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Detection of miR-22, miR-140 and Bone Morphogenetic Proteins (BMP)-2 Expression Levels in Synovial Fluid of Osteoarthritis Patients Before and After Arthroscopic Debridement

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Background: Osteoarthritis (OA) is a degenerative joint disease often present on the surface and edge of the joint and beneath cartilage forming new bone. Arthroscopy had been used for the treatment of knee OA. This study aimed to measure the expression of miR-22, miR-140, and BMP-2 in patients with OA before and after arthroscopy operation.

Material/Methods: The synovial fluid of 80 patients and 60 healthy volunteers were aspirated using a syringe before OA operation and again six months post-operation in patients with OA. The total RNA was extracted and analyzed by quantitative PCR.

Results: The level of miR-22 was elevated in the progression of OA. The expression of miR-140 level in the synovial fluid was significantly reduced in the patients with OA and was negatively correlated with OA severity compared to controls. Expression of miR-22 and miR-120 returned to normal levels post-operatively. BMP-2 expression was reduced in patients with OA, and returned to normal levels post-operatively. Bioinformatics analysis showed that miR-22 and miR-140 closely target with 3'-UTR of BMP-2 in different positions. The correlation between BMP-2 and miR-22 was negative. The correlation between BMP-2 and miR-140 was positive.

Conclusions: The present study identified a change in miR-22, miR-140, and BMP-2 expression in the synovial fluid of patients with OA before and after arthroscopic debridement. Results provide a novel characterization of the pathogenesis and therefore underlying therapeutic target for OA.

MeSH Keywords: **Bone Morphogenetic Proteins • MicroRNAs • Osteoarthritis**

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Background

Osteoarthritis (OA) is a degenerative joint disease often present on the surface and edge of the joint and beneath cartilage forming new bone [1]. Common clinical presentation is joint pain, tenderness, stiffness, swelling, limited motion, and deformity [2,3]. Arthroscopy is used for the treatment of knee OA to reduce injury and promote rapid recovery. Joint debridement can alleviate symptoms rapidly by irrigation of the joint, removal of algesic substances, and smoothing of cartilage lesion [4], but does not stop the progression of OA [5].

MicroRNAs (miR) are a class of small non-coding RNAs with approximately 19 to 23 nucleotides in length; miRNAs exhibit biological functions by binding to the 3' untranslated region (3'-UTR) of a target gene [6]. Previous studies have investigated the role of upregulated or downregulated miRNAs in the development of OA [7–9]. Iliopoulos et al. found that miR-22 acted as a suppressor gene in cartilage destruction. Overexpression of miR-22 blocked inflammatory and catabolic changes in osteoarthritic chondrocytes [8]. Conversely, miR-140 showed a decrease in chondrocytes and synovial fluid in patients with OA, suggesting its expression was negatively correlated with the severity of OA [10].

Bone morphogenetic proteins (BMP) are a significant growth factor promoting osteogenesis [11]. Some studies have suggested that BMP-2 may accelerate the bone repair process in some diseases such as bone grafts and spinal injuries [12,13]. MiR-22 has been shown to inhibit the progression of OA by regulating BMP-7 [8], however, the role of BMP-2 in OA is still unclear.

The present study investigated the progression of OA by examining miR-22, miR-140, and BMP-2 expression in patients with OA before and after arthroscopy.

Material and Methods

Study participants

Informed consent was obtained from all participants who voluntarily joined the study. The Medical Ethics Committee of Tianjin Fifth Central Hospital approved the study methodology. Study participants were: 80 patients with knee OA and 60 healthy volunteers. The patients were diagnosed with knee OA in accordance with diagnostic criteria recommended by the American College of Rheumatology, and were classified with reference to the Kellgren and Lawrence scoring system.

Indications for surgery

Indications for surgery included: complaints of knee pain that was worse when walking up or down, or when squatting; poor

outcome with conservative treatment; tenderness in the joint space and the margin of patellar; limited mobility of patella; positive patella grinding and quadriceps femur retraction tests; articular cartilage degeneration or denudation; free bodies in the joint cavity or meniscus injury; locking; swelling; malignant joint effusion; artificial joint replacement was not indicated; severe valgus or varus deformity; and flexion contracture.

Surgical operation

The patients were supine, and a tourniquet was routinely used as a precautionary measure. An anterolateral portal was established followed by an anteromedial portal to explore suprapatellar bursa, patellofemoral joint, medial and lateral sulcus, lateral tibiofemoral joint compartment, intercondylar fossa, and the medial tibiofemoral joint compartment and detect pathological change in the knee joint. Meniscus tears and damaged chondral areas were repaired. Free bodies and floating articular cartilage were removed, and hyperplasia synovium and cartilage debris were chipped. The articular cavity was irrigated using normal saline to remove algesic substances and any small bone fragments. Dressing and cold packs were applied to stop bleeding and relieve swelling.

Synovial fluid collection

Under sterile conditions, synovial fluid samples from all participants were collected by aspiration using a syringe before the operation and again six months post-operatively. The samples were transported immediately to the laboratory on ice and centrifuged at 4,000 rpm for five minutes at room temperature. The supernatant was stored at -80°C for further analysis.

Real time quantitative PCR analysis

Total RNA was extracted from synovial fluids using TRIzol reagent (Invitrogen, Shanghai, China) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using a reverse transcription kit (Takara Biotechnology, Dalian, China). Relative quantity of cDNA was analyzed by quantitative PCR with SYBR green and the DDCT method. β -actin was used as the internal control gene to normalize the target genes. All primers were designed and synthesized by GenePharma (Shanghai, China) and shown as follows:

miR-140, CAGUGGUUUUACCCUUGGUAG;

miR-22, AAGCUGCCAGUUGAAGAACUGU;

BMP2, GGGACCCGCTGTCTTAGT and

TCAACTCAAATTCGCTGAGGA;

β -actin, CACGATGGAGGGCCGGACTCATC and

TAAAGACCTCTATGCCAACACAGT.

Relative levels of gene expression were expressed relative to β -actin and calculated using the $2^{-\Delta\Delta\text{Ct}}$ method.

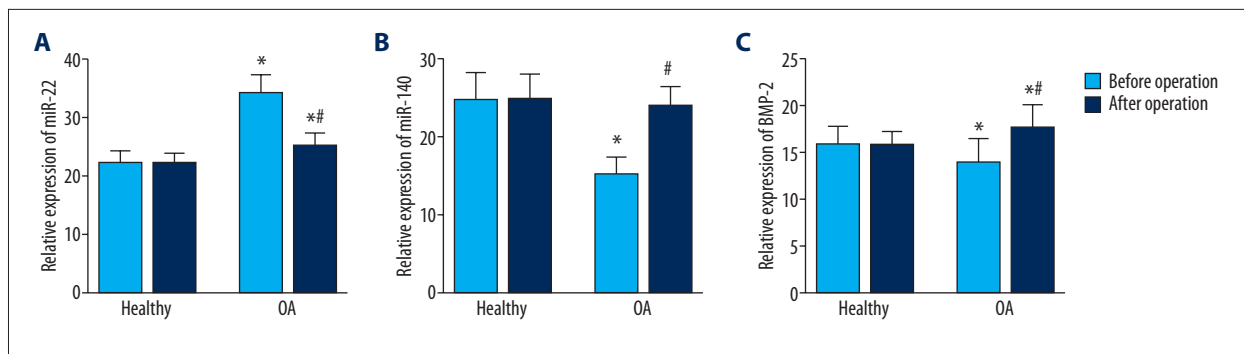


Figure 1. The expression level of miR-22, miR-140, and BMP-2 in OA patients after their operation. (A) The expression level of miR-22 in the OA group was significantly elevated compared with the control group; these findings were reversed following surgical treatment ($p < 0.05$). (B) The expression level of miR-140 in the OA group was significantly lower compared with the control group; these findings were reversed following surgical treatment ($p < 0.05$). (C) The expression level of BMP-2 in the OA group was significantly reducing compared with the control group; these findings were reversed following surgical treatment ($p < 0.05$).

Statistical analysis

All quantitative values are presented as the mean \pm SD. The Student's *t*-test and ANOVA were performed between different groups. All calculations were performed using SPSS 17.0 software (IBM Software, Chicago, IL, USA) and GraphPad (version 6.0, USA). A value of $p < 0.05$ indicated a statistically significant difference.

Result

Expression level of miR-22, miR-140, and BMP-2 in OA patients following surgical treatment

The synovial fluids obtained before and after surgery were used for qPCR. Results showed the expression of miR-22 was significantly increased in patients compared to controls and this expression was significantly decreased post-operatively (Figure 1A; $p < 0.05$). The level of miR-140 was significantly downregulated compared to healthy controls and this was reversed following the operation (Figure 1B; $p < 0.05$). The level of BMP-2 was reduced in patients with OA, this result was also reversed following the operation (Figure 1C; $p < 0.05$).

Level of miR-22, miR-140, and BMP-2 in sub-groups of patients following surgical treatment

Eighty patients (male, 32; female, 48) and 60 healthy volunteers (male, 23; female, 37) were enrolled in the study. Because sex is known to significantly influence the progression of OA, the expression of miR-22, miR-140, and BMP-2 was examined in sub-groups of patients with OA. In male patients, the over-expression of miR-22 was reversed after receiving surgical treatment (Figure 2A; $p < 0.05$). The downregulation of miR-140 and

BMP-2 level increased significantly (Figure 2B, 2C). Similar outcomes were observed in female patients (Figure 2D–2F; $p < 0.05$).

Level of miR-22, miR-140, and BMP-2 in different OA grades of patients receiving surgical treatment

Patients with OA were classified into four grades according to the Kellgren and Lawrence scoring system; the expression of miR-22, miR-140, and BMP-2 was also evaluated (Table 1). The results showed that miR-22 expression was significantly, positively correlated with higher OA scores and expression of miR-140 and BMP-2 were negatively correlated with OA scores. These parameters were reversed significantly following surgical treatment.

MiR-22 and miR-140 participated in the progression of OA by regulating 3'-UTR of BMP-2

Bioinformatics analysis revealed 3'-UTR of BMP-2 had high affinity to bind with miR-22 and miR-140 in different positions, respectively (Figure 3A, 3B). The results indicated that BMP-2 levels in patients with OA were negatively correlated with the concentrations of miR-22 ($r = -0.786$, $p < 0.05$) and positively correlated with the concentration of miR-140 ($r = 0.659$, $p < 0.05$). See Figure 3C and 3D.

Discussion

OA is the most prevalent type of arthritis resulting from the breakdown of joint cartilage and underlying bone [14,15]. The most common symptoms are joint pain and stiffness [16]. In early stages of OA, patients can be treated non-surgically with lifestyle modification and analgesics [17–19]. Once conservative treatments fail, arthroscopic surgery is typically

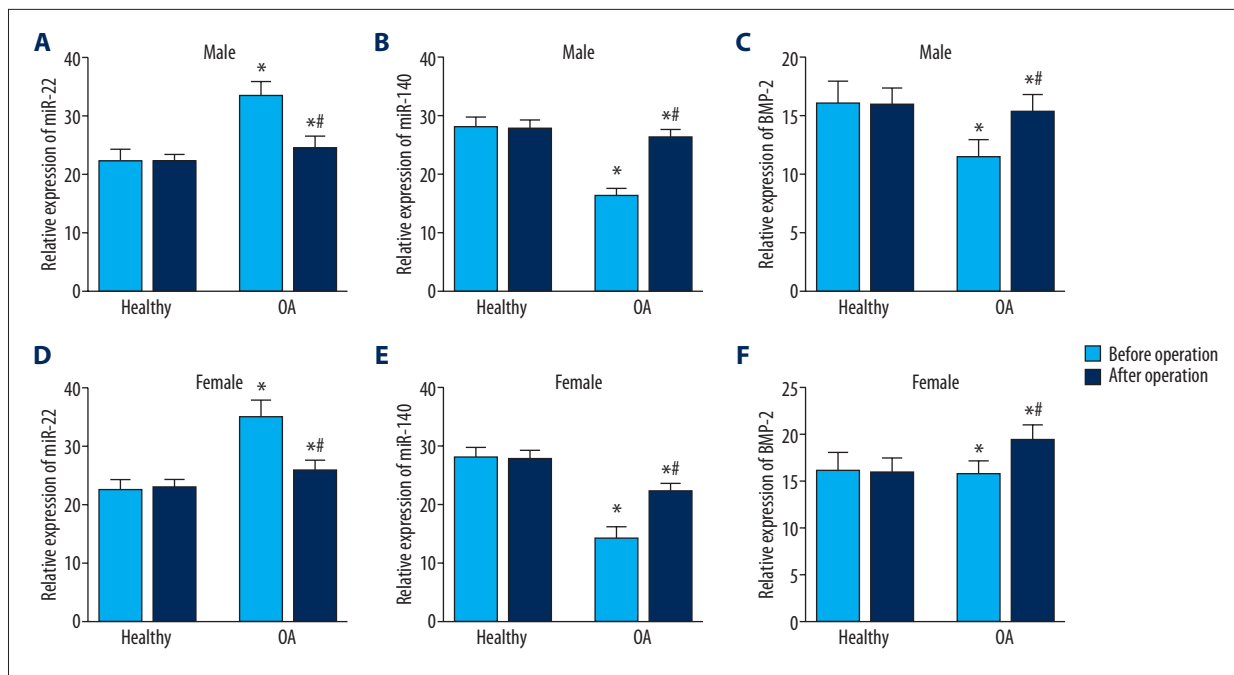


Figure 2. The level of miR-22, miR-140, and BMP-2 in sub-groups of OA patients following their operation for males and females. For males: (A) miR-22 expression in OA patients was upregulated; following surgical treatment it was downregulated ($p < 0.05$); (B) miR-140 expression in OA patients was upregulated; which was downregulated following surgical treatment ($p < 0.05$); (C) BMP-2 expression in OA was upregulated, which was downregulated following surgical treatment ($p < 0.05$). For females: (D) miR-22 expression in OA was upregulated, which was downregulated following surgical treatment ($p < 0.05$); (E) miR-140 expression in OA was upregulated, which was downregulated following surgical treatment ($p < 0.05$); (F) BMP-2 expression in OA was upregulated, which was downregulated following surgical treatment ($p < 0.05$).

Table 1. miRNA-22, miRNA-140 and BMP-2 expressions levels in control and patients groups according to grade of knee osteoarthritis.

Group		miR-22		miR-140		BMP-2	
		Before operation	After operation	Before operation	After operation	Before operation	After operation
OA patients (n=80)	Healthy control (n=60) Grade 0 (n=60)	22.27±1.96	22.25 ±1.69	27.94±1.72	27.92 ±1.55	16.12±1.89	15.86 ±1.48
	Grade 1 (n=9)	30.58±0.62*	22.10 ±1.16	17.90±0.49*	24.08 ±2.54*	17.33±0.36*	20.16 ±0.65*
	Grade 2 (n=23)	32.03±0.44*	23.97±1.77	16.88±0.35*	25.14 ±2.12*	16.30±0.49*	19.18 ±2.01*
	Grade 3 (n=35)	34.06 ±1.03*	25.95 ±1.47*	14.60 ±1.25*	23.70 ±2.63*	13.54±1.31*	17.03 ±2.49*
	Grade 4 (n=13)	38.86±2.56*	26.47±2.09*	12.00±0.45*	21.60 ±0.92*	10.49±0.71*	15.90±2.14

Mean ±S.D. values are shown (* $P < 0.05$).

offered [20]. Joint debridement can relieve pain symptoms and improve range of motion in the knee joint [21]. This procedure provides temporary relief but does not stop the progression of this disease [22].

The development of OA has been shown to be influenced by miRNAs affecting 3'-UTR of target genes. The level of miR-22 has been shown to be elevated in the progression of OA and its inhibition has been shown to prevent inflammatory activity [8,23]. Compared with normal synovial fluid, the expression of miR-140 has been shown to be significantly reduced

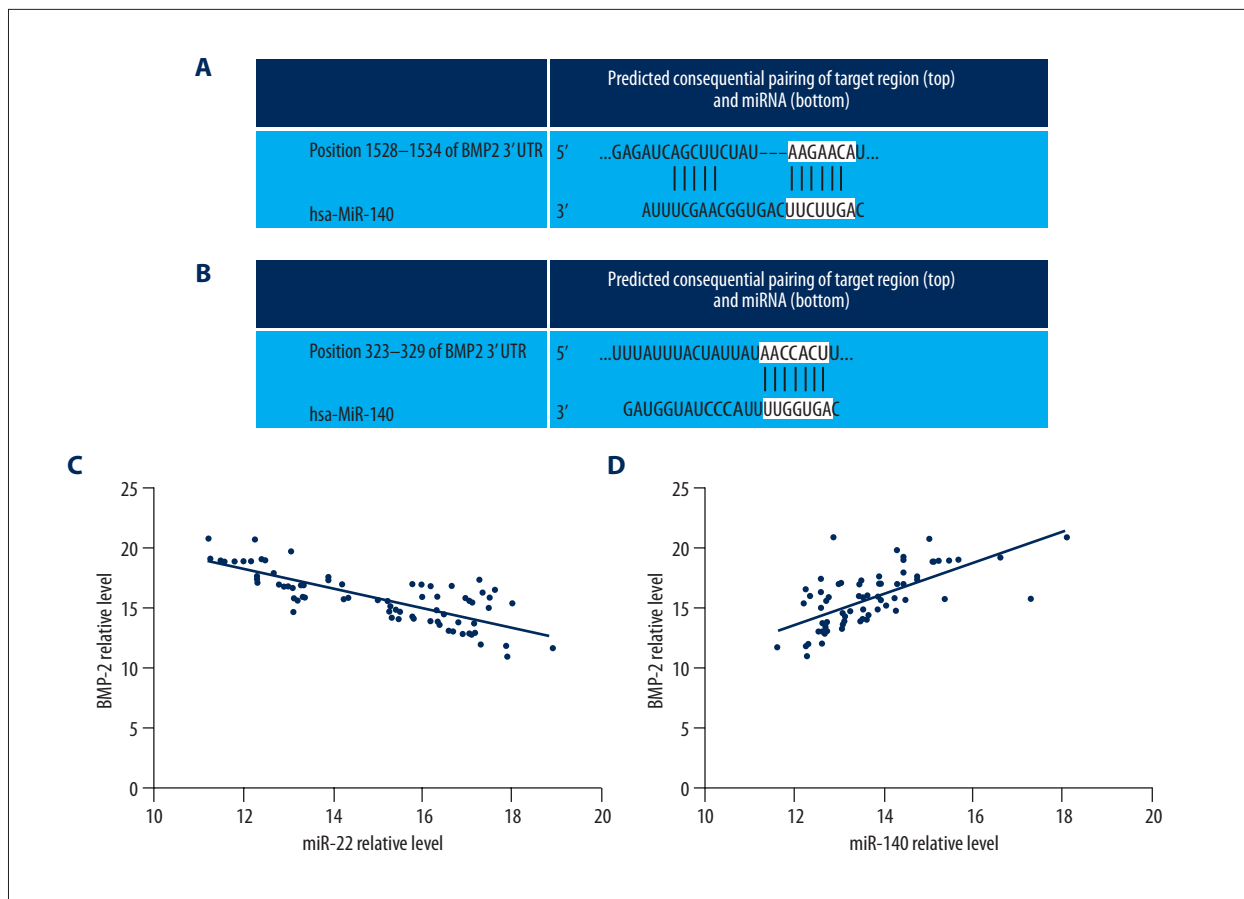


Figure 3. miR-22 and miR-140 participated in the progression of OA by regulating 3'-UTR of BMP-2. Using bioinformatics analysis, 3' UTR of BMP-2 was found to be highly conserved to bind with miR-22 and miR-140 in the different positions. **(A, B)** The 3'-UTR binding sites are shown. **(C)** Correlations between BMP-2 levels and miR-22 in OA patients ($r=-0.786$, $p<0.05$). **(D)** Correlations between BMP-2 levels and miR-140 in OA patients ($r=0.659$, $p<0.05$).

in patients with OA and negatively correlated with the severity of OA [10]. In our study, the results of miRNA expression were nearly reversed post-operatively.

BMPs belong to the transforming growth factor β super family [24] and are best known for their role as bone formation signals. BMP-2 has an essential role in bone regeneration and bones lacking BMP-2 have obvious micro-fracture [25]. In our study, results revealed that BMP-2 expression was reduced in patients with OA, which was reversed after surgical treatment. Utilizing bioinformatics analysis, our study showed that miR-22 and miR-140 closely target with 3'-UTR of BMP-2 in different positions. The correlation between BMP-2 and miR-22 was negative. The correlation between BMP-2 and miR-140 was positive. These data suggest miR-22 and miR-140 play a role in the development of OA by regulating BMP-2.

Conclusions

The present study revealed changes in miR-22, miR-140, and BMP-2 expression in synovial fluid of patients with OA evaluated before and after arthroscopic debridement, thus providing a novel characterization of the pathogenesis and therefore underlying therapeutic target for OA.

Conflict of interest

None.

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