

## Original Article

# miR-1307-5p regulates proliferation and apoptosis of chondrocytes in osteoarthritis by specifically inhibiting transforming growth factor beta-induced gene

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**Abstract:** Objective: To explore the effect of miR-1307-5p which specifically inhibits transforming growth factor beta-induced gene (TGFB1) on the biologic behavior of osteoarthritis (OA) chondrocytes. Methods: We detected miR-1307-5p and TGFB1 expression in the cartilage tissue specimens of OA patients and mice, respectively. RNA22 was applied to predict the target gene of miR-1307-5p, and we further verified the relationship by performing a dual luciferase reporter experiment. Enzyme-linked immunosorbent assay was used to measure the expression of matrix metalloproteinase inhibitor-1 (TIMP-1), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in the culture medium of mouse chondrocytes. Quantitative reverse transcription-polymerase chain reaction and western blot were used to measure the expression of Bax and Bcl-2. MTT method was applied to detect the proliferation activity of chondrocytes, while flow cytometry was implemented to detect the apoptosis of chondrocytes. Results: The expression of miR-1307-5p in cartilage tissue specimens of OA patients was up-regulated, while TGFB1 expression was down-regulated. Compared with normal mice cartilage tissue specimens, the expression of miR-1307-5p in cartilage tissue specimens of OA mouse was increased, while TGFB1 expression was decreased (both  $P < 0.05$ ). The results of the dual luciferase reporter experiment showed that TGFB1 was a target gene of miR-1307-5p. In cell experiments, compared with the normal group, TIMP-1 and Bcl-2 expression, and cell proliferation activities in all model groups were decreased. IL-1 $\beta$ , IL-6, TNF- $\alpha$ , Bax expression, and cell apoptosis rates were increased (all  $P < 0.05$ ). Compared with the blank group, TIMP-1 and Bcl-2 expression, and cell proliferation activities in the miR-1307-5p inhibitor group and the TGFB1 group were increased, while IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and Bax expression, and cell apoptosis rates were decreased (all  $P < 0.05$ ). The changes in all indicators in the miR-1307-5p mimic group were opposite to those of the miR-1307-5p inhibitor group (all  $P < 0.05$ ). There were no significant differences concerning all indicators between the blank group and the NC group, and between the blank group and the miR-1307-5p mimic + TGFB1 group (all  $P > 0.05$ ). Conclusion: The suppression of miR-1307-5p expression can increase TGFB1 expression, promoting the proliferation of chondrocytes in OA mice, while inhibiting their apoptosis.

**Keywords:** miR-1307-5p, TGFB1, osteoarthritis, chondrocytes, proliferation, apoptosis

## Introduction

Osteoarthritis (OA) is also known as degenerative osteoarthrosis. The death of chondrocytes and destruction of joint integrity, which are induced by degenerated articular cartilage, are characteristics of OA [1]. More than 60% of people aged over 60 years old suffer from various degrees of OA. Its prevalence rises with age and life and health are seriously affected [2, 3]. With the development of OA, joint pain and even loss of joint function will develop. The

degeneration of joints is almost irreversible, and there is still no effective treatment. As a result, it is particularly important to explore the pathogenesis and early diagnostic methods of OA [4].

MicroRNA, which has a length of 18-24 nucleotides, is encoded by an endogenous gene. It mainly combines with the 3'-untranslated region of mRNA to promote the degradation of mRNA target genes or induce translational silencing. Accordingly, intracellular activities

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such as cell apoptosis, proliferation, and differentiation are influenced [5, 6]. The proliferation, apoptosis, and inflammatory reactions of chondrocytes, the unique cells in articular cartilage, are important for the development of OA. In the skeletal system, microRNA regulates the proliferation and apoptosis of chondrocytes, and affects the growth and development of cartilage. Wang et al. reported that overexpressed miR-142-3p could inhibit the apoptosis of chondrocytes and the production of inflammatory factors like interleukin-1 (IL-1) and interleukin-6 (IL-6) [7]; Li et al. found that the expression of miR-16-5p in OA chondrocytes was significantly increased when compared to healthy cartilage. In other words, miR-16-5p was involved in the occurrence and development of OA [8]. Previous studies have confirmed that the expression of miR-1307-5p in rheumatoid arthritis synovial cells, which is stimulated by TNF- $\alpha$ , is up-regulated. This is related to the expression of osteoclast-related genes [9]. In addition, it has been found that miR-1307-3p can inhibit the differentiation of adipose stem cells into chondrocytes [10]. However, the role of miR-1307-5p in OA and its effect on the biologic behavior of OA chondrocytes are not clear. Therefore, we aimed to explore the mechanism with miR-1307-5p.

Transforming growth factor beta-induced gene (TGFB1) is also called BIGH3 gene. It is first obtained in lung adenocarcinoma cell lines stimulated by transforming growth factor- $\beta$  (TGF- $\beta$ ). It has been verified that TGFB1 participates in biologic processes like cell growth, differentiation, and damage repair [11]. Ruiz et al. found that the expression of TGFB1 was down-regulated in OA. When TGFB1 was silenced, bone marrow mesenchymal stem cells partially lost their induction of chondrocytes' anabolic markers. Additionally, they found that the protection of bone marrow mesenchymal stem cells provided to chondrocytes was mainly attributed to the existence of TGFB1 mRNA and protein in cell vesicles. Therefore, they suppose that TGFB1 is a chondroprotective factor released by mesenchymal stem/stromal cells and an anabolic regulator of cartilage homeostasis [12]. On the basis of previous studies, we studied the effect of miR-1307-5p specifically inhibiting TGFB1 on the proliferation and apoptosis of OA chondrocytes.

## Materials and methods

### *Screening of gene expression omnibus (GEO) chip and analysis of differential gene expression*

OA was the key word applied to search for OA-related gene expression chips in the GEO database, with GSE79258 chips for subsequent analysis. Based on robust multiarray average algorithm, background correction and standardized preprocessing of the expression data was conducted using Affy package (<http://www.bioconductor.org/packages/release/bioc/html/affy.html>). With limma package in R language (<http://master.bioconductor.org/packages/release/bioc/html/limma.html>). miRNAs that were significantly differentially expressed in OA patients were identified and a heat map of differential gene expression was drawn. Differential genes were those with adj. *P*-value less than 0.05 and |log fold change| over 1.

### *Collection of human tissue specimens*

In total, 23 cartilage tissue specimens were acquired from OA patients who received total knee arthroplasty between March 2019 and March 2020 in our hospital. Additionally, normal articular cartilage tissue specimens were collected from 18 patients with femoral neck fracture. The expressions of miR-1307-5p and TGFB1 in human cartilage tissue specimens were measured using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). This study was approved by the Ethics Committee of our hospital. Informed consent was signed by the patients or their family members.

### *Construction of OA mouse model*

Totally, 45 specific pathogen free mice (male or female) aged 10 to 11 weeks old were selected. They were provided with free drinking water and food. After 7 days of feeding, these mice were randomly allocated to the OA group (30 mice) and the normal group (5 mice). In the OA group, the mice were anesthetized by the intraperitoneally injection of 1% sodium pentobarbital. Thereafter, they were fixed. With the aid of a dissecting microscope, surgery was carried out: (1) cut the inside of patellar ligament using a microblade; (2) find the joint cavity; (3) separate the fat pad between femoral condyles; (4)

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find the medial meniscus tibial ligament of knee joint; (5) cut the tibial ligament with a microblade to create damage on the joint surface; (6) disinfect the surface; (7) suture the skin. Eight mice sutured immediately after incising the skin on the inside of one knee joint, were assigned to the sham group. After operation, the mice were returned to the cage. In order to prevent infection, penicillin was injected daily. The 7 remaining mice were allocated to the normal group, and received no treatment. After 25 days, we closely observed the mice in the OA group. The modeling was considered to be successful when there was increased synovial fluid, obvious synovial hyperplasia in the joint cavity, and severe cartilage defects in the mice [13]. The success rate of modeling in our study was 82.5%.

### *Cultivation, grouping, and transfection of chondrocytes*

The mouse cartilage tissue specimens were cut into small cubes about 1 mm<sup>3</sup>. Thereafter, 1 mg/mL type II collagenase (article number: 17101-015, Shanghai Limin Industrial Co., Ltd., China) was added, and the mixture was incubated in an incubator. Two hours later, 10% fetal bovine serum (article number: 26140079, Shanghai Yingxin Laboratory Instrument Co., Ltd., China) was supplied to terminate the digestion. Undigested tissue pieces were removed by filtering through a 100 mesh nylon screen. After centrifuging, the supernatant was discarded. Meanwhile, the cells were suspended in the growth medium (article number: 1965-084, Thermo Fisher Scientific Inc., USA) provided with 100 U/mL penicillin and streptomycin, and cultured at 37°C and 5% CO<sub>2</sub>. The medium was replaced every 2 to 3 days.

Mouse chondrocytes were divided into 7 groups, including: normal group (normal mouse chondrocytes), blank group (mouse chondrocytes in the modeling group and was transfected with blank plasmid), NC group (mouse chondrocytes in the modeling group and was transfected with miR-1307-5p negative control plasmid), miR-1307-5p mimic group (mouse chondrocytes in the modeling group were transfected with miR-1307-5p mimic plasmid), miR-1307-5p inhibitor group (mouse chondrocytes in the modeling group were transfected with miR-1307-5p inhibitor plasmid), TGFBI group (mouse chondrocytes in the modeling group were transfected with TGFBI overex-

pressed plasmid), and miR-1307-5p mimic combined with TGFBI group (mouse chondrocytes in the modeling group were transfected with both miR-1307-5p mimic and TGFBI overexpressed plasmid). In our study, plasmids used for transfection were bought from Shanghai Jima Pharmaceutical Technology Co., Ltd., China. The cells were transfected in accordance with the instruction of Lipofectamin 3000 transfection kit (Thermo Fisher Scientific Inc., USA). After transfecting for 48 h, the efficiency was measured by qRT-PCR.

### *Verification of target relationship between miR-1307-5p and TGFBI*

With the assistance of biologic information website RNA22, we predicted whether TGFBI was the direct target gene of miR-1307-5p. Thereafter, we conducted a dual luciferase report experiment to verify whether there was a target relationship between miR-1307-5p and TGFBI. Based on the binding site between the 3' untranslated region (3'UTR) of TGFBI and miR-1307-5p, the wild sequence (TGFBI-3'UTR-WT) and mutant sequence (TGFBI-3'UTR-MUT) were designed. The 3'UTR fragment of TGFBI was double-digested and cloned to the upstream of pmirGLO to construct the wild sequence. The mutant sequence was established by mutating the binding site between TGFBI and miR-1307-5p. Both TGFBI-3'UTR-WT and TGFBI-3'UTR-MUT were inserted in a miR-1307-5p mimic and miR-1307-5p negative control plasmid, and the reorganized plasmids were transfected into 293T cells, respectively. The transfection was performed based on the instruction of Lipofectamin 3000 transfection kit (article number: 11668500, Thermo Fisher Scientific Inc., USA). In addition, a dual luciferase gene kit was implemented to detect luciferase activity.

### *Enzyme-linked immunosorbent assay (ELISA)*

After transfecting for 48 h, the mice cells in each group were seeded into 96-well plates. The concentration of matrix metalloproteinase inhibitor-1 (TIMP-1), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in the supernatant were separately measured according to the instructions of TIMP-1 kit (article number: TX20653, Shanghai Yingxin Laboratory Instrument Co., Ltd., China), IL-1 $\beta$  kit (article number: 69-21178, Wuhan Moshake Biological Technology Co.,

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**Table 1.** Primer sequences

Target gene	Primer sequence (5'-3')
miR-1307-5p	F: TGCGGTTTTGGCAATGGTAGAAC R: CCAGTGCAGGGTCCGAGGT
TGFBI	F: CTAAAGCCCACGAAACCTGA R: CACGGAAGAGTCCAAGCCAC
Bax	F: TCCACCAAGAAGCTGAGCGAG R: GTCCAGCCCATGATGGTTCT
Bcl-2	F: CCGCTCGAGGATCAGACCTTTGAATGATTC R: ATAAGAATGCGGCCCTCTGTGAATCCCCTTGAA
GAPDH	F: ATGGTGAAGTCCGAGTGAAC R: CTCGCTCCTGGAAGATGGT
U6	F: GCTTCGGCAGCAATATACTAAAAT R: CGCTTCACGAATTTGCGTGTCAAT

Note: TGFBI: transforming growth factor beta-induced gene.

Ltd., China), IL-6 kit (article number: 69-23048, Wuhan Moshake Biological Technology Co., Ltd., China), and TNF- $\alpha$  kit (article number: 69-99985, Wuhan Moshake Biological Technology Co., Ltd., China).

### qRT-PCR

Following the Trizol method, the total RNA of OA patients' cartilage tissue specimens, which were preserved during joint replacement surgery, was extracted. Its concentration and purity were determined with an ultraviolet spectrophotometer (device number: SP-3803AA, Shanghai Spectrum Instruments Co., Ltd., China). cDNA was synthesized using a reverse transcription kit (article number: 18091050, Thermo Fisher Scientific Inc., USA). Thereafter, the reverse transcriptase was inactivated in a warm bath at 85°C. The reaction system was prepared based on the kit instructions. The reaction conditions are: pre-denaturation (90°C, 30 s), denaturation (90°C, 10 s), annealing (55°C, 30 s), and extension (70°C, 45 s), with a total of 40 cycles. U6 was applied as the internal control of miR-1307-5p, while GAPDH was used as the internal control for the rest of the genes. The primer sequences are displayed in **Table 1**. The relative expression of each target gene was quantified using  $2^{-\Delta\Delta CT}$  method. This method was applicable to both animal and cell specimens.

### Western blot

Liquid nitrogen was added to the mouse cartilage tissue specimens, and the specimens were grinded into powder. Thereafter, appropri-

ate protein lysis solution (article number: 88216, Thermo Fisher Scientific Inc., USA) was supplied to make them lysed. Thirty minutes later, the mixture was centrifuge at 1,500 r/min for 20 minutes. The supernatant was reserved, and a bicinchoninic acid kit was applied to detect the concentration of protein. The protein was separated in a polyacrylamide gel, and transferred to a nitrocellulose membrane with the wet transfer method. The membrane was then immersed in tris-buffered saline with tween (TBST) containing 5% skimmed milk powder for 1 h. The primary antibody of TGFBI (1:1500, article number: ab170874, Abcam, UK), Bax (1:1000, article number:

ab182734, Abcam, UK), and Bcl-2 (1:1,500, article number: ab196495, Abcam, UK) were added drop by drop. After incubating overnight at 4°C, the membrane was rinsed with TBST 3 times. After that, goat anti-rabbit (1:2,500, article number: ab205718, Abcam, UK), the secondary antibody, was put in and the mixture was incubated at room temperature for 1 h. Similarly, the membrane was rinsed with TBST 3 times. Finally, the electrogenerated chemiluminescence reaction solution was applied to image in a dark room. Image J software was implemented to detect the relative expression level of protein. The procedure for cell specimens was the same as above.

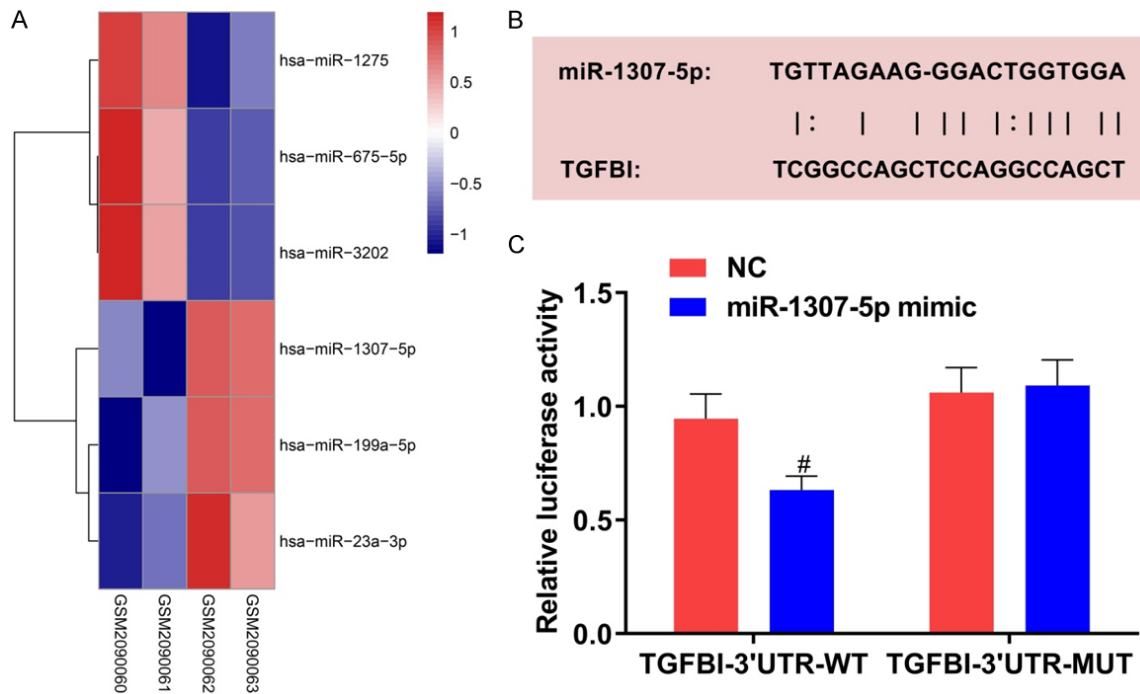
### MTT

After transfecting for 48 h, the transfected cells, which were digested using 0.25% trypsin, were collected. Single cell suspensions were prepared at a concentration of  $3 \times 10^6$  cells/mL and seeded into 96-well plates, with 150  $\mu$ L in each well. The plate was left at 37°C and 5% CO<sub>2</sub>. After culturing for 24 h, 48 h, and 72 h, the plates were taken out, and 15  $\mu$ L of MTT solution (5 mg/mL, article number: M1025, Beijing Solarbio Science & Technology Co., Ltd., China) was added dropwise. The optic density value of every well at 570 nm was obtained using a microplate reader (device number: HBS-1096A, Nanjing Detie Experimental Equipment Co., Ltd., China).

### Flow cytometry

After 48 hours of transfection, the transfected cells were digested using 0.25% trypsin. There-

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**Figure 1.** Screening of differential genes and verification of the target relationship between miR-1307-5p and TGFBI. A: Screening of differential miRNA based on GEO database; B: There is a binding site between TGFBI and miR-1307-5p; C: The dual luciferase report experiment revealed the target relationship between miR-1307-5p and TGFBI. Compared with chondrocytes transfected with NC and TGFBI-3'UTR-WT,  $P < 0.05$ . TGFBI: transforming growth factor beta-induced gene; 3'UTR: 3' untranslated region; GEO: Gene Expression Omnibus.

after, they were centrifuged to remove the supernatant. After washing with phosphate buffer saline, the cell concentration was regulated to  $5 \times 10^5$  cells/mL, and a volume of 1.5 mL cell suspensions was added into 6-well plates. Annexin V/propidium iodide staining solution was used to stain the cells in each well for 20 minutes. The apoptosis of the cells was measured by flow cytometry (device number: Attune Nxt, Thermo Fisher Scientific Inc., USA).

### Statistical methods

All data were analyzed using SPSS statistical software version 21.0. The measurement data were calculated as mean  $\pm$  standard deviation ( $\bar{x} \pm sd$ ); independent sample t test was applied for inter-group comparison, while one-way variance combined with post-hoc Bonferroni or LSD test was used for multiple groups comparison. The difference was significant when  $P$  value was  $< 0.05$ .

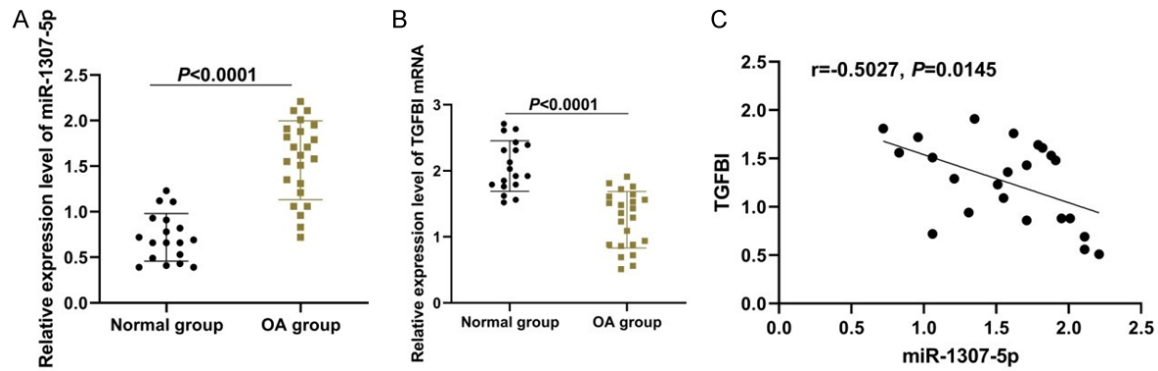
## Results

### Screening of differential genes

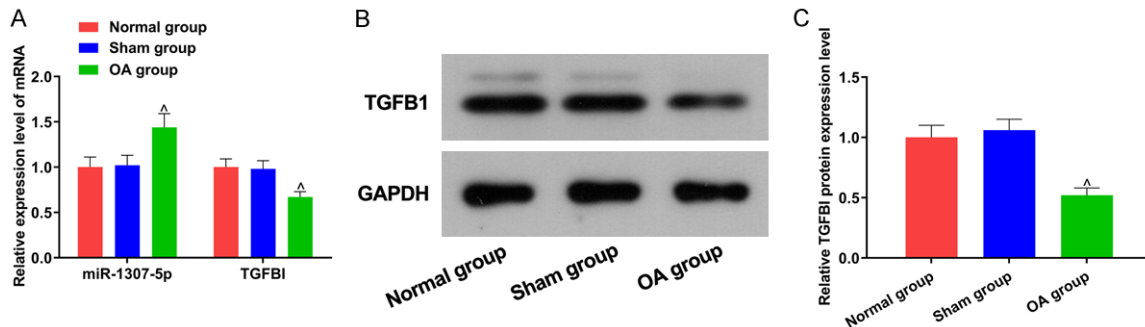
We conducted the analysis with GSE79258 chip. Compared with the normal articular carti-

lage tissue specimens, a total of 3 miRNA expressions in OA patients' cartilage tissue specimens were up-regulated, while 3 miRNA expressions were down-regulated (**Figure 1A**). Referring to related literature, we included miR-1307-5p in the follow-up study. Previous studies showed that miR-1307-5p is dysregulated in rheumatoid arthritis [9]. In addition, it was found that miR-1307-3p can inhibit the differentiation of adipose stem cells into chondrocytes [10]. However, its role in OA, and whether or not it can influence the biologic behavior of OA chondrocytes, are not clear. Here, we aimed to explore the mechanism concerned with miR-1307-5p in OA. Furthermore, we analyzed the downstream genes applying the RNA22 database, and found that there was a specific binding site between miR-1307-5p and TGFBI (**Figure 1B**). TGFBI, which is secreted by mesenchymal stromal cells, can maintain cartilage homeostasis and inhibit the expression of inflammatory factors, displaying a protective effect in OA [12]. Results of dual luciferase reporter experiment showed that miR-1307-5p mimic significantly inhibited the luciferase activity of TGFBI-3'UTR-WT ( $P < 0.05$ ), but exhibited no suppression on the luciferase activity of TGFBI-3'UTR-MUT (**Figure 1C**).

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**Figure 2.** Comparison of miR-1307-5p and TGFBI expression in patients between the two groups. A: Relative miR-1307-5p expression in both groups; B: Relative TGFBI mRNA expression in both groups; C: Correlation between miR-1307-5p and TGFBI mRNA expression. OA: Osteoarthritis; TGFBI: transforming growth factor beta-induced gene.



**Figure 3.** Comparison of miR-1307-5p and TGFBI expression in mice among the three groups. A: Relative miR-1307-5p and TGFBI mRNA expression; B: TGFBI protein expression; C: Relative expression level of TGFBI protein. Compared with the normal mice cartilage tissue specimens,  $\hat{P} < 0.05$ . OA: Osteoarthritis; TGFBI: transforming growth factor beta-induced gene.

### The expression of miR-1307-5p and TGFBI mRNA in OA patients' cartilage tissue specimens

To clarify whether miR-1307-5p participated in the pathogenesis of OA, we firstly measured miR-1307-5p expression in the cartilage tissue specimens of OA patients. As displayed in **Figure 2A**, miR-1307-5p expression in the cartilage tissue specimens of OA patients was significantly higher than that in the normal articular cartilage tissue specimens ( $P < 0.001$ ). Thereafter, we detected the expression of TGFBI mRNA and found that TGFBI mRNA expression in cartilage tissue specimens of OA patients was less than that in normal articular cartilage tissue specimens ( $P < 0.001$ , **Figure 2B**). Additionally, there was a negative correlation between miR-1307-5p and TGFBI mRNA expression in cartilage tissue specimens ( $P < 0.05$ , **Figure 2C**).

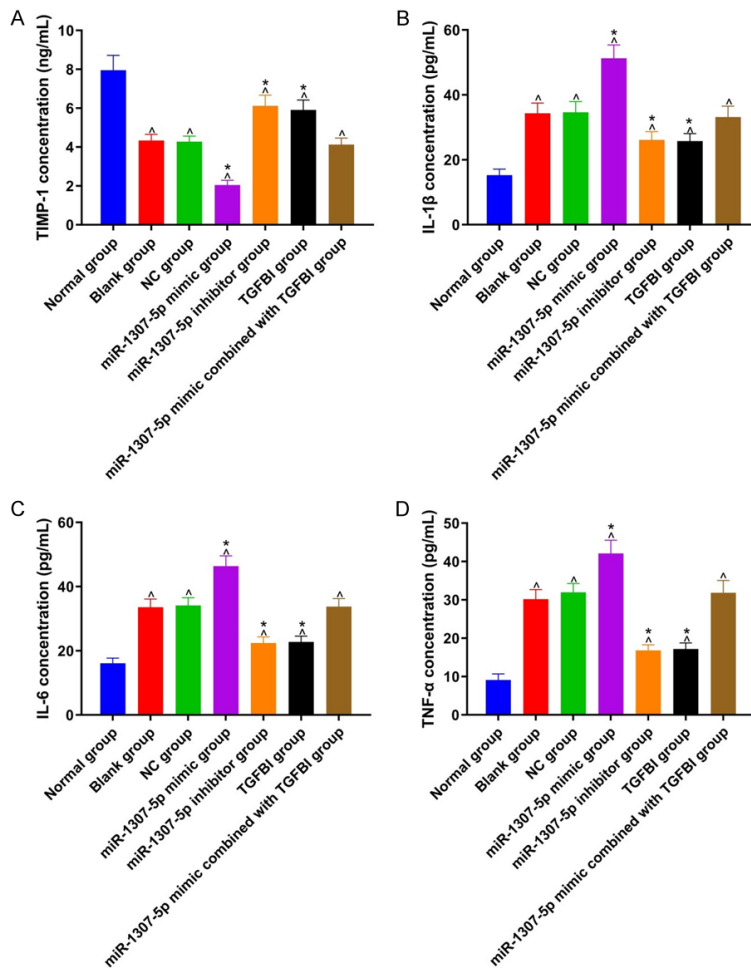
### Expression level of related mRNA and protein in mouse cartilage tissue specimens

As shown in **Figure 3**, compared with the normal mouse cartilage tissue specimens, miR-1307-5p expression in cartilage tissue specimens of OA mouse was significantly increased, while TGFBI expression was decreased (both  $P < 0.05$ ); there were no significant differences in miR-1307-5p and TGFBI expression between the normal group and the sham group (both  $P > 0.05$ ).

### TIMP-1, IL-1 $\beta$ , IL-6, and TNF- $\alpha$ expression

As displayed in **Figure 4**, TIMP-1 expression in the blank group, NC group, miR-1307-5p mimic group, miR-1307-5p inhibitor group, TGFBI group, and miR-1307-5p mimic combined with TGFBI group were decreased when compared with the normal group, while IL-1 $\beta$ , IL-6, and TNF- $\alpha$  expression were increased (all  $P < 0.05$ ).

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**Figure 4.** Comparison of TIMP-1, IL-1 $\beta$ , IL-6, and TNF- $\alpha$  expression among 7 groups. A: TIMP-1 expression in each group; B: IL-1 $\beta$  expression in each group; C: IL-6 expression in each group; D: TNF- $\alpha$  expression in each group. Compared with the normal group,  $^{\wedge}$ P<0.05; compared with the blank group,  $^{\ast}$ P<0.05. TIMP-1: matrix metalloproteinase inhibitor-1; IL-1 $\beta$ : interleukin-1 $\beta$ ; IL-6: interleukin-6; TNF- $\alpha$ : tumor necrosis factor- $\alpha$ ; TGFBI: transforming growth factor beta-induced gene.

Compared with the blank group, TIMP-1 expression in the miR-1307-5p inhibitor group and the TGFBI group were increased, while IL-1 $\beta$ , IL-6, and TNF- $\alpha$  expression were reduced (all P<0.05). There were no significant differences concerning TIMP-1, IL-1 $\beta$ , IL-6, and TNF- $\alpha$  expression between the NC group and the 1307-5p mimic combined with the TGFBI group (all P>0.05). The changes of TIMP-1, IL-1 $\beta$ , IL-6, and TNF- $\alpha$  expression in the miR-1307-5p mimic group were opposite to those in the miR-1307-5p inhibitor group (all P<0.05).

### *Overexpressed TGFBI counteracted the proliferation inhibition induced by miR-1307-5p*

As shown in **Figure 5**, cell proliferation activity in the blank group, NC group, miR-1307-5p

mimic group, miR-1307-5p inhibitor group, TGFBI group, and miR-1307-5p mimic combined with TGFBI group were lower than those in the normal group (all P<0.05). Compared with the blank group, cell proliferation activity in the miR-1307-5p inhibitor group and TGFBI group were increased, but significantly decreased in the miR-1307-5p mimic group (both P<0.05). There were no significant differences on cell proliferation activity between the blank group and the NC group, and between the blank group and the miR-1307-5p mimic combined with TGFBI group (both P>0.05).

### *Inhibited miR-1307-5p or overexpressed TGFBI suppressed chondrocyte apoptosis*

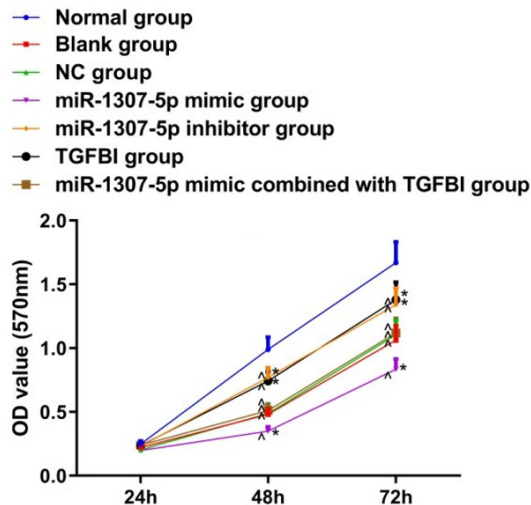
As displayed in **Figure 6**, compared with the normal group, Bcl-2 expression in the blank group, NC group, miR-1307-5p mimic group, miR-1307-5p inhibitor group, TGFBI group, and miR-1307-5p mimic combined with TGFBI group were decreased, while Bax expression and chondrocyte apoptosis were increased (all P<0.05). Compared with the blank group, Bcl-2

expression in the miR-1307-5p inhibitor group and the TGFBI group were increased, while Bax expression and chondrocytes apoptosis were decreased. Compared with the blank group, Bcl-2 expression in the miR-1307-5p mimic group and TGFBI group were decreased, while Bax expression and chondrocyte apoptosis were increased (all P<0.05). There were no significant differences on Bcl-2 expression, Bax expression, and chondrocytes apoptosis between the blank group and the NC group, and between the blank group and the miR-1307-5p mimic combined TGFBI group (all P>0.05).

### **Discussion**

OA occurs in large and small joints throughout the whole body, and is most commonly ob-

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**Figure 5.** Comparison of cell proliferation activity among 7 groups. Compared with the normal group,  $^{\wedge}P < 0.05$ ; compared with the blank group,  $^*P < 0.05$ . OD: optical density; TGFB1: transforming growth factor beta-induced gene.

served in knee and hip joints. It is a disease accompanied by pathological features such as tissue lesions, cartilage degeneration, and cartilage matrix disintegration, which in turn cause inflammation and hyperplasia of bone. As a result, joint pain is developed [14, 15]. It was reported that OA was one of the top four causes of disability. However, there is no effective treatment for OA as a result of its complicated pathology and the adverse effects of drugs [16].

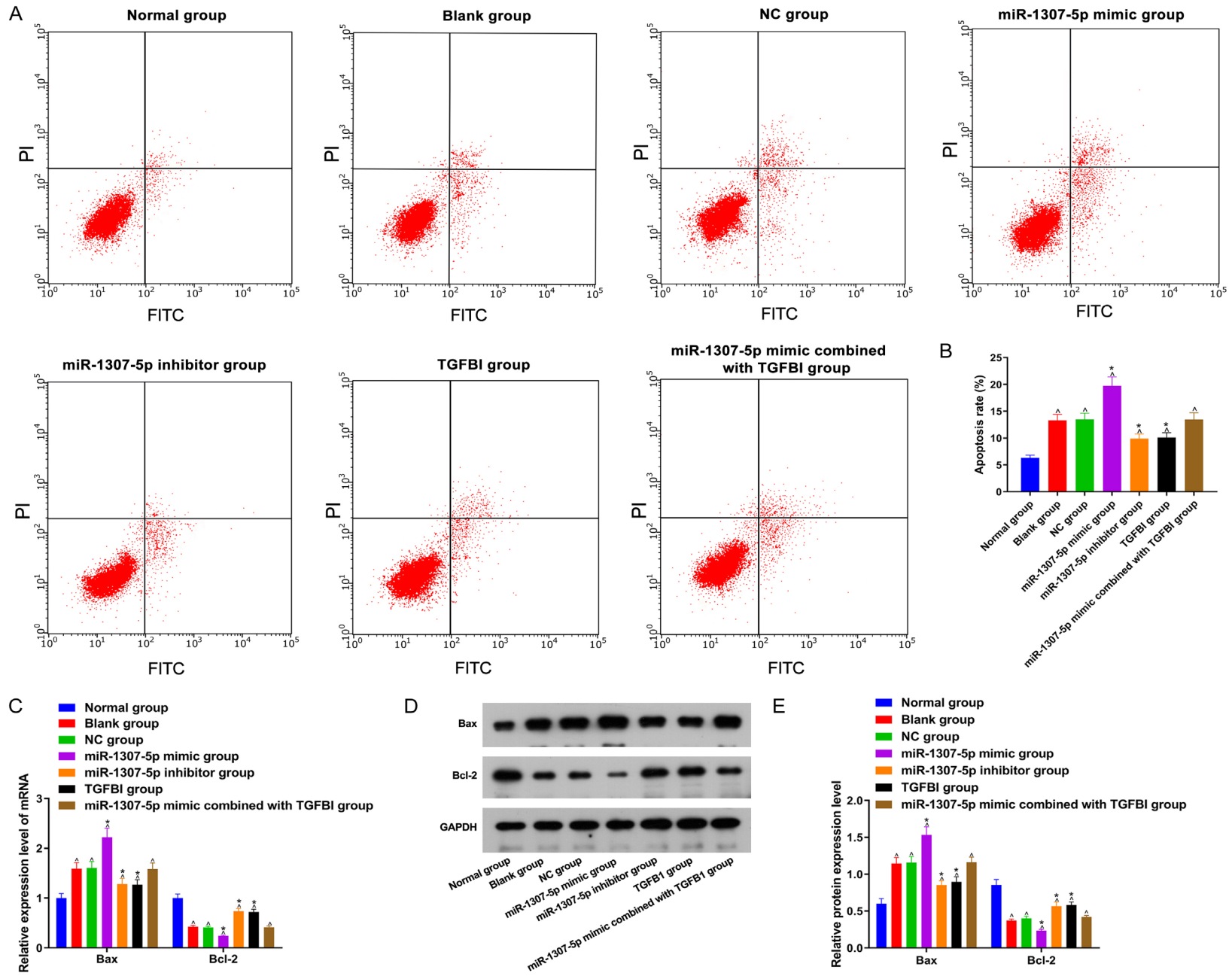
Recently, the role of microRNA in OA has attracted more and more attention. He et al. reported that miR-582-3p could reduce the progression of OA by targeting Yes-associated protein 1 [17]. Cao et al. found that miR-204-5p could inhibit the proliferation of chondrocytes in OA rat model established by surgery, improving the OA-like phenotype of chondrocytes [18]. Here, our results obtained from the tissue and cell specimens revealed that expression of miR-1307-5p in OA was up-regulated, while TGFB1 was down-regulated. In addition, the proliferation and apoptosis of chondrocytes were influenced. The specific role of miR-1307-5p and TGFB1 in OA were thus revealed. The change in inflammatory factors expressed in OA is one of the characteristics of OA. In order to explore these changes in inflammatory factors, we selected four representative ones, including TIMP-1, IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , by consulting relevant literature. In

addition, it was reported that the TIMP-1 expressed in OA was decreased, while IL-1 $\beta$ , IL-6, and TNF- $\alpha$  were increased [19, 20]. In our study, we found the abnormal expression of inflammatory mediators (like TIMP-1, IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) in OA chondrocytes through ELISA. To be specific, the expression of TIMP-1 was decreased, while IL-1 $\beta$ , IL-6, and TNF- $\alpha$  were increased. The role of miR-1307, in diseases has been continuously clarified. Yang et al. reported that the rs7911488-T allele could promote the expression of miR-1307, while suppress the expression of Prrx1, accelerating the proliferation and migration of colorectal cancer cells [21]. Yang et al. also found that the overexpression of miR-1307-3 could inhibit the deposition of cartilage matrix proteoglycans and reduce the expression of cartilage-related markers. Also, the differentiation of human adipose stem cells into chondrocytes was inhibited by specifically targeting BMPR2 and its downstream signaling pathways [10]. Using qRT-PCR, we confirmed that the expression of miR-1307-5p in OA cartilage tissue specimens and cells was increased. Subsequently, we further studied TGFB1, the possible target gene of miR-1307-5p.

Previous studies on TGFB1 are mostly focused on tumors [22, 23]. Iwafuchi et al. confirmed that TGFB1 participated in the cell-collagen interaction [24]. Jung et al. found that the absence of TGFB1 could cause the disintegration of articular cartilage matrix, disclosing a protective role of TGFB1 in bone tissue [25]. In our study, we found that the expression of TGFB1 in OA patients' cartilage tissue specimens was lower than that in normal cartilage tissue specimens. By detecting luciferase activity, we verified that TGFB1 was the target gene of miR-1307-5p. We also verified through experiments that the silencing of miR-1307-5p or overexpression of TGFB1 could promote the expression of Bcl-2 and TIMP-1 and the proliferation of chondrocytes, while inhibit the expression of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and Bax, and the apoptosis of chondrocytes. The influence of overexpressed miR-1307-5p was opposite. Additionally, the effect of overexpressed TGFB1 could be reversed by the excessive expression of miR-1307-5p. Studies on TGFB1 and OA have found that TGF- $\beta$ -induced bone marrow mesenchymal stem cells can suppress chondrocytes' degradation of metabolites and reduce the expression of inflammatory factors. It



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**Figure 6.** Comparison of chondrocyte apoptosis among 7 groups. A: Chondrocyte apoptosis in each group; B: The rate of chondrocyte apoptosis in each group; C: Bcl-2 and Bax mRNA expression in each group; D: Bcl-2 and Bax protein expression in each group; E: Expression of Bcl-2 and Bax protein in each group. Compared with the normal group,  $^{\wedge}P<0.05$ ; compared with the blank group,  $^{*}P<0.05$ . TGFBI: transforming growth factor beta-induced gene.

has also been confirmed that GFBI is synthesized in response to TGF- $\beta$  and can inhibit cell adhesion. These results suggest that GFBI can be used as a chondroprotective factor in OA to maintain the homeostasis of cartilage [12]. Accordingly, we suppose that the regulation of TGFBI in inflammatory factors and the biologic behavior of chondrocytes might be related to TGF- $\beta$  and bone marrow mesenchymal stem cells, which is also the focus of our subsequent study.

However, our results are acquired from in vitro experiments. Subsequently, we will perform animal experiments to verify our conclusion.

In summary, the silencing of miR-1307-5p can inhibit the development of OA inflammation, facilitate the proliferation of chondrocytes, and suppress the apoptosis of chondrocytes. Further studies on miR-1307-5p might contribute to finding of a novel treatment for OA.

### Disclosure of conflict of interest

None.

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