miR-379-5p to influence the proliferation of osteoarthritis chondrocytes and elucidated the regulatory mechanism of miR-379-5p in osteoarthritis. **Methods.** Real time polymerase chain reaction (RT-PCR) was used to detect the expression of miR-379-5p and YBX1 in knee articular cartilages of human. Cell proliferation, inflammatory factors, extracellular matrix (ECM) degradation-associated proteins and proteins in PI3K/Akt pathway were assessed in rat primary chondrocytes treated with interleukin (IL)-1 β or/and miR-379-5p mimics or miR-379-5p inhibitor via cell counting assay kit-8 (CCK-8), enzyme-linked immunosorbent assay (ELISA), immunofluorescence and Western blotting (WB). The target of miR-379-5p predicted by TargetScan and miRwalk software was verified by luciferase reporter assay. Safranin O-fast green staining, immunohistochemistry, and WB were performed to observe the effect of miR-379-5p agomir on development of osteoarthritis in rats. **Results.** MiR-379-5p was down-regulated in human osteoarthritic tissues and negatively correlated with YBX1 expression. High level of miR-379-5p in chondrocytes with IL-1 β stimulated increased cell viability, the expression of proliferation-related protein and extracellular matrix (ECM)-related proteins collagen II and aggrecan. However, the expression of inflammatory factors and ECM-related proteins matrix metalloproteinases (MMP-1) and MMP-13 was decreased. Luciferase reporting assay verified the targeting relationship between miR-379-5p and YBX1. This function of miR-379-5p was exerted through PI3K/Akt pathway and could be blocked by the PI3K/Akt pathway inhibitor LY294002. MiR-379-5p agomir promoted the articular chondrocytes proliferation and alleviated cartilage degradation *in vivo*. **Conclusion.** Our findings reveal that miR-379-5p can promote the articular chondrocytes proliferation in osteoarthritis (OA) by interacting with YBX1 and regulating PI3K/Akt pathway. Restoring miR-379-5p might be a future therapeutic strategy for OA.

Keywords

miR-379-5p, YBX1, IL-1 β , chondrocytes, osteoarthritis

Introduction

Osteoarthritis (OA) is one of the most common joint diseases with degenerative changes in articular cartilage that involve the entire joint tissue. It also involves focal cartilage loss, bone hyperplasia (osteophytes and subchondral sclerosis), thickening of the synovial capsule, and structural changes in the ligaments and surrounding muscles around the joint.¹ OA increases with longevity and has become one of the global clinical problems.² The pathophysiology of OA remains unclear. However, the current view is that mechanical, inflammatory, and metabolic factors are involved in the complex pathogenesis leading to joint destruction and failure.³

MicroRNAs (miRNAs) are a class of non-coding single-stranded RNA molecules encoded by endogenous genes with

a length of about 22 nucleotides. They can regulate gene expression at the transcriptional and post-transcriptional levels by directly binding to specific sequences in target mRNAs.⁴ MiRs have been shown to be regulators of hundreds of genes involved in chondrogenesis, internal environmental stability, and OA pathology.⁵ Mechanical loading is

¹Department of Orthopedics, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, School of Clinical Medicine, Henan University, Zhengzhou, China

Corresponding Author:

Jia Zheng, Department of Orthopedics, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, School of Clinical Medicine, Henan University, No. 7 Weiwu Road, Zhengzhou 450003, Henan, China.

Email: h28938@yeah.net



very important for the regulation of cartilage metabolism and affects miRNAs expression.⁶ Some miRNAs have been shown to regulate chondrocyte hypertrophy, proteolytic enzyme synthesis, and OA-related signaling pathways.⁷ In addition, pathological phenomena generally associated with cartilage degeneration and OA, including inflammation, obesity, apoptosis, and autophagy defects, are also associated with miRNAs specific activity.⁸

Recent research has found that miR-379-5p was lowly expressed in cartilage tissues of patients with knee OA through deep sequencing of microRNAs.⁹ MiR-379-5p is located on chromosome 14q32.31, and it has been found to be lowly expressed in breast cancer and hepatocellular carcinoma.^{10,11} High level of miR-379-5p can inhibit the migration, invasion, and epithelial-mesenchymal transition of hepatocellular carcinoma cells.¹² Another new study showed that A-to-I-edited miR-379-5p inhibited cell proliferation and promoted apoptosis of breast cancer, ovarian cancer, renal cancer, and lung cancer, compared to wild-type miRNA.¹³ In human hepatic cells, miR-379-5p regulation of gasdermin D suppressed arsenite-induced activation of hepatic stellate cells and fibrosis.¹⁴ Although the high level of miR-379-5p has a role in promoting the proliferation of chondrocytes,¹⁵ the possible pathological mechanism between miR-379-5p and OA is still unclear.

We screened out a total of 10 target genes (UBE2E3, EIF4G2, ZBTB26, SLC20AL, LRRC8C, ELMOD2, YBX1, ADAMTS15, HSPA5, TNRC6B) for miR-379-5p through TargetScan 7.2 (http://www.targetscan.org/vert_72/) and miRDB (<http://mirdb.org/>) online tools and all 10 target genes were conserved among human beings, mice and rats. And there was an evidence showed that YBX1 was indeed identified and validated as a direct target of miR-379-5p.¹⁶ Then, we found that Y-box binding protein 1 (YBX1) is a DNA- and RNA-binding protein that contains a highly conserved cold shock domain and is involved in many different cellular processes, such as cell proliferation and development.¹⁷ The expression of YBX1 also can affect the downstream phosphatidylinositol-3-kinase-protein kinase B (PI3K/Akt) signaling pathway and activate it.¹⁸ PI3K/Akt mediated signaling pathway is one of the most critical pathways in the regulation of cell survival, proliferation, differentiation and apoptosis.¹⁹ PI3K/Akt was closely related to the occurrence and development of inflammation.²⁰ It was a signaling pathway that was significantly activated during the pathogenesis of OA.^{21,22} Inhibition of this pathway can significantly alleviate bone damage caused by OA.²³ Therefore, we hypothesize that YBX1 may be involved in the proliferation process of chondrocytes in OA. The purpose of this study was to investigate whether miR-379-5p affects the occurrence and development of OA by targeting YBX1 and thereby inhibiting the PI3K/Akt signaling pathway and promoting chondrocyte proliferation.

Materials and Methods

Cartilage Samples

OA cartilage samples were collected from 32 OA patients (age 64.3 [6.2] years) underwent total knee replacement surgery in the Department of Orthopedics, Henan Provincial People's Hospital, and another 14 normal cartilage samples (age 56.8 [4.4] years) were collected from healthy patient underwent amputation in the Department of Orthopedics, Henan Provincial People's Hospital. These samples were used to detect the level of miR-379-5p and YBX1, and isolate the chondrocytes. The study has been approved by the ethical committee of the Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, School of Clinical Medicine, Henan University. All participants provided their written informed consents.

Cell Culture

We took cartilage from the knee joint of the sacrificed rats under aseptic conditions and placed it in a sterile plate. The cartilage was cut into 1 mm³ pieces and placed in a conical flask. We added 0.2% type II collagenase and shake for 30 min. Then we added 0.25% trypsin and digest for 20 min. The supernatant was filtered through a 200-mesh screen and centrifuged. After supernatant was discarded, the cells were re-suspended in Dulbecco's modified Eagle medium (DMEM) containing 100 U/mL penicillin, 100 mg/L streptomycin, and 10% fetal bovine serum. The cells were collected by centrifugation and cultured in DMEM medium. The cells were subcultured when the bottom of the flask was filled with cells. When the cells were passed on to the third generation, they were used for subsequent experiments.

Cell Treatment

We divided the isolated and cultured rat chondrocytes into the following groups: NC (negative control) group, BC (blank control, without any transfection) group, interleukin (IL)-1 β (adding IL-1 β and adjusting the final concentration to 10 ng/mL) group, IL-1 β +NC mimics (adding IL-1 β and transfecting NC mimics) group, IL-1 β +NC inhibitor (adding IL-1 β and transfecting NC inhibitor) group, IL-1 β +miR-379-5p mimics (adding IL-1 β and transfecting miR-379-5p mimics) group, IL-1 β +miR-379-5p inhibitor (adding IL-1 β and transfecting miR-379-5p inhibitor) group, YBX1 (4 μ g) group, sh-YBX1 (4 μ g) group, IL-1 β +miR-379-5p mimics+LY294002 (with IL-1 β , miR-379-5p mimics and LY294002) group, IL-1 β +miR-379-5p inhibitor+LY294002 (with IL-1 β , miR-379-5p inhibitor and LY294002) group. Before transfection, rat chondrocytes were seeded into 12-well plates with 1×10^5 per well to culture 24 to 48 h. Chondrocytes of 3 to 5 generations were selected and transfected into chondrocytes with

Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and then the corresponding indexes were detected.

Luciferase Reporter Assay

The target of miR-379-5p was predicted using TargetScan and miRwalk software, and the 3'UTR of *YBX1* mRNA may be complementarily paired with the seed sequences of miR-379-5p. The luciferase reporter assay was used to determine that miR-379-5p directly targets to the 3'-UTR of *YBX1* mRNA. The *YBX1* 3'UTR including miR-379-5p binding sites (*YBX1* 3'-UTR-WT) and according mutations (*YBX1* 3'-UTR-MT) were cloned into a pmirGLO vector (Promega, Madison, WI, USA), respectively, between the PmeI and XbaI restriction sites. Then NC mimics (20 nM) or miR-379-5p mimics (20 nM) or NC inhibitor (20 nM) or miR-379-5p inhibitor (20 nM), and 0.5 µg *YBX1* 3'-UTR-WT (20 nM) or 0.5 µg *YBX1* 3'-UTR-MT (20 nM) were cotransfected into chondrocytes using lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). The luciferase activity was measured using the Dual-Glo® Luciferase Assay System (promega, Madison, WI, USA) and a MicroLumatPlus LB96V luminometer (Berthold, Hamburg, Germany) at 24 h after cotransfection. The miR-379-5p mimics sequence (5'-3') was GCGCUGGUAGACU AUGGAAUU; The NC mimics sequence (5'-3') was UUGUACUACACAAAAGUACUG.

The miR-379-5p inhibitor sequence (5'-3') was UUCAUAGUCUACCAGCGCUU. The NC inhibitor sequence (5'-3') was CAGUACUUUUGUGUAGUACAA.

OA Modeling

Twelve-week-old Sprague-Dawley rats were purchased from Shanghai SLAC Laboratory Animal CO. LTD. All rats were divided into 4 groups: sham, OA, OA+NC and OA+ agomir, 6 rats in each group. All rats were anesthetized by intraperitoneal injection of 3% pentobarbital sodium (30 mg/kg) (Tocris, Minneapolis, MN, USA) before surgery. After anesthesia, the articular cavity was exposed through an anterior incision on the medial side of the left knee. The anterior and posterior cruciate ligaments were transected and the medial meniscus was resected. The bleeding was stopped and the surgical wound was sealed layer by layer. Rats in sham group underwent sham surgery only to expose the surface of the right knee cartilage following by suture. Rats in the other 3 groups underwent anterior cruciate ligament transection (ACLT) to induce arthritis before suture. Rats in OA+NC group were injected miR-379-5p NC with a dose of 50 nmol (100 µL saline) into the joint cavity 6 h before ACLT and per week in 12 weeks after surgery. In the OA+agomir group, miR-379-5p agomir was injected in the same way as OA+NC group. We give it by local intra-articular injection after mixed agomir with Lipofectamine™2000 (Invitrogen, Carlsbad,

CA, USA). Six rats in each group were sacrificed every 4 weeks after surgery, and knee articular cartilage were isolated to detect the levels of miR-379-5p and *YBX1* mRNA using real time polymerase chain reaction (PCR) (RT-PCR). Knee articular cartilage samples (with bone) from rats 12 weeks after surgery were used for safranin O-fast green staining. Then, cartilage sample was carefully separated from the bone with a No.10 anatomical blade under aseptic conditions. The whole piece of tissue was fixed with large tweezers during separation. Sterilized phosphate buffered saline (PBS) was added to the surface to keep the cartilage moist. Finally, the isolated cartilage was placed in a culture dish containing PBS for subsequent experiments including RT-PCR, immunohistochemistry (IHC), and western blotting. The severity of articular cartilage degeneration was graded using the Osteoarthritis Research Society International (OARSI) scoring system by blinded observers.²⁴ All animal studies were approved by the Ethics Committee of Zhengzhou University.

Real Time PCR

Once the samples were isolated, they were stored in liquid nitrogen tanks unless we used them right away. Before adding Trizol, we take tissue samples from liquid nitrogen tanks and grind them with liquid nitrogen. Then, total RNAs were isolated from cartilage samples and chondrocytes using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and were reversed transcribed using PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara, Kyoto, Japan). cDNA synthesis was performed with 1 µg of total RNA utilizing oligo dt-primer with a cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA). Real time PCR was performed using TB Green™ Premix Ex Taq™ (Tli RNaseH Plus) (Takara, Kyoto, Japan) on the qTOWER3G real time PCR system (analytikjena, Jena, Germany). Relative gene expression was calculated using $\Delta\Delta C_t$ method and was normalized to U6 or GAPDH.²⁵ The specific primer sequences (5'→3') were as follows: miR-379-5p-F: GCGCTGGTAGACTATGGAA; miR-379-5p-R: GTGCAGGGTCCGAGGT, U6-F: CTCGCTTCGGCAGCACA TATACT;U6-R:ACGCTTCACGAATTTGCGTGTCYBX1, YBX1-F: GGGTGCAGGAGAACAAGGTA; YBX1-R: TCTTCATTGCCGTCCTCTCT, PI3K-F: AGCATGGT CAGCTTTCTTCTT; PI3K-R: GAATGGAAGACGG GAGATTCA, AKT-F: CCAGGATCCATGGGTAGGA; AKT-R: GCAGCCCCTTTGACTTCTT, GAPDH-F: CCA TGTTTCGTCATGGGTGTGAACCA; GAPDH-R: GCCA GTAGAGGCAGGGATGATGTTTCPI3K.

Western Blotting

Total protein was isolated from chondrocytes and rat cartilage samples using Radio-Immunoprecipitation Assay (RIPA) lysis (Beyotime, Shanghai, China) buffer and was qualified with bicinchoninic acid (BCA) Assay Kit (Thermo

Scientific, Waltham, MA, USA). Protein (30 μ g per sample) was separated by SDS-PAGE on a 10% gel and transferred to a polyvinylidene difluoride membrane (Millipore, Boston, MA, USA). The protein-carrying membranes were blocked in 5% nonfat dry milk, and then incubated overnight at 4°C with primary antibodies against ki-67 (ab16667, 1:100), proliferating cell nuclear antigen (PCNA) (ab92552, 1:2,000), IL-6 (ab9324, 1:3000), tumor necrosis factor- α (TNF- α) (ab66579, 1:1,000), IL-8 (ab154390, 1:1000), IL-17 (ab150719, 1:1,000), MMP1 (10371-2-AP, 1:1,000), MMP13 (ab39012, 1:30,000), YBX1 (ab50575, 1:2,000), PI3K (ab86714, 1:10,000), p-PI3K (ab86714, 1:10,000), AKT (ab8805, 1:10,000), p-AKT (ab8805, 1:1,000), collagen II (ab34712, 1:2000), aggrecan (sc-166951, 1: 500) and GAPDH (ab181602, 1:10,000). The membranes were incubated with Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080, 1:5,000) or Rabbit Anti-Mouse IgG H&L (HRP) preadsorbed (ab6728, 1:2,000) for 1 h at room temperature and protein was detected using an enhanced chemiluminescence kit (Thermo Scientific, Waltham, MA, USA). Antibodies against MMP1 and aggrecan were purchased from Applygen Technologies (Beijing, China) and Santa Cruz (CA, USA), respectively, and the other antibodies were purchased from Abcam (Cambridge, UK).

Cell Counting Assay Kit-8 (CCK-8) Assay

Cultured chondrocytes were seeded into a 96-well plate with 1×10^4 cells in each well (150 μ L). The corresponding IL-1 β , NC mimics, miR-mimics, NC inhibitor and miR-inhibitor were added to the well according to the previous group. Then, 20 μ L of 5% CCK-8 solution (PA137267, Pierce, Rockford, IL, USA) was added to each well. The plate was incubated for additional 2.5 h before measuring the absorbance at 450 nm wavelength using a microplate reader (SPECTROstar Nano; BMG Labtech, Ortenberg, Germany).

Immunohistochemistry (IHC) and Safranin O-Fast Green Staining

Cartilage samples were fixed 4 h with formalin and decalcified with formic acid solution followed by embedding with paraffin and cut into 4 μ m slices. For IHC, samples were undergoing a heat mediated antigen retrieval step using citrate buffer and then blocked with 1% bovine serum albumin (BSA) for 10 min at 21°C followed by incubation with the primary antibody, anti-ki-67 antibody (ab16667, 1:200) and anti-PCNA antibody (ab92552, 1:300), for 30 min. An anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) was used as secondary antibody at a 1/1000 dilution, and subsequently the sample was counter-stained with hematoxylin. For safranin O-fast green staining, samples were stained with weigert's iron hematoxylin and safranin O solution according to the instruction of Safranin O Cartilage Stain solution (Solarbio, Beijing, China).

Enzyme Linked Immunosorbent Assay (ELISA)

Centrifuge cell culture media at 2,000 \times g for 10 min to remove debris. Collect supernatants to detect the levels of IL-6, IL-8, IL-17, TNF- α , MMP-1, MMP-13, and aggrecan according to the instructions of Human IL-6 SimpleStep ELISA[®] Kit (No: ab178013, Sensitivity: 1.6 pg/ml, Range: 7.8 pg/ml-500 pg/ml), Human IL-8 SimpleStep ELISA[®] Kit (No: ab214030, Sensitivity: 1.8 pg/ml, Range: 3.91 pg/ml-250 pg/ml), IL-17 Human ELISA Kit (No: ab119535, Sensitivity: 0.5 pg/ml, Range: 1.6 pg/ml-100 pg/ml), Human MMP1 ELISA Kit (No: ab100603, Sensitivity: < 8 pg/ml, Range: 24.69 pg/ml-18000 pg/ml), Human MMP13 ELISA Kit (No: ab221839, Sensitivity: 5.7 pg/ml, Range: 39 pg/ml-2500 pg/ml), and Human Aggrecan ELISA Kit (No: ab213754, Sensitivity: 50 pg/ml, Range: 1.56 ng/ml-100 ng/ml). The level of collagen II in supernatant was detected using sandwich ELISA method with Anti-Collagen II antibody (ab34712, at a dilution of 1: 10000).

Immunofluorescence (IF)

Chondrocytes were fixed with 100% methanol for 5 min, if necessary, permeabilized with 0.1% Triton X-100 for 5 min, and then blocked with 1% BSA in 0.1% PBS-Tween for 1 h. Then cells were incubated with primary antibodies against ki-67 (ab16667, 1:250), PCNA (ab92552, 1:200), IL-6 (ab9324, 1:1,000), TNF- α (ab66579, 1:1,000), MMP13 (ab39012, 1:150) and collagen II (ab34712, 1:200) overnight at 4°C. Finally, cells were incubated with Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed (ab150081, 1:1,000) or Alexa Fluor[®] 488 goat anti-rabbit (ab150077, 1:1,000) 1 h at room temperature followed by counter-staining with 4',6-diamidino-2-phenylindole (DAPI).

Statistical Analysis

All data were expressed as the mean \pm standard deviation (SD). The relationship between the levels of miR-379-5p and *YBX1* mRNA was explored with Pearson correlation analysis. Comparison between different groups was performed using student's t test or 2-way analysis of variance (ANOVA) with Bonferroni posttests, and difference with $P < 0.05$ was considered statistically significant. All analyses were performed using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA).

Results

The Expression Levels of miR-379-5p and YBX1 in OA Cartilage Samples

The results of real time PCR showed that miR-379-5p was down-regulated in OA cartilage samples ($P < 0.001$) (Fig. 1A) while the level of *YBX1* mRNA was up-regulated in OA

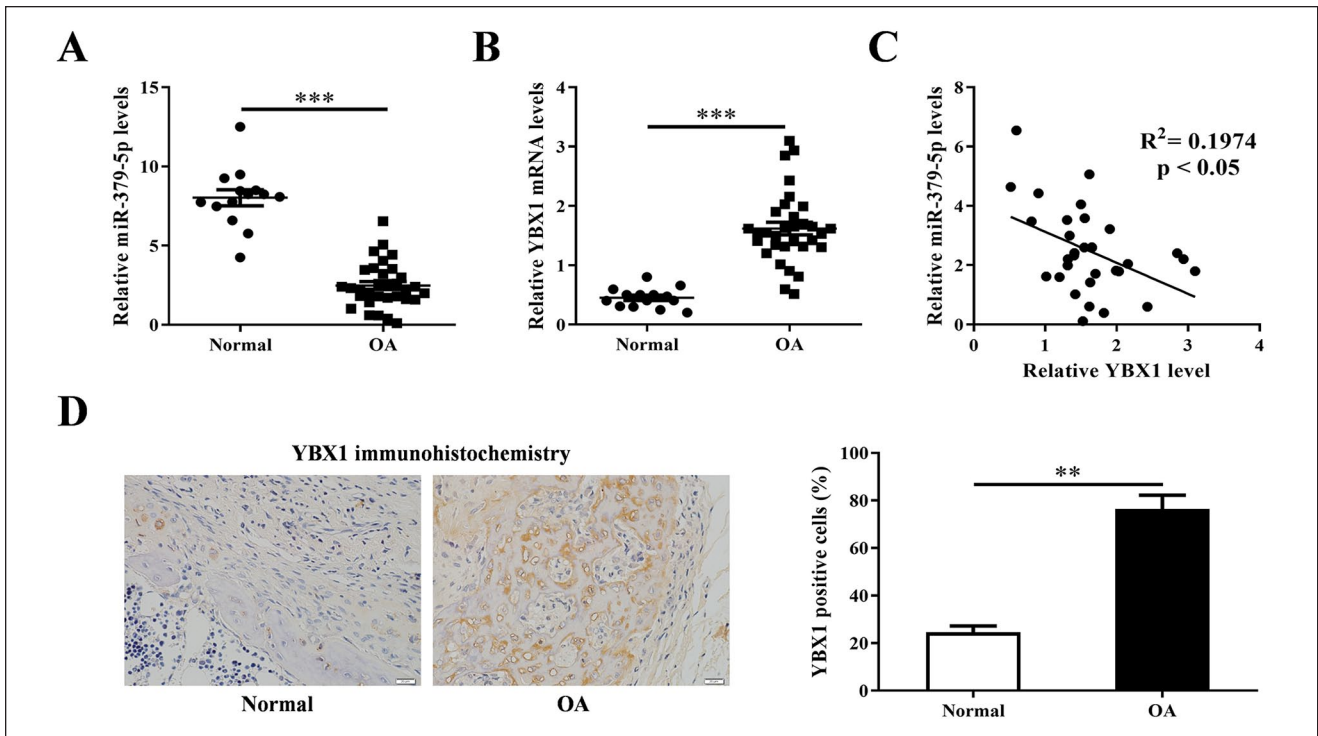


Figure 1. Expression levels of miR-379-5p and YBX1 in cartilage tissues from OA and normal patients. **(A)** Relative miR-379-5p levels in clinical specimens detected by qRT-PCR. **(B)** Relative expression of YBX1 at transcriptional level detected by qRT-PCR. **(C)** The correlation of expression of miR-379-5p and YBX1 was analyzed in the OA cartilage in rats. **(D)** Comparison on the tissues by IHC, the higher level of YBX1 was observed in OA tissues. OA = osteoarthritis. ** $P < 0.01$. *** $P < 0.001$.

cartilage samples ($P < 0.001$) (**Fig. 1B**) compared with those in normal cartilage samples. There was a negative relationship between miR-379-5p and *YBX1* mRNA in OA cartilage samples ($R^2 = 0.1974$, $P < 0.05$) (**Fig. 1C**). Further IHC analysis also showed that YBX1 protein level was significantly increased in OA cartilage samples ($P < 0.01$) (**Fig. 1D**).

The Effect of miR-379-5p on IL-1 β -Mediated Inhibition of Chondrocytes Proliferation

Real time PCR and CCK-8 methods were used to detect the effects of miR-379-5p mimics/inhibitor and IL-1 β on intracellular miR-379-5p expression level and cell proliferation. The results showed that IL-1 β significantly decreased miR-379-5p level in chondrocytes ($P < 0.001$), and this decrease would be rescued by miR-379-5p mimics ($P < 0.001$) rather aggravated by miR-379-5p inhibitor ($P < 0.05$) (**Fig. 2A**). At the same time, IL-1 β weakened the cell viability of chondrocytes, which would be rescued by miR-379-5p mimics ($P < 0.01$) (**Fig. 2B**). The results of IF and western blotting showed that IL-1 β down-regulated the level of ki-67 and PCNA proteins in chondrocytes, which were increased in miR-379-5p mimics + IL-1 β group rather decreased in miR-379-5p inhibitor + IL-1 β group (**Figs. 2C and 3A and B**).

The Effects of miR-379-5p on IL-1 β -Mediated Inflammatory Response and ECM Degradation of Chondrocytes

The results of ELISA assay showed that the levels of inflammatory factors IL-6, IL-8, IL-17, and TNF- α in the cell culture supernatant were increased by IL-1 β , and this would be pulled down by miR-379-5p mimics ($P < 0.001$) rather aggravated by miR-379-5p inhibitor ($P < 0.001$) (**Fig. 4A**). IF again confirmed the effect of IL-1 β and miR-379-5p on the expression of IL-6 and TNF- α in chondrocytes, which was consistent with ELISA results (**Fig. 4B and C**). The levels of ECM degradation-related proteins (MMP-1, MMP-13, collagen II, and aggrecan) in cell culture supernatant were also assessed using ELISA and IF. And the ELISA results showed that IL-1 β promoted the secretion of MMP-1 and MMP-13 ($P < 0.05$), but prevented the secretion of collagen II and aggrecan ($P < 0.001$). However, this change was prevented by miR-379-5p mimics and promoted by miR-379-5p inhibitor ($P < 0.01$) (**Fig. 5A**). This was also verified by IF assay on MMP-13 and collagen II proteins performed in chondrocytes (**Fig. 5B and C**).

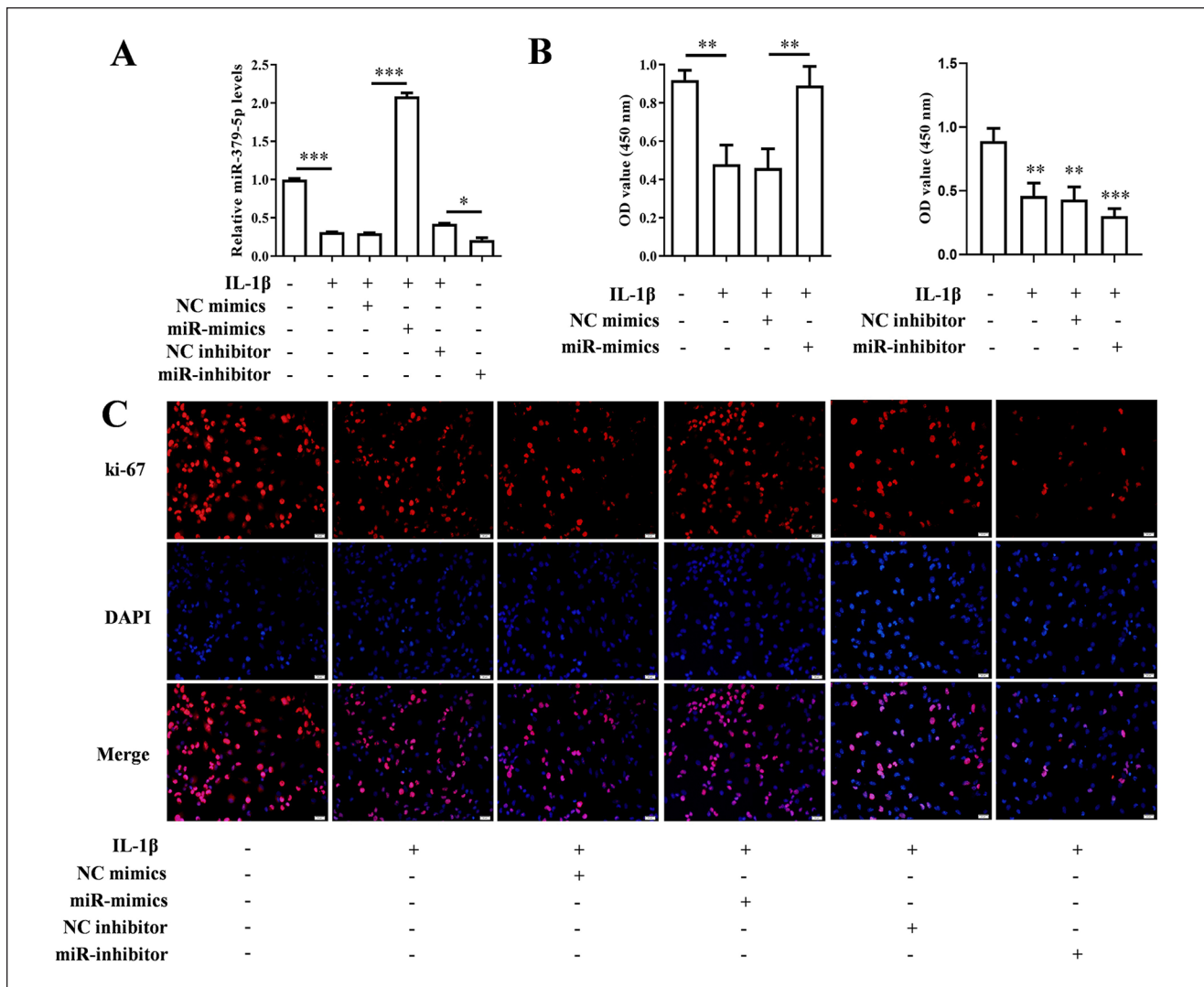


Figure 2. The expression of miR-379-5p increased or decreased, and the changes of cell activity and Ki-67. **(A)** miR-379-5p level in the human chondrocytes after transfected with miR-379-5p mimic or inhibitor was determined by qRT-PCR. **(B)** The activity of the chondrocytes after transfected with miR-379-5p mimic or inhibitor determined by CCK-8 assay. We measure the OD value at 450nm. **(C)** Ki-67 was determined by immunofluorescence staining. CCK-8 = Cell Counting Assay Kit-8; NC = Negative Control; qRT-PCR = Quantitative Real Time Polymerase Chain Reaction; OD = Optical Density; DAPI = 4',6-diamidino-2-phenylindole. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

MiR-379-5p Regulated YBX1 and the Influence on PI3K/Akt Pathway

The results of real-time PCR showed that IL-1 β up-regulated the expression level of YBX1, which was contrary to the effect of miR-379-5p on YBX1 expression ($P < 0.01$) (Fig. 6A). TargetScan and miRwalk software predicted that 3'UTR of YBX1 mRNA was completely paired with the seed sequence of miR-379-5p (Fig. 6B), and luciferase reporter assay verified that miR-379-5p inhibited firefly luciferase by targeting to the 3'UTR of YBX1 mRNA (Fig. 6C). Furthermore, YBX1 protein and PI3K/Akt pathway related proteins (PI3K, p-PI3K, AKT,

p-AKT) were also regulated by miR-379-5p via targeting YBX1 mRNA (Fig. 6D and E).

The Effects of Inhibited PI3K/Akt Pathway on miR-379-5p-Mediated Cell Proliferation and Inflammatory Response

To confirm the effects of inhibited PI3K/Akt pathway on miR-379-5p-mediated cell proliferation and inflammatory response, the PI3K/Akt pathway inhibitor LY294002 was added in miR-379-5p inhibitor + IL-1 β treated chondrocytes. The mRNA levels of PI3K/Akt pathway related proteins (PI3K, p-PI3K, AKT, p-AKT) were up-regulated by

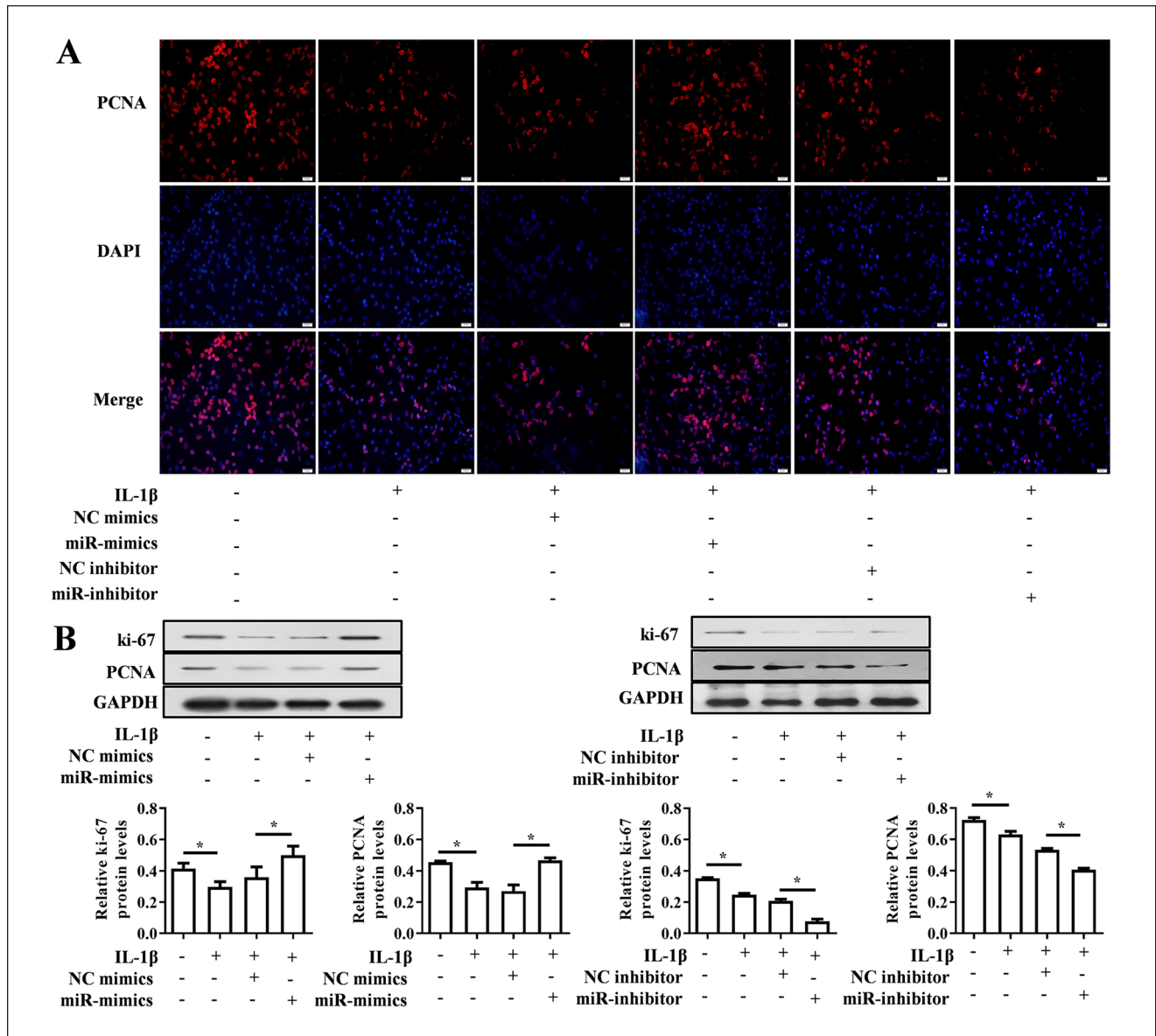


Figure 3. Expression levels of value-added related proteins PCNA and Ki-67 when miR-379-5p up-regulated or down-regulate. **(A)** PCNA was determined by immunofluorescence staining. **(B)** The protein expression of ki-67 and PCNA was determined by Western blotting after transfected with miR-379-5p mimic or inhibitor. NC = Negative Control; PCNA = Proliferating Cell Nuclear Antigen; DAPI = 4',6-diamidino-2-phenylindole; GAPDH = Glyceraldehyde-3-phosphate Dehydrogenase. * $P < 0.05$.

IL-1 β and miR-379-5p inhibitor ($P < 0.001$), which was decreased by LY294002 ($P < 0.001$) (**Fig. 7A**). The cell viability decreased by IL-1 β and miR-379-5p inhibitor was also rescued by LY294002 ($P < 0.001$) (**Fig. 7B**), as well as the expression of ki-67 and PCNA protein ($P < 0.05$) (**Fig. 7C** and **D**). The results of ELISA showed that secretion of inflammatory factors (IL-6, IL-8, IL-17, and TNF- α) and ECM degradation-related proteins (MMP-1 and MMP-13) were increased by IL-1 β and miR-379-5p inhibitor but decreased by LY294002 ($P < 0.01$) (**Fig. 7E**). ECM degradation-related proteins (collagen II and aggrecan) were

decreased by IL-1 β and miR-379-5p inhibitor but increased by LY294002 ($P < 0.01$) (**Fig. 7F**).

The Effect of miR-379-5p on Chondrocyte Proliferation and Cartilage Degradation

The level of miR-379-5p in OA samples of rats were down-regulated and reached the extreme at 4 weeks, while miR-379-5p agomir increased the miR-379-5p level in OA tissues ($P < 0.001$) (**Fig. 8A**). The *YBX1* mRNA was up-regulated reached the extreme at 4 weeks in OA tissues,

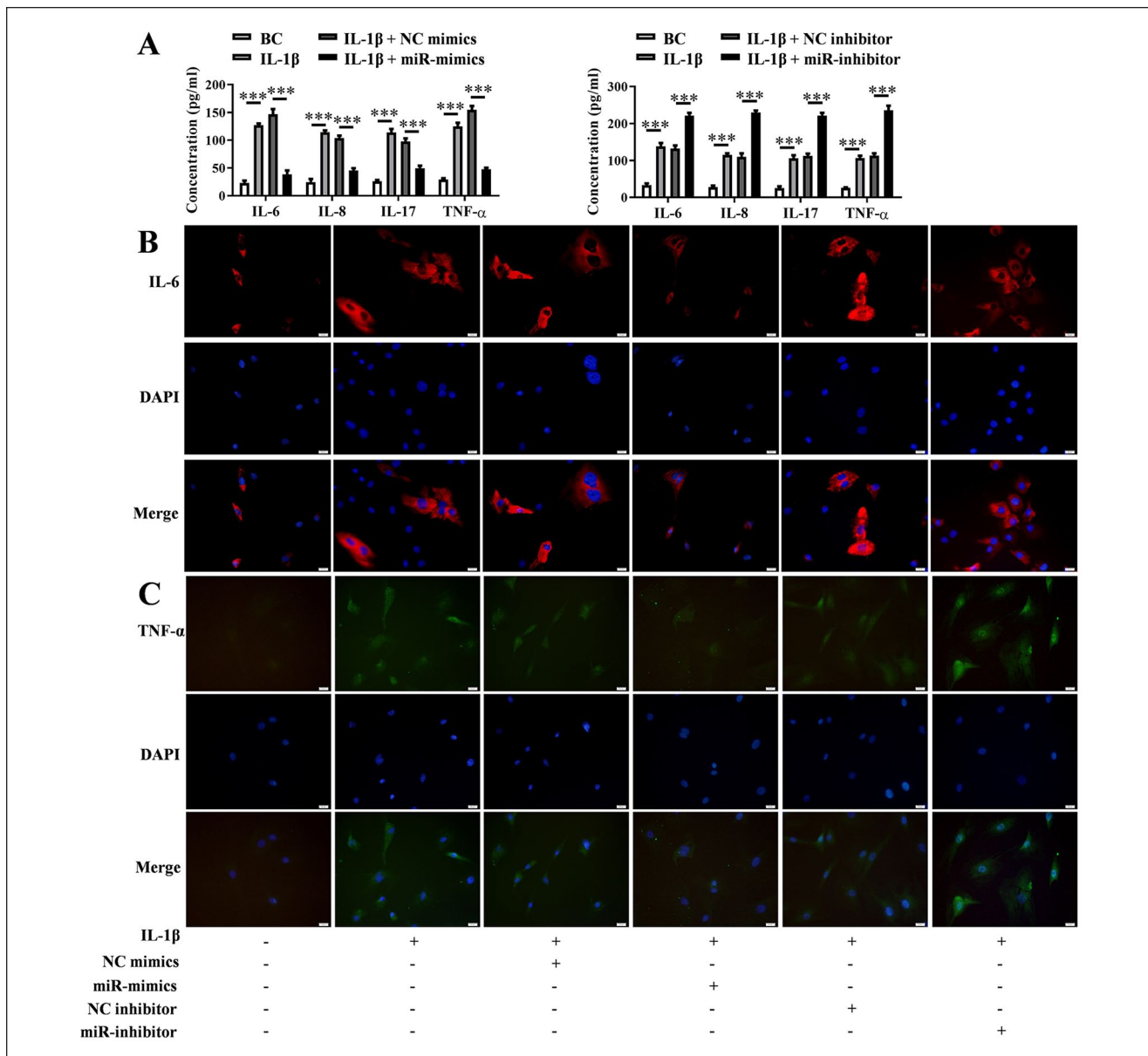


Figure 4. Expression levels of inflammatory cytokines when miR-379-5p up-regulated or down-regulate. **(A)** Concentration of IL-6, IL-8, IL-17, and TNF- α secreted in the media of human chondrocyte after transfection with the miR-379-5p mimic or the inhibitor measured by ELISA assay. **(B)** and **(C)** IL-6 and TNF- α was determined by immunofluorescence staining. ELISA = Enzyme Linked Immunosorbent Assay; BC = Blank Control; TNF- α = Tumor Necrosis Factor- α ; DAPI = 4',6-diamidino-2-phenylindole. *** $P < 0.001$.

which was decreased by miR-379-5p agomir ($P < 0.001$) (**Fig. 8B**). Safranin O-fast green staining showed that there was a severe erosion of cartilage surface and decreased safranin O staining in superficial layer in OA samples, while the erosion of cartilage surface was alleviated and safranin O staining was increased in OA samples with miR-379-5p agomir injection (**Fig. 8C**). The severity of articular cartilage degeneration in each rat was graded according to OARSI scoring system (**Fig. 8D**). Furthermore, miR-379-5p agomir increased the ki-67 and PCNA protein levels in OA

tissues (**Fig. 8E**). And up-regulated IL-6, IL-8, IL-17, TNF- α , MMP-1, and MMP-13 in OA tissues were decreased by miR-379-5p agomir while down-regulated collagen II and aggrecan were increased (**Fig. 8F** and **G**).

Discussion

Cartilage damage and inflammatory response are important features of OA.²⁶ IL-1 β signaling pathway is involved in the progression of OA.²⁷ Therefore, IL-1 β -treated cartilage

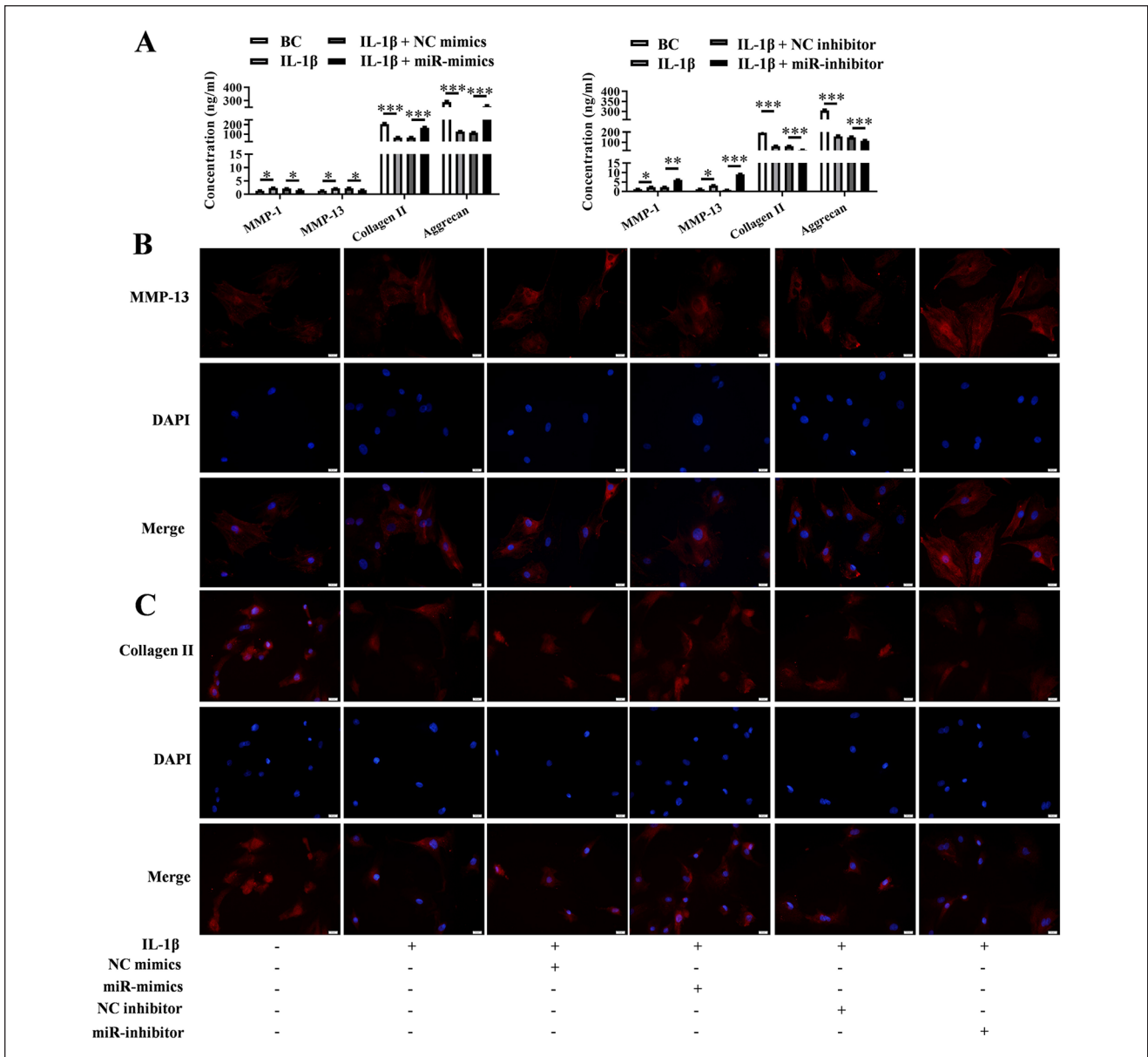


Figure 5. Expression levels of ECM-related proteins when miR-379-5p up-regulated or down-regulate. **(A)** Concentrations MMP-1, MMP-13, Collagen II, and aggrecan secreted in the media of human chondrocyte after transfection with the miR-379-5p mimic or the inhibitor measured by ELISA assay. **(B, C)** Collagen II and aggrecan were determined by immunofluorescence staining. ECM = extracellular matrix; MMP = matrix metalloproteinase; ELISA = Enzyme Linked Immunosorbent Assay; BC = Blank Control; NC = Negative Control; DAPI = 4',6-diamidino-2-phenylindole. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

cells were used as in vitro OA model in this study. Inflammation of articular tissues, degradation of ECM and apoptosis of chondrocytes were important factors in the pathogenesis of OA.²⁸ Chondrocytes in articular cartilage were cells that produce ECM components²⁹ and chondrocytes, stimulated by the pro-inflammatory cytokine IL-1 β , produce matrix metalloproteinases and other inflammatory cytokines,³⁰ causing cellular inflammation and ECM degradation. In this study, we found that elevated level of

miR-379-5p promoted the proliferation of IL-1 β stimulated chondrocytes, reduced the production of inflammatory factors and matrix metalloproteinases, and promoted extracellular ECM formation. These effects might be exerted by targeting YBX1 and inhibiting the activation of PI3K/Akt pathway in IL-1 β stimulated chondrocytes. In addition, intra-articular injection of miR-379-5p agomir was an effective means of administering miRNA-379-5p in OA rats, which promoted the proliferation of chondrocytes in

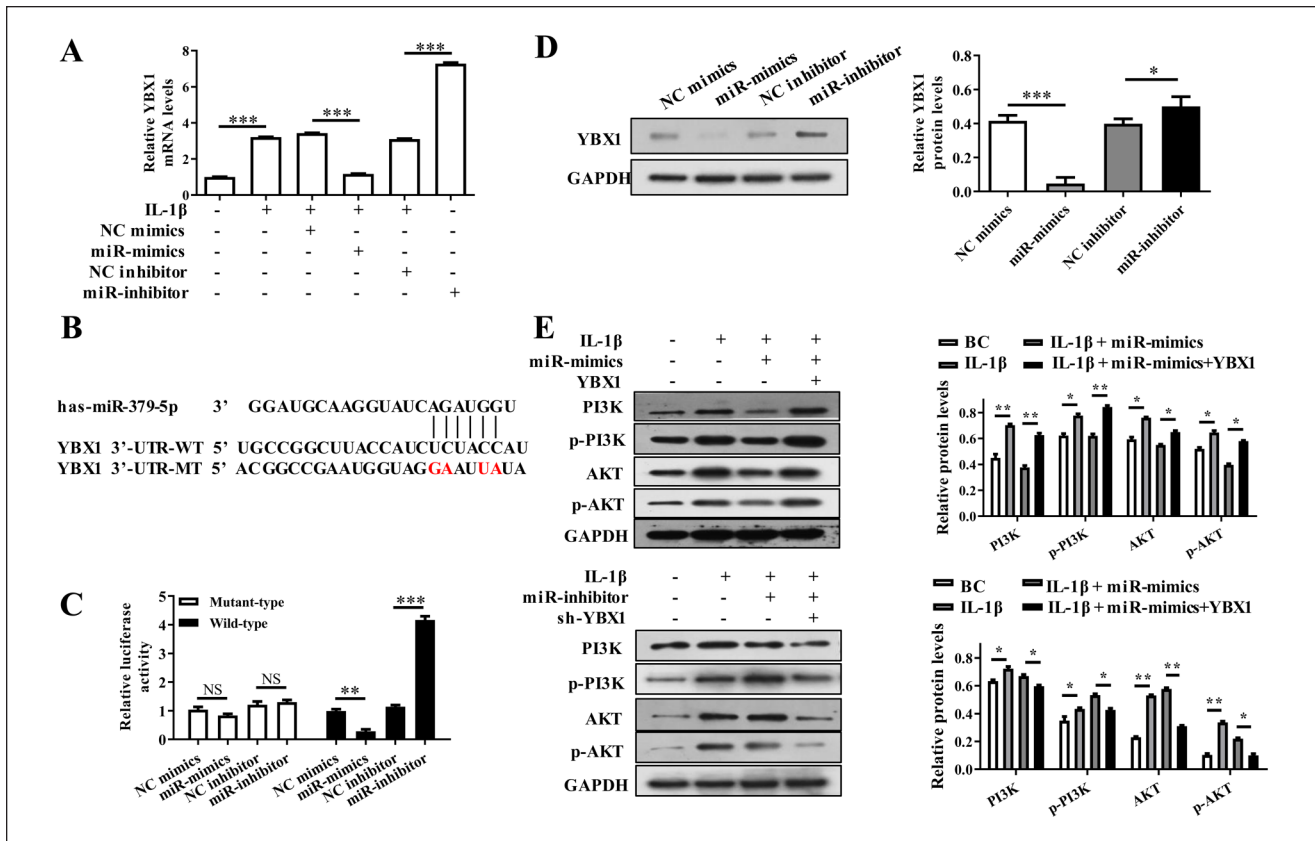


Figure 6. MiR-379-5p inhibited the activation of PI3K/Akt signaling by targeting YBX1 in IL-1 β stimulated chondrocytes. **(A)** YBX1 mRNA levels in the human chondrocytes after transfected with miR-379-5p mimics or inhibitor were determined by qRT-PCR. **(B)** The binding sequence between miR-379-5p and YBX1 in the 3'UTR predicted by TargetScan software. **(C)** The relative luciferase activity after co-transfection of miR-379-5p mimic/inhibitor and 3' UTR of YBX1 mRNA. **(D)** YBX1 protein levels in the chondrocyte after transfected with miR-379-5p mimic or inhibitor were determined by Western blotting. **(E)** The relative protein expression of PI3K/Akt pathway -related genes in the chondrocyte after transfected with miR-379-5p mimic and YBX1/sh-YBX1. NC = Negative Control; BC = Blank Control; qRT-PCR = Quantitative Real Time Polymerase Chain Reaction; 3' UTR = 3' Untranslated Regions; GAPDH = Glyceroldehyde-3-phosphate Dehydrogenase; AKT = Protein Kinase B; NS = Not Significant. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

the knee articulation of OA rats and alleviated the inflammation and ECM degradation in OA tissue.

High level of miR-379-5p could promote chondrocyte proliferation.¹⁵ While in this study, miR-379-5p was down-regulated in human knee OA tissues, which was consistent with the result of miRNA sequencing.⁹ This indicated that inhibition of miR-379-5p was associated with the progression of OA. Ki-67 protein was associated with cell cycle and PCNA protein participant in the process of DNA replication. Ki-67 and PCNA were standard markers of cell proliferation.^{31,32} Up-regulated miR-379-5p promoted both cell viability and levels of ki-67 and PCNA in IL-1 β stimulated chondrocytes, indicating miR-379-5p promotion of cell proliferation was associated with up-regulation of ki-67 and PCNA. Inflammatory cytokines IL-6, IL-8 and IL-17 were implicated in OA.³³ TNF- α was related to the produce of matrix metalloproteinase (MMPs) degrading all components of the extracellular matrix, MMP-13 was also degrading aggrecan, which aggravated the matrix destruction.³⁴

The present results showed that up-regulated miR-379-5p inhibited the expression and secretion of inflammatory cytokines IL-6, IL-8, IL-17, and TNF- α and matrix metalloproteinase MMP-1 and MMP-13, and the inhibition of collagen II and aggrecan was relieved in IL-1 β stimulated chondrocytes. This indicated that miR-379-5p, in addition of promoting cell proliferation, protected cells against IL-1 β stimulated inflammation and ECM degradation in chondrocytes. The rescue by miRNA mimics after IL-1 β treatment was sometimes clearly exceeding the control level, and this was because there were other pathways effects as well. The regulatory amplitude of miRNA mimics to different downstream molecules was different.

YBX1 is a modulator of MIA/CD-RAP-dependent chondrogenesis.³⁵ YBX1 also plays an important role in signaling DNA damage and cell proliferation.³⁶ YBX1 expression can affect the downstream PI3K/Akt signaling pathway and activate it.¹⁸ Therefore, YBX1 plays an important role in PI3K/Akt. Akt also plays an important role in preventing

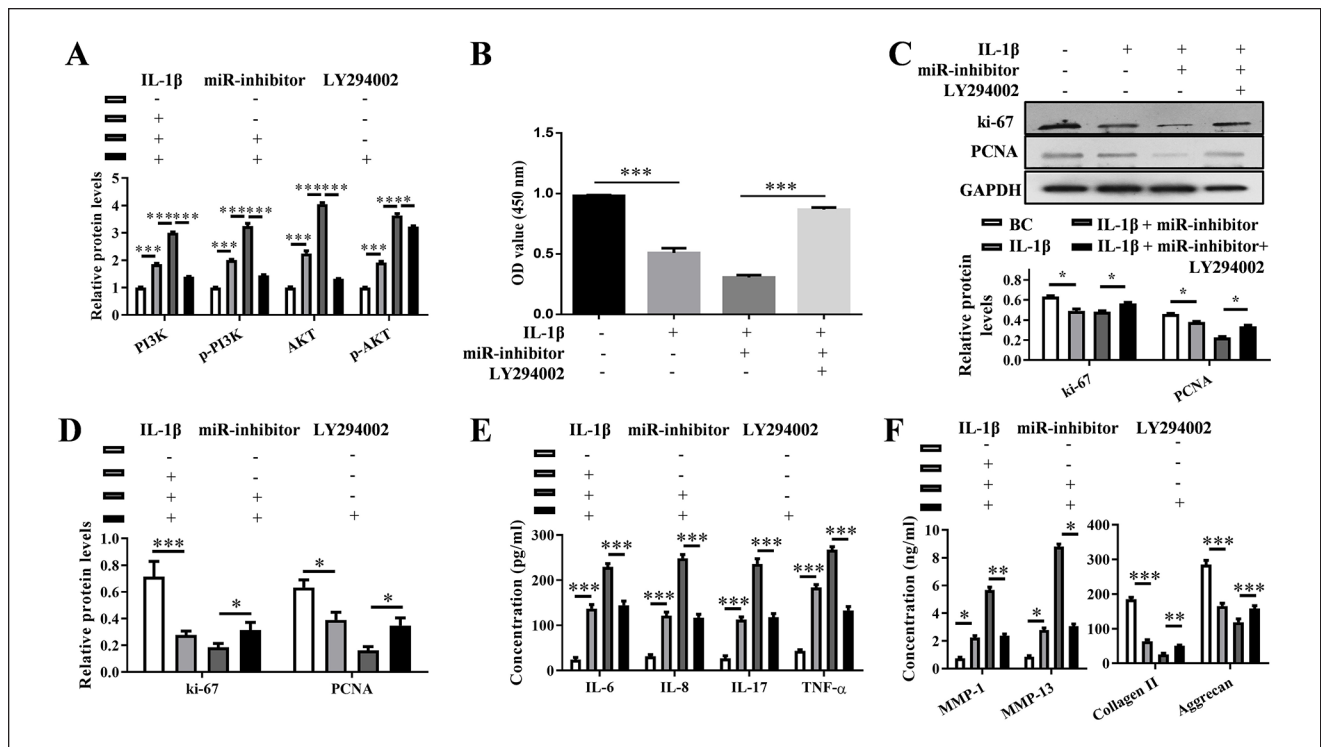


Figure 7. Blocking PI3K/Akt pathway attenuates the effect of miR-379-5p inhibitor on OA progression. **(A)** The relative protein expression of PI3K/Akt pathway-related genes in the human chondrocytes after transfected with miR-379-5p inhibitor/LY294002 (the inhibitor of PI3K/Akt pathway) were determined by qRT-PCR. **(B)** The activity of the chondrocytes determined by CCK-8 assay. We measure the OD value at 450 nm. **(C and D)** The protein expression of ki-67 and PCNA was determined by Western blotting. **(E)** Concentration of IL-6, IL-8, IL-17, and TNF- α measured by ELISA assay. **(F)** Concentration of MMP-1, MMP-13, Collagen II, and aggrecan was measured by ELISA assay. OA = Osteoarthritis; CCK-8 = Cell Counting Assay Kit-8; ELISA = Enzyme Linked Immunosorbent Assay; MMP = matrix metalloproteinase; OD = Optical Density; PCNA = Proliferating Cell Nuclear Antigen; TNF- α = Tumor Necrosis Factor- α . * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

cell proliferation, migration, and invasion.³⁷ In this study, YBX1 was targeted by miR-379-5p in chondrocytes. PI3K, p-PI3K, AKT, p-AKT, key proteins in PI3K/Akt signaling, were elevated in IL-1 β - or YBX1-induced chondrocytes, while this effort was reversed by miR-379-5p. Furthermore, we demonstrated that inhibition of PI3K/Akt signaling in miR-379-5p and IL-1 β treated chondrocytes elevated ki-67, PCNA, collagen II and aggrecan, and decreased IL-6, IL-8, IL-17, TNF- α , MMP-1, and MMP-13. These suggested that miR-379-5p regulated the cell proliferation and ECM homeostasis in chondrocytes by targeting YBX1 and PI3K/Akt signaling. The PI3K/Akt pathway has also been shown to be an important regulator of bone formation and remodeling, and its reduced expression can induce delayed ossification, short-bone and the formation of secondary ossification centers.³⁸ In addition, we performed the intra-articular injection of miR-379-5p agomir in OA rats, and demonstrated that miR-379-5p agomir elevated the miR-379-5p level in OA tissue, promoted cell proliferation and inhibited inflammation and ECM degradation, this alleviated the erosion of cartilage surface caused by MMPs and inflammatory cytokines.^{34,39}

In addition to PI3K/Akt, AMP-activated protein kinase (AMPK) activation is also associated with YBX1.⁴⁰ It is a critical cellular energy sensor, which could take part in the tumorigenesis and OA development.^{41,42} At transcriptional level, AMPK has been reported to be regulated by miR-379-5p.⁴³ However, due to limited funds and time, we only confirmed that miR-379-5p can inhibit PI3K/Akt pathway and promote chondrocyte proliferation by targeting YBX1, but did not pay much attention to AMPK signaling in chondrocytes, which may also serve as a key regulator in OA development.

Taken together, our results suggested that miR-379-5p was lowly expressed in human knee OA tissues. The up-regulation of miR-379-5p in chondrocytes promoted cell proliferation, decreased the expression and secretion of inflammatory cytokines, and inhibited the degradation of ECM by inhibiting the PI3K/Akt signaling pathway. Intra-articular injection of miR-379-5p agomir also alleviated the progression of OA in rats with OA. Thus, miR-379-5p may be used for the treatment of human OA in clinical. Moreover, there are few studies on miR-379-5p and OA. Our study provides a theoretical basis for subsequent studies on the

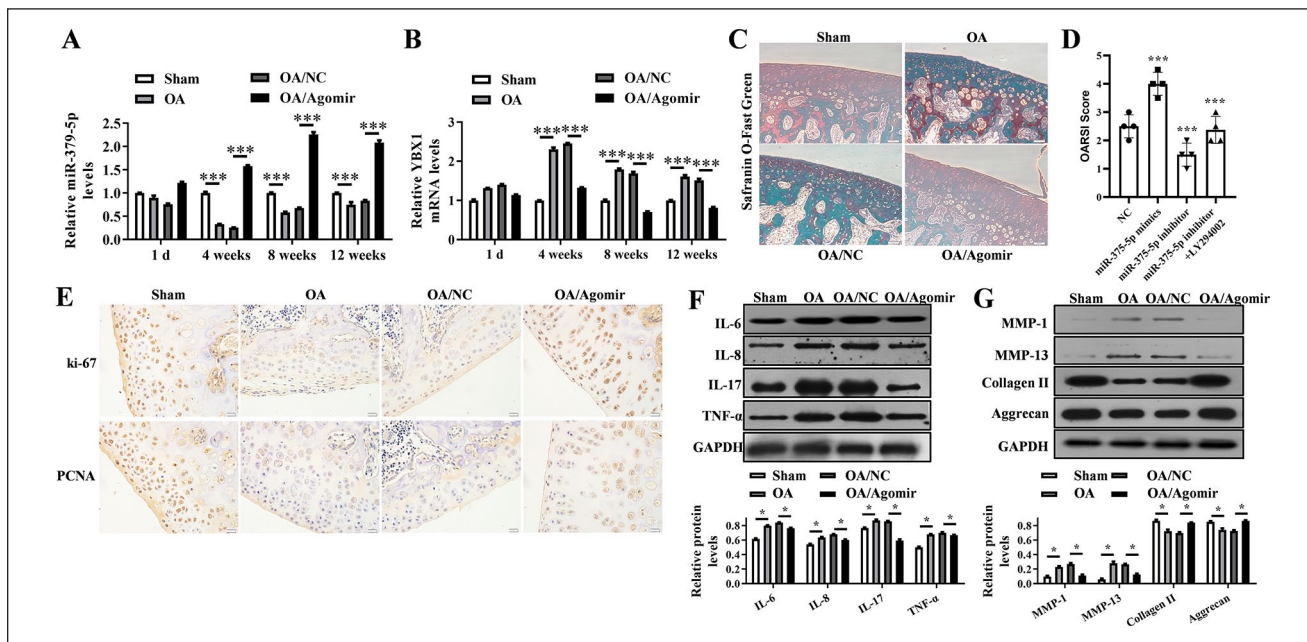


Figure 8. MiR-379-5p agomir promotes the articular chondrocytes proliferation and alleviates cartilage degradation in vivo. **(A and B)**. One day, 3, 4 or 6 weeks after surgery, miR-379-5p and YBX1 level in the tibial cartilage was detected by qRT-PCR. **(C)** Representative safranin O-fast green staining of the knee-joint from each group and analysis of degenerative changes by Mankin's scoring. **(D)** Severity of articular cartilage degeneration in each rat was graded according to OARSI scoring system. **(E)** The expression of ki-67 and PCNA was determined by immunohistochemical staining. **(F)** The protein level of IL-6, IL-8, IL-17, and TNF- α measured by Western blotting. **(G)** The protein level of MMP-1, MMP-13, Collagen II, and aggrecan was measured by Western blotting. OARSI = Osteoarthritis Research Society International; MMP = matrix metalloproteinase; BC = Blank Control; qRT-PCR = Quantitative Real Time Polymerase Chain Reaction; PCNA = Proliferating Cell Nuclear Antigen; TNF- α = Tumor Necrosis Factor- α ; OD = Optical Density; GAPDH = Glyceraldehyde-3-phosphate Dehydrogenase. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

role of miR-379-5p in OA. MiR-379-5p via inhibition of PI3K/Akt pathway by targeting YBX1 may represent a novel and promising target for the treatment of OA.

Author Contributions

Jia Zheng made substantial contributions to research design; Hongjun Zhang and Wendi Zheng were responsible for the acquisition and interpretation of data; Wendi Zheng and Du Li performed statistical analysis and data visualization; Hongjun Zhang drafted the paper; and Jia Zheng revised it critically for important intellectual content. All authors have read and approved the final manuscript.

Acknowledgments and Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by Ministry of Science and Technology National Key Research and Development Program (Grant No.: 122300411149), Natural Science Foundation of Henan Province (Grant No.: 182300410349), and the Joint Construction Project of Henan Provincial Health Committee and Ministry of Health (Grant Nos.: 201701017 and B20190316).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This study was approved by the Ethics Committee of the Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, School of Clinical Medicine, Henan University. All animal studies were approved by the Ethics Committee of Zhengzhou University.

ORCID iD

Jia Zheng  <https://orcid.org/0000-0003-2484-6513>

References

- Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. *Lancet* (London, England). 2019 Apr 27;393(10182):1745-59. doi:10.1016/s0140-6736(19)30417-9.
- Ondresik M, Azevedo Maia FR, da Silva Morais A, Gertrudes AC, Dias Bacelar AH, Correia C, *et al.* Management of knee osteoarthritis. Current status and future trends. *Biotechnol Bioeng.* 2017 Apr;114(4):717-39. doi:10.1002/bit.26182.
- Zheng L, Zhang Z, Sheng P, Mobasheri A. The role of metabolism in chondrocyte dysfunction and the progression of osteoarthritis. *Ageing Res Rev.* 2021 Mar;66:101249. doi:10.1016/j.arr.2020.101249.
- Catalanotto C, Cogoni C, Zardo G. MicroRNA in control of gene expression: an overview of nuclear functions. *Int J Mol Sci.* 2016 Oct 13;17(10):1712. doi:10.3390/ijms17101712.

5. Malemud CJ. MicroRNAs and osteoarthritis. *Cells*. 2018 Aug 1;7(8):92. doi:10.3390/cells7080092.
6. De Palma A, Cheleschi S, Pascarelli NA, Tenti S, Galeazzi M, Fioravanti A. Do MicroRNAs have a key epigenetic role in osteoarthritis and in mechanotransduction? *Clin Exp Rheumatol*. 2017 May-Jun;35(3):518-26.
7. Wu C, Tian B, Qu X, Liu F, Tang T, Qin A, et al. MicroRNAs play a role in chondrogenesis and osteoarthritis (review). *Int J Mol Med*. 2014 Jul;34(1):13-23. doi:10.3892/ijmm.2014.1743.
8. Xie F, Liu YL, Chen XY, Li Q, Zhong J, Dai BY, et al. Role of MicroRNA, LncRNA, and exosomes in the progression of osteoarthritis: a review of recent literature. *Orthop Surg*. 2020 Jun;12(3):708-16. doi:10.1111/os.12690.
9. Badalamenti G, Fanale D, Incorvaia L, Barraco N, Listi A, Maragliano R, et al. Role of tumor-infiltrating lymphocytes in patients with solid tumors: can a drop dig a stone? *Cell Immunol*. 2019;343:103753.
10. Khan S, Brougham CL, Ryan J, Sahrudin A, O'Neill G, Wall D, et al. miR-379 regulates cyclin B1 expression and is decreased in breast cancer. *PLoS ONE*. 2013;8(7):e68753. doi:10.1371/journal.pone.0068753.
11. Chen JS, Li HS, Huang JQ, Dong SH, Huang ZJ, Yi W, et al. MicroRNA-379-5p inhibits tumor invasion and metastasis by targeting FAK/AKT signaling in hepatocellular carcinoma. *Cancer Lett*. 2016 May 28;375(1):73-83. doi:10.1016/j.canlet.2016.02.043.
12. Chen JS, Huang JQ, Dong SH, Huang XH. [Effects of microRNA-379-5p on proliferation, migration and invasion of hepatocellular carcinoma cell line]. *Zhonghua Yi Xue Za Zhi*. 2016 May 17;96(18):1450-3. doi:10.3760/cma.j.issn.0376-2491.2016.18.013.
13. Xu X, Wang Y, Mojumdar K, Zhou Z, Jeong KJ, Mangala LS, et al. A-to-I-edited miRNA-379-5p inhibits cancer cell proliferation through CD97-induced apoptosis. *J Clin Invest*. 2019 Dec 2;129(12):5343-56. doi:10.1172/JCI123396.
14. Li J, Xue J, Wang D, Dai X, Sun Q, Xiao T, et al. Regulation of gasdermin D by miR-379-5p is involved in arsenite-induced activation of hepatic stellate cells and in fibrosis via secretion of IL-1beta from human hepatic cells. *Metallomics*. 2019 Feb 20;11(2):483-95. doi:10.1039/c8mt00321a.
15. Jee YH, Wang J, Yue S, Jennings M, Clokie SJ, Nilsson O, et al. mir-374-5p, mir-379-5p, and mir-503-5p regulate proliferation and hypertrophic differentiation of growth plate chondrocytes in male rats. *Endocrinology*. 2018 Mar 1;159(3):1469-78. doi:10.1210/en.2017-00780.
16. Zhang F, Duan C, Yin S, Tian Y. MicroRNA-379-5p/YBX1 axis regulates cellular EMT to suppress migration and invasion of nasopharyngeal carcinoma cells. *Cancer Manag Res*. 2020;12:4335-46. doi:10.2147/CMAR.S253504.
17. Ladomery M, Sommerville J. A role for Y-box proteins in cell proliferation. *Bioessays*. 1995 Jan;17(1):9-11. doi:10.1002/bies.950170104.
18. Troiano A, Lomoriello IS, di Martino O, Fusco S, Pollice A, Vivo M, et al. Y-box binding protein-1 is part of a complex molecular network linking $\Delta Np63\alpha$ to the PI3K/akt pathway in cutaneous squamous cell carcinoma. *J Cell Physiol*. 2015 Sep;230(9):2067-74. doi:10.1002/jcp.24934.
19. Song HP, Chu ZG, Zhang DX, Dang YM, Zhang Q. PI3K-AKT pathway protects cardiomyocytes against hypoxia-induced apoptosis by MitoKATP-mediated mitochondrial translocation of pAKT. *Cell Physiol Biochem*. 2018;49(2):717-27. doi:10.1159/000493037.
20. Li T, Mo H, Chen W, Li L, Xiao Y, Zhang J, et al. Role of the PI3K-Akt signaling pathway in the pathogenesis of polycystic ovary syndrome. *Reprod Sci*. 2017 May;24(5):646-55. doi:10.1177/1933719116667606.
21. Lin C, Shao Y, Zeng C, Zhao C, Fang H, Wang L, et al. Blocking PI3K/AKT signaling inhibits bone sclerosis in subchondral bone and attenuates post-traumatic osteoarthritis. *J Cell Physiol*. 2018 Aug;233(8):6135-47. doi:10.1002/jcp.26460.
22. Chen C, Yang M, Chen Y, Wang Y, Wang K, Li T, et al. Astilbin-induced inhibition of the PI3K/AKT signaling pathway decelerates the progression of osteoarthritis. *Exp Ther Med*. 2020 Oct;20(4):3078-83. doi:10.3892/etm.2020.9048.
23. Zhang Q, Lai S, Hou X, Cao W, Zhang Y, Zhang Z. Protective effects of PI3K/Akt signal pathway induced cell autophagy in rat knee joint cartilage injury. *Am J Transl Res*. 2018;10(3):762-70.
24. Altman RD, Gold GE. Atlas of individual radiographic features in osteoarthritis, revised. *Osteoarthr Cartil*. 2007; 15(suppl A):A1-56. doi: 10.1016/j.joca.2006.11.009.
25. Bakhshalizadeh S, Rabiee F, Shirazi R, Ghaedi K, Amidi F, Nasr-Esfahani MH. Assessment of PGC1 α -FNDC5 axis in granulosa cells of PCOS mouse model. *J Reprod Infertil*. 2018 Apr-Jun;19(2):89-94.
26. Pap T, Korb-Pap A. Cartilage damage in osteoarthritis and rheumatoid arthritis—two unequal siblings. *Nat Rev Rheumatol*. 2015 Oct;11(10):606-15. doi:10.1038/nrrheum.2015.95.
27. Jenei-Lanzl Z, Meurer A, Zaucke F. Interleukin—1beta signaling in osteoarthritis—chondrocytes in focus. *Cell Signal*. 2019 Jan;53:212-23. doi:10.1016/j.cellsig.2018.10.005.
28. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis. *Nat Rev Rheumatol*. 2015 Jan;11(1):35-44. doi:10.1038/nrrheum.2014.162.
29. Yan S, Wang M, Zhao J, Zhang H, Zhou C, Jin L, et al. MicroRNA-34a affects chondrocyte apoptosis and proliferation by targeting the SIRT1/p53 signaling pathway during the pathogenesis of osteoarthritis. *Int J Mol Med*. 2016 Jul;38(1):201-9. doi:10.3892/ijmm.2016.2618.
30. Sun FF, Hu PF, Xiong Y, Bao JP, Qian J, Wu LD. Tricetin protects rat chondrocytes against IL-1beta-induced inflammation and apoptosis. *Oxid Med Cell Longev*. 2019;2019:4695381. doi:10.1155/2019/4695381.
31. Sun X, Kaufman PD. Ki-67: more than a proliferation marker. *Chromosoma*. 2018 Jun;127(2):175-86. doi:10.1007/s00412-018-0659-8.
32. Jurikova M, Danihel L, Polak S, Varga I. Ki67, PCNA, and MCM proteins: markers of proliferation in the diagnosis of breast cancer. *Acta Histochem*. 2016 Jun;118(5):544-52. doi:10.1016/j.acthis.2016.05.002.
33. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology

- of osteoarthritis. *Nat Rev Rheumatol*. 2011 Jan;7(1):33-42. doi:10.1038/nrrheum.2010.196.
34. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. *Front Biosci*. 2006 Jan 1;11:529-43. doi:10.2741/1817.
 35. Schmid R, Meyer K, Spang R, Schitteck B, Bosserhoff AK. YBX1 is a modulator of MIA/CD-RAP-dependent chondrogenesis. *PLoS ONE*. 2013;8(12):e82166. doi:10.1371/journal.pone.0082166.
 36. Uramoto H, Izumi H, Ise T, Tada M, Uchiumi T, Kuwano M, *et al*. p73 interacts with c-Myc to regulate Y-box-binding protein-1 expression. *J Biol Chem*. 2002 Aug 30;277(35):31694-702. doi:10.1074/jbc.M200266200.
 37. Faber AC, Li D, Song Y, Liang MC, Yeap BY, Bronson RT, *et al*. Differential induction of apoptosis in HER2 and EGFR addicted cancers following PI3K inhibition. *Proc Natl Acad Sci U S A*. 2009 Nov 17;106(46):19503-8. doi:10.1073/pnas.0905056106.
 38. Gu YX, Du J, Si MS, Mo JJ, Qiao SC, Lai HC. The roles of PI3K/Akt signaling pathway in regulating MC3T3-E1 preosteoblast proliferation and differentiation on SLA and SLActive titanium surfaces. *J Biomed Mater Res A*. 2013 Mar;101(3):748-54. doi:10.1002/jbm.a.34377.
 39. Blasioli DJ, Kaplan DL. The roles of catabolic factors in the development of osteoarthritis. *Tissue Eng Part B Rev*. 2014 Aug;20(4):355-63. doi:10.1089/ten.TEB.2013.0377.
 40. Su W, Wang L, Zhao H, Hu S, Zhou Y, Guo C, *et al*. LINC00857 interacting with YBX1 to regulate apoptosis and autophagy via MET and phosphor-AMPKa signaling. *Mol Ther Nucleic Acids*. 2020 Dec 4;22:1164-75. doi:10.1016/j.omtn.2020.10.025.
 41. Ashrafian H. Cancer's sweet tooth: the Janus effect of glucose metabolism in tumorigenesis. *Lancet (London, England)*. 2006 Feb 18;367(9510):618-21. doi:10.1016/s0140-6736(06)68228-7.
 42. Zhou S, Lu W, Chen L, Ge Q, Chen D, Xu Z, *et al*. AMPK deficiency in chondrocytes accelerated the progression of instability-induced and ageing-associated osteoarthritis in adult mice. *Sci Rep*. 2017 Feb 22;7:43245. doi:10.1038/srep43245.
 43. Yu Q, Li P, Weng M, Wu S, Zhang Y, Chen X, *et al*. Nano-vesicles are a potential tool to monitor therapeutic efficacy of carbon ion radiotherapy in prostate cancer. *J Biomed Nanotechnol*. 2018 Jan 1;14(1):168-78. doi:10.1166/jbn.2018.2503.