Identification of miR-338-5p and miR-4776-3p as mediators of articular cartilage degeneration

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Abstract

Purpose

Osteoarthritis is one of the most common chronic diseases nowadays, and can cause serious physical illness and economic burden. Cartilage degeneration is one of primary reasons for osteoarthritis, but there were not thorough studies on cartilage lesions.

Methods

miR-338-5p and miR-4776-3p were overexpressed and knocked down in SW1353 cells. Dual luciferase assay is used to detect the binding of miR-338-5p, miR-4776-3p and *SOX6*. The expression of *MMP13*, *TNFA*, *IL6*, *MCP1*, *PARP*, *Runx2*, *ALP*, *OPN*, *WNT4*, *WNT16*, *TRACP* and *CTSK* was quantified by qPCR. And the expression of WNT4 at the protein level was quantified by WB.

Results

In this study, we found miR-338-5p and miR-4776-3p showed significantly high expression on cartilage in osteoarthritis through bioinformatics methods, and verified miR-338-5p and miR-4776-3p can improve catabolism, and trigger biological processes such as inflammation and apoptosis. Meanwhile we validated miR-338-5p and miR-4776-3p may play negative regulation in the process of bone biology.

Conclusions

Our study showed that miR-338-5p and miR-4776-3p can be regarded as medium of articular cartilage degeneration, and it provided new biomarker of early diagnosis in cartilage disease and osteoarthritis.

Introduction

With growth of aging people, osteoarthritis is increasingly become main source of pain, disability and socioeconomic consumption worldwide [1]. During osteoarthritis period, cartilage tissue in the joints will corrode, thereby causing pain and serious loss of patients' joint function [2]. Cartilage degeneration is one of main reasons in osteoarthritis, but we have not fully understood the cellular and molecular mechanisms of its occurrence and development [3]. At present, there is no better method which can treat osteoarthritis caused by cartilage degeneration. Therefor disease prevention and early diagnosis appear to be necessary.

Micro-RNA (miRNA) is a kind of endogenous, small RNA with a length of about 20–24 nucleotide, and it has multiple important regulation role in cells. It enables to target most protein-coding transcripts, nearly participating in animals' various development and pathological processes, such as cell proliferation,

migration, apoptosis and autophagy [4, 5]. miRNA expression also causes many diseases, such as osteoporosis, diabetes, obesity and cancers [6–9], while some miRNA directly participate in inflammatory reaction and apoptosis process [10–13], which provides good reference for studies on osteoarthritis. Recent studies have showed that miR-140 played an important role in cartilage degeneration [14], however, similar studies on miRNA are rarely seen.

In this study, we tried to explore and verify new miRNA which plays an important role in cartilage degeneration. First, we downloaded 2,100 miRNA expression data of patients with lumbar disc herniation (LDH) and patients with osteoarthritis of spine (FJOA) from NCBI GEO database, and made bioinformatics analysis, which found miR-4776-3p and miR-338-5p showed significantly high expression in FJOA. Then, we explored functions of miR-4776-3p and miR-338-5p by utilizing miRNA mimics and miRNA inhibitors, which obtained these two miRNAs participated in inflammatory reaction, catabolism and apoptosis process. Finally, we predicted target genes of miR-4776-3p and miR-338-5p via Target scan website, and verified target binding of miR-4776-3p with *SOX2* and miR-338-5p to *SOX9* through experiments. This study, through bioinformatics analysis and experimental verification, proved initially the role of miR-4776-3p and miR-338-5p in cartilage degeneration, providing new target and treatment regimen for early diagnosis of osteoarthritis.

Materials And Methods

Data download

At initial stage, we searched miRNA expression database related to cartilage in GEO database of NCBI (<https://www.ncbi.nlm.nih.gov/geo/>). We only selected samples of patients with cartilage disease and healthy persons, and ruled out databases derived from animals and human cells, finally only GSE79258 was found to conform to our requirements and used as subsequent analysis and verification. GSE79258 is GPL21599 (miRCURY LNA micro-RNA Array, 7th generation, miRBase 20) sequencing platform based data, including 2 cartilage samples from LDH patients and 2 cartilage samples from FJOA patients.

Identification of differently expressed miRNA (DE-miRNA)

We made differential analysis for data downloaded by using "Limma" package in R language, so as to obtain differently expressed miRNA in LDH and FJOA. We set $|\log_2FC| > 1$ and $P \leq 0.05$ as threshold of identifying DE-miRNA. Heat Map was created by "pheatmap" package in R language, and Volcano Plot was created by Graph Pad Prism 7.

Prediction of potential transcription factors

The upstream transcription factors of DE-miRNAs were predicted by FunRich software, which is a stand-alone software tool mainly used for functional enrichment and interaction network analysis of genes and proteins. We DE-miRNAs to obtain their upstream transcription factors, and present the top ten transcription factors according to *P* value.

GO annotation and KEGG pathway enrichment analysis

The GO functional annotation and KEGG pathway enrichment analysis for the DE-genes were conducted by Enrichr, which is a comprehensive resource for curated miRNA sets and a search engine that accumulates biological knowledge for further biological discoveries (<http://amp.pharm.mssm.edu/Enrichr/>). We input DE-miRNAs, and conducted biological process (BP), cellular component (CC), and molecular function (MF) functional annotation and KEGG pathway enrichment analysis. The top ten results are presented according to *P* value, with *P* < 0.05 considered as statistically significant.

Predication of potential target gene of DE-miRNA

We predicated downstream target gene of DE-miRNA by using TargetScanHuman (http://www.targetscan.org/vert_72/). TargetScanHuman is a website which predicts miRNA target gene, including miRNA target gene results of 10 species such as human, mouse, rats, opossum, chimpanzees, rhesus monkeys, bovine, dogs, chicken and frog, and it can clearly see binding of miRNA and target gene. TargetScan predicted biological target of miRNA by searching conserved 8mer, 7mer and 6mer loci which match with each miRNA seed region [15].

Cell culture

Human chondrosarcoma cell line SW1353 was purchased from the cell bank of Chinese Academy of Sciences (Shanghai, China). SW1353 cell was cultured in L-15 medium (HyClone, Waltham, MA, USA), in which added 10% high-quality fetal bovine serum (Invitrogen, Carlsbad, California, USA), 100U/ml penicillin and 100µg/ml streptomycin (Invitrogen). All cells were cultured in a wet incubator containing 5% CO₂ at 37°C.

Primer design and vector construction

Through whole-genome synthesis method, *SOX6* complete coding sequence (63~2688bp) was synthesized and constructed into pIRES2-EGFP carrier, *SOX6* 3'UTR region 1000bp (4300~5300bp) fragment was synthesized and constructed into psi-check-2 carrier and marked as wild type carrier (Wild type). After successful construction of wild type carrier, mutant type carrier (Mutant) was constructed by using point mutation method. All miRNA mimics and inhibitors were synthesized by Genepharma. See Table 1 for sequence:

Cell transfection and luciferase reporter gene assay

Wild type and mutant type carriers were co-transfected into SW1353 cells together with miRNA mimics and mimic NC in a concentration of 20nM by Lipofectamine 3000 transfection reagents (Invitrogen, Carlsbad, CA, USA), to complete miRNA over expression cotransfection. Wild type and mutant type carriers were co-transfected into SW1353 cells together with miRNA inhibitors and inhibitor NC in a concentration of 20nM to complete miRNA over expression cotransfection. Cells were lysed after 48h of

culturing, and activities of firefly luciferase and ranilla luciferase in cell lysis buffer were measured by dual-luciferase reporter gene assay kit (E1910) (Promega, Madison, WI, USA).

RNA extraction and real-time polymerase chain reaction (qPCR)

miRNA mimics, mimic NC, miRNA inhibitors and inhibitor NC were transfected into SW1353 cells by Lipofectamine 3000 transfection reagents with a concentration of 20 nM, and then cells were lysed after 48h of culturing; total RNA was extracted by TRIzol reagent (Invitrogen), then transcribed as c DNA by ReverAid (Thermo Scientific). Real-time PCR was conducted by using qPCR SYBR Green Master Mix (High Rox Plus) (Thermo Scientific). qPCR system was 20ul, including 1ul upstream primer, 1ul downstream primer, 0.4ul cDNA (1000ng/ul), 10ul Green Master Mix and 7.6ul ultra-pure water. This system was reacted for 60s at 95°C, then 30s at 95°C; 30s at annealing temperature and 30s at 72°C; 40 cycles were done. Finally DNA was detected for 30s at 95°C and 35s at 5°C. 2- $\Delta\Delta C_t$ method was used to determine relative gene expression level. See Table 2 for primer sequence:

Western blot analysis (WB)

After treating the cells with miRNA mimics, miRNA inhibitors, *SOX6* overexpression vector and *SOX6* siRNA for 48 h, the cells were collected. $1 \times (10^6)$ cells were cracked by 1 ml of RIPA and 10 μ l of PMSF, then centrifuged at 12,000 r/min at 4°C for 4 min. The intermediate protein layer solution was removed, and the BCA protein quantification kit was used for protein quantification. Samples of each group were diluted to 50 μ g/ml, and the diluted protein was mixed with Sample Buffer at the ratio of 4:1 and heated at 100°C for 5 min. Then Mixing Acrylamide, Resolving Buffer, Starcking Buffer, distilled water, 10% APS, and TEMED were mixed in proportion to make SDS-PAGE separation gel and stacking gel, and poured into the gel plate. The Prestained Protein Ladder and the sample were separately added into the sample wells of the gel plate, and the proteinloaded SDS-PAGE gel was subjected to vertical gel electrophoresis for 50 min. The polyvinylidene difluoride (PVDF) membrane was activated by methanol for 1 min and then transmembrane was performed. After that, the PVDF membrane was blocked by 5% fat-free milk containing TBST solution for 1 h. After blocking, the PVDF membrane was washed by TBST. The WNT antibody (Thermo Fisher Scientific, rabbit polyclonal antibody-Catalog Number PA5-27321) was incubated at 25°C for 2 h at a dilution of 1:500, and the second antibody was incubated at 25°C for 1 h. Finally, Supersignal West Pico PLUS was used to fill the PVDF membrane and was placed in the iBright FL1000 (Thermo Fisher Scientific) for observation.

Statistical analysis

The data were presented as mean value $\pm$ SD. Statistical difference between groups was determined by two-tailed T test. Statistical difference between groups was analyzed by one-way variance, then Student-Neuman-Keuls multiple comparative test was used to make analysis. All experiments were conducted at least 3 times independently, with similar results; also, typical experiments were shown. It was statistically significant if $P < 0.05$. NS> 0.05 $\square$ $P < 0.05$ $\square$ $P < 0.01$ $\square$ $P < 0.001$.

Results

Differently expressed miRNA

By data analysis, we found 55 differently expressed miRNA in LDH and FJOA, including 21 highly expressed miRNAs in FJOA, 29 lowly expressed miRNAs in FJOA. miRNA details are presented in attached table 1. Expression quantity of miR-338-5p and miR-4776-3p in FJOA was nearly 4 times of that in LDH, which was selected as target miRNA for subsequent experiments (Figure 1A, B).

Prediction of Upstream Transcription Factors of DE-miRNAs.

We predicted the upstream transcription factors of DE-miRNAs through FunRich software, and the top 10 transcriptions are presented in Figures 2. The top 10 transcription factors of DE-miRNAs were EGR1, SP1, SP4, MEF2A, POU2F1, ZFP161, HOXB9, POU6F1, RORA, SOX6.

Functional annotation and pathway enrichment analysis

The Enrichr database was utilized to perform GO functional annotation and KEGG pathway enrichment analysis. The GO functional annotation included three categories, namely, biological process (BP), cellular component (CC), and molecular function (MF). The top 10 enriched GO items are listed in Figure 3. The biological process analysis results show that the biological process of DE-miRNAs is mainly concentrated in regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism (Figure 3A), the cellular component is mainly concentrated in nucleus and cytoplasm (Figure 3B), the molecular function is mainly concentrated in transcription factor activity and transcription regulator activity (Figure 3C). The biological process of DE-miRNAs is mainly concentrated in S1P, VEGFP, ErbB signaling network and pathway (Figure 3D).

miRNA target gene prediction

miR-338-5p and miR-4776-3p target genes were predicted by TargetScanHuman website, which found miR-338-5p binds 4813 genes naturally and miR-4776-3p binds 4175 genes naturally; the details are presented in attached table 2. Various references reported transcription factors SOX5/SOX6 and SOX9 together promoted chondrogenesis in whole genome by super-enhancers [16,17], miR-338-5p and miR-4776-3p made target binding with *SOX6*, so *SOX6* was chosen as study target (Figure 4 A, B).

miR-338-5p and miR-4776-3p bind with SOX6

Through dual-luciferase reporter gene assay, we found exogenous miR-338-5p mimics can bind with 3'UTR of *SOX6*, allowing its activity to reduce by about 30%, while miR-4776-3p mimics can bind with 3'UTR of *SOX6*, allowing its activity to reduce by about 30%. However, it was found SOX6 activity would rise by about 40% after transfection of miR-338-5p inhibitors and also about 40% after transfection of miR-4776-3p inhibitors (Figure 5A-D).

Overexpression of miR-338-5p and miR-4776-3p mimics can increase expression of catabolism, inflammation and apoptosis markers.

By bioinformatics method, it was found expression quantity of miR-338-5p and miR-4776-3p in FJOA was about 4 times of that in control group, and then we further explored how these two miRNAs worked in cartilage degeneration. After miR-338-5p mimics and miR-4776-3p mimics were transfected to SW1353 cells, it was found expression level of catabolism genes (*MMP13*), inflammation marker gene (*TNFA*, *IL6*, and *MCP1*) and apoptosis marker genes (*PARP*) was significantly improved. This indicated miR-338-5p and miR-4776-3p can promote catabolism, inflammation and apoptosis, thereby promoting chondrocyte degeneration (Figure 6A, B).

Knock-down of miR-338-5p and miR-4776-3p can inhibit expression of catabolism, inflammation and apoptosis markers

The experiments showed miR-338-5p and miR-4776-3p mimics enabled to improve catabolism, and trigger inflammation and apoptosis; to further verify whether it was reversed after knock-down of miR-338-5p and miR-4776-3p, miR-338-5p and miR-4776-3p inhibitors were transfected to SW1353 cells. qPCR assay found that miR-338-5p and miR-4776-3p inhibitors can inhibit expression of *MMP13*, *TNFA*, *IL6*, *MCP1* and *PARP*. These expressions miR-338-5p and miR-4776-3p played an important role in chondrocyte degeneration (Figure 7A, B).

Impact of miR-338-5p and miR-4776-3p on osteogenesis

To further verify whether miR-338-5p and miR-4776-3p can affect osteogenesis directly, we conducted over expression and inhibition of miR-338-5p and miR-4776-3p, and found that miR-338-5p and miR-4776-3p mimics can inhibit expression of *Runx2*, *ALP*, *OPN*, *WNT4* and *WNT16* of bone marker genes, and improve expression of osteoclastogenesis marker genes *TRACP* and *CTSK* (Figure 8A, B). Yet, miR-338-5p and miR-4776-3p inhibitors can improve expression of *Runx2*, *ALP*, *OPN*, *WNT4* and *WNT16*, and inhibit expression of *TRACP* and *CTSK* (Figure 8C, D). WB results showed that miR-338-5p and miR-4776-3p overexpression can inhibit expression of WNT4 and miR-338-5p and miR-4776-3p knockdown can improve expression of WNT4 at the protein level (Figure 8E). This indicated miR-338-5p and miR-4776-3p can directly affect osteogenesis.

SOX6 mediates the expression of osteogenic genes and osteoclast genes

To verify whether the changes in the expression levels of osteogenic and osteoclast markers upon overexpression or inhibition of miR-338-5p and miR-4776-3p are directly caused by changes in *SOX6*, we overexpressed and knockdown *SOX6* in human SW1353 cells. By qRT-PCR, we found that the expression of *Runx2*, *ALP*, *OPN*, *WNT4* and *WNT16* were significantly upregulated and the expression of *TRACP* and *CTSK* was significantly downregulated when *SOX6* overexpression (Fig. 9A). The expression of *Runx2*, *ALP*, *OPN*, *WNT4* and *WNT16* were significantly downregulated and the expression of *TRACP* and *CTSK* was significantly upregulated when *SOX6* knockdown (Fig. 9B). Overexpression of miR-338-5p and

miR-4776-3p can reduce the expression of WNT4 at the protein level, while knockdown of miR-338-5p and miR-4776-3p can increase the expression of WNT4 (Fig. 9C).

Discussion

The most challenging aspect in the treatment of articular cartilage injury mainly includes determination of matrix changes and cells and molecular mechanism of chondrocyte differentiation and of dedifferentiation behavior, and understanding of how to affect structural integrity and tissue remodeling of articular cartilage [18]. Several studies have showed that miRNA in tissues played a vital role in cellular processes (such as apoptosis, cell proliferation, differentiation, metabolism and cell cycle control) [19]. Several existing studies have shown that miRNA may be related to premature degradation of cartilage [14, 20], and some studies also have indicated multiple miRNA had higher expression quantity in cartilage with arthritis [21–23]. However, most studies involved comparison of miRNA expression quantity between samples of osteoarticular cartilage and normal samples, and did not go into the molecular mechanism of cartilage degeneration caused by miRNA. In this paper, we preliminarily studied the molecular mechanism of cartilage degeneration caused by miR-338-5p and miR-4776-3p, providing new ideas for further studying cartilage injury and degeneration.

Throughout the study, we intended to find miRNA with significantly high expression of cartilage in osteoarthritis, and study the role of miRNA in cartilage lesions. We downloaded 2100 miRNAs expression data of LDH patients and FJOA patients from NCBI GEO database, and made bioinformatics analysis, which found miR-4776-3p and miR-338-5p showed significantly high expression in FJOA. To determine miR-338-5p and miR-4776-3p have physiological and pathological actions action in articular cartilage degeneration or not, miR-338-5p and miR-4776-3p were over expressed and knocked down in chondrocyte SW1353, so as to determine their impact on cartilage. Through detection of expression quantity of catabolism, inflammatory reaction and apoptosis marker genes, we found miR-338-5p and miR-4776-3p can improve catabolism and trigger apoptosis, thereby promoting cartilage degeneration. Through dual-luciferase reporter gene assay, miR-338-5p and miR-4776-3p can bind with cartilage-related gene *SOX6* naturally, but further study on the molecular mechanism of cartilage lesion and the role of *SOX6* in in vivo is required.

Related studies have shown that cartilage in osteoarthritis usually accompanies bone lesions. Joint integrity comes from balance of physiological interaction between skeletons and cartilages. Accumulated evidences in the bodies also indicated the increase of bone absorption occurred at early stage of osteoarthritis progression, and cytokines blocking bone absorption can prevent cartilage injury, thereby confirming the role of bone factors in crosstalk of the two tissues [24]. In this study, we also preliminarily studied the role of miR-338-5p and miR-4776-3p in bone formation and absorption, which found they can inhibit osteogenesis and promote osteoclastogenesis. However, we did not study correlation and molecular mechanism of miR-338-5p and miR-4776-3 between bone formation and absorption in cartilage lesions, which will be the focus in future studies.

Conclusions

To sum up, we, by using bioinformatics methods in the whole study, found miR-338-5p and miR-4776-3p had significantly different high expression quantity of cartilage in osteoarthritis, and verified miR-338-5p and miR-4776-3p can improve catabolism and trigger inflammation and apoptosis by experiments. At the same time, they can promote osteoclastogenesis and inhibit osteogenesis. These study results showed that miR-338-5p and miR-4776-3p played an important role in cartilage lesion, and can be used as diagnostic marks of early cartilage lesions.

Declarations

Authors' contributions

Wang J and Guan X analyzed the data and drafted the manuscript. Zhang L, Shao S, Gao B, Li H, Huo L, Huang S done the experiments and preformed statistical analysis of the data. Wu J and Cui X revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Oligonucleotide sequences synthesized in the present study

Name	Sequences (5'–3')
miR-338-5p mimics-F	AACAAUAUCCUGGUGCUGAGUG
miR-338-5p mimics-R	CUCAGCACCAGGAUAUUGUUUU
miR-4776-3p mimics-F	CUUGCCAUCCUGGUCCACUGCAU
miR-4776-3p mimics-R	GCAGUGGACCAGGAUGGCAAGUU
miR-338-5p inhibitors	CACUCAGCACCAGGAUAUUGUU
miR-4776-3p inhibitors	AUGCAGUGGACCAGGAUGGCAAG
NC mimics	UUCUUCGAACGUGUCACGUTT
NC inhibitors	CAGUACUUUUGUGUAGUACAA

Table 2. PCR primer sequences in the present study

Name	Sequences (5'–3')	Temperature
hsa- <i>β-actin</i> -F	GAGAAAATCTGGCACCACACCT	—
hsa- <i>β-actin</i> -R	GCACAGCCTGGATGCAACGTA	—
has-MMP13-F	ACTGAGAGGCTCCGAGAAATG	63°C
has-MMP13-R	GAACCCCGCATCTTGGCTT	63°C
has-TNFA-F	CCTCTCTCTAATCAGCCCTCTG	60°C
has-TNFA-R	GAGGACCTGGGAGTAGATGAG	60°C
has-IL-6-F	ACTCACCTCTTCAGAACGAATTG	63°C
has-IL-6-R	CCATCTTTGGAAGGTTTCAGGTTG	63°C
has-MCP1-F	CAGCCAGATGCAATCAATGCC	60°C
has-MCP1-R	TGGAATCCTGAACCCACTTCT	60°C
has-PARP-F	CGGAGTCTTCGGATAAGCTCT	62°C
has-PARP-R	TTTCCATCAAACATGGGCGAC	62°C
hsa- <i>Runx2</i> -F	TCTTCACAAATCCTCCCC	52°C
hsa- <i>Runx2</i> -R	TGGATTAAAAGGACTTGG	52°C
hsa- <i>Alp</i> -F	GACAAGAAGCCCTTCACTGC	59°C
hsa- <i>Alp</i> -R	AGACTGCGCCTGGTAGTTGT	59°C
hsa- <i>OPN</i> -F	ACTCGAACGACTCTGATGATGT	57°C
hsa- <i>OPN</i> -R	GTCAGGTCTGCGAACTTCTTA	57°C
hsa- <i>WNT4</i> -F	AGGAGGAGACGTGCGAGAAA	60°C
hsa- <i>WNT4</i> -R	CGAGTCCATGACTTCCAGGT	60°C
hsa- <i>WNT16</i> -F	TTCAGACACGAGAGATGGAACT	63°C
hsa- <i>WNT16</i> -R	CCAGCCTTCACTTGCTGAG	63°C
hsa- <i>TRACP</i> -F	GACTGTGCAGATCCTGGGTG	63°C
hsa- <i>TRACP</i> -R	GGTCAGAGAATACGTCCTAAAAG	63°C
hsa- <i>CTSK</i> -F	ACTCAAAGTACCCCTGTCTCAT	62°C
hsa- <i>CTSK</i> -R	CCACAGAGCTAAAAGCCCAAC	62°C

Figures

Figure 1

Differently expressed miRNA. (A) Volcano plot of miRNA expression. (B) Differently expressed miRNA heat map.

Figure 2

The top ten upstream transcription factors of upregulated DE-miRNAs.

Figure 3

GO functional annotation and KEGG pathways of DE-miRNAs. (A) The top 10 enriched BP items of DE-miRNA. (B) The top 10 enriched CC items of DE-miRNA. (C) The top 10 enriched MF items of DE-miRNA candidate target genes. (D) The top 10 enriched KEGG pathways of DE-miRNA.

Figure 4

miR-338-5p and miR-4776-3p bind with SOX6 naturally. (A) miR-338-5p bind with SOX6 naturally. (B) miR-4776-3p bind with SOX6 naturally.

Figure 5

miR-338-5p and miR-4776-3p bind with SOX6 and inhibit SOX6 expression. (A) miR-338-5p mimics bind with SOX6 and inhibit SOX6 expression. (B) miR-338-5p inhibitors improve SOX6 expression. (C) miR-4776-3p mimics bind with SOX6 and inhibit SOX6 expression. (D) miR-4776-3p inhibitors improve SOX6 expression.

A **B** **Figure 6**

Over expressed miR-338-5p and miR-4776-3p improve catabolism, and trigger inflammation and apoptosis. (A) miR-338-5p mimics improve gene expression of MMP13, TNFA, IL6, MCP1 and PARP. (B) miR-4776-3p mimics improve gene expression of MMP13, TNFA, IL6, MCP1 and PARP.

A **B** **Figure 7**

Knock-down of miR-338-5p and miR-4776-3p inhibit catabolism, and trigger inflammation and apoptosis. (A) miR-338-5p inhibitors reduce gene expression of MMP13, TNFA, IL6, MCP1 and PARP. (B) miR-4776-3p inhibitors reduce gene expression of MMP13, TNFA, IL6, MCP1 and PARP.

Figure 8

miR-338-5p and miR-4776-3p affect osteogenesis. (A) miR-338-5p mimics inhibit gene expression of Runx2, ALP, OPN, WNT4 and WNT16, and promote expression of osteoclastogenesis genes TRACP and CTSK. (B) miR-4776-3p mimics inhibit gene expression of Runx2, ALP, OPN, WNT4 and WNT16, and promote expression of osteoclastogenesis genes TRACP and CTSK. (C) miR-338-5p inhibitors promote gene expression of Runx2, ALP, OPN, WNT4 and WNT16, and inhibit expression of osteoclastogenesis

genes TRACP and CTSK. (D) miR-4776-3p inhibitors promote gene expression of Runx2, ALP, OPN, WNT4 and WNT16, and inhibit expression of osteoclastogenesis genes TRACP and CTSK. (E) miR-338-5p mimics and miR-4776-3p mimics inhibit the expression of WNT4, miR-338-5p inhibitors and miR-4776-3p inhibitors increased the expression of WNT4 at the protein level.

Figure 9

SOX6 mediates the expression of osteogenic genes and osteoclast genes. (A) Knockdown of SOX6 significantly suppressed the expression the expression of Runx2, ALP, OPN, WNT4, WNT16 and increased the expression of TRACP and CTSK. (B) Overexpression of SOX6 significantly increased the expression of Runx2, ALP, OPN, WNT4, WNT16 and suppressed the expression of TRACP and CTSK. (C) Knockdown of miR-338-5p and miR-4776-3p can increase the expression of WNT4, while overexpression of miR-338-5p and miR-4776-3p can reduce the expression of WNT4 at the protein level (Fig. 9C).