Upregulation of microRNA-590 in rheumatoid arthritis promotes apoptosis of bone cells through transforming growth factor-β1/phosphoinositide 3-kinase/Akt signaling

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Abstract. The aim of the present study was to further define the role of microRNA (miR)-590 in osteoarthritis (OA) and to investigate the underlying mechanism. In brief, reverse transcription-quantitative polymerase chain reaction was used to analyze miR-590 expression in bone tissue samples from rats with OA. Results indicated the expression of miR-590 was increased. miR-590 upregulation induced apoptosis in bone cells, whereas miR-590 downregulation reduced apoptosis of bone cells. Furthermore, miR-590 upregulation suppressed the protein expression levels of transforming growth factor (TGF)-β1, phosphoinositide 3-kinase (PI3K) and phosphorylated (p)-Akt in bone cells. However, downregulation of miR-590 induced the protein expression levels of TGF-β1, PI3K and p-Akt in bone cells. In addition, TGF-β1 attenuated the effects of miR-590 upregulation on bone cell apoptosis and the inactivation of PI3K inhibited the effects of miR-590 downregulation on bone cell apoptosis. Taken together, the present data suggested that miR-590 promoted apoptosis in bone cells from rats with OA by regulating the TGF-β1/PI3K signaling pathway.

Introduction

Osteoarthritis (OA) is a chronic degenerative osteoarticular disease that is severely damaging to human health (1). Its degenerative changes manifest as degeneration and destruction of articular cartilage, subchondral bone sclerosis or cystic degeneration, hyperostosis in the joint edge, hypertrophic synovium, capsular contracture, ligament laxity, and muscular atrophy and weakness (2). OA is associated with high morbidity, and is frequently observed in middle-aged and elder patients (3). Typically, the number of female cases is greater than that of male cases (3). The morbidity among persons aged <40, 60-75 or >75 years old is ~5, 50 or 80%, respectively (3). These findings suggest a distinctly increasing trend with age. The disability rate of OA is as high as 53%, and the final outcomes of OA include joint deformities and dysfunction if it is left untreated (3). Patients with OA partly or completely lose their ability to live independently. This severely affects the quality of life of patients and causes tremendous economic and social burdens. Therefore, investigating the etiology and pathogenesis of OA is of great value for the early prevention, early diagnosis and effective intervention of OA.

MicroRNAs (miRs) are a class of highly conserved non-coding, single-strand, small, molecular RNA that were identified in recent years (4). They are 20-25 nucleotides in length (4). Notably, miRs are extensively distributed in multiple eukaryotes and primarily achieve their functions through two modes, namely, induction of target mRNA degradation and targeted inhibition of mRNA translation (5). Furthermore, miRs serve important regulatory roles in processes such as cell growth and division (6).

Transforming growth factor (TGF)-β is a polypeptide factor that can promote cell growth, proliferation and synthesis (7). In addition, it can regulate vascular endothelial cell growth, inflammatory cell chemotaxis, fibroblast proliferation, extracellular matrix synthesis and degradation (7). Furthermore, it serves an important role in immune regulation and tissue repair (7). TGF-β1 is a member of the TGF-β family that has been extensively studied. Research on the association between the Smad canonical signal pathway and OA has been performed. Notably, previous results have emphasized the importance of the role of the Smad signaling pathway in OA genesis (7). Thus, Smad signaling pathway has become a novel hotspot in research on the pathogenesis of OA (8). Wu *et al* (9) suggested that melatonin-mediated miR-590-5p upregulation promotes chondrogenic differentiation of human mesenchymal stem cells. In the present study, the role of miR-590 in OA and the underlying mechanism were investigated.

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Materials and methods

Induction of OA. A total of 16 male Sprague Dawley rats (200-230 g and 8-10 weeks old) were purchased from the Animal Experiment Center of Kunming Medical University (Kunming, China). Rats were housed under standard conditions (22-23˚C with 50-60% humidity) and had free access to diet and water. All rats were randomly assigned to two groups: Sham (n=8) and OA (n=8) groups. Rats in the OA group were injected with 300 μl of chicken CII (100 mg; Chondrex Inc., Redmond, WA, USA). Rats were sacrificed using decollation at 12 days post-chicken CII injection under 35 mg/kg of pentobarbital sodium (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Animal experiments were approved by the Institutional Animal Care and Welfare Committee of Yuxi Municipal Hospital of Traditional Chinese Medicine (Yuxi, China).

Histopathology. The ankle joints were acquired, washed with PBS and fixed with 10% formalin for 2-3 days at room temperature. The tissues samples were decalcified in 10% formic acid and embedded in paraffin. Following this, tissues samples were cut into 4-μm thick sections and stained with hematoxylin for 2 min and stained with eosin for 1 min at room temperature. The tissues were observed using light microscopy (magnification, x100). H&E staining was used to confirm the OA model.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was harvested using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) from serum samples or cell samples. Subsequently, SuperScript RT system (Takara Bio Inc., Otsu, Japan) was used to reverse transcribe the RNA to cDNA. RT-qPCR was performed using the Applied Biosystems 7900 Fast Real-Time PCR system and TaqMan Universal PCR Master Mix (both Applied Biosystems, Thermo Fisher Scientific, Inc.). Primers used for RT-qPCR were as follows: miR-590, 5'-GGAATTCTTCAGTTGTAACCCAG-3' and 5'-CGGGAT CCTTGAGATGTCACCAA-3'; and U6, 5'-CTCGCTTCG GCAGCACA-3' and 5'-AACGCTTCACGAATTTGCGT-3'. The reaction condition included pre-denaturation at 95˚C for 5 min, followed by 40 cycles of denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 30 sec. Relative miR-590 expression was calculated using the $2^{-\Delta\Delta Cq}$ method (10).

Microarray analysis. Total RNA (500 ng) from serum samples or cell samples was hybridized to the SurePrint G3 Mouse Whole Genome GE Microarray (G4852A Stratagene; Agilent Technologies, Inc., Santa Clara, CA, USA). Data was analyzed using A.10.7.3.1 Agilent Feature Extraction Software (Agilent Technologies, Inc.).

Culture of bone cells and transfection. Mouse embryo osteoblast precursor MC3T3-E1 cells were purchased from the Cell Bank of Chinese Academy of Sciences of Shanghai, (Shanghai, China) and maintained in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (both Invitrogen; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37˚C in an atmosphere containing 5% CO_2. A total of 100 ng of miR-590 (forward primer, 5'-GGC GTCGACACAGTTCAGACAGAAGTCACAAAAA-3' and reverse primer, 5'-GGCTCTAGACACCATCTAGTACTT TTGCAATGAA-3'), 100 ng of anti-miR-590 (5'-GACGUA AAAUACUUAUUCGAG-3' and 5'-GAGCUUAUUCAU AAAAUGCAG-3'), 100 ng of TGF-β1 (5'-CTCCCCCATGCC GCCCTCGG-3' and 5'-AGTGCAGCTGAAGCCCCGCC-3') and 100 ng of negative mimics (5'-CCCCCCCCCCCCC-3' and 5'-CCCCCCCCCCC-3') were purchased from Sangon (Shanghai, China). MC3T3-E1 cells were transfected using Lipofectamine 2,000 (Gibco; Thermo Fisher Scientific, Inc.) according to the instructions of the manufacturer. Following anti-miR-590 transfection at 4 h, 100 μg/ml disitertide, a PI3K inhibitor (MedChemExpress, Shanghai, China), was added to cells for 44 h.

Cell proliferation assay. MC3T3-E1 cells were transfected for 0, 24, 48 and 72 h and then 20 μl of MTT was added to cells and incubated for 4 h at 37˚C. Dimethyl sulfoxide was added to cells once the medium was removed. Following this, the solution was shaken for 20 min at 37˚C. The optical density at 490 nm was measured using a plate reader (ELX-800; Biotek Instruments, Inc., Winooski, VT, USA).

Lactate dehydrogenase (LDH) activity levels. LDH activity levels were measured using a LDH kit (cat. no. C0017; Beyotime Institute of Biotechnology, Haimen, China) according to the manufacturer's instructions. The optical density was measured using a plate reader (ELX-800; Biotek Instruments, Inc.) at 450 nm.

Flow cytometry. MC3T3-E1 cells ($1x10^6$ cells/ml) were washed with PBS, fixed with 4% paraformaldehyde for 10 min at room temperature and suspended in 1X binding buffer (BD Biosciences, San Jose, CA, USA). Cell was stained with 5 μl of Annexin V-fluorescein isothiocyanate and propidium iodide (both BD Biosciences) in the dark for 15 min. Apoptotic cells were assayed using a Flow cytometer (C6; BD Biosciences) and quantified using Flowjo 7.6.1 (FlowJo, LLC, Ashland, OR, USA).

Caspase-3 and caspase-9 activity levels. MC3T3-E1 cell ($1x10^6$ cells/ml) protein was lysed with radioimmunoprecipitation assay lysis buffer and the protein concentration of each sample was quantified via the BCA protein assay (both Beyotime Institute of Biotechnology). Total protein (50 μg per lane) was used to measure the activity of caspase-3 and caspase-9 with caspase-3 and caspase-9 activity kits (cat. nos. C1115 and C1157; Beyotime Institute of Biotechnology) according to the manufacturer's instructions.

Dual luciferase report system. The 3'-untranslated region (UTR) of TGF-β1- pMD18-T vector was designed by GeneCopoeia, Inc. (Guangzhou, China). MC3T3-E1 cells were co-transfected with the 3'-UTR of TGF-β1, a dual luciferase reporter vector and miR-590 mimics or negative mimics using Lipofectamine 2000 (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) according to the manufacturer's instructions. Following 48 h of transfection, the luciferase activity

was measured using dual luciferase reporter gene assay kit (Beyotime Institute of Biotechnology). The absolute values of Firefly luminescence were normalized to those of *Renilla* luciferase activity.

Western blot analysis. MC3T3-E1 cells ($1x10^6$ cells/ml) were lysed in radioimmunoprecipitation assay lysis (cat. no. P0013B, Beyotime Institute of Biotechnology) buffer on ice for 30 min and centrifuged at 4˚C at 10,000 x g for 8 min. Total protein was centrifuged and quantified with BCA assay reagent (Beyotime Institute of Biotechnology). A total of 50 μg of Proteins were electrophoresed on 8-12% SDS gels and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% bovine serum albumin (Beyotime Institute of Biotechnology) for 1 h at room temperature and incubated with Collagen I (sc-25974; 1:1,000), Runt-related transcription factor 2 (Runx2; sc-8566; 1:1,000), TGF-β1 (sc-13034; 1:1,000; all Santa Cruz Biotechnology, Inc.), phosphorylated (p)-Smad (ab53100; 1:1,000; Abcam, Cambridge, MA, USA) and GAPDH (sc-51631; 1:5,000; Santa Cruz Biotechnology, Inc.) primary antibodies at 4˚C overnight. Horseradish peroxidase-conjugated antibodies against rabbit IgG (sc-2004; 1:5,000, Santa Cruz Biotechnology, Inc.) was incubated at 4˚C for 1 h following a wash step. Following this, bands were visualized with SuperSignal West Pico chemiluminescencesubstrate (Thermo Fisher Scientific, Inc.) and analyzed using Image_Lab_3.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Immunofluorescence. MC3T3-E1 cells ($1x10^5$ cells/ml) were washed with PBS and fixed with 4% paraformaldehyde for 10 min at room temperature. MC3T3-E1 cells were treated with 0.1% Triton X-100 in PBS for 10 min and blocked with 5% bovine serum albumin (Beyotime Institute of Biotechnology) in PBS for 1 h at 37˚C. MC3T3-E1 cells were then incubated with TGF-β1 (sc-130348; 1:100; Santa Cruz Biotechnology, Inc.) at 4˚C overnight. Following this, MC3T3-E1 cells were incubated with goat anti-rabbit IgG-CFL 555 (sc-362272; 1:5,000, Santa Cruz Biotechnology, Inc.) for 1 h at 37˚C and stained with 4',6-diamidino-2-phenylindole for 15 min. Cells were visualized using confocal microscopy (LSM 510 Meta; Zeiss, Göttingen, Germany).

Statistical analysis. Data were expressed as the mean ± standard deviation. Statistical analyses were performed with the Student's t-test or one-way analysis of variance followed by Tukey's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

miR-590 is upregulated in the serum of rats with OA. To evaluate whether miR-590 impacted on the onset of OA, serum miR-590 expression levels were determined in rats with OA. As indicated in Fig. 1A, signs of OA were indicated in rats with OA. Serum miR-590 expression levels were upregulated in rats with OA compared with the sham group (Fig. 1B and C). These results indicated that miR-590 may serve an important role in OA.

miR-590 regulates cell growth and apoptosis of MC3T3-E1 cells. To explore the mechanism of miR-590 on apoptosis of MC3T3-E1 cells, miR-590 mimics were used to increase the expression of miR-590 in MC3T3-E1 cells. Notably, miR-590 mimics significantly increased the expression of miR-590, decreased cell proliferation and increased LDH activity, the apoptotic rate, caspase-3 activity and caspase-9 activity in MC3T3-E1 cells compared with the control group (Fig. 2). However, anti-miR-590 mimics significantly inhibited the expression of miR-590, promoted cell proliferation and decreased LDH activity, the apoptotic rate, caspase-3 activity and caspase-9 activity in MC3T3-E1 cells compared with the control group (Fig. 3). Therefore, the findings indicated that miR-590 could regulate bone cell growth and apoptosis in MC3T3-E1 cells.

miR-590 regulates the TGF-β1 signaling pathway of MC3T3-E1 cells. To evaluate the underlying mechanism in miR-590-mediated bone cell apoptosis in arthritis, gene chip analysis was used to analyze the expression levels of TGF-β1 signaling pathway components. As indicated in Fig. 4, the mRNA expression levels of TGF-β1 and PI3K were significantly reduced in MC3T3-E1 cells treated with miR-590 when compared with the control group. Furthermore, miR-590 targeted the 3'-UTR of TGF-β1 mRNA. Notably, miR-590 overexpression led to significantly decreased luciferase reporter levels compared with the control group (Fig. 4D and E). In addition, immunofluorescence revealed that overexpression of miR-590 suppressed the protein expression levels of TGF-β1 in MC3T3-E1 cells compared with the control group (Fig. 4F). Western blot analysis revealed that upregulation of miR-590 significantly suppressed the protein expression levels of TGF-β1, PI3K and p-Akt, and induced the expression levels of B-cell lymphoma-2 associated X protein (Bax) in MC3T3-E1 cells compared with the control group (Fig. 5A-E). However, downregulation of miR-590 significantly induced the protein expression of TGF-β1, PI3K and p-Akt, and suppressed the expression of Bax in MC3T3-E1 cells, compared with control group (Fig. 5F-J). These results indicated that miR-590 induced bone apoptosis through TGF-β1/PI3K/Akt signaling.

The promotion of TGF-β1 attenuates the effects of miR-590 on bone cell apoptosis in MC3T3-E1 cells by the TGF-β1 signaling pathway. To investigate the role of TGF-β1 on the effects of miR-590 in bone cell apoptosis in MC3T3-E1 cells, TGF-β1 plasmid was utilized to increase the protein expression of TGF-β1 in MC3T3-E1 cells. As indicated in Fig. 6A-E, the promotion of TGF-β1 significantly induced the protein expression of TGF-β1, PI3K and p-Akt, and suppressed that of Bax in MC3T3-E1 cells when compared with the miR-590 group. As indicated in Fig. 6F-L, the promotion of TGF-β1 significantly attenuated the effects of miR-590 on the inhibition of cell proliferation and the promotion of LDH activity, apoptotic rate and caspase-3/9 activity in MC3T3-E1 cells compared with those in miR-590 group.

The inhibition of PI3K attenuates the effects of miR-590 on bone cell apoptosis in MC3T3-E1 cells by the PI3K signaling

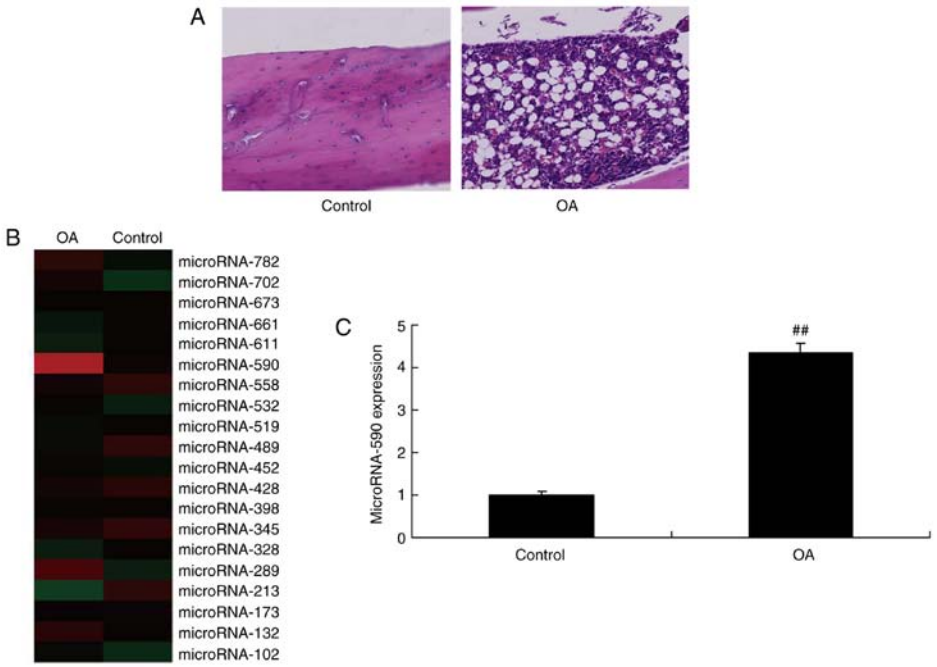

Figure 1. MicroRNA-590 overexpression in the serum of rats with OA. (A) Hematoxylin and eosin staining for OA tissues. MicroRNA-590 analysis by (B) gene chip and (C) quantitative polymerase chain reaction. ##P<0.01 vs. control group. OA, osteoarthritis rat group.

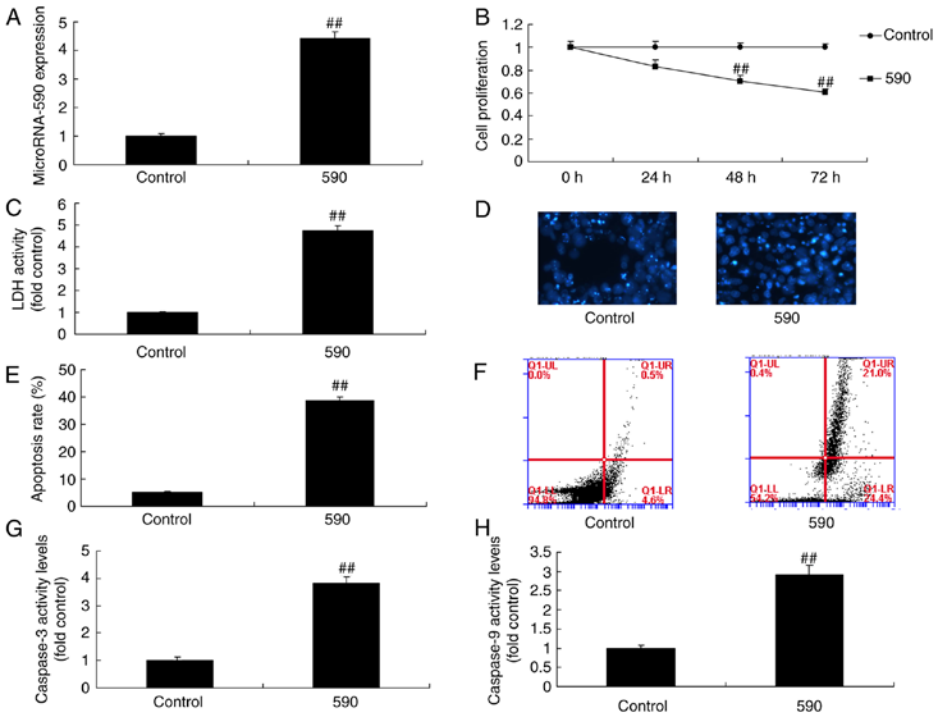

Figure 2. MicroRNA-590 overexpression on cell growth and apoptosis of MC3T3-E1 cells. (A) MicroRNA-590 expression, (B) cell proliferation, (C) LDH activity, (D) 4',6-diamidino-2-phenylindole staining (magnification, x100), (E and F) apoptosis rate, and (G) caspase-3 and (H) caspase-9 activity. ##P<0.01 vs. control group. 590, microRNA-590 overexpression group; LDH, lactate dehydrogenase.

pathway. The present study assessed the function of PI3K on the effects of miR-590 on adipogenic differentiation. Disitertide, a PI3K inhibitor, significantly suppress the protein expression levels of PI3K and p-Akt, and induce the protein expression of Bax in MC3T3-E1 cells compared with the miR-590 group (Fig. 7A-D). Furthermore, PI3K inhibitor attenuated the effect of miR-590 on cell proliferation and LDH activity, apoptotic rate and caspase-3/9 activity compared with the miR-590 group (Fig. 7E-K).

Discussion

OA is the most common degenerative disease in human axial joints and peripheral dynamic joints (11). It can involve the

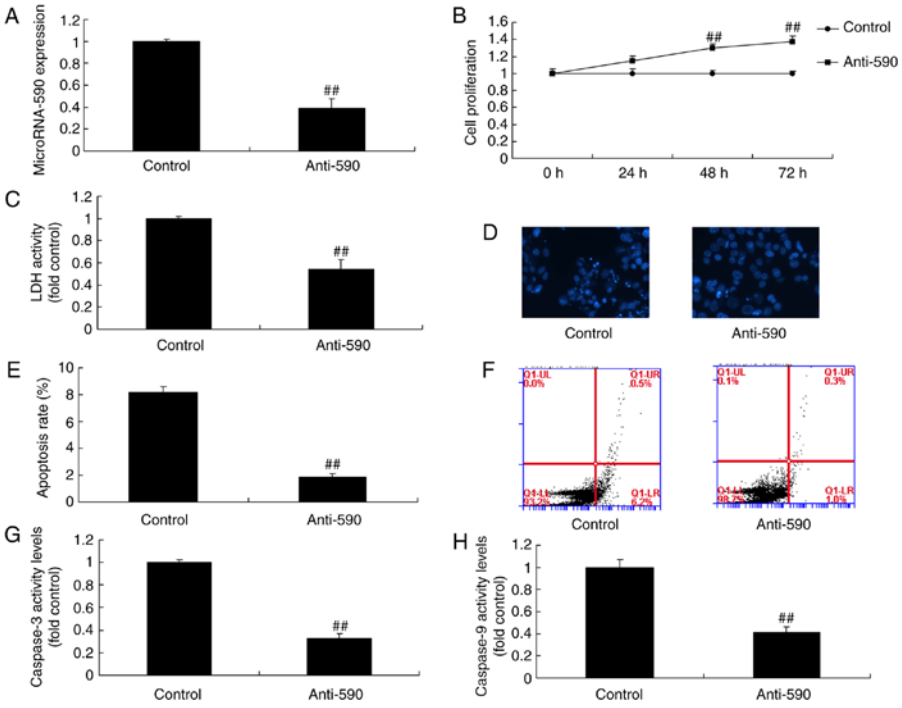

Figure 3. Anti-microRNA-590 effects on cell growth and apoptosis of MC3T3-E1 cells. (A) MicroRNA-590 expression, (B) cell proliferation, (C) LDH activity, (D) 4',6-diamidino-2-phenylindole staining (magnification, x100), (E and F) apoptosis rate, and (G) caspase-3 and (H) caspase-9 activity. ##P<0.01 vs. control group. Anti-590, anti-microRNA-590 group; LDH, lactate dehydrogenase.

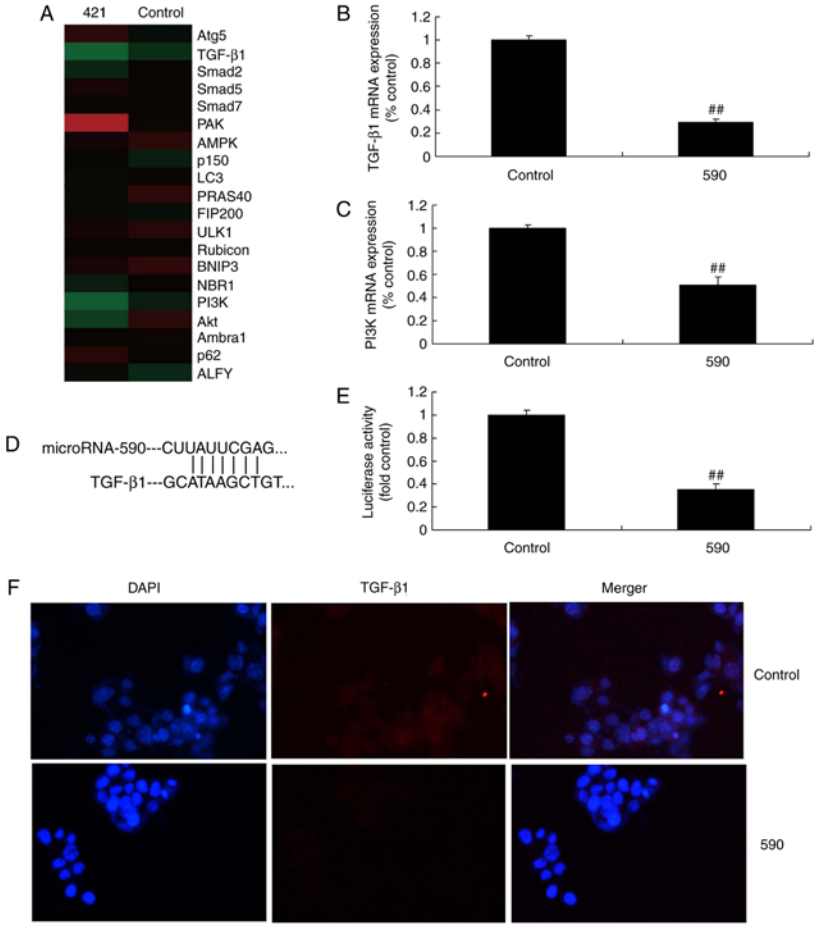

Figure 4. MicroRNA-590 regulates TGF-β1 protein expression of MC3T3-E1 cells. (A) Gene chip analysis of signaling pathways. mRNA expression levels of (B) TGF-β1 and (C) PI3K. (D) Interaction of microRNA-590 targeted the 3'-untranslated region of TGF-β1. (E) Luciferase reporter levels and (F) immunofluorescence analysis of TGF-β1 protein (magnification, x100). ##P<0.01 vs. control group. 590, microRNA-590 overexpression group; TGF, transforming growth factor; PI3K, phosphoinositide 3-kinase.

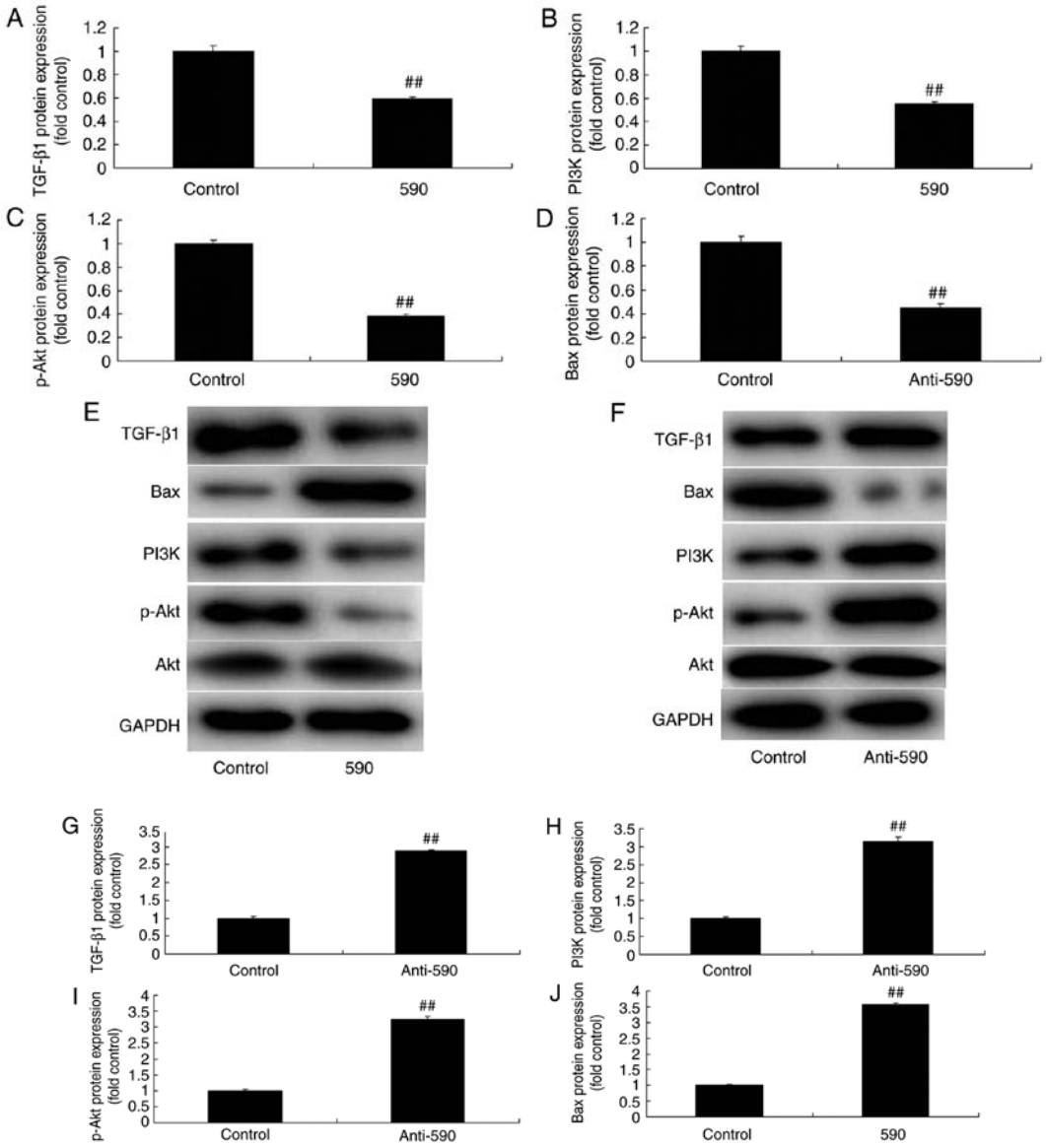

Figure 5. MicroRNA-590 regulates the TGF-β1/Smad pathway in MC3T3-E1 cells. (A-J) TGF-β1, PI3K, p-Akt and Bax protein expression levels were analyzed in microRNA-590-overexpressed and anti-microRNA-590-treated MC3T3-E1 cells. ##P<0.01 vs. control group. 590, microRNA-590 over-expression group; Anti-590, anti-microRNA-590 group; PI3K, phosphoinositide 3-kinase; p, phosphorylated; TGF, transforming growth factor; Bax, B-cell lymphoma-2-associated X protein.

articular cartilage, synovial membrane, articular capsule and muscles surrounding joints (11). OA is a type of irreversible articular damage induced by different factors. Major OA-induced pathological changes include cartilage degeneration and disappearance, reactive hyperplasia of ligament attachment points at the articular edge and subchondral bone, and osteophyte formation (12). At present, no effective treatment is available in clinic, and the therapeutic effects are inadequate. Therefore, exploring potential OA treatment options is of great importance (12). Involvement of miRs in the pathology and physiology of OA has been verified through methods of gene network and epigenetics (12) Further understanding of the action of targets and regulatory factors during molecular research on OA may facilitate the development of novel treatments.

miRs can control joint destruction and stimulate repair (13,14). Furthermore, miRs regulate protein expression and can thus can be used to treat and intervene in the molecular targets of OA (13,14). The present study revealed that serum miR-590 expression was significantly upregulated in rats with OA. Additionally, the results indicated that miR-590 mimics significantly increased the expression of miR-590 in MC3T3-E1 cells, decreased cell proliferation and increased LDH activity, the apoptosis rate and caspase-3/9 activity in MC3T3-E1 cells. Wu *et al* (9) suggested that melatonin-mediated miR-590-5p upregulation promotes chondrogenic differentiation in human mesenchymal stem cells. These results Imply that the upregulation of miRNA-590 in rheumatoid arthritis promotes apoptosis of bone cells.

TGF-β1 is a type of polypeptide involved in the formation of bone and cartilage (15). It can also stimulate chondrocytes to produce proteoglycan and promotes excessive expression of type II collagen and aggrecan (15). Previous results

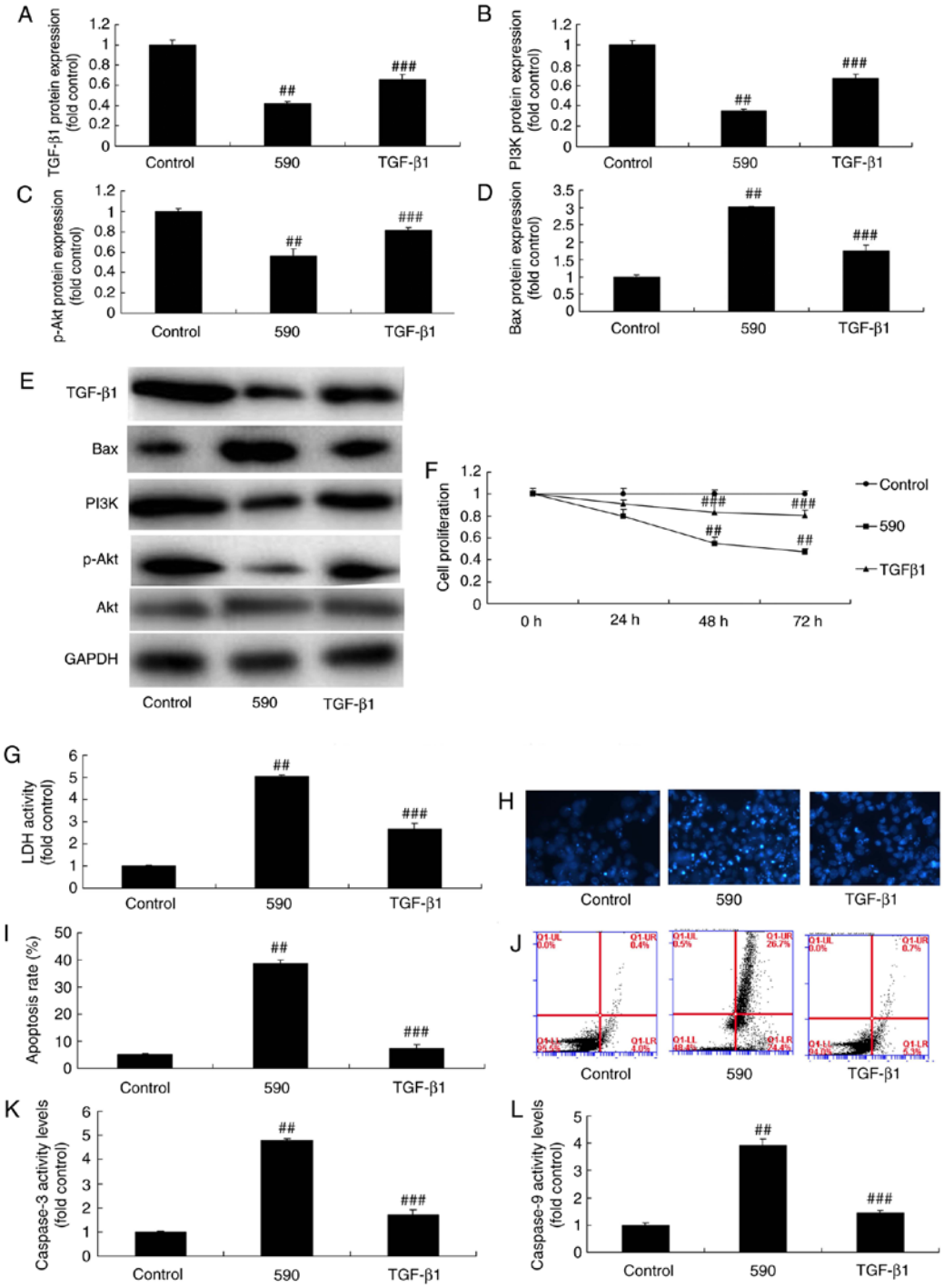

Figure 6. The promotion of TGF-β1 reduces the effect of microRNA-590 on bone cell apoptosis in MC3T3-E1 cells through TGF-β1 signaling. (A-E) TGF-β1, PI3K, p-Akt and Bax protein expression levels were analyzed using western blot analysis. (F) Cell proliferation, (G) LDH activity, (H) 4',6-diamidino-2-phenylindole stained images (magnification, x100), (I and J) apoptosis rate, and (K) caspase-3 and (L) caspase-9 activity were indicated. $^{##}$P<0.01 vs. control group, $^{###}$P<0.01 vs. microRNA-590 group. 590, microRNA-590 group; TGF-β1, microRNA-590 and TGF-β1 group; PI3K, phosphoinositide 3-kinase; p, phosphorylated; TGF, transforming growth factor; Bax, B-cell lymphoma-2-associated X protein.

have suggested that the expression of TGF-β1, TGF-β2 and TGF-β3 genes in OA chondrocytes exhibit various degrees of upregulation (15). This is consistent with the percentage of positive TGF-β chondrocytes (15). TGF-β2 can inhibit chondrocyte hypertrophy and downregulate the expression of inflammatory cytokines interleukin-1β, tumor necrosis factor-α, matrix metalloproteinase (MMP)-9 and MMP-13 in OA chondrocytes cultured *in vitro* (16,17). Thus, TGF-β2 can prevent the excessive degradation of type II cartilage collagen. TGF-β1 can increase the expression of tissue inhibitor of metalloproteinase (TIMP)-1 in OA chondrocytes, whereas TIMP-1 is also an angiogenic inhibitor (16,17). Therefore, TGF-β1 can antagonize vascular invasion of OA (16,17). In the present study, upregulation of miR-590 significantly suppressed the protein expression of TGF-β1, PI3K and p-Akt, and induced Bax protein expression in MC3T3-E1 cells. Notably, Ekhteraei-Tousi *et al* (18) suggested that miR-590-5p regulates cardiosphere-derived

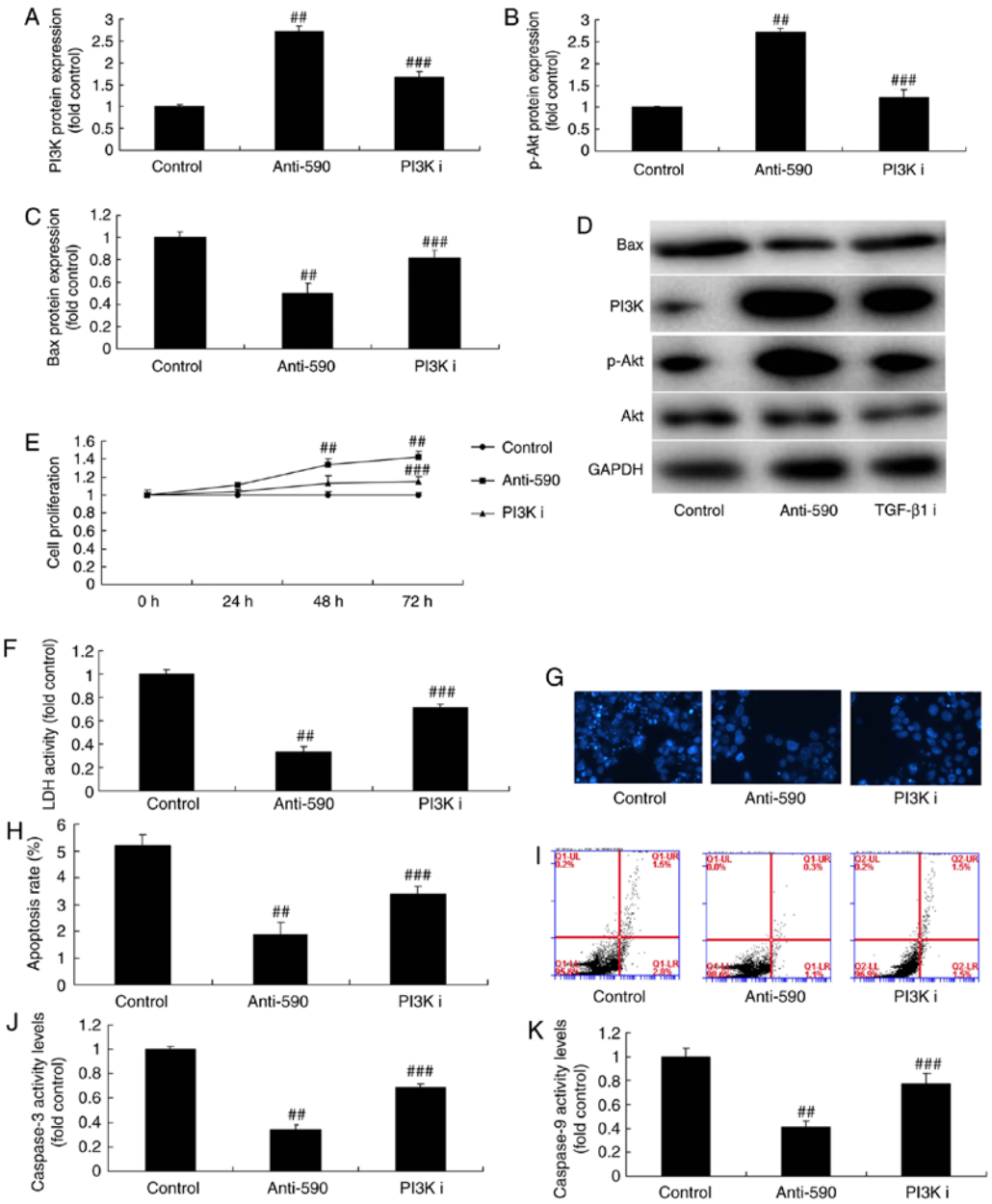

Figure 7. The inhibition of PI3K reduces the effect of microRNA-590 on bone cell apoptosis in MC3T3-E1 cells through PI3K signaling. (A-D) PI3K, p-Akt and Bax protein expression levels were analyzed using western blot analysis. (E) Cell proliferation, (F) LDH activity, (G) 4',6-diamidino-2-phenylindole staining images, (H and I) apoptosis rate, (J) caspase-3 and (K) caspase-9 activity were indicated. $^{\#\#}$P<0.01 vs. control group; $^{\#\#\#}$P<0.01 vs. microRNA-590 group. 590, microRNA-590 group; PI3K I, microRNA-590 and PI3K inhibitor group; TGF, transforming growth factor p, phosphorylated; TGF, transforming growth factor; Bax, B-cell lymphoma-2-associated X protein.

stem cells differentiation through downregulation of TGFB signaling. Based on the above information, it may be concluded that miR-590-5p regulates TGF-β1/PI3K/Akt signaling in bone cells.

It has been indicated in recent research that the PI3K/Akt signal transduction pathway is also involved in the pathogenesis and progression of OA (19). Expression levels of PI3K/Akt are increased in cartilage in OA. Activation of the PI3K/Akt signaling pathway can aggravate degradation of cartilage matrix protein (20). Furthermore, activation of the PI3K/Akt signaling pathway can also enhance the expression of MMPs in chondrocytes (20). Consequently, Smad signaling serves an important role in cartilage degeneration. Notably, gene knockout can induce increased expression of PI3K/Akt signaling molecules in nervous system cells (20). In the present study it was reported that TGF-β1 significantly reduced the effects of miR-590 upregulation on bone cell apoptosis. Furthermore, the inactivation of PI3K significantly inhibited the effects of miR-590 on bone cell apoptosis. Li *et al* (21) revealed that YKL-40-induced inhibition of miR-590-3p promotes angiogenesis of endothelial progenitor cells via PI3K/Akt signaling pathway.

In conclusion, the findings suggest that miR-590 can mediate OA and regulate bone cell apoptosis through TGF-β1/PI3K/Akt signaling (Fig. 8), which may be a critical factor for identifying potential OA treatments in the clinic. Further clinical studies are warranted to investigate the efficacy of miR-590 in OA.

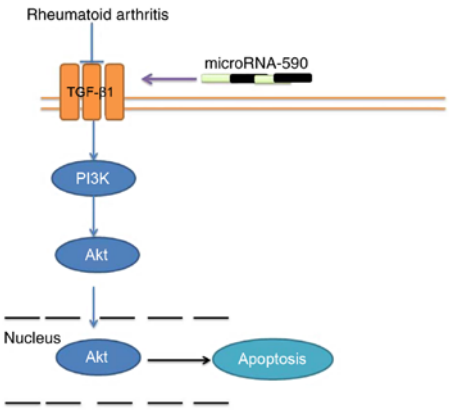

Figure 8. Upregulation of microRNA-590 in rheumatoid arthritis promotes apoptosis of bone cells through TGF-β1/PI3K/Akt signaling. TGF, transforming growth factor; PI3K, phosphoinositide 3-kinase.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

JY designed the experiment; YZ and JL performed the experiment; JY and YZ analyzed the data; and JY wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Animal experiments were approved by the Institutional Animal Care and Welfare Committee of Yuxi Municipal Hospital of Traditional Chinese Medicine.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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