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Original Article

Involvement of miR-145 in the pathogenesis of osteoarthritis by mediating the expression of osteoprotegerin

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Abstract: Objective: To investigate the role of miR-145 in the pathogenesis of osteoarthritis by mediating the expression of osteoprotegerin. Method: Articular cartilage tissues of 43 patients with osteoarthritis (OA) undergoing knee arthroplasty or arthroscopic debridement in our hospital were selected. Meanwhile, articular cartilage tissues of 12 amputation patients caused by emergency trauma were collected. All the patients were divided into normal group (group A) (n = 12), mild group (group B) (n = 15), moderate group (group C) (n = 14) and severe group (group D) (n = 14) according to modified ManKin articular cartilage pathological grading standard. A total of 60 C57BL/6 mice were randomly divided into five groups: miR-145-mimic group (n = 12), miR-145-inhibitor group (n = 12), model group (n = 12), operation group (n = 12) and normal group (n = 12). The expression of miR-145 was detected by qRT-PCR. The expression of OPG protein in mouse tissues was detected by western blot. The OA of the knee joint in the mouse models was observed by X-ray. Results: The expression of miR-145 in group C and group D was significantly lower than that in group A and B ($P < 0.05$). The expression of miR-145 in the normal group was significantly lower than that in miR-145-mimic group, and higher than that in miR-145-inhibitor group, operation group and model group ($P < 0.05$) in mouse tissues after injection. The expression of OPG protein in the normal group and miR-145-mimic group was significantly lower than that in operation group, model group and miR-145-inhibitor group ($P < 0.05$). The relative expression of miR-145 and OPG was negatively correlated. Conclusion: miR-145 can regulate the expression of OPG and improve the progression of OA.

Keywords: MiR-145, osteoprotegerin, osteoarthritis

Introduction

Osteoarthritis (OA) is a common chronic disease in clinical orthopedics. The clinical features of patients include cartilage degeneration and secondary peripheral bone hyperplasia, mostly in the elderly [1]. In a US report [2], it is estimated that by 2020, more than 50 million Americans will be affected by OA. Another study showed that [3] by 2012, about 200,000 elderly patients in China had undergone knee arthroplasty due to knee osteoarthritis, rheumatoid arthritis, traumatic arthritis, severe pain or dysfunction caused by bone necrosis or tumor. Early symptoms of OA are not easily realized by patients. As the disease progresses, the patient will experience symptoms such as distorted joints and increased pain while bearing weight. Moreover, severe illness may affect walking ability and daily life of patients, and may even lead to disabilities [4].

The pathogenesis of OA is not clear, but it is generally believed that it is controlled by a complex regulatory network comprised of various factors, which include transcription factors, signaling pathways and related proteins involved in the process of cartilage degenerative diseases [5]. MicroRNA (miR) is a type of non-coding short-chain RNA of approximately 22 nt in length. It can inhibit the translation of target genes by binding to its downstream target gene mRNA 3'untranslated region (UTR) [6]. Studies have shown that [7-9] miRs play a regulatory role in many diseases such as cardiovascular and cerebrovascular diseases, tumors, immune diseases and so on. MiR-145 is an important member of the miR family. In the study of Yang

et al. [10], it was shown that the low expression of miR-145 can promote the directional differentiation of MSCs cells into chondrocytes in TGF-β3 induced chondrogenic differentiation of MSCs. This suggests that miR-145 may be involved in the progress of the differentiation of MSCs into chondroblasts. Recent studies have shown that osteoprotegerin (OPG) plays an important role in the development of OA [11]. However, it is unclear whether there is a correlation between miR-145 and OPG. Therefore, the expression and mechanism of miR-145 and OPG in OA were explored in this study to provide references for clinicians.

Materials and methods

A total of 43 patients with OA undergoing knee arthroplasty or arthroscopic debridement in our hospital were selected, including 23 males and 20 females, with an age range of 45~72 years and an average age of 62.7±6.1 years. Meanwhile, 12 amputation patients caused by emergency trauma but not diseases were enrolled, including 7 males and 5 females, with an age range of 25~47 years and an average age of 35.5±5.9 years. They had no congenital disease, no malignant tumors, and no history of infection and arthritis. All the patients were divided into normal group (group A) (n = 12), mild group (group B) (n = 15), moderate group (group C) (n = 14) and severe group (group D) (n = 14) according to modified ManKin articular cartilage pathological grading standard. The femoral condylar full thickness cartilage and a small amount of subchondral bone were collected. Full consent of patients and their families was obtained before sample collection, and this study was approved by the Medical Ethics Committee in our hospital.

Inclusion and exclusion criteria for OA patients

Inclusion criteria: patients who signed informed consent; patients who met the OA diagnostic criteria of *Guidelines for the Diagnosis and Treatment of Osteoarthritis* issued by the Chinese Medical Association in 2007 [12]; patients with complete clinical data.

Exclusion criteria: patients with rheumatic arthritis, suppurative arthritis and rheumatoid arthritis; malignant tumors; or a history of severe systemic infection, were excluded.

Sources of mice and cells

Sixty C57BL/6 mice (Beijing Vital River Laboratory Animal Technology Co., Ltd., batch number. SYXK (Beijing), 2012-0036, 213) aged 3 weeks and weighing 10 g, were used in this study. The mice were separately raised in cages, drinking water and eating freely. Housing conditions for mice are temperature of 21±2°C with 45±10% relative humidity and 24-hour illumination system. They were randomly divided into five groups: miR-145-mimic group (n = 12), miR-145-inhibitor group (n = 12), model group (n = 12), operation group (n = 12) and normal group (n = 12). RAW-264.7 cells were purchased from American type culture collection, ATCC® TIB-71.

Main reagents

The main reagents used in the study include: total RNA extraction kit EasyPure miRNA Kit, TransScript Green miRNA Two-Step qRT-PCR SuperMix (Beijing TransGen Biotech Corporation, China, ER601-01, AQ202-01), FBS (fetal bovine serum), Penicillin streptomycin double-resistance, DMEM medium, RIPA, BCA protein kit, Lipofectamine™ 3000 transfection kit (Shanghai Thermo Fisher, China, 10099141, 15070063, 89900, 23225, L3000015), OPG mice monoclonal antibody, GAPDH, HRP labeled goat anti-mouse IgG secondary antibody (MAB459, AF5718, HAF007), Macrophage colony stimulating factor (Suzhou Cyagen Biotechnology Co., Ltd., China, MEOPP-0702), Dual luciferase reporter gene detection kit (Shanghai Beyotime Biotechnology, China, RG028), PCR instrument (ABI, USA 7500), miR-145 and U6 primers designed and synthesized by Shanghai Jima Technology Co., Ltd.

Cell culture and transfection

RAW-264.7 cells (mouse mononuclear macrophage leukemia cells) were placed in DMEM medium (10% FBS, 100 IU/mL penicillin streptomycin double-resistance), cultured in a constant-temperature incubator (37°C, 5% CO_2), and transfected with miR-145 mimic and inhibitor. Unrelated sequences were treated as the model group. Lipofectamine 3000 transfection kit was used to transfect the cells, and the transfection process was performed in strict accordance with the manufacturer's instructions. MiR-145 mimic, inhibitor and model were transfected into RAW-264.7 cells for 24 h.

Table 1. Primer sequence

Gene	Upstream primer	Downstream primer
miR-145	5'-GTCCAGTTTTCCCAGGAATCCCT-3'	5'-TGGTGTCGTGGAGTCG-3'
U6	5'-GCGCGTCGTGAAGCGTTC-3'	5'-GTGCAGGGTCCGAGGT-3'

Meanwhile, the untransfected RAW-264.7 cells were cultured as normal cells in the untransfected group for 24 h. The original medium for all cells was replaced with 50 ng/mL macrophage colony stimulating factor twice a day. In addition, pGL3-OPG-WT and miR-145 mimics, pGL3-OPG-WT and miR-145 unrelated sequences, pGL3-OPG-MUT and miR-145 mimics and pGL3-OPG-MUT and miR-145 irrelevant sequence, were transfected as double luciferase reporter samples.

Establishment of mouse models

The knee joint OA models were established in 48 mice randomly, and the remaining 12 mice that received no surgery were treated as normal group. The 48 mice were injected intraperitoneally with 400 μL Avertin for anesthesia. After anesthesia, the skin of the knee joint was cut, the joint capsule was opened with a scalpel, the joint cavity was exposed, the meniscus was cut off, and then the joint capsule and the skin were sutured. After the surgery, the mice were placed on a heating table at 25°C for 20~30 minutes, until they regained consciousness. Then iodine was used to wipe the limbs. The mice were set to original cages when they could move freely, and after their limbs were observed by X-ray. After 2 weeks of modeling, 60 mice were divided into 5 groups with 12 mice in each group. There were five groups: miR-145-mimic group, miR-145-inhibitor group, model group, operation group and normal group. The RAW-264.7 cells transfected with miR-145-mimic were injected into the mice of the miR-145-mimic group (0.1 mL $1*10^7$/cell); the RAW-264.7 cells transfected with miR-145-inhibitor were injected into the mice of the miR-145-inhibitor (0.1 mL $1*10^7$/cell); the RAW-264.7 cells transfected with unrelated sequences were injected into the mice of the model group (0.1 mL $1*10^7$/cell); the untransfected RAW-264.7 cells were injected into the mice of the operation group and normal group (0.1 mL $1*10^7$/cell). All the injections were performed on the tail vein of the mice. After 2 weeks of injection, the mice were sacrificed after anesthesia with ether, and the knee joints were observed by X-ray before death. The cartilage tissues of the knee joint of the mice were collected for follow-up experiment.

PCR detection

EasyPure miRNA Kit kit was used to extract total RNA from the collected femoral condyle cartilage and a small amount of subchondral bone from human the specimens, cartilage tissues of the knee joint from the mice and RAW-264.7 cells. The purity, concentration and integrity of the extracted total RNA were determined by UV spectrophotometer and agarose gel electrophoresis. MiR-145 and U6 primers were designed and synthesized by Shanghai GenePharma Co., Ltd (**Table 1**). TransScript® miRNA RT Enzyme Mix and 2× TS miRNA Reaction Mix were used for reverse transcription of total RNA in accordance with the instructions of the kits. Then PCR amplification was carried out. PCR reaction system: cDNA 1 μL, upstream primer, downstream primer, 0.4 μL each, 2× TransTaq® Tip Green qPCR SuperMix 10 μL, Passive Reference Dye (50×) 0.4 μL, dd H_2O was complemented to 20 μL. PCR reaction condition: 40 cycles of predenaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing and elongation at 60°C for 30 s. Each sample had 3 repeat wells, and the experiment was carried out 3 times. U6 was used as an internal reference and $2^{-\Delta c}$ was used to analyze the data in this study.

Western blot detection

Total proteins were extracted from the collected cartilage tissues of the knee joint from the mice by RIPA cleavage method, and the concentration was detected by BCA assay, then adjusted to 4 μg/μL. The proteins were separated by SDS-PAGE electrophoresis. After separation, they were transferred to the PVDF membrane, stained with Ponceau fluid, immersed in PBST for 5 min, blocked with 5% nonfat dry milk for 2 h, then mixed with OPG mice monoclonal antibody (1:1000) and incubated overnight at 4°C. The membrane was washed to remove the primary antibody, and horseradish peroxidase-labelled sheep anti-mouse secondary antibody (1:5000) was added, incubated at 37°C for 1 h. TBST was used to rinse for 3 times, 5 min each time. The proteins were visualized in a dark

Table 2. Relative Expression of miR-145 in patients' femoral condyle cartilage and a small amount of subchondral bone

Group	miR-145 expression	F value	*P* value
A Group (n=12)	1.254±0.315	45.317	< 0.001
B Group (n=15)	1.184±0.184		
C Group (n=14)	0.927±0.157*,#		
D Group (n=14)	0.462±0.084*,#,Δ		

Note: *represented that there were differences compared with the group A (P < 0.001); #represented that there were differences compared with the group B (P < 0.001); Δrepresented that there were differences compared with the group C (P < 0.001).

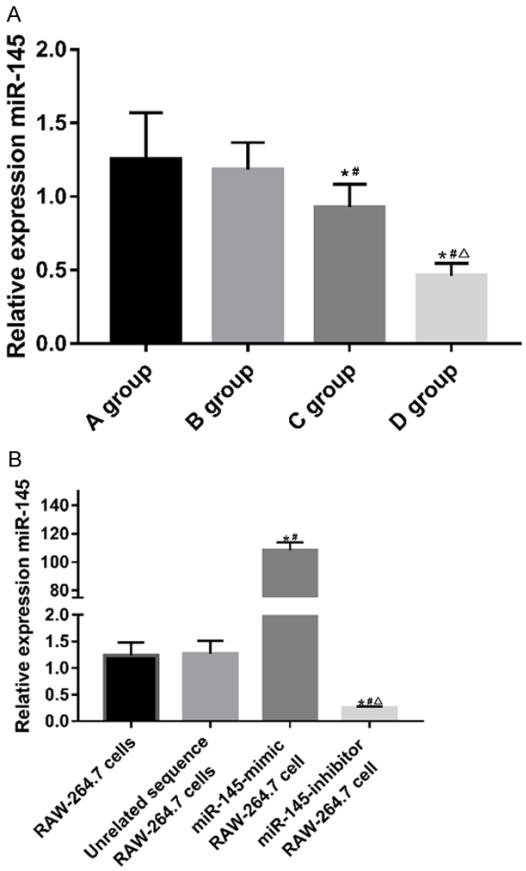

Figure 1. Expression of miR-145. A. Expression of miR-145 in subchondral bone tissue of patients. *represented that there were differences compared with the group A (P < 0.001); #represented that there were differences compared with the group B (P < 0.001); Δrepresented differences compared with the group C (P < 0.001). B. Expression of miR-145 in transfected cell. *represented differences compared with the untransfected RAW-264.7 cells (P < 0.001); #represented differences compared with the unrelated sequences NC RAW-264.7 cells (P < 0.001); Δrepresented differences compared with the miR-145-mimic RAW-264.7 cells (P < 0.001).

room, and filter paper was used to absorb excess liquid on the membrane. ECL was used for luminescent and visualization. The protein bands were scanned and the gray values were analyzed by Quantity One software. The relative expression level of the protein = the gray value of the target protein band/the gray value of the GAPDH protein band.

Dual luciferase reporter assay

The miR-145 target gene was screened by the online miR target gene prediction software (Targetscan), and the 3'-utranslated regions (UTR) binding site of miR-145 on OPG was predicted. The 3'-UTR promoter sequence of miR-145 binding site on OPG was inserted into pGL-control vector (Promega Corporation, PCI-NEO, USA) by Kpn I and Bgl II site, and then the plasmid was named pGL3-OPG-WT. At the same time, a plasmid containing 3'-UTR promoter sequence of miR-145 mutant binding site on OPG was constructed, called pGL3-OPG-MUT. pGL3-OPG-WT + miR-145 mimic, pGL3-OPG-WT + miR-145 negative control, pGL3-OPG-MUT + miR-145 mimics, pGL3-OPG-MUT + miR-145 negative control, pGL3-OPG-WT/pGL3-OPG-MUT (100 ng) + miR-145 mimetic (50 ng) + miR-145 negative control (50 ng) were transfected into cells. After transfection for 24 hours, cells were rinsed with PBS. Then 100 μL cell lysis buffer was added to each well and the plate was shaken until the cells were completely dissociated. At that time, the cell lysis buffer of each well was transferred to 1.5 mL centrifuge tube and all tubes were centrifuged at 12,000 rpm for 20 minutes. Thereafter, the relative activity of luciferase in the supernatant was detected using a dual luciferase kit. The above experiment was repeated three times.

Statistical analysis

The data in this study were statistically analyzed by SPSS 20.0 software package; the measurement data were expressed by mean ± standard deviation (Mean ± SD); one-way ANOVA was used for multigroup comparison, and LSD-t test was used for pairwise comparison; Pearson test was used to analyze the correlation between miR-145 and OPG protein in tissues of the miR-145-mimic group and the miR-145-inhibitor group after transfection. P < 0.05 indi-

Table 3. Relative Expression of miR-145 in cells of each group

Group	miR-145 expression	F value	*P* value
Untransfected RAW-264.7 cells	1.243±0.237	1118.456	< 0.001
Unrelated sequence NC RAW-264.7 cells	1.273±0.234		
miR-145-mimic RAW-264.7 cell	108.272±5.55$^{*,\#}$		
miR-145-inhibitor RAW-264.7 cell	0.256±0.020$^{*,\#,\Delta}$		

Note: *represented that there were differences compared with the untransfected RAW-264.7 cells (P < 0.001); $^{\#}$represented that there were differences compared with the unrelated sequences NC RAW-264.7 cells (P < 0.001); $^{\Delta}$represented that there were differences compared with the miR-145-mimic RAW-264.7 cells (P < 0.001).

Table 4. Comparison of miR-145 Relative expression in mice of each group

Group	miR-145 expression	F value	*P* value
Normal group (n=12)	1.294±0.138	288.151	< 0.001
Operation group (n=12)	0.509±0.095*		
Model group (n=12)	0.534±0.086*		
miR-145-mimic group (n=12)	1.847±0.221$^{*,\#,\Delta}$		
miR-145-inhibitor group (n=12)	0.325±0.051$^{*,\#,\Delta,\blacktriangle}$		

Note: *represented that there were differences compared with the normal group (P < 0.001); # represented that there were differences compared with the operation group (P < 0.001); $^{\Delta}$represented that there were differences compared with the model group (P < 0.001); $^{\blacktriangle}$represented that there were differences compared with the miR-145-mimic group (P < 0.001).

cated statistical difference between the two groups.

Results

Expression of miR-145 in patients' tissues

There were significant differences in the expression of miR-145 in the tissues of each group (P < 0.05). There was no significant difference in miR-145 expression between group A and group B (P > 0.05). The expression of miR-145 in the group C and group D was significantly lower than that in the group A and B (P < 0.05). The expression of miR-145 in the group D was lower than that in the group C (P < 0.05) (**Table 2** and **Figure 1A**).

The condition of mice after modeling

There were degenerative diseases of articular cartilage, defects and uneven articular surface in each group, indicating the OA models were established successfully. Then the mice were injected with cells, and no death occurred. X-ray detection showed that the cartilage tissues in the normal group and miR-145-mimic group were intact, with no degeneration, and with even articular surface. While the cartilage tissues in the operation group, model group and miR-145-inhibitor group developed degenerative diseases of the articular cartilage, with obvious defects and uneven articular surface.

Expression of miR-145 in transfected cells

There were significant differences in the expression of miR-145 in the cells of each group (P < 0.05). There was no significant difference in the expression of miR-145 between the untransfected RAW-264.7 cells and the model group transfected with unrelated sequences (P > 0.05). While the expression of miR-145 in RAW-264.7 cells transfected with miR-145-mimic was significantly increased compared with untransfected cells and model group transfected with unrelated sequences (P < 0.05). The expression of miR-145 in RAW-264.7 cells transfected with miR-145-inhibitor was significantly lower than that in the other three groups, (P < 0.05) (**Table 3** and **Figure 1B**).

Expression of miR-145 in mouse tissues

There were significant differences in the expression of miR-145 in the mouse tissues of each group (P < 0.05). The expression of miR-145 in the normal group was significantly lower than that in the miR-145-mimic group, and significantly higher than that in the miR-145-inhibitor group, operation group and model group (P < 0.05). The expression of miR-145 in the operation group and model group was significantly lower than that in the miR-145-mimic group, and higher than that in the miR-145-inhibitor group, there was significant difference (P < 0.05). The expression of miR-145 in the operation group and model group showed no significant difference (P > 0.05). The expression of miR-145 in the miR-145-inhibitor group was significantly lower than that in the miR-145-mimic group (P < 0.05) (**Table 4** and **Figure 2A**).

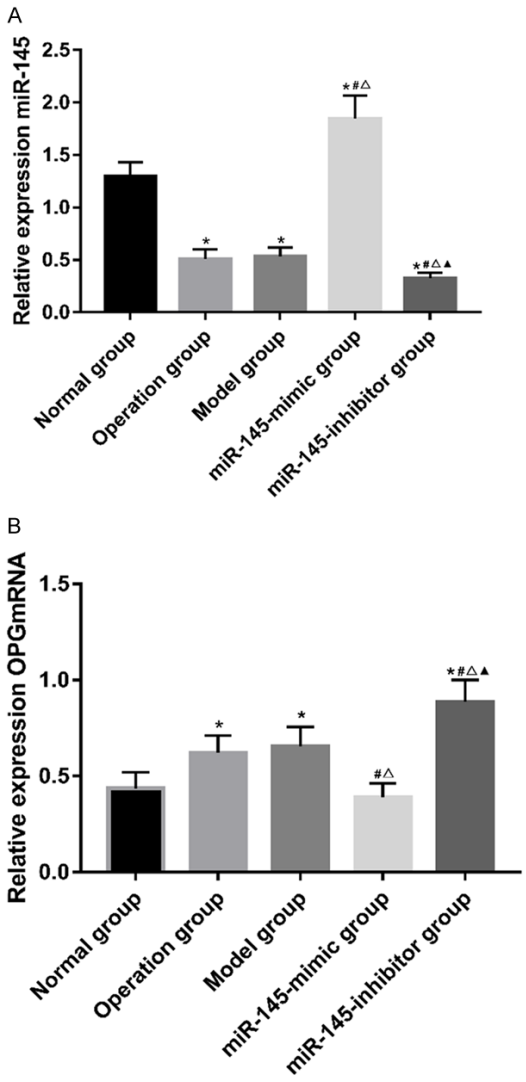

Figure 2. The expression of miR-145 and OPG in mouse tissues. A. The expression of miR-145 in mouse tissues. B. The expression of OPG protein in mouse tissues, *represented that there were differences compared with the normal group ($P < 0.05$); #represented that there were differences compared with the model group ($P < 0.05$); △represented that there were differences compared with the model group ($P < 0.05$); ▲represented that there were differences compared with the miR-145-mimic group ($P < 0.05$).

Expression of OPG protein in mouse tissues

That there were significant differences in the expression of OPG protein in mouse tissues of each group ($P < 0.05$). The expression of OPG protein in the normal group and miR-145-mimic group was significantly lower than that in the operation group, model group and miR-145-inhibitor group ($P < 0.05$). There was no significant difference in the expression of OPG protein between the normal group and the miR-145-mimic group ($P > 0.05$), but the expression in the miR-145-inhibitor group was significantly lower than that in the model group ($P < 0.05$). The expression of OPG protein in the operation group and model group showed no significant difference ($P > 0.05$) (**Table 5** and **Figure 2B**).

Correlation analysis of miR-145 and OPG in mouse tissues of the miR-145-mimic group and the miR-145-inhibitor group

The relative expression of miR-145 and OPG was negatively correlated in the two groups. The expression of OPG protein decreased gradually with the increase of miR-145 in mouse tissues of the miR-145-mimic group, while the OPG protein increased gradually with the decrease of miR-145 in mouse tissues of the miR-145-inhibitor group (**Table 6** and **Figure 3**).

Target gene prediction and dual luciferase reporter

The presence of a targeting binding site for OPG and miR-145 was found using online miR prediction software (Targetscan) (**Figure 4A**). According to the dual luciferase reporter assay, transfection of pGL3-OPG-WT + miR-145 mimics resulted in decreased activity compared to the transfection of miR-145 unrelated sequence ($P < 0.05$). However, changes in relative luciferase activity were observed after transfection of pGL3-OPG-MUT between the miR-145 mimetic and the miR-145 unrelated sequence (**Figure 4B**). This was consistent with the predictions, confirming that miR-145 can bind to sequence in the 3'-UTR of OPG, thereby regulating OPG.

Discussion

OA is also a chronic degenerative disease caused by complicated etiology [13]. Research has shown that [14] hip and knee OA is ranked 11th out of 291 disabling diseases worldwide. A Chinese study has shown that [15] about 50% of the elderly over the age of 60 say that they are repeatedly suffering from arthritis.

MiR-145, located on the human 5q32 chromosome, is thought to be a tumor suppressor with an low expression in breast cancer [16]. Other studies have shown that [17] miR-145 was closely related to the regulation of chondrogenic differentiation of stem cells and the maintenance of normal physiological state of carti-

Table 5. Comparison of OPG protein expression in mouse tissues of each group

Group	OPG protein expression	F value	*P* value
Normal group (n=12)	0.435±0.084	53.355	< 0.001
Operation group (n=12)	0.621±0.089*		
Model group (n=12)	0.654±0.102*		
miR-145-mimic group (n=12)	0.389±0.073#,△		
miR-145-inhibitor group (n=12)	0.885±0.115*,#,△,▲		

Note: *represented that there were differences compared with the normal group (P < 0.001); #represented that there were differences compared with the model group (P < 0.001); △represented that there were differences compared with the model group (P < 0.001); ▲represented that there were differences compared with the miR-145-mimic group (P < 0.001).

Table 6. Correlation analysis of miR-145 and OPG in transfected tissues of mice

Group	r value	*P* value
miR-145-mimic (miR-145 VS OPG)	-0.673	0.016
miR-145-inhibitor (miR-145 VS OPG)	-0.650	0.022

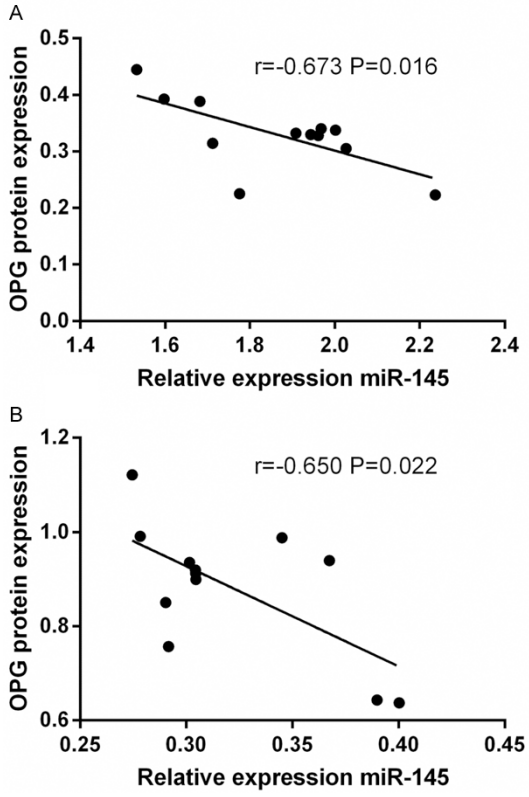

Figure 3. Correlation analysis of miR-145 and OPG in mouse tissues. A. The relative expression of miR-145 and OPG protein in mouse tissues of the miR-145-mimic group was negatively correlated (r = -0.673, P = 0.016). B. The relative expression of miR-145 and OPG protein in mouse tissues of the miR-145-inhibitor group was negatively correlated (r = -0.650, P = 0.022).

lage. However, there are few studies on the specific introduction of the expression of miR-145 in OA patients. Therefore, the effect of miR-145 on the development of OA was investigated. In this study, the OA patients were divided into groups by modified ManKin articular cartilage pathological grading standard, and the expression of miR-145 in the tissues of each group was detected. The results showed that the expression of miR-145 in patient tissues of the group C and group D was significantly lower than that of the group A and B, suggesting that miR-145 is involved in the occurrence and development of OA (**Figure 5**). In *Effects of of miR on OA and cartilage formation* published by Le et al. [18], it was noted that miR-145 was minimally expressed in OA. Moreover, in the report of Karlsen et al. [19], miR-145 was found to be low expressed in human primary osteoarticular chondrocytes by gene microarray screening. PCR analysis of clinical samples in this study confirmed the low expression of miR-145 in cartilage tissues of OA patients, which proved their research. However, how miR-145 affects the occurrence and development of OA has not been studied.

OPG, a secretable soluble protein, belongs to the tumor necrosis factor receptor superfamily and widely exists in cardiovascular, digestive and skeletal muscle systems. Studies by Logar et al. [20] showed that the expression of OPG in femur OA tissues was significantly higher than that in peripheral tissues. In the study by Chen et al. [21], it was found that miR-145 could inhibit OPG to regulate the osteoclast number *in vitro*. However, it is unclear whether miR-145 and OPG show similar regulatory effects *in vivo*. Therefore, OA models were established in our study to observe the expression of miR-145 and OPG in OA mouse models. The monocyte or macrophage-like cell line RAW 264.7 has been one of the most commonly used bone marrow cell lines and has existed for more than 40 years. Although the phenotype and functional stability of RAW 264.7 are often discussed in the literature or in various scientific discussion groups, their stability during serial passaging has not been confirmed in any reliable study [22]. The mouse OA models were established,

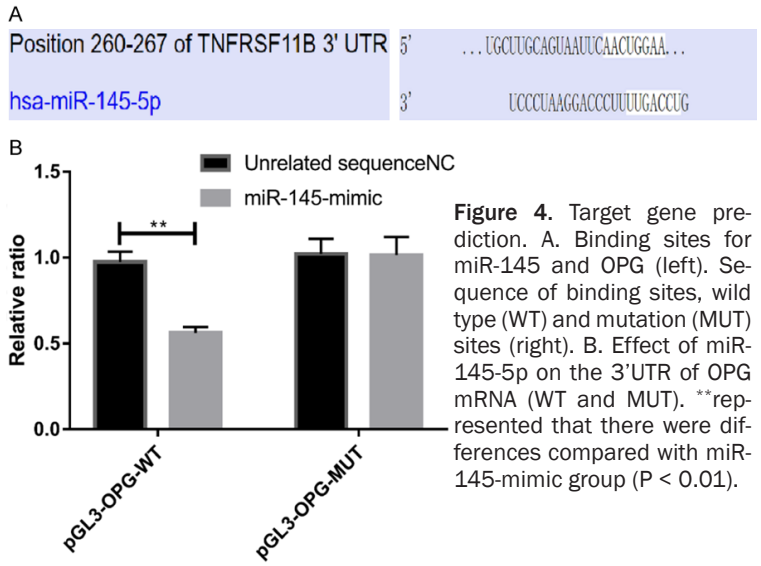

Figure 4. Target gene prediction. A. Binding sites for miR-145 and OPG (left). Sequence of binding sites, wild type (WT) and mutation (MUT) sites (right). B. Effect of miR-145-5p on the 3'UTR of OPG mRNA (WT and MUT). **represented that there were differences compared with miR-145-mimic group ($P < 0.01$).

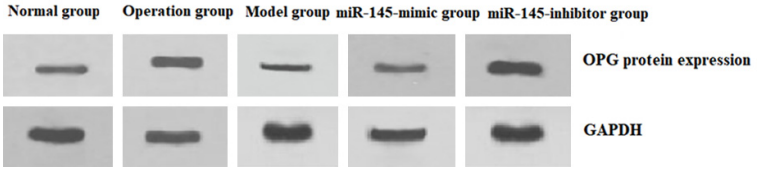

Figure 5. OPG protein expression in mouse tissues of each group. Comparison of OPG protein expression in mouse tissues of normal group, operation, model group, miT-145-mimic group and miR-145-inhibitor group by western blot.

and the transfected and untransfected RAW-264.7 cells were transplanted into mice by tail vein injection. X-ray results of mice two weeks after injection found that, the articular cartilage tissues of mice in the miR-145-mimic group and normal group injected with miR-145-inhibitor RAW-264.7 cells were intact, with no degeneration, and with even articular surface. While the articular cartilage tissues of mice in the model group and the miR-145-inhibitor group developed degenerative diseases of articular cartilage, with obvious defects and uneven articular surface. Furthermore, through PCR and WB detection, it was found that the expression of miR-145 in the mouse cartilage tissues of the miR-145-mimic group increased, but the expression of OPG decreased. While the expression of miR-145 in the mouse cartilage tissues of the miR-145-inhibitor group decreased significantly, and the expression of OPG increased significantly. Study by Pilichou et al. [23] showed that the expression of OPG in synovial fluid of patients with knee OA was significantly higher than that in normal controls. Our findings suggested that overexpression of miR-145 could inhibit the expression of OPG, indicating that miR-145 may be a potential target for clinical therapy. Pearson analysis found that there was a negative correlation between miR-145 and OPG in mouse cartilage tissues of the miR-145-mimic group and the miR-145-inhibitor group, indicating that there is a regulatory relationship between them.

This study found that overexpression of miR-145 could inhibit the expression of OPG, suggesting that injection of miR-145-mimic can effectively inhibit the expression of OPG. However, there are still some limitations in this study. First of all, chondrocytes in mice were not stained, it was not clear how the cartilage tissues changed. Secondly, no clinical pilot trials were conducted, whether miR-145 can become a target for the treatment of OA still needs to be further verified. Finally, all samples were cartilage tissues, and whether there are same expressions in other samples needs further experimental verification. Therefore, we hope to add more experimental targets, carry out clinical trials, collect a variety of samples (blood, joint fluid, etc.) in the future to verify the results of this study.

In conclusion, miR-145 can regulate the expression of OPG and improve the progress of OA, which is expected to be a potential therapeutic target in clinic.

Disclosure of conflict of interest

None.

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References

[1] Sokolove J and Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: lat-

est findings and interpretations. Ther Adv Musculoskelet Dis 2013; 5: 77-94.
[2] Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi HK, Deyo RA, Gabriel SE, Hirsch R, Hochberg MC and Hunder GG. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis Rheum 2008; 58: 26-35.
[3] Chen Wei, Lv Hongzhi, Zhang Xiaolin, Yin Bin, Song L and Yingze Z. Epidemiological study on prevalence of knee osteoarthritis in middle-aged and elderly Chinese. Journal of Hebei Medical University 2015; 487-490.
[4] Palmierismith RM, Cameron KL, Distefano LJ, Driban JB, Pietrosimone B, Thomas AC and Tourville TW. The role of athletic trainers in preventing and managing posttraumatic osteoarthritis in physically active populations: a consensus statement of the athletic trainers' osteoarthritis consortiuma. J Athl Train 2017; 52: 610-623.
[5] Hosseinzadeh A, Kamrava SK, Joghataei MT, Darabi R, Shakerizadeh A, Shahriari M, Reiter RJ, Ghaznavi H and Mehrzadi S. Apoptosis signaling pathways in osteoarthritis and possible protective role of melatonin. J Pineal Res 2016; 61: 411-425.
[6] Ha M and Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15: 509-524.
[7] Samanta S, Balasubramanian S, Rajasingh S, Patel U, Dhanasekaran A, Dawn B and Rajasingh J. MicroRNA: a new therapeutic strategy for cardiovascular diseases. Trends Cardiovasc Med 2016; 26: 407-419.
[8] Vescovo D and Denti MA. microRNA and lung cancer. Adv Exp Med Biol 2015; 889: 153-177.
[9] Mehta A and Baltimore D. MicroRNAs as regulatory elements in immune system logic. Nat Rev Immunol 2016; 16: 279-294.
[10] Yang B, Guo H, Zhang Y, Chen L, Ying D and Dong S. MicroRNA-145 regulates chondrogenic differentiation of mesenchymal stem cells by targeting Sox9. PLoS One 2011; 6: e21679.
[11] Ramos YF, Bos SD, van der Breggen R, Kloppenburg M, Ye K, Lameijer EW, Nelissen RG, Slagboom PE and Meulenbelt I. A gain of function mutation in TNFRSF11B encoding osteoprotegerin causes osteoarthritis with chondrocalcinosis. Ann Rheum Dis 2015; 74: 1756-1762.
[12] X QG. Guidelines for the diagnosis and treatment of osteoarthritis (2007 edition) Chinese Journal of Arthrosurgery (Electronic version) 2007; 1: 281-285.
[13] Glyn-Jones S, Palmer A, Agricola R, Price A, Vincent T, Weinans H and Carr A. Osteoarthritis. Lancet 2015; 386: 376-387.
[14] Cross M, Smith E, Hoy D, Nolte S, Ackerman I, Fransen M, Bridgett L, Williams S, Guillemin F and Hill CL. The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis 2014; 73: 1323-1330.
[15] Xue Q, Wang K, Pei F, Zhu H, Jin D, Tao T, Li E, Li N, Zhang Y and Wang X. The survey of the prevalence of primary osteoarthritis in the population aged 40 years and over in China. Chinese Journal of Orthopaedics 2015; 12: 1206-1212.
[16] Götte M, Mohr C, Koo CY, Stock C, Vaske AK, Viola M, Ibrahim SA, Peddibhotla S, Teng YH and Low JY. MiR-145-dependent targeting of junctional adhesion molecule A and modulation of fascin expression are associated with reduced breast cancer cell motility and invasiveness. Oncogene 2010; 29: 6569-6580.
[17] Martinezsanchez A, Dudek KA and Murphy CL. Regulation of human chondrocyte function through direct inhibition of cartilage master regulator SOX9 by microRNA-145 (miRNA-145). J Biol Chem 2012; 287: 916-924.
[18] Le LT, Swingler TE and Clark IM. Review: the role of microRNAs in osteoarthritis and chondrogenesis. Arthritis Rheum 2013; 65: 1963-1974.
[19] Karlsen TA, Shahdadfar A and Brinchmann JE. Human primary articular chondrocytes, chondroblasts-like cells, and dedifferentiated chondrocytes: differences in gene, microRNA, and protein expression and phenotype. Tissue Eng Part C Methods 2010; 17: 219-227.
[20] Logar DB, Komadina R, Preželj J, Ostanek B, Trost Z and Marc J. Expression of bone resorption genes in osteoarthritis and in osteoporosis. J Bone Miner Metab 2007; 25: 219-225.
[21] Chen Y, Wang X, Yang M, Ruan W, Wei W, Gu D, Wang J, Guo X, Guo L and Yuan Y. MiR-145-5p increases osteoclast numbers in vitro and aggravates bone erosion in collagen-induced arthritis by targeting osteoprotegerin. Med Sci Monit 2018; 24: 5292.
[22] Taciak B, Białasek M, Braniewska A, Sas Z, Sawicka P, Kiraga Ł, Rygiel T and Król M. Evaluation of phenotypic and functional stability of RAW 264.7 cell line through serial passages. PLoS One 2018; 13: e0198943.
[23] Pilichou A, Papassotiriou I, Michalakakou K, Fessatou S, Fandridis E, Papachristou G and Terpos E. High levels of synovial fluid osteoprotegerin (OPG) and increased serum ratio of receptor activator of nuclear factor-κB ligand (RANKL) to OPG correlate with disease severity in patients with primary knee osteoarthritis. Clin Biochem 2008; 41: 746-749.