European Review for Medical and Pharmacological Sciences 2019; 23: 7247-7255

MiR-373 promotes the osteogenic differentiation of BMSCs from the estrogen deficiency induced osteoporosis

L.-Y. LI[1], X.-L. WANG[2], G.-S. WANG[3], H.-Y. ZHAO[4]

[1]Anesthesia Operating Room, Wuwei People's Hospital, Wuwei, P.R. China
[2]Department of Thoracic Surgery, Wuwei People's Hospital, Wuwei, P.R. China
[3]Department of Orthopedics, Wuwei People's Hospital, Wuwei, P.R. China
[4]Department of Orthopedics, The First Hospital of Lanzhou University, Lanzhou, P.R. China

Abstract. – OBJECTIVE: This study aimed to investigate the expression of miR-373 in osteoporosis patients and rat models induced by estrogen deficiency and to detect whether miR-373 can regulate the ability of osteogenic differentiation of bone marrow mesenchymal stem cells in the osteoporosis microenvironment caused by estrogen deficiency.

PATIENTS AND METHODS: Bone tissues and blood samples were collected from 20 osteoporotic patients and 20 controls. PCR analysis was used to detect the expression of miR-373 in bone tissue and serum from postmenopausal osteoporotic patients and normal patients. 120 SD rats were purchased and randomly divided into sham operation group and OVX group. Rat models of sham-operated and bilateral oophorectomy mice models were constructed. The expression of miR-373 in bone tissue, cells, and serum of the mice was tested. Then, bone marrow mesenchymal stem cells from sham-operated rats and bilaterally ovariectomized rats were isolated and cultured. After 10 days of osteogenic induction, alkaline phosphatase staining and alizarin red staining were performed to test the osteogenic differentiation ability of bone marrow mesenchymal stem cells, and whether miR-373 affects this ability.

RESULTS: PCR results showed that the expression of miR-373 in the bone tissue and the serum of patients with postmenopausal osteoporosis was significantly reduced. The expression of miR-373 was markedly decreased in the bone tissue, cells, and serum from the rats of bilateral ovariectomy group. Alkaline phosphatase staining and alizarin red staining showed that miR-373 could promote the differentiation of bone marrow mesenchymal stem cells into osteoblasts and reverse the decreased osteogenic differentiation of bone marrow mesenchymal stem cells caused by osteoporosis.

CONCLUSIONS: The expression of miR-373 is decreased in osteoporotic patients and rat models caused by estrogen deficiency, and it can promote the differentiation of bone marrow mesenchymal stem cells into the osteogenic direction. This work provides a new direction and experimental basis for clinical diagnosis and treatment of osteoporosis.

Key Words:
MiRNA, Osteoporosis, Bone marrow mesenchymal stem cells, Osteogenic differentiation.

Introduction

Osteoporosis (OP) is a systemic metabolic disease with reduced bone density, impaired bone microstructure, reduced bone mass, decreased bone strength, and increased bone fragility and resolution[1,2]. According to epidemiological statistics, there are up to 100 million people over the age of 60 in China. With the aging of the population, the incidence of osteoporosis is increasing and it has become a serious public health problem in China[3]. As women age, especially in postmenopausal women, estrogen levels are significantly reduced, osteoblast dysfunction and massive bone loss occur, bone remodeling is impaired, resulting in bone resorption greater than bone formation and eventually osteoporosis[4]. Osteoblasts mediate bone formation in the bone marrow microenvironment and thus play an important role in bone development[5,6]. Osteoblast viability and proliferative capacity are significantly reduced in postmenopausal osteoporosis patients, which in turn affects bone formation. Most of the osteoblasts in the human bone microenvironment are derived from bone marrow mesenchymal stem cells (BMSCs). Since BMSCs have the advantages of multidirectional differentiation, easy access, easy proliferation, and small immune rejection, it is used for bone reconstruction and bone repair and should be a new method for the treatment of osteoporosis[7,8].

Corresponding Author: Gengshen Wang, MM; e-mail: dreamer1980@126.com

MicroRNAs (miRNAs) are a class of non-coding RNAs composed of approximately 22 nucleotides present in all eukaryotic cells with tissue-specific and developmental stage specificity[9,10]. miRNAs specifically bind to their target mRNA, degrade or inhibit their translation, thereby regulating the expressions of genes, and ultimately play an important regulatory role in cell proliferation, differentiation, apoptosis, individual growth, development, as well as the occurrence and development of diseases[11,12]. Some reports[13-15] showed that miR-373 can participate in the occurrence and development of cancer, liver, kidney, and other organ diseases. In addition, Liu et al[16] found that miR-373 can promote osteosarcoma cell survival and invasion by activating the PI3K/AKT pathway. Huang et al[17] found that miR-373 can target the trichorhinophalangeal syndrome 1 gene (TRPS1) and play an important role in bone development and formation. Palmieri et al[18] revealed that miR-373 had a certain relationship with the translation process of bone formation in osteoblasts. However, no researches have shown the relation between miR-373 and osteoporosis or osteogenic differentiation of bone marrow mesenchymal stem cells.

This investigation collected bone tissue and serum from normal bone and postmenopausal osteoporosis patients to detect the expression of miR-373. The results were verified in bone tissue and serum of normal mice and ovariectomy-induced osteoporosis rats. The effect of miR-373 on osteogenic differentiation of bone marrow mesenchymal stem cells was also examined to find key miRNAs that affect osteoporosis caused by estrogen deficiency, providing a new direction for clinical diagnosis and treatment of osteoporosis.

Patients and Methods

Collection of Clinical Bone Tissue and Serum Samples

Bone tissue of 20 patients who were diagnosed with postmenopausal osteoporosis in Wuwei People's Hospital from January 2017 to October 2017 (microCT detected bone density T-score $\leq$ -2.5) was collected and stored in liquid nitrogen or –80°C ultra-low temperature freezer (Thermo Fisher Scientific, Waltham, MA, USA). The study was approved by the Ethics Committee of Wuwei People's Hospital. 5 mL of the patient's fasting venous blood was taken in the morning and immediately sent to the test or stored in a –80°C refrigerator. The bone tissue and serum of 20 patients with normal bone tissue (microCT detected bone density T-score $\geq$ 1, hospitalized for trauma) were collected from Wuwei People's Hospital.

PCR Detection of MiRNAs and Osteogenic Gene Expression

According to the miRNA extraction kit (Foregene, China) instructions, the miRNA in the sample was extracted, the concentration and purity were determined for future use. RNA, kit components and distilled water were added to a 200 μL Eppendorf (EP) tube (Hamburg, Germany) according to the instructions of the Reverse Transcription Kit (TaKaRa, Otsu, Shiga, Japan), and the total system was 10 μL. The RNA was reversely transcribed into cDNA using a reverse transcription apparatus (Roche, Mannheim, Germany) and stored at –20°C until use. 10 μL of SYBR Green (Norvezan, China), 7 μL of distilled water, 10 nmol of the target gene primer (Jima, China) and 1 μL of cDNA were added to the PCR plate, and the total system was 20 μL. The reaction was carried out on a PCR machine (Roche, Mannheim, Germany) under the following conditions: 95°C for 30 s, 95°C for 5 s, 60°C for 30 s, 72°C for 30 s for 40 cycles; 72°C for 30 s. Using U6 as an internal reference, the expression level of miRNAs was quantified according to the $2^{-\Delta\Delta Ct}$ method. The sequences of all primers in this experiment are shown in Table I.

Construction of a Rat Model of Osteoporosis Caused by Estrogen Deficiency and Collection of Bone Tissue and Serum

120 SD rats (12 weeks, 230 to 270 g) were purchased and randomly divided into 2 groups, with 60 in each group. Ovariectomy is currently recognized as a method for constructing a rat model of osteoporosis caused by estrogen deficiency. One week after the animals were acclimated to the environment, OVX group (ovariectomy group) underwent bilateral ovariectomy. Sham group (sham operation group) underwent the same operation as OVX group, except for the ovaries which were not removed. The feeding environment was the same in both groups except for the difference in the operation. After all, rats were fed for 8 weeks, microCTL was performed to measure bone mineral density indicators such as the number of trabecular bone (Tb.N), bone separation (Tb.SP), bone mineral density (BMD), and structural model index (SMI) of the two groups to determine whether the model was successful. In addition, SD rats in the Sham and OVX groups were

Table I. The sequences of primers.

Gene	Forward	Reverse
Human U6	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTCAT
Human miR-146a	CAGTGCGTGTCGTGGAGT	GGGTGAGAACTGAATTCCA
Human miR-142	GTCACCTGTAGTGTTTCCTACTT	TATGGTTGTTCTGCTCTCTGTCTC
Human miR-28	AAGGAGCUCACAGUCUAUUGAG	CAAUAAGACUGUGAGCUCCUUU
Human miR-373	CGCGCGAAGTGCTTCGATTT	GTGCAGGGTCCGAGGT
Human miR-101	TGGGCTACAGTACTGTGATA	TGCGTGTCGTGGAGTC
Mouse miR-373	GTGCTTCGATTTTGGGG	GTGCAGGGTCCGAGGT
Mouse ALP	ACAACCTGACTGACCCTTCG	TCATGATGTCCGTGGTCAAT
Mouse Runx2	AGAAGGCACAGACAGAAGCTTGA	AGGAATGCGCCCTAAATCACT
Mouse BMP2	CCTTGCTGACCACCTGAACT	AACATGGAGATTGCGCTGA
Mouse BMP4	TCGTTACCTCAAGGGAGTGG	ATGCTTGGGACTACGTTTGG
Mouse GAPDH	CATCACTGCCACCCAGAAGAC	CCAGTGAGCTTCCCGTTCAG

intraperitoneally injected with phenobarbital (Sigma-Aldrich, St. Louis, MO, USA) for anesthesia, and blood was taken from the orbit. The eyeballs were removed using ophthalmic forceps and the whole blood was collected using a 1.5 mL EP tube, and centrifuged at 12,000 r/min for 10 min under low-temperature conditions. Next, the serum was separated and stored in a –80°C refrigerator. At the same time, the femur and tibia were separated, the muscle tissue was scraped, and the bone tissue was stored in a refrigerator at –80°C.

Rat BMSCs Culture

SD rats in the Sham and OVX groups were sacrificed by cervical dislocation, and the femur and tibia were separated in a sterile environment after cervical dislocation. The femur and tibia were scraped with sterile ophthalmic scissors and forceps to exfoliate other tissues. The femur and tibia were repeatedly washed with the cell culture medium in a clean bench, and the culture solution was collected with a sterile ampoule. Then, the culture solution was transferred to a cell bottle (Nest, Rahway, NJ, USA) by a pipette (Hyclone, South Logan, UT, USA) and placed in an incubator at 37°C and 5% CO_2. After 24 h, the fresh medium was replaced to remove unattached cells. The culture was continued for about a week. When the cell confluence reached 80% to 90%, the cells were digested with trypsin digestive solution (Yantian, China) and passaged. The cells were passed to the third generation for subsequent investigations.

Rat BMSCs Transfection

When the cell density reached 40% to 50%, transfection reagent and serum-free Opti medium (Hyclone, South Logan, UT, USA) were used for cell transfection and were grouped into miR-373 mimics group and negative control (NC) group, respectively. The final concentration of miR-373 mimics and its NC was 50 nM. Fresh culture medium was replaced after 6 h of transfection to maintain normal cell growth.

Osteogenic Differentiation of Rat BMSCs

After 24 h of cell transfection, the osteogenic induction solution (Saiye Company, China) was added for differentiation, and the fresh medium was replaced every three days. After 10 days of differentiation, subsequent investigations were performed.

ALP Staining and ARS Staining

After 10 days of osteogenic differentiation of BMSCs, it was gently washed 3 times with phosphate-buffered saline (PBS), fixed in 500 μL of paraformaldehyde for 30 min, and alkaline phosphatase (ALP) staining solution and alizarin red (ARS) staining solution were added and incubated at 37°C. ALP staining solution (Nanjing Jiancheng Bioengineering Institute, China) and ARS staining solution (Cyagen, China) were incubated for 1 h and 30 min, respectively. After washing the impurities with PBS, the plates were placed under an inverted optical microscope and photographed. The staining area and color depth of BMSCs in Sham and OVX groups after osteogenic induction were compared, the larger the staining area and the darker the color, the stronger the osteogenic differentiation ability, and the smaller the staining area and the lighter the color, the weaker the ability of osteogenic differentiation.

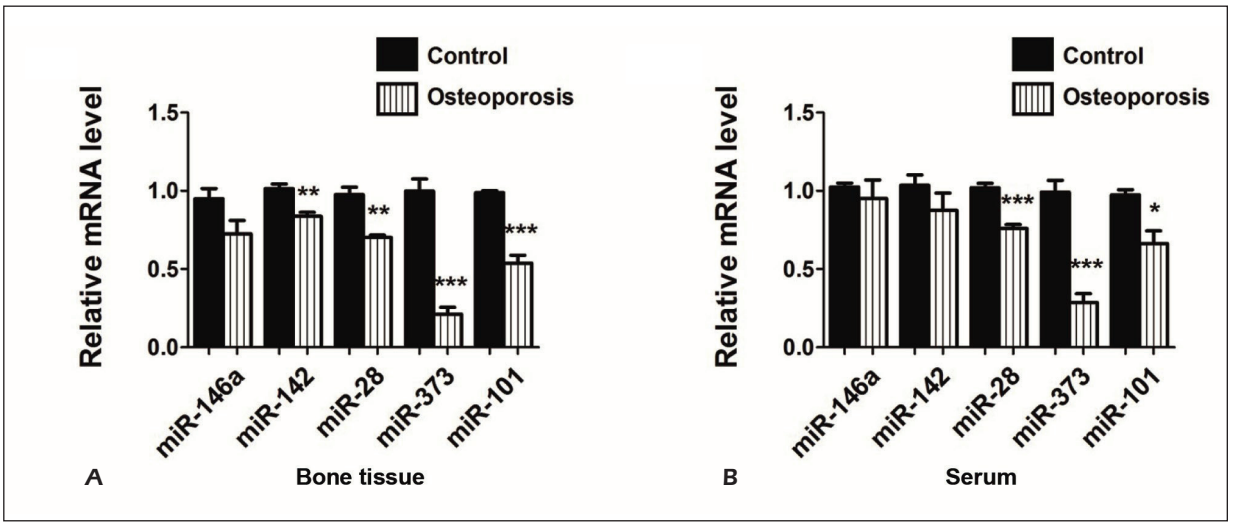

Figure 1. Expression of five miRNAs in bone tissue and serum of normal and postmenopausal osteoporosis patients. ***A,*** Expression of miRNAs in bone tissue of two groups of patients. ***B,*** expression of miRNAs in serum of two groups of patients.

Statistical Analysis

All data were statistically processed using GraphPad software (La Jolla, CA, USA) and analyzed for statistical significance, $*p< 0.05$, $**p< 0.01$, and $***p<0.001$, which were statistically significant.

Results

Detection of Expression of MiRNAs in Clinical Bone Tissue and Serum

PCR results showed that the expressions of miR-146a, miR-142, miR-28, miR-373, and miR-101 in the bone tissue of postmenopausal osteoporosis patients were decreased compared with normal group (Figure 1A). As shown in Figure 1B, the expressions of miR-146a and miR-142 in the serum of postmenopausal osteoporosis patients were unchanged compared with control group, and the expressions of miR-28, miR-373, and miR-101 were decreased.

Detection of MiR-373 Expression in Bone Tissue, Serum, and Cells of Sham and OVX Rats

Compared with Sham group, the expression of miR-373 was significantly decreased in OVX group, and the difference was statistically significant (Figure 2A). In addition, the expression of serum miR-373 in Sham and OVX groups is shown in Figure 2B, and the expression of miR-373 in

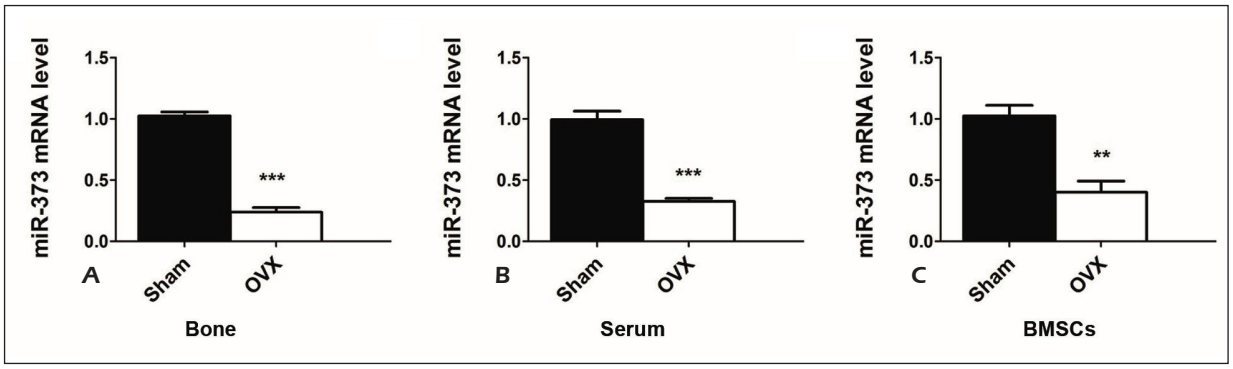

Figure 2. Detection of miR-373 expression levels in femur, serum and cells of Sham and OVX rats. The results indicate that miR-373 expression is significantly reduced in OVX rats, and the results are consistent with the trend of Figure 1. ***A,*** Expression of miR-373 in bone tissue of two groups of rats. ***B,*** Expression of miR-373 in serum of two groups of rats. ***C,*** Expression of miR-373 in BMSCs of two groups of rats.

OVX was significantly lower than that in Sham group. The expression of miR-373 in Sham and OVX cells was significantly different, the expression was significantly decreased in OVX, and the difference was statistically significant (Figure 2C).

Detection of Bone Microstructural Indexes of the Two Groups of Rats

MicroCT results showed that the number of trabecular bone (Tb.N) and bone mineral density (BMD) of OVX rats was significantly lower than that of Sham group, and the structural model index (SMI), Bone resolution (Tb.SP) was significantly increased than that of Sham group (Figures 3A-3D).

Detection of the Expression of Osteogenic Related Genes in Bone Tissue of the Two Groups

PCR results showed that the expressions of osteogenic related genes ALP, Runx2, BMP2, and BMP4 in OVX rats were significantly lower than that in Sham group (Figures 4A-4D).

ALP Staining and ARS Staining

ALP results showed that (Figure 5), compared with Sham group, the mineralization of OVX group was significantly shallower, and the area of mineralized nodules decreased, indicating that the osteogenic differentiation ability was weakened. Compared with transfected NC group, the color of the transfected miR-373 mimics group increased and the area of mineralized nodules increased, indicating that the osteogenic differentiation ability of cells transfected with miR-373 mimics increased. As shown by ARS staining in Figure 5, compared with Sham group, the orange-red color of the OVX group was significantly lighter, and the calcium deposition was significantly decreased, while the transfection of miR-373 mimics significantly promoted the osteogenic differentiation of OVX rats. The above results showed that compared with Sham group, the osteogenic differentiation ability of OVX group was decreased, and miR-373 mimics promoted the osteogenic differentiation of BMSCs.

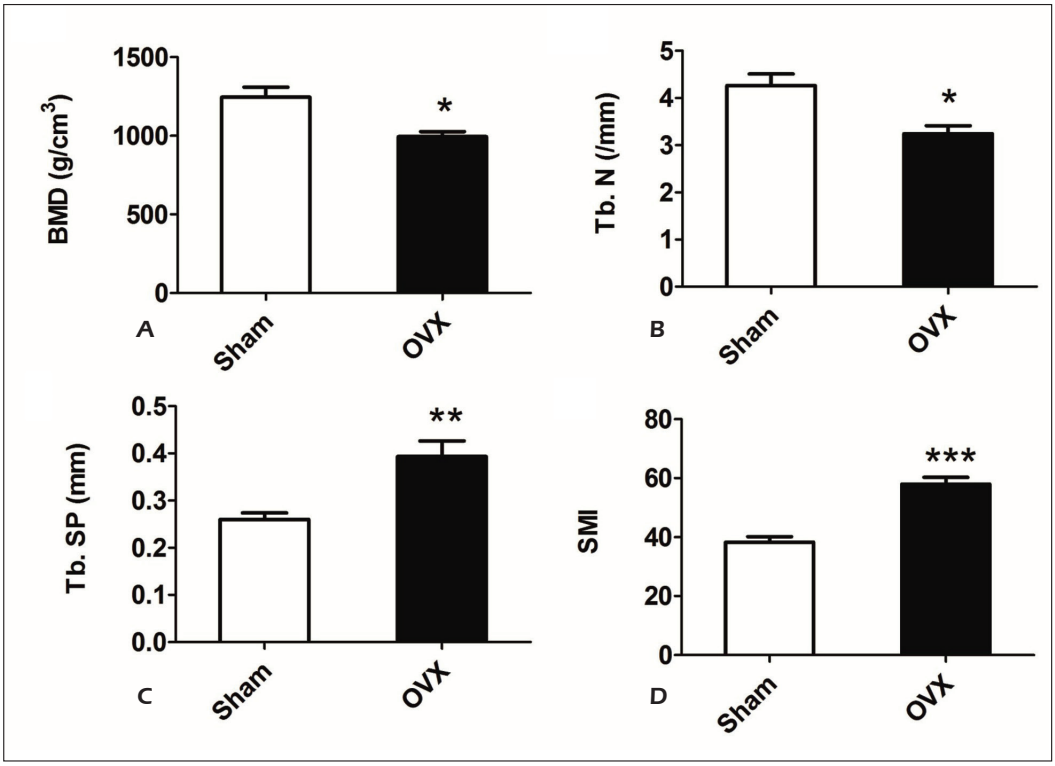

Figure 3. Bone microstructure indicators of Sham and OVX rats. ***A,*** Comparison of bone mineral density (BMD) between the two groups of rats. ***B,*** Comparison of trabecular bone number (Tb. N) between the two groups of rats. ***C,*** Comparison of trabecular bone separation (Tb. SP) between the two groups of rats. ***D,*** Comparison of structural model indices (SMI) between the two groups of rats.

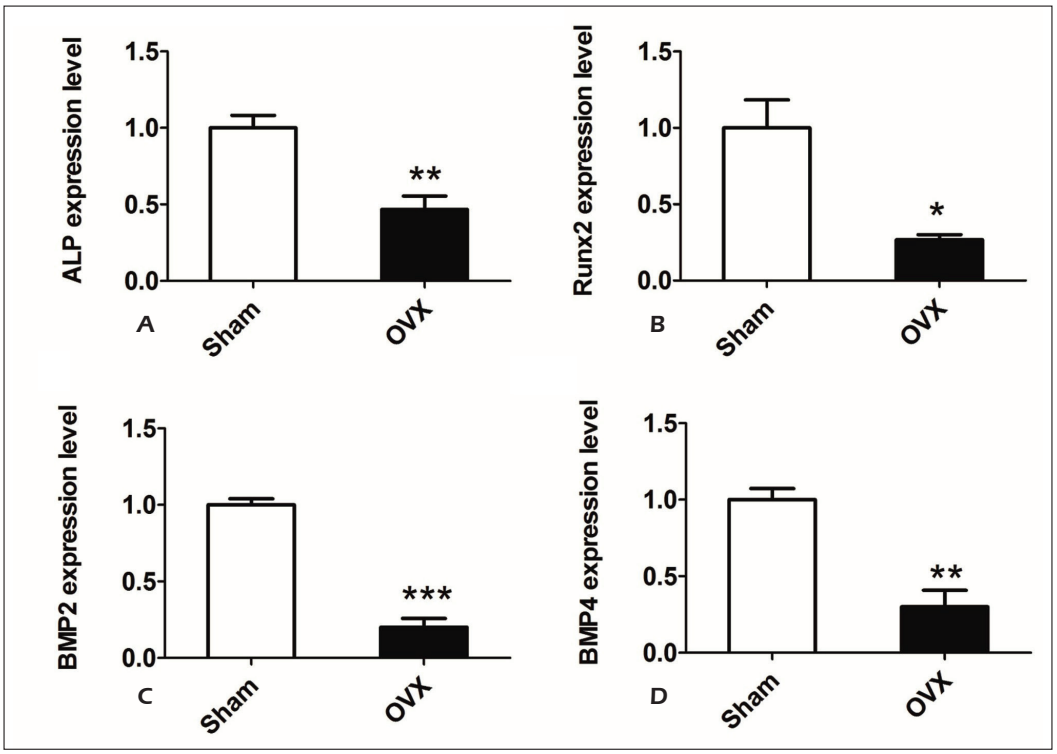

Figure 4. Osteogenesis-related gene expression in bone tissue of Sham and OVX rats. ***A,*** expression of ALP in bone tissue of two groups of rats. ***B,*** expression of Runx2 in bone tissue of two groups of rats. ***C,*** expression of BMP2 in bone tissue of two groups of rats. ***D,*** expression of BMP4 in bone tissues of two groups of rats.

Detection of Osteogenesis-Related Gene Expression

PCR results (Figure 6) showed that compared with Sham group, the expressions of the bone-related genes ALP, Runx2, BMP2, and BMP4 were significantly decreased compared with Sham group, indicating that the osteogenic differentiation ability was weakened. Compared with transfected NC group, the expressions of bone-related genes ALP, Runx2, BMP2, and BMP4 were significantly increased in transfected miR-373 mimics, indicating that the osteogenic differentiation ability of cells after transfection with miR-373 mimics was increased. The above results showed that compared with Sham group, the osteogenic differentiation ability of OVX group was decreased, and miR-373 mimics promoted the differentiation of BMSCs into osteoblasts.

Discussion

We found that miR-373 was significantly reduced in clinical postmenopausal osteoporosis patients. Subsequent reports showed that miR-373 was also down-regulated in estrogen-deficient rats with osteoporosis. Also, overexpression of miR-373 significantly promoted the osteogenic differentiation of bone marrow interstitial stem cells, and miR-373 can reverse the osteogenic differentiation of bone marrow mesenchymal stem cells caused by osteoporosis. Therefore, we believe that miR-373 can be an important target for the treatment of postmenopausal osteoporosis, and has important clinical significance.

Guo et al[19] and Zhang et al[20] found that miR-373 can reduce the radiosensitivity of lung cancer cells by targeting TIMP2. MiR-373 regulates inflammatory cytokine-mediated chondrocyte proliferation by acting on the P2X7 receptor, thereby participating in the occurrence and development of osteoarthritis. However, no works have reported whether miR-373 is associated with the development and progression of postmenopausal osteoporosis. In this investigation, we examined the expressions of five miRNAs in postmenopausal osteoporosis patients. The results showed that the expression of miR-373 in bone tissue and serum of postmenopausal osteoporosis patients was significantly decreased, the difference was statistically

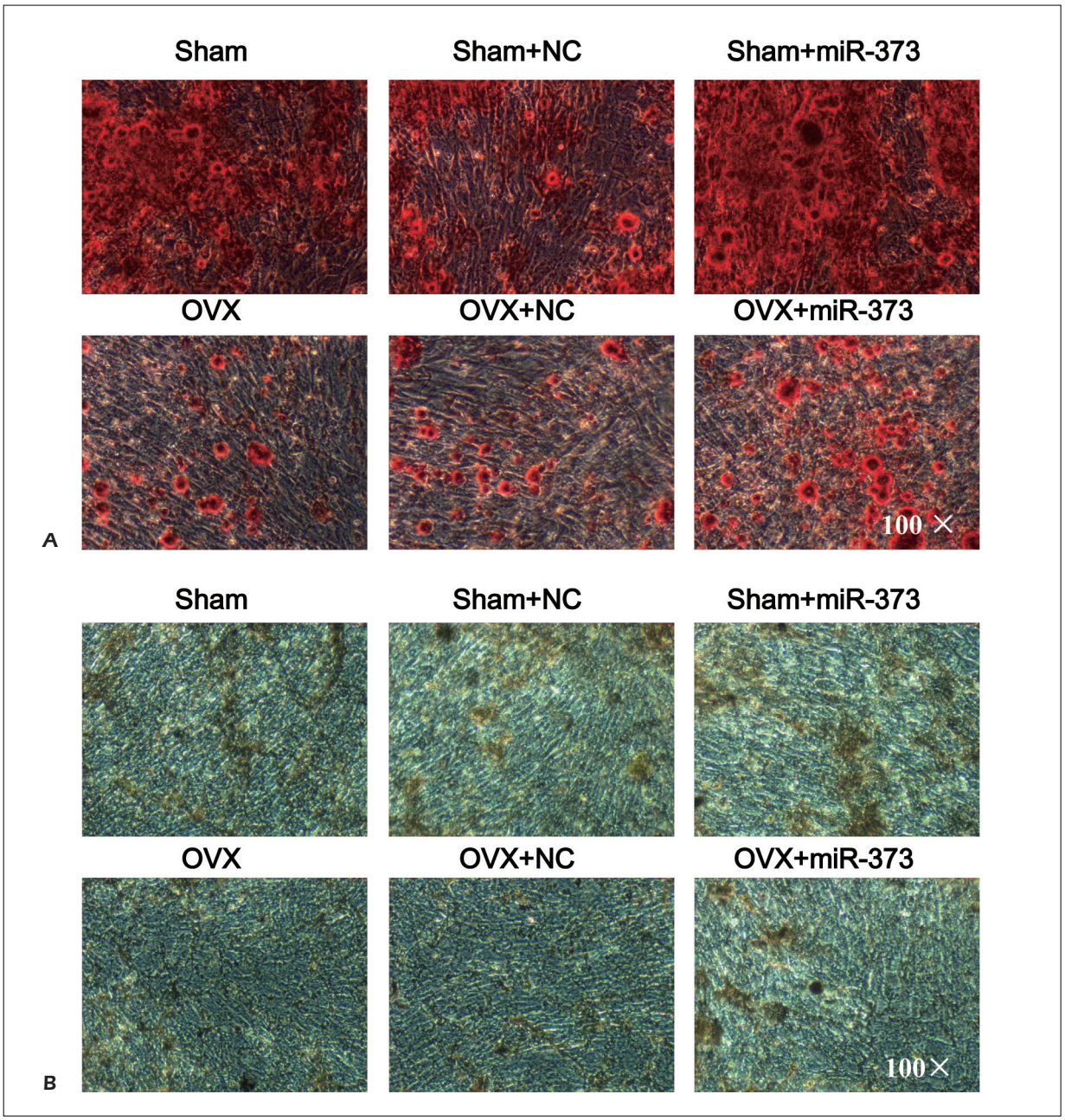

Figure 5. Compared with the Sham group, the osteogenic differentiation ability of the OVX group was weakened, and miR-373 mimics could promote the osteogenic differentiation of BMSCs in Sham and OVX rats. ***A,*** Effect of overexpression of miR-373 on the osteogenic differentiation of BMSCs in the two groups was detected by ARS (magnification 100x). ***B,*** Effect of overexpression of miR-373 on the osteogenic differentiation of BMSCs in the two groups was detected by ALP (magnification 100x).

significant, and the trend was consistent in bone tissue and serum. We suspect that estrogen levels in postmenopausal women with osteoporosis are markedly reduced, which may lead to decreased expression of miR-373. Therefore, miR-373 plays an important regulatory role in bone remodeling, bone development, bone metabolism after estrogen reduction. Therefore, we continue to explore whether miR-373 is involved in the development of osteoporosis caused by estrogen deficiency.

Wang et al[21] found that miR-373 was more conserved in humans and rats. Hence, we explored the expression of miR-373 in a rat model of osteoporosis caused by estrogen deficiency, and the results showed that miR-373 expression was also reduced in a rat model of osteoporosis

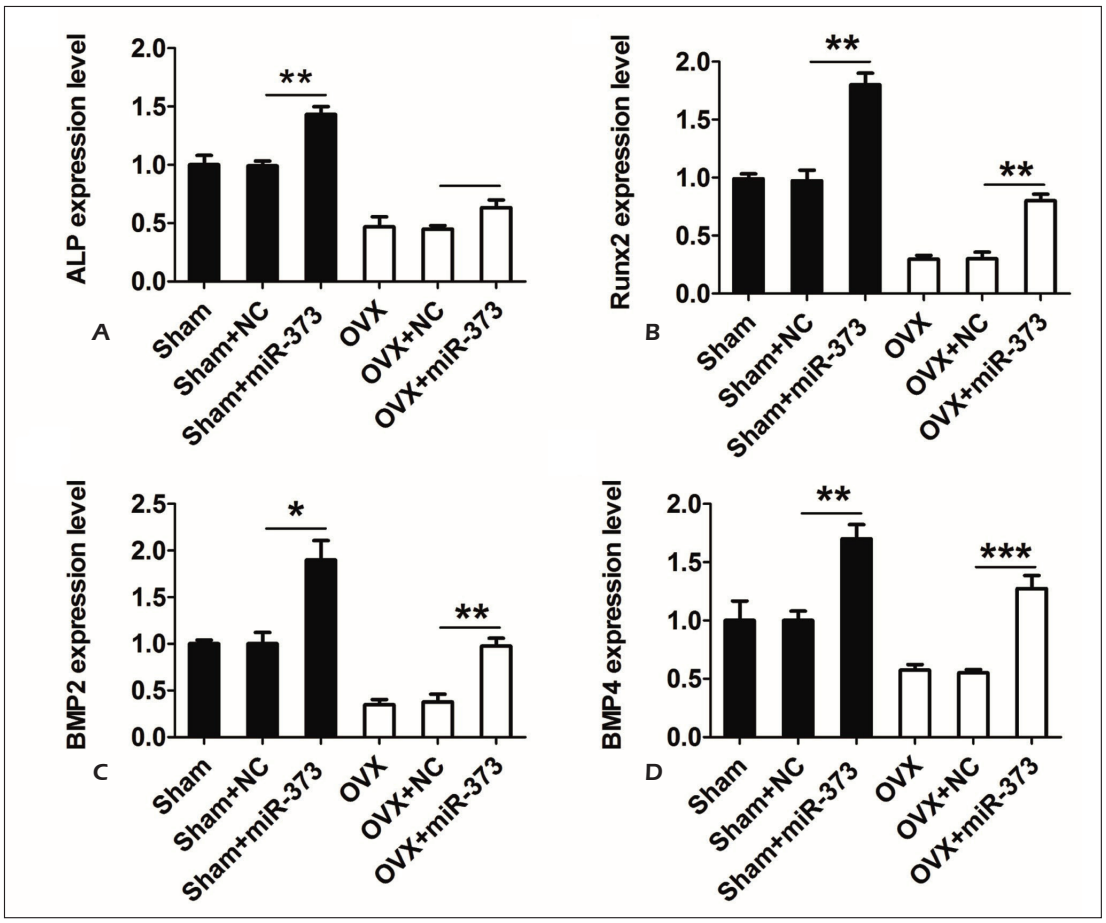

Figure 6. Detection of expressions of osteogenic related genes after administration of miR-373 in Sham and OVX groups. ***A,*** Effect of overexpression of miR-373 on the expression of osteogenic gene ALP in BMSCs of two groups of rats. ***B,*** Effect of overexpression of miR-373 on the expression of osteogenic gene Runx2 in BMSCs of two groups of rats. ***C,*** Effect of overexpression of miR-373 on the expression of osteogenic gene BMP2 in BMSCs of two groups of rats. ***D,*** Effect of overexpression of miR-373 on the expression of osteogenic gene BMP4 in BMSCs of two groups of rats.

caused by estrogen deficiency. Therefore, we observed that miR-373 was down-regulated in osteoporosis caused by estrogen deficiency. Further *in vitro* researches demonstrated that miR-373 can significantly improve the ability of rat bone marrow mesenchymal stem cells to differentiate into osteoblasts. Besides, it can reverse the osteogenic differentiation of bone marrow mesenchymal stem cells due to osteoporosis. The innovation of this work is that miR-373 is reduced in osteoporosis patients and rat models caused by estrogen deficiency. Therefore, miR-373 can be used as an early predictor and diagnosis of osteoporosis caused by estrogen deficiency. This study found that miR-373 can promote the differentiation of bone marrow mesenchymal stem cells into the osteogenic direction, and it can reverse the osteogenic differentiation of bone marrow mesenchymal stem cells caused by osteoporosis. Therefore, miR-373 may be a new method and a new target for clinical treatment of osteoporosis caused by estrogen deficiency and provide a theoretical basis for the clinical application of miRNAs in the treatment of osteoporosis caused by estrogen deficiency.

Conclusions

The expression of miR-373 in osteoporosis caused by estrogen deficiency is reduced. Overexpression of miR-373 can promote the differentiation of bone marrow mesenchymal stem cells into osteoblasts, and can reverse the reduced osteogenic differentiation ability of bone marrow mesenchymal stem cells due to osteoporosis. This

study provides new targets and ideas for clinical treatment and diagnosis of osteoporosis, and provides an experimental basis for the application of miRNA in the treatment of osteoporosis. However, it is unclear why the expression level of miR-373 is reduced in postmenopausal osteoporosis patients. We will further clarify the mechanism leading to the decrease of miR-373 expression in future experiments, and collect more clinical samples for verification.

Conflict of Interests

The Authors declared that they have no conflict of interests.

References

1) Yang F, Yang L, Li Y, Yan G, Feng C, Liu T, Gong R, Yuan Y, Wang N, Idiiatullina E, Bikkuzin T, Pavlov V, Li Y, Dong C, Wang D, Cao Y, Han Z, Zhang L, Huang Q, Ding F, Bi Z, Cai B. Melatonin protects bone marrow mesenchymal stem cells against iron overload-induced aberrant differentiation and senescence. J Pineal Res 2017; 63. doi: 10.1111/jpi.12422.
2) Dede AD, Callan M. Treatment of osteoporosis: whom, how and for how long? Br J Hosp Med (Lond) 2018; 79: 259-264.
3) Sun W, Wang YQ, Yan Q, Lu R, Shi B. Effects of Er-Zhi-Wan on microarchitecture and regulation of Wnt/beta-catenin signaling pathway in alveolar bone of ovariectomized rats. J Huazhong Univ Sci Technolog Med Sci 2014; 34: 114-119.
4) Yin C, Tian Y, Yu Y, Wang H, Wu Z, Huang Z, Zhang Y, Li D, Yang C, Wang X, Li Y, Qian A. A novel long noncoding RNA AK016739 inhibits osteoblast differentiation and bone formation. J Cell Physiol 2019; 234: 11524-11536.
5) Wu ZH, Huang KH, Liu K, Wang GT, Sun Q. DGCR5 induces osteogenic differentiation by up-regulating Runx2 through miR-30d-5p. Biochem Biophys Res Commun 2018; 505: 426-431.
6) Kim SH, Zhang Z, Moon YJ, Park IW, Cho YG, Jeon R, Park BH. SPA0355 prevents ovariectomy-induced bone loss in mice. Korean J Physiol Pharmacol 2019; 23: 47-54.
7) Liu F, Tan F, Tong W, Fan Q, Ye S, Lu S, Teng Z, Han M, Zhang M, Chai Y. Effect of Zuoguiwan on osteoporosis in ovariectomized rats through RANKL/OPG pathway mediated by beta2AR. Biomed Pharmacother 2018; 103: 1052-1060.
8) Cao Y, Yang H, Jin L, Du J, Fan Z. Genome-wide DNA methylation analysis during osteogenic differentiation of human bone marrow mesenchymal stem cells. Stem Cells Int 2018; 2018: 8238496.
9) Camp E, Pribadi C, Anderson PJ, Zannettino ACW, Gronthos S. MiRNA-376c-3p mediates TWIST-1 inhibition of bone marrow-derived stromal cell osteogenesis and can reduce aberrant bone formation of TWIST-1 haploinsufficient calvarial cells. Stem Cells Dev 2018; 27: 1621-1633.
10) Zhang S, Liu Y, Zheng Z, Zeng X, Liu D, Wang C, Ting K. MicroRNA-223 suppresses osteoblast differentiation by inhibiting DHRS3. Cell Physiol Biochem 2018; 47: 667-679.
11) Zhang L, Tang Y, Zhu X, Tu T, Sui L, Han Q, Yu L, Meng S, Zheng L, Valverde P, Tang J, Murray D, Zhou X, Drissi H, Dard MM, Tu Q, Chen J. Overexpression of miR-335-5p promotes bone formation and regeneration in mice. J Bone Miner Res 2017; 32: 2466-2475.
12) Long H, Sun B, Cheng L, Zhao S, Zhu Y, Zhao R, Zhu J. MiR-139-5p represses BMSC osteogenesis via targeting Wnt/beta-catenin signaling pathway. DNA Cell Biol 2017; 36: 715-724.
13) Wang L, Wang L, Chang W, Li Y, Wang L. MicroRNA-373 promotes the development of esophageal squamous cell carcinoma by targeting LATS2 and OXR1. Int J Biol Markers 2019: 34: 148-155.
14) Sur S, Sasaki R, Devhare P, Steele R, Ray R, Ray RB. Association between microRNA-373 and long noncoding RNA NORAD in hepatitis C virus-infected hepatocytes impairs wee1 expression for growth promotion. J Virol 2018; 92. pii: e01215-18.
15) Yang H, Liao D, Tong L, Zhong L, Wu K. MiR-373 exacerbates renal injury and fibrosis via NF-kappaB/MatrixMetalloproteinase-9 signaling by targeting Sirtuin1. Genomics 2019; 111: 786-792.
16) Liu Y, Cheng Z, Pan F, Yan W. MicroRNA-373 promotes growth and cellular invasion in osteosarcoma cells by activation of the PI3K/AKT-Rac1-JNK pathway: the potential role in spinal osteosarcoma. Oncol Res 2017; 25: 989-999.
17) Huang JZ, Chen M, Zeng M, Xu SH, Zou FY, Chen D, Yan GR. Down-regulation of TRPS1 stimulates epithelial-mesenchymal transition and metastasis through repression of FOXA1. J Pathol 2016; 239: 186-196.
18) Palmieri A, Pezzetti F, Brunelli G, Zollino I, Scapoli L, Martinelli M, Arlotti M, Carinci F. Differences in osteoblast miRNA induced by cell binding domain of collagen and silicate-based synthetic bone. J Biomed Sci 2007; 14: 777-782.
19) Guo Y, Jiang Y, Sang M, Xu C. Down-regulation of miR-373 increases the radiosensitivity of lung cancer cells by targeting TIMP2. Int J Biochem Cell Biol 2018; 99: 203-210.
20) Zhang W, Zhong B, Zhang C, Luo C, Zhan Y. MiR-373 regulates inflammatory cytokine-mediated chondrocyte proliferation in osteoarthritis by targeting the P2X7 receptor. FEBS Open Bio 2018; 8: 325-331.
21) Wang H, Li J, Liu YC, Chen X, Chai T. [Mechanism and clinical significance of miR-373 in elderly patients with multiple myeloma]. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2018; 26: 829-835.