Pathology - Research and Practice 215 (2019) 152423

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

MiR-671 ameliorates the progression of osteoarthritis *in vitro* and *in vivo*

Baoxin Zhang[1], Mingqi Sun[1], Jianzhong Wang, Chao Ma, Ting Hao, Guanghui Liu, Guoyu Bao*, Yong Zhu*

Department of Orthopedics, The Second Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia, 010059, China

ARTICLE INFO

Keywords:
miR-671
Osteoarthritis
Chondrocyte
Pathogenesis

ABSTRACT

Objectives: Expression of miR-671 was reported to be downregulated in articular cartilage of patients with OA compared to healthy individuals, indicating it may serve as potential biomarker for OA. However, the mechanism by which miR-671 regulates the progression of OA remains unclear. Here, we aimed to investigate the role of miR-671 in cartilage from patients with OA.

Methods: The expression of miR-671 and inflammation mediators in cartilage from patients with OA was analyzed by RT-PCR. *In vitro*, chondrocytes CHON-001 were stimulated with IL-1β for 24 h for OA model establishment. Protein expression of MMP-13, aggrecan, and collagen II was measured by western blot. *In vivo*, the severity of OA in mice was determined by histological analysis.

Results: We found that the level of miR-671 was downregulated in OA tissues, plasma and IL-1β treated CHON-001 cells, compared with control. MiR-671 mimics ameliorated IL-1β-induced proliferation inhibition and apoptosis stimulation, as well as decreased protein levels of collagen II and aggrecan in CHON-001 cells. *In vivo* study showed miR-671 mimics alleviated the progression of OA in mice.

Conclusion: These results indicated miR-671 play an important role during the pathogenesis of OA. Therefore, miR-671 may serve as a potential therapeutic target for the treatment of OA.

1. Introduction

The pathology of osteoarthritis (OA) involves the disease process of the whole joint, including local and progressive loss of hyaline articular cartilage with concomitant changes in subchondral skeleton, including the development of marginal growth, osteophyte and periosteal thickening (osteosclerosis). Several factors such as genetic reasons, overweight, repetitive movements or injuries to joints, metabolic disorders and acromegaly, may initiate various pathological pathways of OA. In recent years, owing to the discovery of a central role of inflammation in OA, the view of the pathophysiologic features of OA has been transformed from non-inflammatory causes to inflammatory reasons [1]. Interleukin-1β (IL-1β), the major pro-inflammatory and pro-catabolic cytokine, could induce the release of matrix metalloproteinase (MMP) and other inflammatory mediators into the articular cavity and ultimately leading to cartilage degradation [2]. Therefore, suppression of these inflammatory mediators will be potential therapeutic strategy for the treatment of OA.

MicroRNA (abbreviated miRNA), a small non-coding RNA molecule, functions in RNA silencing and post-transcriptional regulation of gene expression [3–5]. Many miRNAs are evolutionarily conserved [6], which implies that they have important biological functions such as lipid metabolism [7], apoptosis [8], differentiation [9], organ development [10] and malignant tumors [11]. Beyond that, several miRNAs were reported to associate with OA. MiR-146a was intensely expressed in OA cartilage and its expression could be induced by stimulation of IL-1β [12]. Silencing miR-34a inhibited chondrocyte apoptosis in a rat OA model *in vivo* [13]. Recently, a microRNA array analysis identifies that miR-671 expression level was consistently downregulated in articular cartilage of OA patients compared to healthy individuals, indicating its function as potential OA biomarkers [14]. However, the underlying mechanisms of miR-671 in OA have not been reported.

Therefore, in this study, we interrogated the role of miR-671 during the pathogenesis of OA *in vitro* and *in vivo*.

* Corresponding authors.
E-mail addresses: baoxinzhang59@hotmail.com (B. Zhang), mingqisun59@hotmail.com (M. Sun), jianzhongwang59@yandex.com (J. Wang), chaoma59@yandex.com (C. Ma), tinghao59@yandex.com (T. Hao), guanghuiliu59@yandex.com (G. Liu), guoyu_bao59@126.com (G. Bao), yongzhu59@hotmail.com (Y. Zhu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.prp.2019.04.015
Received 3 January 2019; Received in revised form 7 April 2019; Accepted 26 April 2019

2. Materials and methods

2.1. Cell culture

Human chondrocyte cell line CHON-001 was purchased from American type culture collection (ATCC, Manassas, VA, USA). CHON-001 cells were seed in RPMI-1640 supplemented with 10% FBS and 1X penicillin-streptomycin-glutamine (10378-016; Invitrogen) at 37 °C in a humidified incubator with 95% air and 5% CO_2. CHON-001 cells were stimulated with IL-1β (10 ng/ml) for 24 h treatment for OA model establishment.

2.2. Knee tissue collection

Osteoarthritis specimens were obtained from 20 patients undergoing surgery at the Second Affiliated Hospital of Inner Mongolia Medical University. Control cartilage samples were obtained from 20 nonarthritic knee joints of donors without known history of joint disease. The diagnosis of OA met the American College of Rheumatology (ACR) classification criteria [15]. The study was approved by the Ethics Committee of the Second Affiliated Hospital of Inner Mongolia Medical University and followed the guidelines of the Declaration of Helsinki. All the patients provided written informed consent.

2.3. Quantitative real-time-PCR (qRT-PCR)

Quantitative RT-PCR was conducted to detect miR-671 expression. Total RNAs were extracted from human knee tissue/plasma samples (healthy or patients) or CHON-001 lysate using RNA extract kit (Tiangen Biotech Co., Ltd., Beijing, China). The cDNA synthesis was performed using SuperScript II RNase H Reverse Transcriptase Kit (Life Technologies) according to the manufacturer's protocol. Real-time PCR was performed with SYBR Green PCR Mix reagent (Thermo Fisher) and samples were analyzed using an ABI Prism 7700 Sequence Detection System (Applied Biosystems). Primers for miR-671 and U6 were purchased from Ambion (Life Technologies, CA, USA). The sequences of primers were listed as below: miR-671-forward, 5′ TGTAAAACGACGG CCAGT; miR-671-reverse, 5′ CAGGAAACAGCTATGACC; U6-forward, 5′ TGTAAAACGACGGCCAGT; U6-reverse, 5′ CAGGAAACAGCTATGACC. The qPCR procedure was performed as follows: initial denaturation at 95 °C for 10 min; 40 cycles of denaturation at 95 °C for 15 s and annealing extension at 60 °C for 30 s. The relative expression was determined by the 2-ΔΔCt method with U6 as control (Ct refers to cycle threshold). Experiments were triplicated.

2.4. Western blot

Total protein was extracted from CHON-001 cells with SDS sample buffer (50 mM Tris-HCl pH 6.8, 5 mM EDTA, 2% SDS and 5% glycine). Protein from each sample was separated in a 10% polyacrylamide SDS gel at 80 V for 20 min and 100 V for 1 h, followed by transferring onto PVDF membranes (Millipore, MA, USA) at 300 mA for 1 h. The membranes were blocked for 1 h with 5% non-fat milk in TBST (20 mM Tris pH 7.6, 150 mM NaCl and 0.05% Tween-20) followed by primary antibodies at 4 °C overnight. The primary antibodies included MMP-13(1:1000), aggrecan (1:1000), collagen II (1:1000) and beta-actin (1:2000) from Santa Cruz (Santa Cruz, CA, USA). Then, the membranes were washed three times with TBST and incubated with HRP (horseradish peroxidase)-conjugated goat anti-rabbit secondary antibody (Santa Cruz, dilution 1:5000) for 1 h at room temperature。

2.5. MiR-671 mimics

MiR-671 mimics are chemically synthesized in GenePharma company (Shanghai, China). The sequence was: UCCGGUUCUCAGGGCUC CACC. MiRNA mimics are small, chemically modified double stranded RNAs designed to mimic endogenous mature miRNA molecules. A total of 2 × 10 [5] chondrocytes were incubated to 80% confluence in a 6-well plate. Then, cells were incubated in serum-free DMEM for another 12 h. The transfection of miRNA was performed according to the manufacturer's instruction. Briefly, 100 nM miRNA mimic or 100 nM scrambled 22 nt nucleotides (miR-Scr, with no homology to mammal genome) were mixed with Lipofectamine 2000 solution (Invitrogen) at room temperature for 20 min. After that, cells were incubated with mixture for 12 h at 37°. And then, a total of 10 ng/ml IL-1β (PeproTech, Rocky Hill, NJ, USA) or PBS (Invitrogen) was added to each well for an appropriate period.

2.6. Cell proliferation assay

Cell proliferation was measured using cell counting kit 8 (CCK-8, Cat. 96992, Sigma Aldrich, Louis, MO, USA) according to manufacturer's protocol. Briefly, CHON-001 cells were seeded in 96-well plate at a density of 3000 cells per well overnight and treated with miR-671 mimics or/and IL-1β. Then, 1/10th volume of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt solution was added to the plates for 2 h. The optical density (OD) value of cell was read at 450 nm using a microplate reader. All experiments were performed in triplicates.

2.7. Apoptosis

Apoptosis of CHON-001 cell was detected with Annexin V-FITC and propidium iodide (PI) apoptosis kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. In brief, CHON-001 cells were treated with miR-671 mimics or/and IL-1β. After treatments, cells were resuspended in Binding buffer and incubated with Annexin V/PI. Finally, the cell apoptosis was analyzed with the imaging flow cytometer BD Celesta.

2.8. OA mice model

C57BL/6 male wild-type mice (ten-week-old) were purchased from Animal Center of Chinese Academy of Sciences (Shanghai, China). The experimental OA mice were surgically induced by destabilization of the medial meniscus as reported [16]. Mice were anesthetized with peritoneal injection of 4% chloral hydrate; the cranial attachment of the medial meniscus to the tibial plateau was transected with a microsurgical knife. The animal study has been approved by the Ethics Committee of the Second Affiliated Hospital of Inner Mongolia Medical University.

2.9. Immunohistochemistry

Knee samples were fixed in 4% paraformaldehyde at 4 °C for 24 h, followed by decalcification in 10% EDTA solution at 4 °C for two weeks. Then, the samples were dehydrated and embedded in paraffin blocks. Frontal serial sections (5 μm thick) across entire joints were obtained and 10 slides per joint at every 50 μm were selected and stained with Safranin-O/Fast Green to evaluate cartilage destruction. In addition, multiple separate scoring systems were used to assess the extent of cartilage degeneration. The destruction of articular cartilage was evaluated using the Osteoarthritis Research Society International (OARSI) scoring system (0–12) for medial femoral condyle and medial tibial plateau [17]. The severity of synovitis was measured using a scoring system which was previously described [18]. AxioVision software was applied to measure the thickness of the medial subchondral bone plate according to Safranin O stained sections.

2.10. Statistical analysis

Graphs were analyzed with GraphPad Prism 7 software (La Jolla,

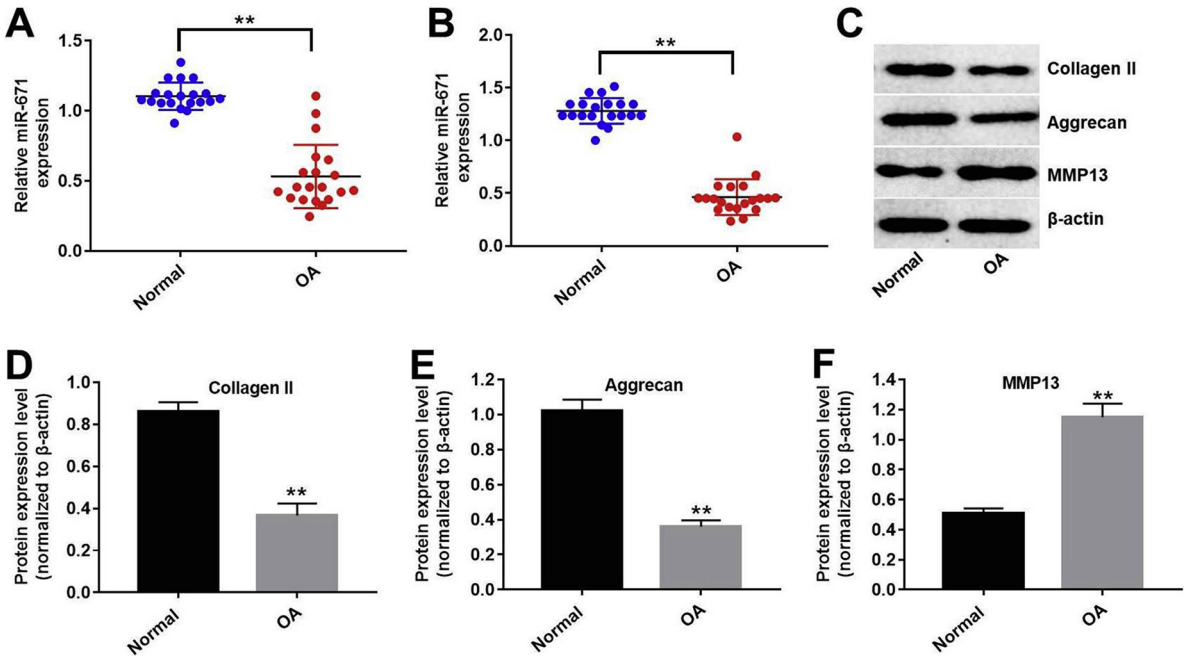

Fig. 1. Levels of miR-671 and inflammatory mediators in OA tissue and plasma. (A) Quantitative PCR was used to determine the level of miR-671 in plasma samples from patients with OA (n = 20) and health donors (n = 20). * indicates $P < 0.05$, as compared to the non-OA donors. (B) Quantitative PCR was used to assess the level of miR-671 in tissue samples from patients with OA (n = 20) and health donors (n = 20). * indicates $P < 0.05$, as compared to the non-OA donors. (C) The expressions of MMP13, collagen II and aggrecan in tissues from patients with OA (n = 20) and health donors (n = 20) were measured with western blot. (D–F) Quantification of protein expressions in OA tissues. ** indicates $P < 0.01$, compared to non-OA group. Experiments were performed in triplicate.

CA, USA). All data are expressed as the means ± SEM unless specified. The comparison between two groups was conducted by Student's t-test. $P < 0.05$ was set as statistical significance.

3. Results

3.1. Differentiate expressions of miR-671 and inflammatory mediators in human osteoarthritic cartilage

To interrogate the potential role of miR-671 in human OA, we examined the level of miR-671 in both plasma and cartilage tissues obtained from patients with OA or healthy donors without any joint diseases. We found that miR-671 level was significantly lower in patients with OA compared with healthy donors in both plasma and cartilage tissues (Fig. 1A, B), indicating the important role of miR-671 during the pathogenesis of OA. Next, we compared the expressions of inflammatory mediators in both patients with OA and healthy donors. The western blot results revealed that the protein levels of Collagen II and Aggrecan were markedly decreased in tissue from patients with OA compared with that from normal donors, whereas protein level of MMP13 was obviously upregulated in OA tissues (Fig. 1C–F). These observations demonstrated miR-671 might play an important role during the progression of OA.

3.2. Establishment of in vitro OA model by treating chondrocytes with IL-1β

Interleukin-1β (IL-1β), the major proinflammatory and pro-catabolic cytokine, has been reported to contribute to destruction of articular cartilage (ref). In order to establishment of *in vitro* OA model, we used IL-1β to induce inflammatory injury in human chondrocyte cell lines (CHON-001 cells). The results displayed the level of miR-671 in cells was significantly decreased by 10 ng/ml IL-1β (Fig. 2A). In addition, western blot data showed that IL-1β treatment markedly downregulated the protein levels of Collagen II and Aggrecan (Fig. 2B–D), while upregulated the expression of MMP13 (Fig. 2B, E). Above data indicated that *in vitro* OA model was successfully established by treating chondrocytes with IL-1β, which could be used in the subsequent experiments.

3.3. MiR-671 mimics alleviated IL-1β-induced proliferation inhibition in CHON-001 cells via decreasing apoptosis

In order to evaluate the effect of miR-671 on IL-1β-treated chondrocytes CHON-001, the viability of cells was determined with CCK-8 assay. The result showed that IL-1β markedly reduced the cell viability compared with control group, which was reversed by miR-671 mimics (Fig. 3A). Similar results were observed when using immuno-staining of Ki67 to evaluate the effect of miR-671 mimics on cell proliferation in CHON-001 cells (Fig. 3B, C). In addition, the data of apoptosis assay revealed that IL-1β-treated cells exhibited higher apoptosis rate than control cells, while the addition of miR-671 mimics could attenuated the cell apoptosis induced by IL-1β (Fig. 3D, E). In summary, the results above suggested that miR-671 mimics alleviated IL-1β-induced proliferation inhibition in CHON-001 cells *via* decreasing apoptosis.

3.4. The effects of miR-671 mimics on the expressions of inflammatory mediators in IL-1β-treated CHON-001 cells

We next investigated the effects of miR-671 mimics on the expressions of inflammatory mediators in IL-1β-treated CHON-001 cells. We found that miR-671 mimics slightly increased the levels of Collagen II and Aggrecan in cells, whereas IL-1β treatment significantly inhibited the expressions of them (Fig. 4A–C). As we expected the decreased protein levels of Collagen II and Aggrecan induced by IL-1β treatment were completely reversed by miR-671 mimics (Fig. 4A–C). These results indicated that miR-671 mimics could potentially alleviate the progression of OA.

3.5. MiR-671 mimics alleviated the progression of OA in mice

Next, we interrogated whether miR-671 could alleviated the progression of OA *in vivo* using OA mice models. The Safranin O staining

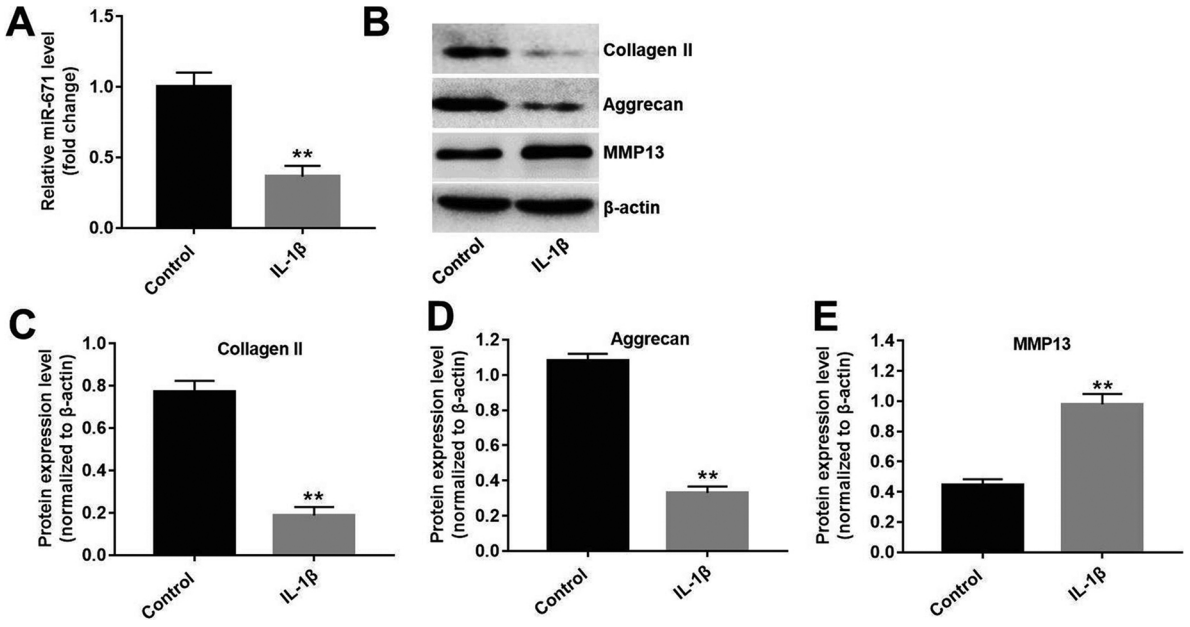

Fig. 2. Levels of miR-671 and inflammatory mediators in IL-1β-induced OA cell model. CHON-001 cells were treated with 10 ng/ml IL-1β for 24 h. (A) The level of miR-671 in IL-1β-induced CHON-001 cells were determined with qRT-PCR. (B) Expressions of MMP13, collagen II and aggrecan in IL-1β-treated CHON-001 cells were determined by western blot. (C–E) Quantification of protein expressions in CHON-001 cells. * indicates $P < 0.05$, compared to control group. Each experiment was performed in triplicate.

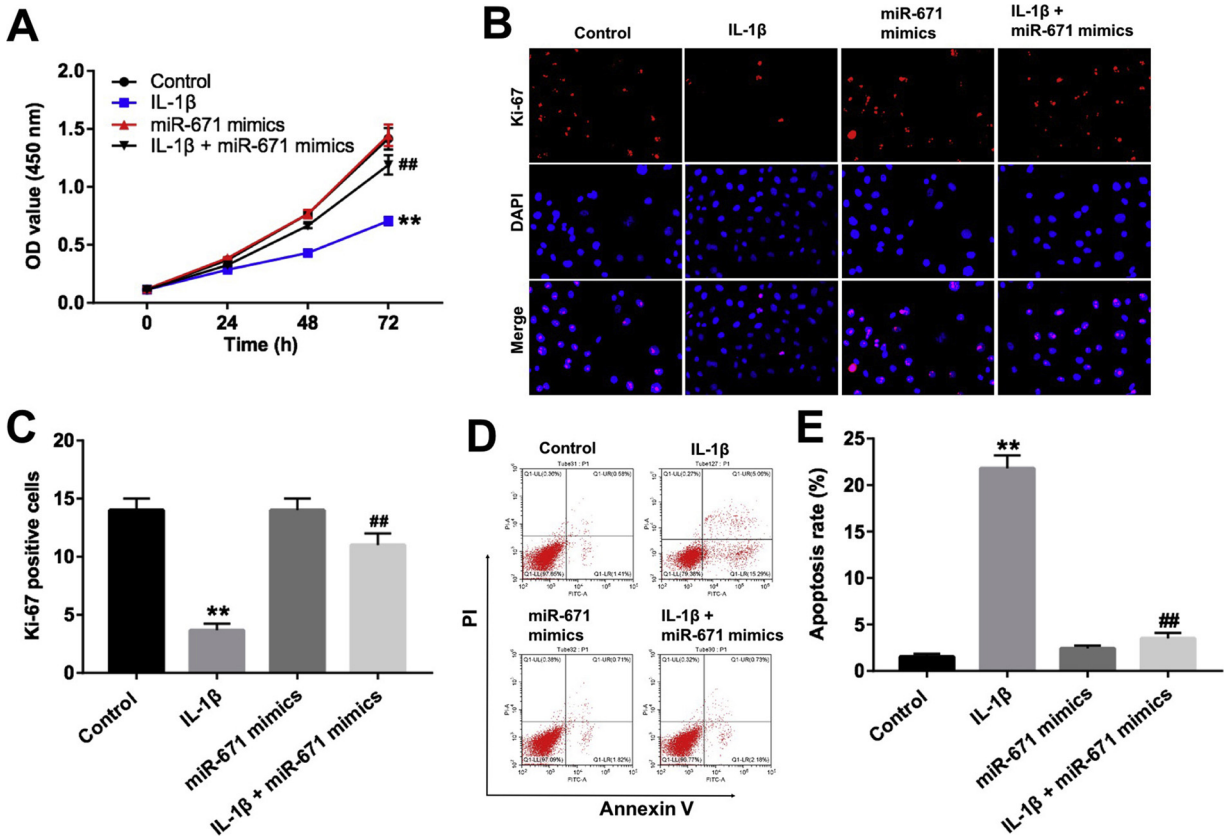

Fig. 3. MiR-671 mimics alleviated IL-1β-induced proliferation inhibition in CHON-001 cells *via* decreasing apoptosis. CHON-001 cells were transfected with 100 nM miRNA mimic or 100 nM scrambled 22 nt nucleotides for 12 h, then treated with 10 ng/ml IL-1β for 24, 48 and 72 h. (A) Cell viability were detected with CCK8 assay. (B) The cells were stained with Ki-67 and DAPI. (C) Quantification of Ki-67 positive cells. * indicates $P < 0.05$, compared to NC group; # indicates $P < 0.05$, compared to IL-1β group. (D) CHON-001 cells were transfected with 100 nM miRNA mimic for 12 h, followed by 10 ng/ml IL-1β treatment. Cell apoptosis were detected with Annexin V/PI staining. * indicates $P < 0.05$, compared to NC group; # indicates $P < 0.05$, compared to IL-1β group. Each experiment was performed in triplicate.

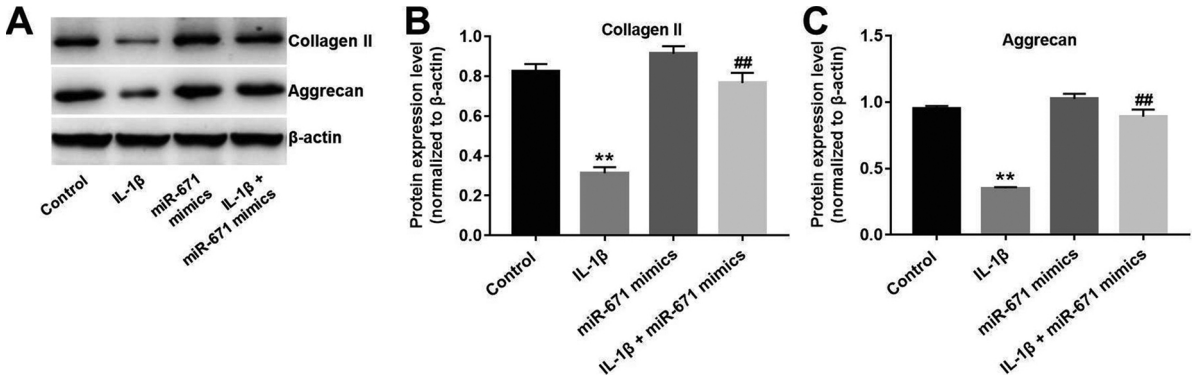

Fig. 4. The effects of miR-671 mimics on the expressions of inflammatory mediators in IL-1β-treated CHON-001 cells. (A) Protein expressions of collagen II and aggrecan in IL-1β-treated CHON-001 cells were determined by western blot. (B–C) Quantification of collagen II and aggrecan expressions in CHON-001 cells. * indicates $P < 0.05$, compared to control group. Each experiment was performed in triplicate.

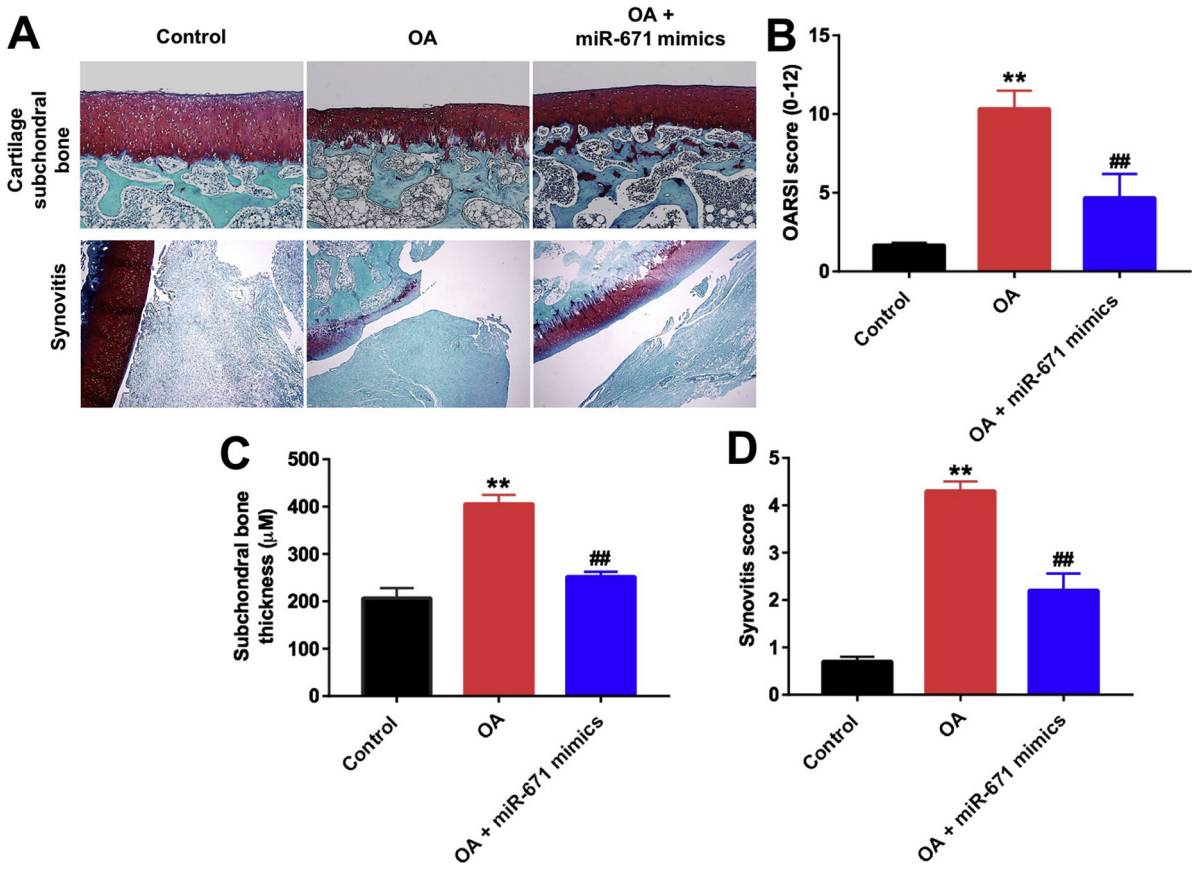

Fig. 5. MiR-671 mimics alleviated the progression of OA in mice. Mice were randomly divided into three groups: control group, OA group, and OA + miR-671 mimics group. After surgery, the miR-671 mimics group received an intraperitoneal injection of miR-671 mimics daily, while the OA group received vehicle (DMSO) for 8 weeks. Histological analysis of OA was determined by Safranin O staining (A), Osteoarthritis Research Society International (OARSI) scores (B), subchondral bone plate thickness (C), and synovitis scores (D). The lower panel shows the enlarged part of the upper black panel. * indicates $P < 0.05$, compared to NC group; # indicates $P < 0.05$, compared to OA group. $n = 5$ for each group. Each experiment was performed in triplicate.

showed that cartilage superficial destruction, cartilage erosion, vast proteoglycan loss, and apparent hypocellularity was observed in OA group, compared with smooth and integrate cartilage surface in the control group. Such patterns in OA group were ameliorated by addition of miR-671 mimics (Fig. 5A). Consistent with the results of Safranin O staining, the OARSI scores of the OA group were significantly higher than those of control group ($P < 0.01$), which could be reversed by miR-671 mimics ($P < 0.01$) (Fig. 5B). Similar results were also observed in terms of subchondral bone plate thickness and ameliorated synovitis (Fig. 5C, D). We found that an obviously increased subchondral bone plate thickness and decreased synovitis were observed in OA group, which could be reversed by treatment of miR-671 mimics as well. Taken together, these results suggested that miR-671 had the ability to attenuate the progression of OA in mice.

4. Discussion

Currently, drugs for OA treatment are mainly for pain and inflammation relief of the joint, and effective drugs for prevention of progression of OA were limited [19,20]. It raised an unmet medical

need that safe and effective agents were urgent for the treatment of patients with OA. In this study, we revealed that miR-671 were downregulated in OA tissue, plasma and IL-1β-treated CHON-001 cells. MiR-671 mimics alleviated IL-1β-induced proliferation inhibition and apoptosis in CHON-001 cells *via* decreasing expressions of collagen II and aggrecan. In addition, we also demonstrated that miR-671 mimics alleviated the progression of OA in mice. Thus, we hypothesized that miR-671 could be a potential therapeutic target for OA, and stimulation of miR-671 probably could improve the proliferation of cartilage cell.

OA is a multi-factorial de-generative disease, and its progression is stimulated by proinflammatory cytokine signaling [2]. For the inflammation-triggered modulations in chondrocytes, microRNA show markedly effects on OA. It was reported that the inflammatory circuitry of miR-149 functioned as a pathological mechanism in OA [21], and introduction of miR-21 into the cartilage of OA mice significantly stimulated cartilage destruction [22]. A miRNA profiling using high-density miRNA-arrays in serum of OA patients revealed that miR-671 were significantly downregulated in patients with OA compared to healthy articular cartilage [23]. Besides, there are no other reports on involvement of miR-671 in OA pathogenesis. In addition to the role of miR-671 in regulating cancer cell migration [24], it has recently been reported to regulate apoptotic genes such as caspase 8, p38, Myc-associated factor X, and Ras protein-specific guanine nucleotide releasing factor 1 in neurons of mice [24]. In addition, it could inhibit macrophage-mediated inflammation in orbital fat-derived stem cells by enhancing the expression of IL-IRA and TNFRII [25,26], indicating its potential role in apoptosis and inflammation, both of which are related to OA. Based on these above studies, we confirmed that levels of miR-671 were decreased in OA tissue, plasma and IL-1β-treated CHON-001 cells. MiR-671 mimics could alleviate the progression of OA, indicating the potential role of miR-671 in OA pathogenesis.

Degradation of cartilage is a hallmark of OA, and OA joints were proved to have higher level of matrix-degrading enzymes such as MMP13 than normal ones [27,28]. MMP-13 has a broad substrate specificity and can cleave collagen (types I, II, III, IV, X, and XIV), aggrecan, and fibronectin [29,30]. Induced expression of MMP13 resulted in pathologic changes in the joints in a transgenic mouse model, which was similar to human OA [31], providing evidence of its key roles in the pathogenesis of OA. There are mounting evidences that inhibition of MMP13 expression could prevent the pathogenesis of OA [32]. Akhtar et al. revealed that overexpression of miR-27b induced by IL-1β could inhibit the expression of MMP13 protein in human chondrocytes derived from normal and OA cartilage [33]. In this study, we demonstrated that IL-1β markedly increased the expression of MMP-13 in CHON-001 cells. The underlying correlation of miR-671 and MMP13 in the pathogenesis of OA needs further exploration.

Chondrocytes in OA cartilage produce high levels of type II collagen and aggrecan, which can provide tensile strength and shock absorption under mechanical damage [34]. Uncoordinated synthesis of the two proteins could be a crucial event in the pathogenesis of OA. Therefore, the inhibition of the degradation of collagen-II and aggrecan may have positive effects on OA treatment. In our study, we confirmed that collagen II and aggrecan were depressed in both human OA tissue andIL-1β-induced OA cell model. Moreover, miR-671 mimics could reverse IL-1β induced downregulation of collagen II and aggrecan.

In conclusion, we demonstrated that the level of miR-671 was downregulated in OA tissues, plasma and IL-1β treated CHON-001 cells. In addition, miR-671 mimics ameliorated IL-1β-induced proliferation inhibition and apoptosis stimulation. *In vivo* assay also suggested that miR-671 mimics alleviated the progression of OA in mice. Therefore, our results might give new insights for the understanding of the pathogenesis of OA.

Conflict of interest

The authors declare there is no conflict of interest.

References

[1] M.B. Goldring, M. Otero, Inflammation in osteoarthritis, Curr. Opin. Rheumatol. 23 (5) (2011) 471–478.
[2] M. Kapoor, J. Martel-Pelletier, D. Lajeunesse, J.P. Pelletier, H. Fahmi, Role of proinflammatory cytokines in the pathophysiology of osteoarthritis, Nat. Rev. Rheumatol. 7 (1) (2011) 33–42.
[3] V. Ambros, The functions of animal microRNAs, Nature 431 (7006) (2004) 350–355.
[4] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2) (2004) 281–297.
[5] D.P. Bartel, Metazoan MicroRNAs, Cell 173 (1) (2018) 20–51.
[6] B. Fromm, T. Billipp, L.E. Peck, M. Johansen, J.E. Tarver, B.L. King, et al., A uniform system for the annotation of vertebrate microRNA genes and the evolution of the human microRNAome, Annu. Rev. Genet. 49 (2015) 213–242.
[7] C. Esau, S. Davis, S.F. Murray, X.X. Yu, S.K. Pandey, M. Pear, et al., miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting, Cell Metab. 3 (2) (2006) 87–98.
[8] L. He, X. He, S.W. Lowe, G.J. Hannon, microRNAs join the p53 network–another piece in the tumour-suppression puzzle, Nat. Rev. Cancer 7 (11) (2007) 819–822.
[9] C.Z. Chen, L. Li, H.F. Lodish, D.P. Bartel, MicroRNAs modulate hematopoietic lineage differentiation, Science 303 (5654) (2004) 83–86.
[10] T.E. Callis, J.F. Chen, D.Z. Wang, MicroRNAs in skeletal and cardiac muscle development, DNA Cell Biol. 26 (4) (2007) 219–225.
[11] J. Takamizawa, H. Konishi, K. Yanagisawa, S. Tomida, H. Osada, H. Endoh, et al., Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival, Cancer Res. 64 (11) (2004) 3753–3756.
[12] K. Yamasaki, T. Nakasa, S. Miyaki, M. Ishikawa, M. Deie, N. Adachi, et al., Expression of MicroRNA-146a in osteoarthritis cartilage, Arthritis Rheum. 60 (4) (2009) 1035–1041.
[13] M.M. Abouheif, T. Nakasa, H. Shibuya, T. Niimoto, W. Kongcharoensombat, M. Ochi, Silencing microRNA-34a inhibits chondrocyte apoptosis in a rat osteoarthritis model in vitro, Rheumatology (Oxford) 49 (11) (2010) 2054–2060.
[14] E. Ntoumou, M. Tzetis, M. Braoudaki, G. Lambrou, M. Poulou, K. Malizos, et al., Serum microRNA array analysis identifies miR-140-3p, miR-33b-3p and miR-671-3p as potential osteoarthritis biomarkers involved in metabolic processes, Clin. Epigenetics 9 (2017) 127.
[15] R. Altman, E. Asch, D. Bloch, G. Bole, D. Borenstein, K. Brandt, et al., Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association, Arthritis Rheum. 29 (8) (1986) 1039–1049.
[16] F. Vasheghani, Y. Zhang, Y.H. Li, M. Blati, H. Fahmi, B. Lussier, et al., PPARgamma deficiency results in severe, accelerated osteoarthritis associated with aberrant mTOR signalling in the articular cartilage, Ann. Rheum. Dis. 74 (3) (2015) 569–578.
[17] K.P. Pritzker, S. Gay, S.A. Jimenez, K. Ostergaard, J.P. Pelletier, P.A. Revell, et al., Osteoarthritis cartilage histopathology: grading and staging, Osteoarthr. Cartil. 14 (1) (2006) 13–29.
[18] J.S. Lewis, W.C. Hembree, B.D. Furman, L. Tippets, D. Cattel, J.L. Huebner, et al., Acute joint pathology and synovial inflammation is associated with increased intra-articular fracture severity in the mouse knee, Osteoarthr. Cartil. 19 (7) (2011) 864–873.
[19] D.J. Hunter, Pharmacologic therapy for osteoarthritis–the era of disease modification, Nat. Rev. Rheumatol. 7 (1) (2011) 13–22.
[20] X. Chevalier, F. Eymard, P. Richette, Biologic agents in osteoarthritis: hopes and disappointments, Nat. Rev. Rheumatol. 9 (7) (2013) 400–410.
[21] P. Santini, L. Politi, P.D. Vedova, R. Scandurra, A. Scotto d'Abusco, The inflammatory circuitry of miR-149 as a pathological mechanism in osteoarthritis, Rheumatol. Int. 34 (5) (2014) 711–716.
[22] J. Song, C. Ahn, C.H. Chun, E.J. Jin, A long non-coding RNA, GAS5, plays a critical role in the regulation of miR-21 during osteoarthritis, J. Orthop. Res. 32 (12) (2014) 1628–1635.
[23] D.T. Felson, Clinical practice. Osteoarthritis of the knee, N. Engl. J. Med. 354 (8) (2006) 841–848.
[24] A. Nan, L. Chen, N. Zhang, Z. Liu, T. Yang, Z. Wang, et al., A novel regulatory network among LncRpa, CircRar1, MiR-671 and apoptotic genes promotes lead-induced neuronal cell apoptosis, Arch. Toxicol. 91 (4) (2017) 1671–1684.
[25] X. Tan, Y. Fu, L. Chen, W. Lee, Y. Lai, K. Rezaei, et al., miR-671-5p inhibits epithelial-to-mesenchymal transition by downregulating FOXM1 expression in breast cancer, Oncotarget 7 (1) (2016) 293–307.
[26] G.S. Lien, J.F. Liu, M.H. Chien, W.T. Hsu, T.H. Chang, C.C. Ku, et al., The ability to suppress macrophage-mediated inflammation in orbital fat stem cells is controlled by miR-671-5p, Stem Cell Res. Ther. 5 (4) (2014) 97.
[27] M.B. Goldring, The role of cytokines as inflammatory mediators in osteoarthritis: lessons from animal models, Connect. Tissue Res. 40 (1) (1999) 1–11.
[28] J.P. Pelletier, J. Martel-Pelletier, S.B. Abramson, Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets, Arthritis Rheum. 44 (6) (2001) 1237–1247.
[29] V. Knauper, S. Cowell, B. Smith, C. Lopez-Otin, M. O'Shea, H. Morris, et al., The role of the C-terminal domain of human collagenase-3 (MMP-13) in the activation of procollagenase-3, substrate specificity, and tissue inhibitor of metalloproteinase interaction, J. Biol. Chem. 272 (12) (1997) 7608–7616.
[30] V. Knauper, C. Lopez-Otin, B. Smith, G. Knight, G. Murphy, Biochemical characterization of human collagenase-3, J. Biol. Chem. 271 (3) (1996) 1544–1550.
[31] L.A. Neuhold, L. Killar, W. Zhao, M.L. Sung, L. Warner, J. Kulik, et al., Postnatal

expression in hyaline cartilage of constitutively active human collagenase-3 (MMP-13) induces osteoarthritis in mice, J. Clin. Invest. 107 (1) (2001) 35–44.
[32] Y. Yoshihara, H. Nakamura, K. Obata, H. Yamada, T. Hayakawa, K. Fujikawa, et al., Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis, Ann. Rheum. Dis. 59 (6) (2000) 455–461.
[33] N. Akhtar, Z. Rasheed, S. Ramamurthy, A.N. Anbazhagan, F.R. Voss, T.M. Haqqi, MicroRNA-27b regulates the expression of matrix metalloproteinase 13 in human osteoarthritis chondrocytes, Arthritis Rheum. 62 (5) (2010) 1361–1371.
[34] H.J. Suh, H. Lee, B.J. Min, S.U. Jung, E.Y. Jung, Effects of gangliosides from deer bone extract on the gene expressions of matrix metalloproteinases and collagen type II in interleukin-1beta-induced osteoarthritic chondrocytes, Nutr. Res. Pract. 10 (6) (2016) 569–574.