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MiR-103-3p regulates chondrocyte autophagy, apoptosis, and ECM degradation through the PI3K/Akt/mTOR pathway by targeting CPEB3

Jun Li[1], Farui Sun[1], Yuanjin Zhang[1], Xian Pan[1], Bo Li[1], Guofu Zhang[1] and Qian Zhou[2*]

Abstract

Background Chondrocyte apoptosis is associated with the severity of cartilage destruction and matrix degeneration in the progression of osteoarthritis. Increasing evidence indicates that autophagy has a significant cytoprotective effect against chondrocyte apoptosis. Here, we investigated the role of microRNA-103-3p (miR-103-3p) in regulating chondrocyte function and elucidated the underlying mechanism.

Methods MiR-103-3p expression in interleukin-1β (IL-1β)-stimulated chondrocytes was evaluated using RT-qPCR. The targets of miR-103-3p predicted by online databases were verified using biotin-based pulldown assay and luciferase reporter assay. IL-1β stimulated-chondrocytes were transfected with miR-103-3p inhibitor along with siRNA targeting cytoplasmic polyadenylation element-binding protein3 (siCPEB3), the autophagy inhibitor 3-MA, or the PI3K agonist 740 Y-P. Chondrocyte proliferation was evaluated using cell counting kit-8. Apoptosis was detected by flow cytometry. The levels of apoptosis-, extracellular matrix (ECM)-, autophagy-, and the PI3K/Akt/mTOR pathway-related proteins in chondrocytes were detected using immunoblotting or immunofluorescence.

Results We found that IL-1β stimulation upregulated miR-103-3p and downregulated CPEB3 in mouse chondrocytes. Inhibiting miR-103-3p reduced IL-1β-induced apoptosis and ECM macromolecule degradation while enhancing autophagy in chondrocytes. MiR-103-3p targeted CPEB3, and its downregulation rescued the expression of level in IL-1β stimulated-chondrocytes. MiR-103-3p downregulation inhibited the PI3K/Akt/mTOR pathway in IL-1β stimulated-chondrocytes by upregulating CPEB3. 3-MA, 740 Y-P, or CPEB3 knockdown counteracted the effect of miR-103-3p downregulation on chondrocyte apoptosis, ECM macromolecule degradation, and autophagy.

Conclusion Overall, inhibition of miR-103-3p reduces IL-1β-induced apoptosis and ECM macromolecule degradation in chondrocytes by enhancing autophagy through the CPEB3/PI3K/Akt/mTOR pathway.

Keywords Osteoarthritis, Chondrocyte, Autophagy, miR-103-3p, CPEB3, PI3K

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Introduction

Knee osteoarthritis is an inflammatory joint disease characterized by cartilage degeneration, with an age-associated increase in prevalence worldwide [1]. Both aging and obesity can increase the incidence of osteoarthritis [2]. This condition can lead to joint pain, swelling, and impaired mobility [3]. The management of osteoarthritis remains a challenge due to its complex etiology [4]. Current treatments including pharmacologic and nonpharmacologic interventions are mainly applied to relieve the symptoms associated with pain, but they cannot fundamentally reverse the pathologic process of the disease [5]. Most patients with severe symptoms have to undergo joint replacement or arthroscopic surgery [6]. Therefore, elucidating the underlying mechanisms of osteoarthritis may help develop novel therapeutic strategies.

Accumulating studies indicate that the mechanisms of osteoarthritis include increased expression of inflammatory cytokines, mediators of apoptosis, and extracellular matrix (ECM) catabolic enzymes, in addition to decreased expression of anabolic molecules and modification of homeostatic process, including autophagy [7–10]. These alterations lead to synovial inflammation, cartilage degradation, and subchondral bone sclerosis [11, 12]. An increase in the apoptosis of chondrocytes is associated with the destruction of articular cartilage and the pathologic process of osteoarthritis [13]. Autophagy is an intracellular degradation mechanism responsible for eliminating dysfunctional cytoplasmic components. In the cartilage of osteoarthritis patients and experimental models, the levels of autophagy-related proteins including unc-51 like autophagy activating kinase 1 (ULK1), microtubule-associated protein light chain 3 (LC3), and Beclin 1 were reduced, accompanied by an increase in apoptosis [14]. Evidence shows that autophagy is a homeostatic or protective mechanism in normal cartilage. It helps cells survive under adverse conditions and may be a potential target for preventing chondrocyte apoptosis and mitigating the severity of osteoarthritis [15].

MicroRNAs (miRNAs), a class of short noncoding RNAs, can negatively regulate gene expression through binding to specific complementary sequences in the 3'UTR of mRNAs [16]. MiRNA could be useful for diagnostic or management purposes in musculoskeletal conditions [17, 18]. The difference in miRNA expression patterns in osteoarthritis patients compared with healthy controls highlights the important role of miRNAs in the pathophysiology of the disease [19–21]. Previous studies have identified the regulatory function of miRNAs on ECM, chondrogenesis, inflammation, and other processes crucial for joint function and homeostasis [22–24]. MiR-103-3p was reported to drive cancer progression [25, 26]. MiR-103-3p inhibited neural stem cell proliferation and enhanced apoptosis [27]. It also could restrain the differentiation of myoblasts and prevent the production of autolysosomes [28]. It was found that miR-103 was highly expressed in patient-derived osteoarthritic cartilage in comparison to normal cartilage [29]. Additionally, Chen et al. reported that miR-103 contributed to osteoarthritis progression [30]. However, the specific function and mechanism of miR-103-3p in osteoarthritis are largely unknown.

In this study, IL-1β-treated chondrocytes were used as an in vitro model of osteoarthritis to investigate the role of miR-103-3p in regulating chondrocyte apoptosis, ECM, and autophagy and to clarify the underlying mechanism.

Materials and methods

Chondrocyte culture

Chondrocytes were isolated from the knee of neonatal C57BL/6 mice (Beijing Vital River Co. Ltd., Beijing, China) as described by Tang et al. [31]. The animal experiments were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Briefly, articular cartilage pieces were digested with 0.25% trypsin (AC15L811; Shanghai Life-iLab, Shanghai, China) in a culture dish for 30 min at 37 °C and further digested with 0.05% collagenase IV (C5138; Sigma-Aldrich, MO, USA) for 6 h at 37 °C. The suspension was filtered through a 70 μm nylon mesh followed by centrifugation for 5 min at 1000 rpm. The resulting primary chondrocytes were incubated in DMEM (AC01L043; Shanghai Life-iLab) containing 10% foetal bovine serum (F0193; Sigma-Aldrich), 100 U/mL penicillin, and 100 mg/L streptomycin (Sigma-Aldrich) with 5% CO_2 at 37 °C. Experiments were conducted using chondrocytes at passage 2.

Chondrocyte transfection and treatment

The miR-103-3p mimic (5'-AGCAGCAUUGUACAGGG CUAUGA-3')/inhibitor (5'-UCAUAGCCCUGUACAAU GCUGCU-3') and negative control (NC) mimic (5'-UU UGUACUACACAAAAGUACUG-3')/inhibitor (5'-CAG UACUUUUGUGUAGUACAAA-3') were obtained from Ribobio (Guangzhou, China). The siRNA targeting cytoplasmic polyadenylation element-binding protein3 (siCPEB3) (5'-AGAUUGUUACCAUUAUCGGUC-3') and siNC (5'-AUAAUGAUUCCAUGGUGUCUC-3') were obtained from Genechem (Shanghai). 2 mL suspension of chondrocytes (2.5×10^5 cells/mL) was incubated in 6-well plates overnight at 37 °C. Then, chondrocytes were transfected with miR-103-3p mimic/inhibitor (100 nM), siCPEB3 (50 nM), or their controls for 48 h using Lipofectamine 3000 (L3000015; Invitrogen, CA, USA). After 48 h, chondrocytes were treated with 10 ng/mL IL-1β (JN0342; Beijing Biolab, Beijing) for 24 h. To investigate

the mechanism, chondrocytes were pretreated with the autophagy inhibitor 3-MA (MG9192; Shanghai MesGen, Shanghai) or the PI3K agonist 740 Y-P (M00988; Beijing Biolab) 2 h before IL-1β stimulation.

RT-qPCR

RNAs were isolated from chondrocytes using FreeZol Reagent (R711; Nanjing Vazyme, Nanjing, China) and reverse transcribed into cDNA using HiScript III 1st Strand cDNA Synthesis Kit (R312; Nanjing Vazyme) following the manufacturer's instructions. The qPCR was performed using AceQ Universal SYBR qPCR Master Mix (Q511; Nanjing Vazyme) on the QuantStudio™ Real-Time PCR System (Thermo Fisher Scientific, MA, USA). The values were normalized to GAPDH and analyzed by the $2^{-\Delta\Delta Ct}$ method. The primer sequences are as follows (5′-3′): miR-103-3p forward ACACTCCAGCTGG GAGCAGCATTGTAC, reverse TGGTGTCGTGGAGT CG; CPEB3 forward CCCAAGCCCGAAGACAGTAG, reverse GGAAGAAGCTGTCCTCCACC; U6 forward G ATGACACGCAAATTCGTGAA, reverse GCTGTCAA CGATACGCTACG; GAPDH forward TGGCCTTCCGT GTTCCTAC, reverse GAGTTGCTGTTGAAGTCGCA.

Cell counting kit-8 (CCK-8)

The cultured chondrocytes were seeded on 96-well plates (1×10^3/well). After treatment, 20 μL CCK-8 solution (MA0225; Dalian MeilunBio, Dalian, China) was added to each well and incubated for 2.5 h at 37 °C. The absorbance at 450 nm was measured using a SPECTROstar Nano microplate reader (BMG Labtech, Germany).

Flow cytometry

Chondrocyte apoptosis was detected according to the instructions of Annexin V-FITC/PI Apoptosis Detection Kit (BL110B; Hefei Biosharp Life Science, Hefei, China). The treated chondrocytes were suspended in a binding buffer (4×) and incubated with 5 μL Annexin V for 10 min. Afterwards, cells were incubated with 10 μL PI for 15 min. Cells were then resuspended in 400 μL phosphate buffer saline (PBS) and analyzed by flow cytometry (FACScan flow cytometer; Becton, Dickinson and Company, NJ, USA).

Immunoblotting

Immunoblotting was conducted as described by Xu et al. [32]. Primary antibodies included cleaved caspase3 (1:2,000, AF7022; Affinity), Bax (1:2,000, AF0120; Affinity), Bcl-2 (1:2,000, AF6139; Affinity), ADAMTS5 (1:2,000, DF13268; Affinity), MMP13 (1:2,000, AF5355; Affinity), collagen II (1:2,000, AF0135; Affinity), aggrecan (1:2,000, DF7561; Affinity), LC3 (1:2,000, AF5402; Affinity), Beclin 1 (1:2,000, AF5128; Affinity), ATG5 (1:2,000, DF6010; Affinity), p62 (1:2,000, AF5384; Affinity), CPEB3 (1:500, ab10883; Abcam), p-PI3K (1:2,000, AF3242; Affinity), PI3K (1:2,000, AF6241; Affinity), p-Akt (1:2,000, AF0016; Affinity), Akt (1:2,000, AF6261; Affinity), p-mTOR (1:2,000, AF3308; Affinity), mTOR (1:2,000, AF6308; Affinity), and GAPDH (1:2,500, ab9485; Abcam). Protein signals were detected using Ultra-High Sensitivity ECL Substrate Kit (JY226423; Nanjing Jiangyuanbio, Nanjing) and imaged by ChemiDoc XRS + System (Bio-Rad, CA). Data were quantified using ImageJ software. Uncropped, full immunoblot images and gels are provided in Supplementary materials.

Immunofluorescence

Chondrocytes were fixed with 4% paraformaldehyde (MM1514; Shanghai MKbio, Shanghai) for 30 min. Then cells were washed in PBS containing 0.5% Triton X-100 (R22017; Zhengzhou Leyebio, Zhengzhou, China) for 15 min, blocked with 10% goat serum (LM803001C; Shanghai LMAI Bio) for 30 min, and washed with PBS for 10 min. Afterwards, cells were indicated overnight at 4 °C with Rabbit polyclonal antibody to LC3 (1:250, AF5402; Affinity), and subsequently with secondary antibody (Fluor594-conjugated) (1:100, S0006; Affinity) for 1 h at room temperature. After counterstaining with DAPI, cells were observed under a Nikon Eclipse 50i microscope (Nikon, Japan).

Biotin-based pulldown assay

The biotin-labeled probe specific for miR-103-3p was commercially synthesized by RiboBio. Briefly, the Beyo-Mag™ streptavidin magnetic beads (P2151; Shanghai Beyotime, Shanghai) were incubated with the biotin-labeled probe for 60 min at room temperature. Subsequently, the probe-beads were added to the cell lysates and incubated overnight at 4 °C. After washing, the RNAs bound to the beads were extracted and detected by qPCR analysis.

Luciferase reporter assay

CPEB3 3'UTR sequence at miR-103-3p binding sites (CPEB3-Wt) or its mutant version (CPEB3-Mut) was cloned into the pmirGLO vector (Promega, MI, USA). Then NC mimic (20 nM) or miR-103-3p mimic (20 nM) and 0.5 μg of CPEB3-Wt/Mut plasmids were co-transfected into chondrocytes using Lipofectamine 3000. After 48 h, chondrocytes were harvested and lysed. Luciferase activity was detected using Luciferase Reporter Assay Kit (K801-200; Biovision, CA).

Statistical analysis

All analyses were performed with Prism 9.0 (GraphPad Software). Data are expressed as the mean ± standard deviation. Comparisons among groups were performed using analysis of variance with Bonferroni posttests. All

results were obtained from three repeated experiments. $p < 0.05$ was considered statistically significant.

Results

Inhibiting miR-103-3p reduces IL-1β-induced apoptosis and ECM macromolecule degradation in chondrocytes

As shown by RT-qPCR, miR-103-3p expression in mouse chondrocytes was upregulated by IL-1β stimulation (Fig. 1A). The data indicated that miR-103-3p expression was markedly reduced by transfection of miR-103-3p inhibitor in IL-1β-stimulated chondrocytes (Fig. 1B). The influence of miR-103-3p inhibition on chondrocyte viability was evaluated, indicating that miR-103-3p inhibition restored IL-1β-inhibited cell viability (Fig. 1C). In chondrocytes stimulated by IL-1β, apoptosis was notably increased compared with control group. Suppression of miR-103-3p significantly inhibited the apoptosis-promoting effect of IL-1β (Fig. 1D-E). Immunoblotting also showed upregulated cleaved caspase3 and Bax levels and downregulated Bcl-2 levels in chondrocytes stimulated by IL-1β, while their levels were restored by miR-103-3p inhibitor (Fig. 1F-I). ADAMTS5 and MMP13 protein levels were increased whereas collagen II and aggrecan were reduced after L-1β stimulation. By contrast, miR-103-3p inhibitor downregulated ADAMTS5 and MMP13 expression and upregulated collagen II and aggrecan expression in IL-1β-treated cells (Fig. 1J-N).

Overexpressing miR-103-3p promotes IL-1β-induced apoptosis and ECM macromolecule degradation in chondrocytes

The qPCR results demonstrated that miR-103-3p mimic notably increased miR-103-3p expression in IL-1β-stimulated chondrocytes (Fig. 2A). After overexpressing miR-103-3p, chondrocyte viability under IL-1β stimulation was further inhibited (Fig. 2B). Additionally, the effect of IL-1β on chondrocyte apoptosis and ECM macromolecule degradation was further enhanced by miR-103-3p overexpression, demonstrated by flow cytometry and immunoblotting (Fig. 2C-M).

Inhibiting miR-103-3p reduces apoptosis and ECM macromolecule degradation in chondrocytes through enhancing autophagy

As shown by immunoblotting, IL-1β stimulation markedly induced downregulation of autophagy markers LC3, Beclin 1, and ATG5, and upregulation of p62 in chondrocytes, which were reversed by miR-103-3p inhibitor (Fig. 3A-E). Immunofluorescence also showed decreased LC3-stained intensity in IL-1β-treated chondrocytes, while LC3 expression was restored after miR-103-3p inhibition (Fig. 3F-G). As expected, miR-103-3p mimic further inhibited autophagy in chondrocytes stimulated by IL-1β, exerting an opposite effect to miR-103-3p inhibitor (Fig. 3H-L). To investigate the underlying mechanism, 3-MA (an autophagy inhibitor) was used. The data indicated that the inhibitory effect of miR-103-3p inhibitor on chondrocyte apoptosis and ECM macromolecule degradation was significantly reversed by 3-MA (Fig. 3M-S).

MiR-103-3p activates the PI3K/Akt/mTOR pathway by targeting CPEB3

We identified nine possible targets of miR-103-3p using online prediction tools, including ZHX1, AGFG1, ARMC1, RNF38, ZNRF2, SUN2, FAM117B, FERMT2, and CPEB3 (Fig. 4A). Among these genes, only CPEB3 was significantly enriched in the biotinylated miR-103-3p probe in chondrocytes (Fig. 4B), suggesting that CPEB3 may bind to miR-103-3p. CPEB3 expression was found to be downregulated in mouse chondrocytes stimulated by IL-1β compared with control cells (Fig. 4C). Inhibition of miR-103-3p restored the expression of CPEB3 in IL-1β-stimulated chondrocytes (Fig. 4D). Wild-type or mutant CPEB3 3'UTR sequence was inserted into the luciferase vector (Fig. 4E). Subsequent luciferase reporter assay showed a reduction in the luciferase activity of wild-type CPEB3 3'UTR reporter after co-transfection with miR-103-3p mimic. However, the luciferase activity of mutant reporter exhibited no significant change (Fig. 4F). Furthermore, CPEB3 was reported to suppress PI3K/Akt signaling [33]. We evaluated whether this signaling can be regulated by CPEB3 in chondrocytes. As immunoblotting indicated, IL-1β stimulation notably downregulated CPEB3 expression, and increased the phosphorylation levels of PI3K, Akt, and mTOR in chondrocytes, which were reversed by miR-103-3p downregulation. Interestingly, knocking down CPEB3 reversed the miR-103-3p downregulation's effect on the PI3K/Akt/mTOR signaling under IL-1β stimulation (Fig. 4G-K).

MiR-103-3p regulates chondrocyte apoptosis, ECM, and autophagy via the CPEB3/PI3K/Akt/mTOR pathway

For rescue experiments, IL-1β stimulated-chondrocytes were transfected with miR-103-3p inhibitor along with siCPEB3 or the PI3K agonist 740 Y-P. Following transfection, the reduction in chondrocyte apoptosis induced by miR-103-3p inhibitor was reversed by CPEB3 knockdown or 740 Y-P treatment (Fig. 5A-B). The data indicated that the effect of miR-103-3p inhibitor on the levels of chondrocyte apoptosis-and ECM-related molecules was significantly reversed by CPEB3 knockdown or 740 Y-P treatment (Fig. 5C-G). Immunofluorescence revealed an increase in LC3 intensity due to miR-103-3p overexpression, which was markedly mitigated by CPEB3 knockdown or 740 Y-P treatment (Fig. 5H-I).

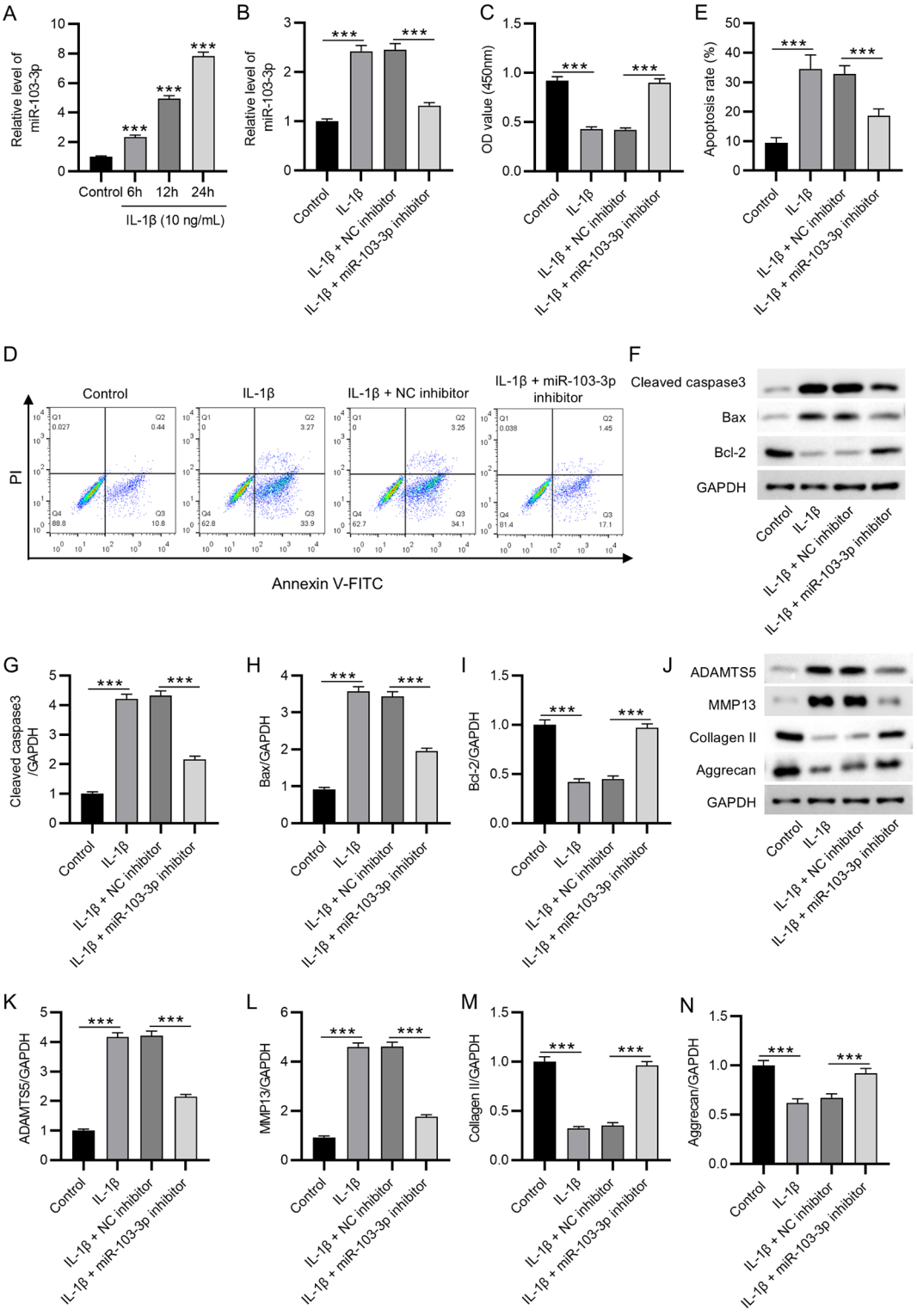

Fig. 1 Inhibiting miR-103-3p reduces IL-1β-induced apoptosis and ECM macromolecule degradation in chondrocytes. (**A**) RT-qPCR of miR-103-3p expression in mouse chondrocytes stimulated by IL-1β. (**B**) RT-qPCR of miR-103-3p expression in chondrocytes stimulated by IL-1β or/and transfected with miR-103-3p inhibitor. (**C**) CCK-8 assay evaluated chondrocyte viability. (**D**-**E**) Flow cytometry evaluated chondrocyte apoptosis. (**F**-**I**) Western blotting of cleaved caspase3, Bcl-2, and Bax protein levels in chondrocytes. (**J**-**N**) Western blotting of ADAMTS5, MMP13, collagen II, and aggrecan protein levels in chondrocytes. $N = 3$. $^{***}p < 0.001$

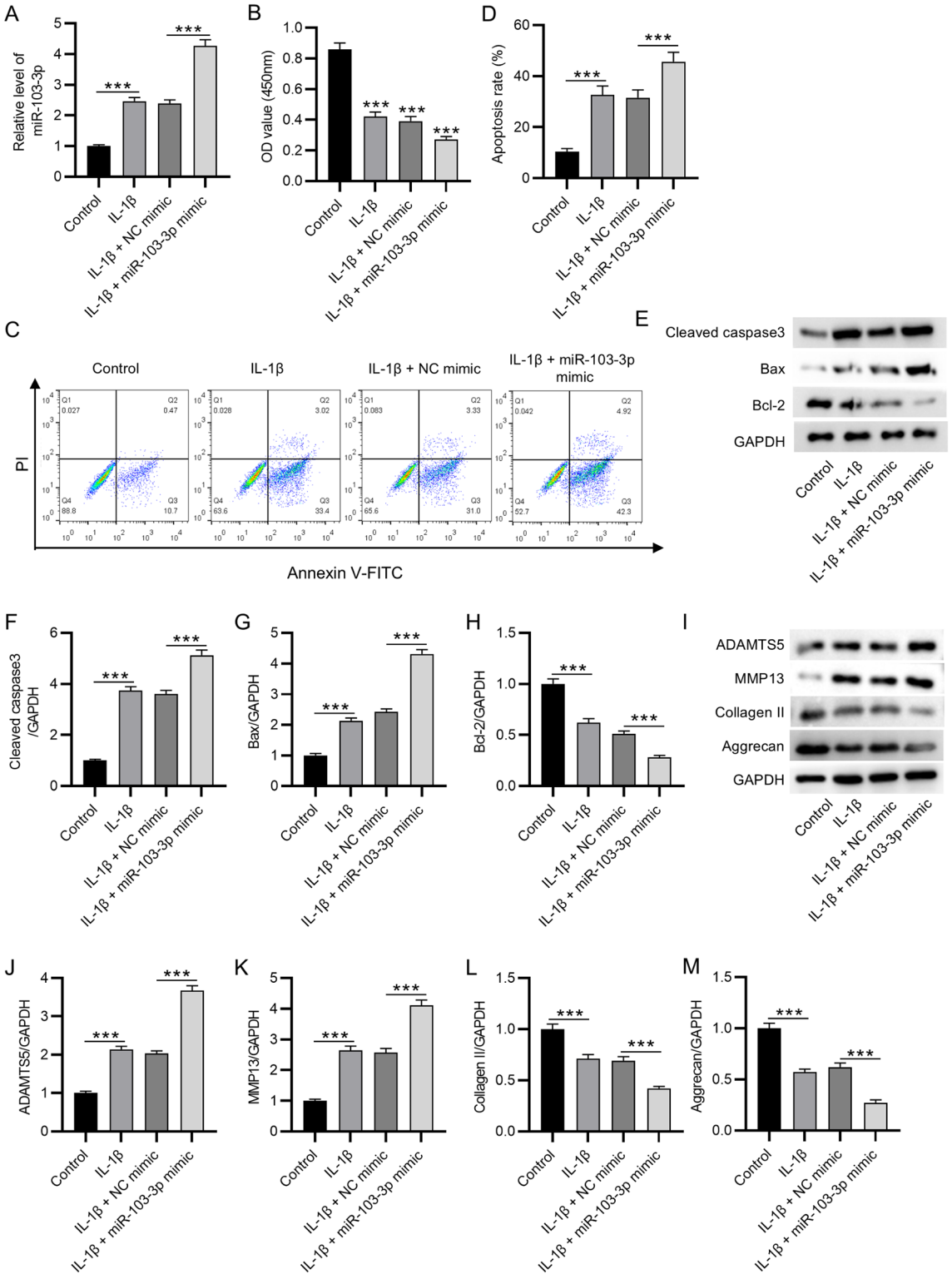

Fig. 2 Overexpressing miR-103-3p promotes IL-1β-induced apoptosis and ECM macromolecule degradation in chondrocytes. (**A**) RT-qPCR of miR-103-3p expression in chondrocytes stimulated by IL-1β or/and transfected with miR-103-3p mimic. (**B**) CCK-8 assay evaluated chondrocyte viability. (**C**-**D**) Flow cytometry evaluated chondrocyte apoptosis. (**E**-**H**) Western blotting of cleaved caspase3, Bcl-2, and Bax protein levels in chondrocytes. (I-M) Western blotting of ADAMTS5, MMP13, collagen II, and aggrecan protein levels in chondrocytes. $N = 3$. $^{***}p < 0.001$

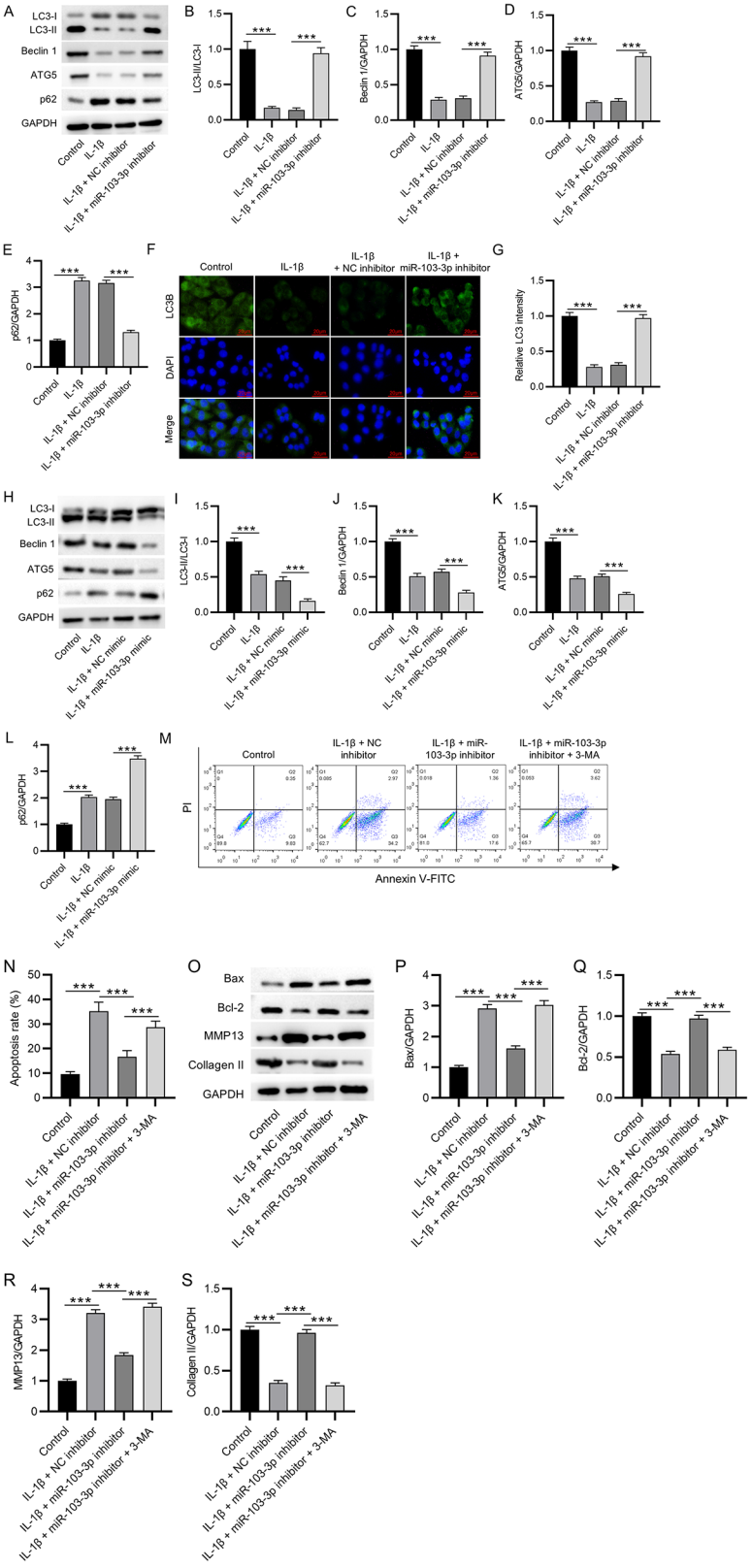

Fig. 3 (See legend on next page.)

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Fig. 3 Inhibiting miR-103-3p reduces apoptosis and ECM macromolecule degradation in chondrocytes through enhancing autophagy. (**A**-**E**) Western blotting of LC3, Beclin 1, ATG5, and p62 levels in chondrocytes stimulated by IL-1β or/and transfected with miR-103-3p inhibitor. (**F**-**G**) Immunofluorescence staining of LC3 in chondrocytes. (**H**-**L**) Western blotting of LC3, Beclin 1, ATG5, and p62 protein levels in chondrocytes stimulated by IL-1β or/and transfected with miR-103-3p mimic. (**M**-**N**) Flow cytometry evaluated apoptosis in IL-1β-stimulated chondrocytes treated with miR-103-3p mimic or 3-MA. (**O**-**S**) Western blotting of Bax, Bcl-2, MMP13, and collagen II protein levels in chondrocytes. $N = 3$. $^{***}p < 0.001$

Discussion

Studies indicate that apoptosis is a key feature in osteoarthritis cartilage degeneration [34], and autophagy is chondroprotective through repairing chondrocyte damage and regulating apoptosis [35]. In osteoarthritis cartilage, autophagy is inhibited, and apoptosis is enhanced [36]. This study showed that miR-103-3p was upregulated in the in vitro model of osteoarthritis. Inhibiting miR-103-3p reduced IL-1β-induced apoptosis and ECM macromolecule degradation in chondrocytes by enhancing autophagy. These effects were associated with the upregulation of CPEB3 and inhibition of the PI3K/Akt/mTOR pathway.

In recent years, the involvement of miRNAs in osteoarthritis pathogenesis has been reported [37]. For example, inhibition of miR-199b-5p protected chondrocytes against damage under inflammatory conditions [38]. MiR-25-3p promoted chondrocyte proliferation and restrained apoptosis to ameliorate osteoarthritis [39]. Collagen and proteoglycan play a key role in maintaining the mechanical properties of cartilage [40]. Collagen II and aggrecan are the most abundant cartilage proteins [41], and their degradation is a hallmark of osteoarthritis pathology [42]. ADAMTS metalloproteinase, consisting of 19 kinds of zinc endopeptidases, is a secretory zinc endopeptidase. ADAMTS5 is involved in the proteolytic cleavage of aggrecan [43]. MMP13 is a major matrix metalloproteinase that contributes to cartilage degeneration through its capacity of cleaving collagen II [44]. Here, miR-103-3p was found to be upregulated in IL-1β-treated chondrocytes, consistent with previous studies [30]. Its inhibition reduced IL-1β-induced ECM macromolecule degradation, suggesting that inhibiting miR-103-3p may be protective against osteoarthritis. Cytoplasmic polyadenylation element binding proteins (CPEBs) are a type of RNA binding proteins and translational regulators that contain four members CPEB1, CPEB2, CPEB3 and CPEB4 [45]. CPEB1 was reported to participate in osteoarthritis [46, 47]. The role of CPEB3 in osteoarthritis is unknown. CPEB3 was considered a key gene in inhibiting cancer progression [48, 49]. CPEB3 could modulate the activities of matrix metalloproteinases MMP2 and MMP9 in endometrial stromal cells [50]. CPEB3 expression was also related to inflammation-induced neuronal damage [51]. Various studies have used siRNAs, for example, to identify the target molecules, or as a therapeutic target, or to highlight the efficacy of a given drug [52–54]. In this study, we demonstrated that IL-1β decreased CPEB3 expression in chondrocytes. Furthermore, CPEB3 knockdown by siRNA counteracted the effect of miR-103-3p downregulation on ECM macromolecule degradation. It suggests the important role of the miR-103-3p/CPEB3 axis in osteoarthritis.

Chondrocyte death is the central feature in the progression of osteoarthritis [55]. Both autophagy and apoptosis are programmed cell deaths and key mechanisms of cell fate regulation [56]. Decreased expression of key ECM molecules in cartilage induces chondrocyte apoptosis, and in turn, chondrocyte death mediated by apoptosis aggravates matrix damage [57]. Our results revealed that IL-1β-stimulated chondrocytes exhibited increased expression of proapoptotic proteins cleaved caspase3 and Bax. Inhibiting miR-103-3p led to a substantial decrease in apoptosis by upregulating CPEB3. In the early stages of osteoarthritis, autophagy acts as an adaptive mechanism to aid chondrocyte survival. As the disease progresses, chondrocyte autophagy gradually decreases, driving cell apoptosis [58]. Caramés B et al. indicated that pharmacologic activation of autophagy prevented cell and matrix damage, representing a new approach for chondroprotection [59]. Evidence suggests that early activation of autophagy may be beneficial for osteoarthritis treatment [60]. In this study, miR-103-3p increased the levels of autophagy indicators (LC3 II, ATG5, and Beclin 1) in IL-1β-stimulated chondrocytes, and the inhibitor of autophagy dramatically counteracted the effect of miR-103-3p downregulation on chondrocyte apoptosis, suggesting that miR-103-3p downregulation attenuates chondrocyte apoptosis through enhancement of autophagy. The PI3K/Akt/mTOR signaling plays a key role in cartilage degradation, synovial inflammation, and subchondral bone dysfunction [61]. Inhibition of this signaling pathway could enhance autophagy of osteoarthritic chondrocytes and attenuate inflammation [62, 63]. It was reported that CPEB3 could suppress PI3K/Akt signaling [33, 64]. Interestingly, we identified that miR-103-3p activated PI3K/Akt/mTOR signaling in osteoarthritic chondrocytes by targeting CPEB3. Furthermore, PI3K agonist or CPEB3 knockdown counteracted the effect of miR-103-3p downregulation on chondrocyte apoptosis, ECM macromolecule degradation, and autophagy. These results suggest that the CPEB3/PI3K/Akt/mTOR signaling may be involved in the chondroprotective effect of miR-103-3p.

In summary, this study shows that miR-103-3p negatively regulates CPEB3 expression, which is involved

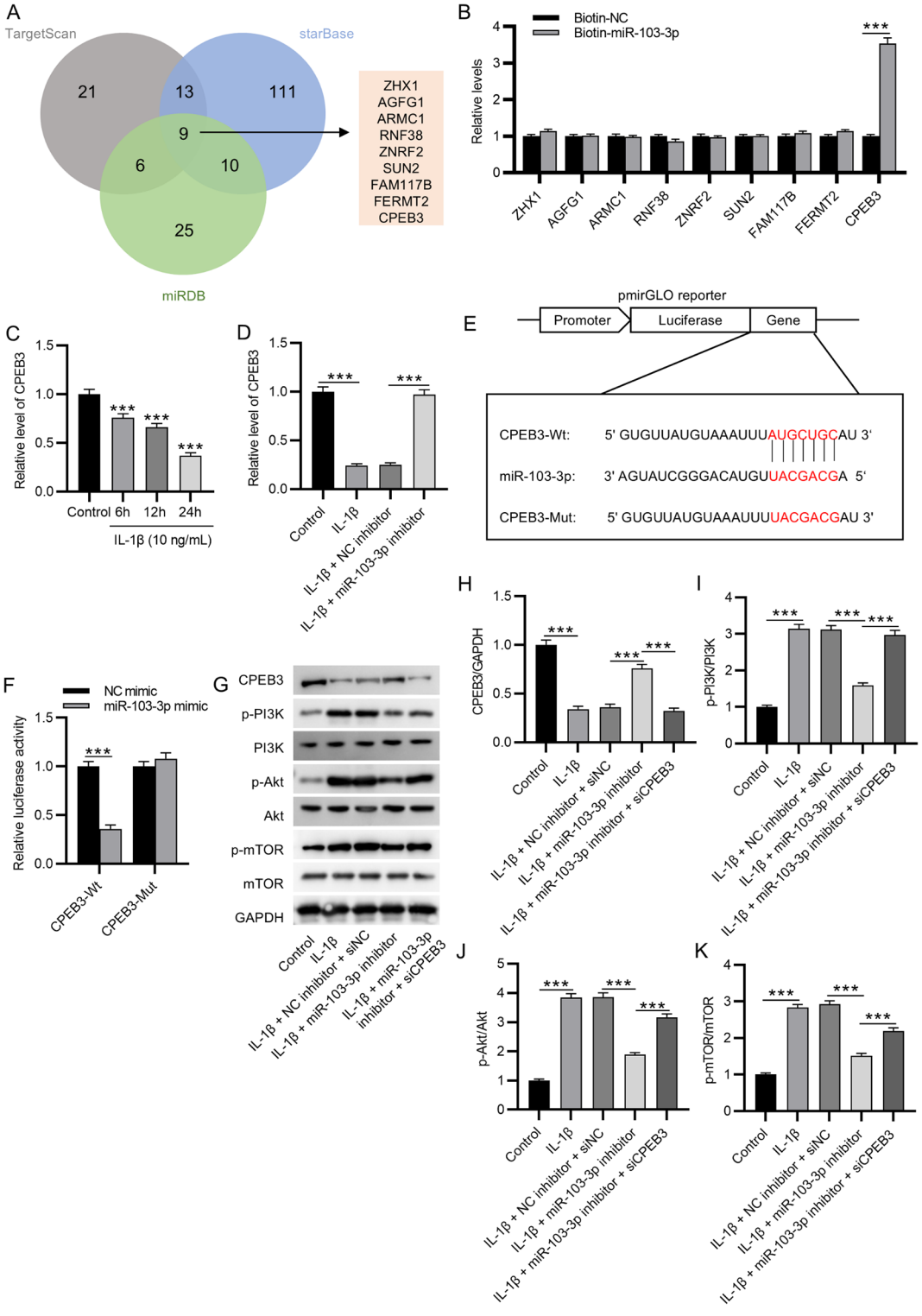

Fig. 4 MiR-103-3p activates the PI3K/Akt/mTOR pathway by targeting CPEB3. (**A**) Venn diagram showing possible targets of miR-103-3p predicted by online tools. (**B**) RNA pulldown assay and RT-qPCR results showing enrichment of ZHX1, AGFG1, ARMC1, RNF38, ZNRF2, SUN2, FAM117B, FERMT2, and CPEB3 bound to miR-103-3p probe in chondrocytes. (**C**) qPCR of CPEB3 level in mouse chondrocytes stimulated by IL-1β. (**D**) RT-qPCR of CPEB3 expression in chondrocytes stimulated by IL-1β or/and transfected with miR-103-3p inhibitor. (**E**) Binding sites where miR-103-3p interacts with CPEB3. (**F**) Luciferase activity of CPEB3 3′UTR-Wt/Mut reporters in chondrocytes following co-transfection with miR-103-3p mimic. (**G**-**K**) Western blotting of CPEB3, PI3K, Akt, and mTOR protein levels in IL-1β-stimulated chondrocytes transfected with miR-103-3p inhibitor or/and siCPEB3. $N = 3$. $^{***}p < 0.001$

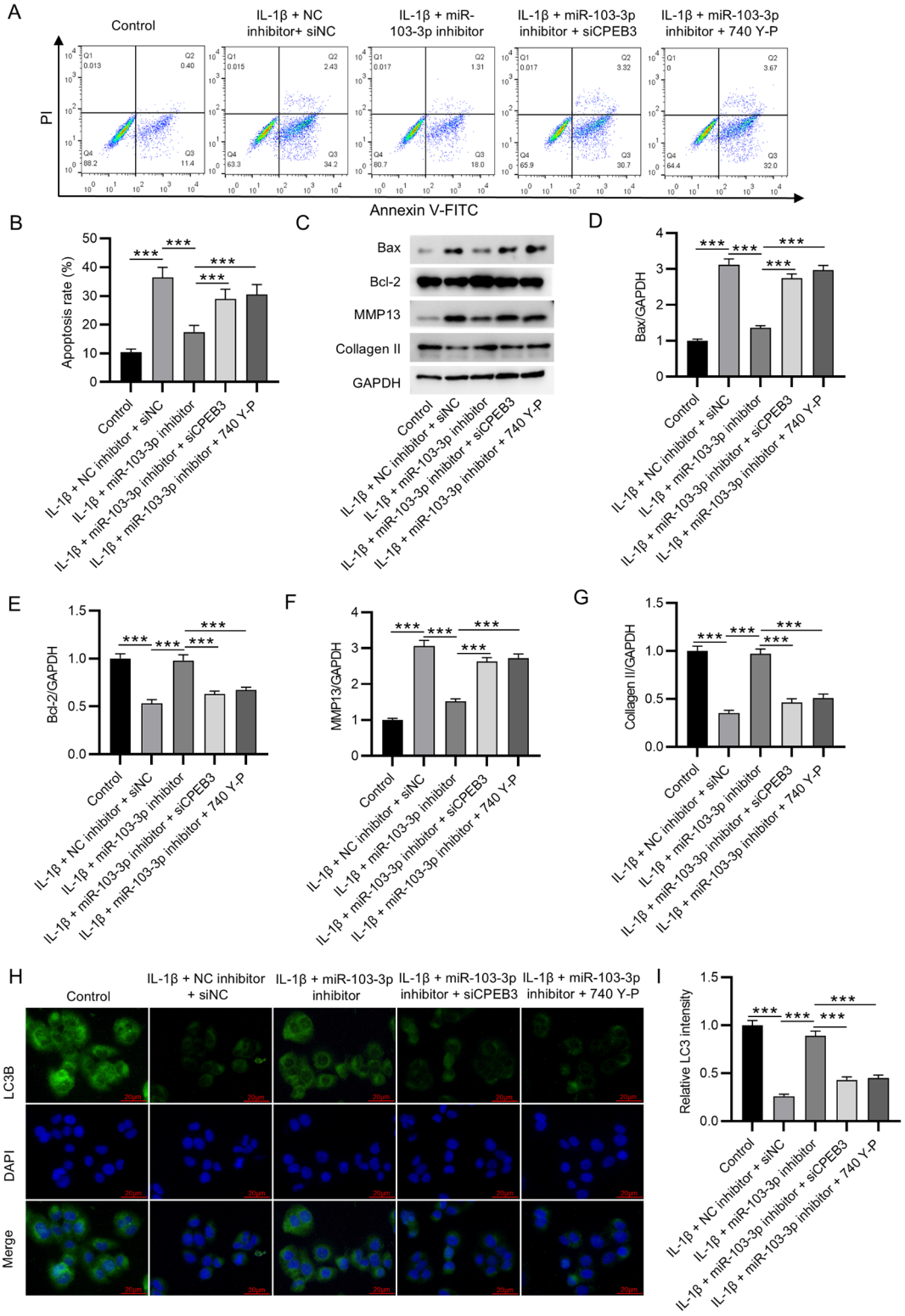

Fig. 5 MiR-103-3p regulates chondrocyte apoptosis, ECM, and autophagy via the CPEB3/PI3K/Akt/mTOR pathway. IL-1β-stimulated chondrocytes were transfected with miR-103-3p inhibitor along with siCPEB3 or the PI3K agonist 740 Y-P. (**A**-**B**) Flow cytometry evaluated chondrocyte apoptosis. (**C**-**G**) Western blotting of Bax, Bcl-2, MMP13, and collagen II protein levels in chondrocytes. (**H**-**I**) Immunofluorescence staining of LC3 in chondrocytes. $N = 3$. $^{***}p < 0.001$

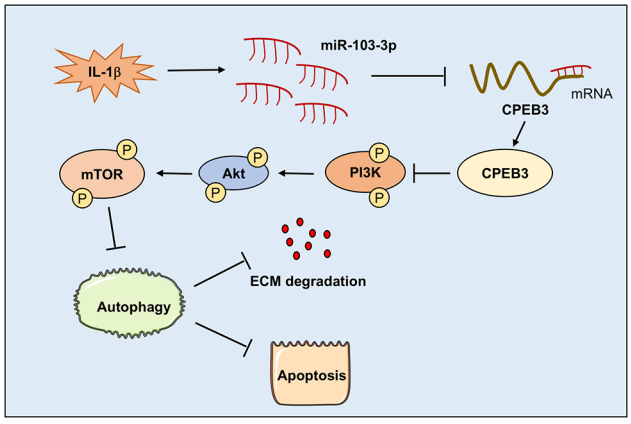

Fig. 6 Schematic diagram illustrating how miR-103-3p regulates chondrocyte apoptosis, ECM, and autophagy through the CPEB3/PI3K/Akt/mTOR pathway

in chondrocyte apoptosis, autophagy, and ECM macromolecule degradation through the PI3K/Akt/mTOR signaling (Fig. 6). Our results confirm that inhibition of miR-103-3p is protective against chondrocyte damage and these findings provide novel clues for understanding the mechanism of chondrocyte autophagy in osteoarthritis. However, to support our conclusion, animal experiments are needed in future work.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13018-025-05719-x.

Supplementary Material 1: Uncropped, full immunoblot images and gels

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Author contributions

Jun Li conceived and designed the experiments. Jun Li, Farui Sun, Yuanjin Zhang, Xian Pan, Bo Li, Guofu Zhang and Qian Zhou carried out the experiments and analyzed the data. Jun Li, Farui Sun, Yuanjin Zhang and Qian Zhou drafted the manuscript. All authors agreed to be accountable for all aspects of the work. All authors have read and approved the final manuscript.

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Data availability

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

Declarations

Competing interests

The authors declare no competing interests.

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