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MicroRNA-579-3p promotes the progression of osteoporosis by inhibiting osteogenic differentiation of mesenchymal stem cells through regulating Sirt1

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Abstract. – OBJECTIVE: To explore whether microRNA-579-3P was involved in the development of osteoporosis, and to investigate the possible molecular mechanisms.

PATIENTS AND METHODS: The messenger RNA (mRNA) expression levels of microRNA-579-3P, alkaline phosphatase (ALP), runt-related transcription factor 2 (RUNX2) and bone sialoprotein (BSP) in serum samples of osteoporosis patients and normal controls were detected by quantitative Real-time polymerase chain reaction (qRT-PCR), respectively. Meanwhile, the expressions of the above genes during osteogenic differentiation of human bone marrow mesenchymal stem cells (hMSCs) were examined as well. To investigate the effect of microRNA-579-3P on osteogenesis, microRNA-579-3P was overexpressed and knocked down in hMSCs. Subsequently, the mRNA and protein expression levels of osteogenesis-related genes, such as ALP, RUNX2 and BSP, were detected by qRT-PCR and Western blot, respectively. In addition, ALP activity and mineralization forming ability were evaluated by ALP staining and alizarin red staining. Bioinformatics predicted that Sirt1 was the target gene of microRNA-579-3P. Subsequent luciferase reporter gene assay was performed to verify the binding relationship of microRNA-579-3P to Sirt1. Meanwhile, qRT-PCR and Western blot were used to detect the changes in the mRNA and protein expression levels of Sirt1, respectively. After overexpression of microRNA-579-3P and Sirt1, qRT-PCR, Western blot, ALP staining and alizarin red staining assays were performed to detect the osteogenic differentiation of hMSCs.

RESULTS: The expression of microRNA-579-3P in serum of patients with osteoporosis was significantly higher than that of normal controls. Meanwhile, the expression of microRNA-579-3P decreased gradually during osteogenic differentiation of hMSCs. Overexpression of microRNA-579-3P significantly reduced the expressions of osteogenic related genes, including ALP, RUNX2 and BSP. Besides, ALP activity and mineralized nodule formation ability decreased obviously as well. Luciferase reporter gene assay showed that microRNA-579-3P could bind to Sirt1. After overexpression of microRNA-579-3P, the mRNA and protein expression levels of Sirt1 were significantly reduced, which were reversed after silence of microRNA-579-3P. Simultaneous overexpression of microRNA-579-3P and Sirt1 could reverse the inhibition of osteogenic differentiation of hMSCs caused by overexpression of microRNA-579-3P alone.

CONCLUSIONS: MicroRNA-579-3P could inhibit osteogenic differentiation of hMSCs by regulating Sirt1, thereby promoting the development of osteoporosis.

Key Words:
Osteoporosis, MicroRNA-579-3P, Sirt1, Human bone marrow mesenchymal stem cells (hMSCs), Osteogenic differentiation.

Introduction

Osteoporosis is a systemic disease, in which bone mass is low and the microstructure of bone tissue is destroyed. This may result in increased bone fragility and fracture[1]. Due to the lack of typical symptoms, early stages of osteoporosis are often overlooked until the occurrence of brittle fractures of the hip, spine, proximal humer-

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us, pelvis and distal forearm[2]. Therefore, it is of great significance to investigate the development of osteoporosis. Boyce et al[3] have shown that osteoblasts play an important role in bone metabolism, which also participate in the process of bone resorption and bone remodeling. Therefore, osteoblast proliferation and differentiation play important roles in the maintenance of normal bone structure[4]. In recent years, Di Bella et al[5] have found that mesenchymal stem cells (MSCs) are stromal cells with constant self-renewal and multi-directional differentiation potential. Under specific induction conditions, MSCs can differentiate into osteoblasts, chondrocytes, fat cells, etc. Therefore, this provides a new direction and strategy for the treatment of bone-related diseases in cell regeneration medicine. Furthermore, we have explored the mechanism of osteoporosis development by establishing model of osteogenic differentiation of human bone marrow mesenchymal stem cells (hMSCs) *in vitro*, with far-reaching significance. MicroRNAs (miRNAs) are a type of single-stranded RNA molecules with about 20-25 nt in length. They are regulated by base pairing with target genes to regulate their expression[6]. In recent years, Zhang et al[7] have shown that miRNAs promote or inhibit the differentiation of bone precursor cells and osteoblasts by regulating key transcription factors and osteogenic markers on the osteogenic signaling pathway. Fattore et al[8] have confirmed that microRNA-579-3P is associated with the healing of melanoma. However, few studies have investigated the exact role of microRNA-579- 3P in osteoporosis. SIRT1 is a deacetylase in mammals, and is widely expressed in mature tissues recently becoming a hot spot in the medical research[9,10]. It is known to all that SIRT1 is expressed in MSCs. With the passage and differentiation of MSCs *in vitro*, the expression of SIRT1 decreases and the activity weakens. At the same time, the multi-potential differentiation of MSCs decreases as well[11]. This indicates that SIRT1 may have important regulation during differentiation of MSCs. Moreover, studies[12,13] have confirmed that SIRT1 plays an important role in the differentiation of MSCs into osteoblasts, adipocytes, as well as chondrocytes. In this work, we identified differentially expressed miRNAs by detecting differences in gene expression in osteoporotic patients and normal controls. Our results found microRNA-579-3P was aberrantly expressed in osteoporotic patients. Besides, bioinformatics predicted that microRNA-579-3P could target bind to SIRT1. Using hMSCs as a research model, we investigated whether microRNA-579- 3P and its regulation on SIRT1 could affect osteogenic differentiation of hMSCs. Our findings might provide new clues and ideas for the research and clinical treatment of osteoporosis.

Patients and Methods

Cell Culture

This study was approved by the Ethics Committee of The Affiliated Hospital (Traditional Chinese Medicine) of Southwest Medical University. Informed consent was obtained from each subject before the study. Serum samples of osteoporosis patients and normal controls were collected and placed in ethylenediaminetetraacetic acid (EDTA) anticoagulation tubes. Bone marrow of normal controls was added to 10 mL of serum-free low-glucose α-modified Eagle's medium (α-MEM) medium (HyClone, South Logan, UT, USA), followed by centrifugation at 400 g for 10 min at room temperature. Next, fat layer was aspirated, and cells were re-suspended in low-sugar α-MEM medium. An equal volume of Fircoll isolate was slowly added along the tube wall. After centrifugation for 30 min, the layer of mononuclear cells was washed and re-suspended in low sugar alpha-MEM medium containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA). Cell density was then adjusted to 2×10^6 /mL. After that, the cells were inoculated into culture flasks, and cultured in a 37°C and 5% CO_2 incubator. After 24 h, fresh culture medium was replaced to remove non-adherent cells. The medium was changed every day, and the cells were routinely digested and passaged with 0.25% trypsin every 3 to 5 days.

Cell Osteogenic Differentiation Induction

hMSCs in logarithmic growth phase were routinely digested and seeded into 24-well culture plates at a density of 2×10^4 /well. When the cells grew to 80% of confluence, the medium was changed to osteogenic induction medium. The culture medium was an osteogenic induction culture containing dexamethasone 10 nM, sodium β-glycerophosphate 10 mM, ascorbic acid Vc 0.2 mM, and 10% FBS in H-DMEM.

Cell Transfection

Logarithmic growth phase hMSCs were first seeded into 24-well culture plates (2×10^4 /well). When the cells grew to 80% of confluence, they

were transfected with microRNA-579-3P mimics, microRNA-579- 3P inhibitor as well as pcDNA-SIRT1 according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 6 hours, the medium was replaced by conventional medium for another 48 hours of culture.

Alkaline Phosphatase (ALP) Staining

Cells were first washed three times with phosphate buffered saline (PBS), and then fixed for 30 s at room temperature. After washing twice with PBS, the cells were added with 1.5 mL of ALP stain solution (Sigma-Aldrich, St. Louis, MO, USA) at room temperature for 15 min in dark. After discarding the staining solution, the cells were washed 3 times with distilled water, air-dried and observed.

ALP Activity Detection

Osteoblast suspension was first inoculated into 96-well sterile cell culture plates at a density of 1×10^5/mL. ALP activity was determined in strict accordance with alkaline phosphatase kit.

Alizarin Red Staining

Cells were first washed twice with PBS buffer, and fixed in 95% ethanol for 10 min. After washing twice with double distilled water, the cells were incubated with 1% alizarin red-Tris-HCl (pH 8.3) at 37°C for 30 min. After rinsing with double distilled water and dried in an oven at 65°C, photographs were taken under an inverted fluorescence microscope. Images were taken at 200 times.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA in serum samples and cells was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The concentration and purity of RNA were determined by ultraviolet spectrophotometer. The expression levels of microRNA-579-3P, SIRT1, ALP, runt-related transcription factor 2 (RUNX2) and bone sialoprotein (BSP) were detected using a PCR detection kit. Primer sequences used in this study were as follows: microRNA-579-3p: upstream: 5'-ACACTCCAGCTGGGTTCATTTGGTATAAACC-3', downstream: 5'-TGGTGTCGTGGAGTCG-3'; U6: upstream 5'-CGCTTCGGCAGCACATATACTAAAATTGGAAC-3', downstream 5'-GCTTCACGAATTTGCGTGTCATCCTTGC-3'. SIRT1: upstream 5'-CAT AGA CAC GCT GGA ACA GG-3'; downstream 5'-TTGAGGGAAGACCCAATAACA-3'; ALP: upstream: 5'-AACATCAGGGACATTGACGTG-3', downstream: 5'-GTATCTCGGTTTGAAGCTCT-3'; RUNX2: Upstream: 5'-GGAGCGGACGAGGCAAGAGT-3', downstream 5'-AGGAATGCGCCCTAAATCAC-3'; BSP, upstream: 5'-'GCGTGCTTCTTAGACGGACTG-3'; Downstream: 5'-CGTCAGAGGGCTGGGATG-3'; GAPDH Upstream: 5'-TTCTTTTGCGTCGCCAGCCGA-3'; Downstream: 5'-GTCACCACCCGCCCAATACGA-3'.

Western Blot

Total proteins in cells were first extracted. The concentration of extracted protein was determined by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). 50 μg of total protein was electrophoresed by 8% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and transferred onto membranes. After blocking with 5% skim milk for 1 h, the membranes were incubated with primary antibodies of GAPDH (Abcam, Cambridge, MA, USA) or RUNX2 (Abcam, Cambridge, MA, USA) diluted 1:1000 for 12 h in a 4°C shaker. After washing with Tris-buffered saline and Tween 20 (TBST) for 3 times (10 min / time), the membranes were incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit or anti-mouse IgG secondary antibody. Finally, immune-reactive bands were detected using hypersensitive enhanced chemiluminescence (ECL) reagent (Thermo Fisher Scientific, Waltham, MA, USA).

Luciferase Reporter Gene Assay

The miRNA precursor sequence and the 3'-untranslated region (3'-UTR) of SIRT1 gene were cloned into pSUPER vector and psi-CHECK 2 vector by PCR. Cloned results were confirmed by sequencing. Subsequently, two plasmids (0.5 μg 3'-UTR and l μg miRNA per well) were co-transfected into HEK 293 cells. Finally, luciferase activity was performed 32 h later.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 statistical software (IBM, Armonk, NY, USA) was used for all statistical analysis. The results were expressed as mean ± standard deviation ($\bar{x}\pm s$). *t*-test was used to compare the differences between two groups. One-way analysis of variance was used to compare the differences among different groups, followed by Post-Hoc Test LSD (Least Significant Difference). $p<0.05$ was considered statistically significant.

Results

Expression of microRNA-579-3P in Serum of Osteoporosis Patients and Osteogenic Differentiation of hMSCs

To preliminarily determine whether there was a close relationship between microRNA-579-3P and osteoporosis, we first extracted total RNA from serum of osteoporosis and normal controls. Next, microRNA-579-3P expression was detected by qRT-PCR. Results showed that microRNA-579-3P expression in serum of osteoporosis patients was significantly higher than that of normal controls (Figure 1A). The expression of microRNA-579-3P decreased gradually after induction of osteogenic differentiation of hMSCs for 1 d, 3 d, 7 d, and 10 d (Figure 1B). Meanwhile, the expressions of osteogenesis-related genes, including ALP, RUNX2 and BSP, gradually increased after osteogenic differentiation of hMSCs for 1 d, 3 d, 7 d and 10 d (Figure 1C). The above results indicated that microRNA-579-3P might be associated with osteoporosis.

High Expression of microRNA-579- 3P Inhibited Osteogenic Differentiation of hMSCs

To further verify the relationship between microRNA-579-3P and osteogenic differentiation of hMSCs, we overexpressed microRNA-579-3P in cells. QRT-PCR and Western blot found that the

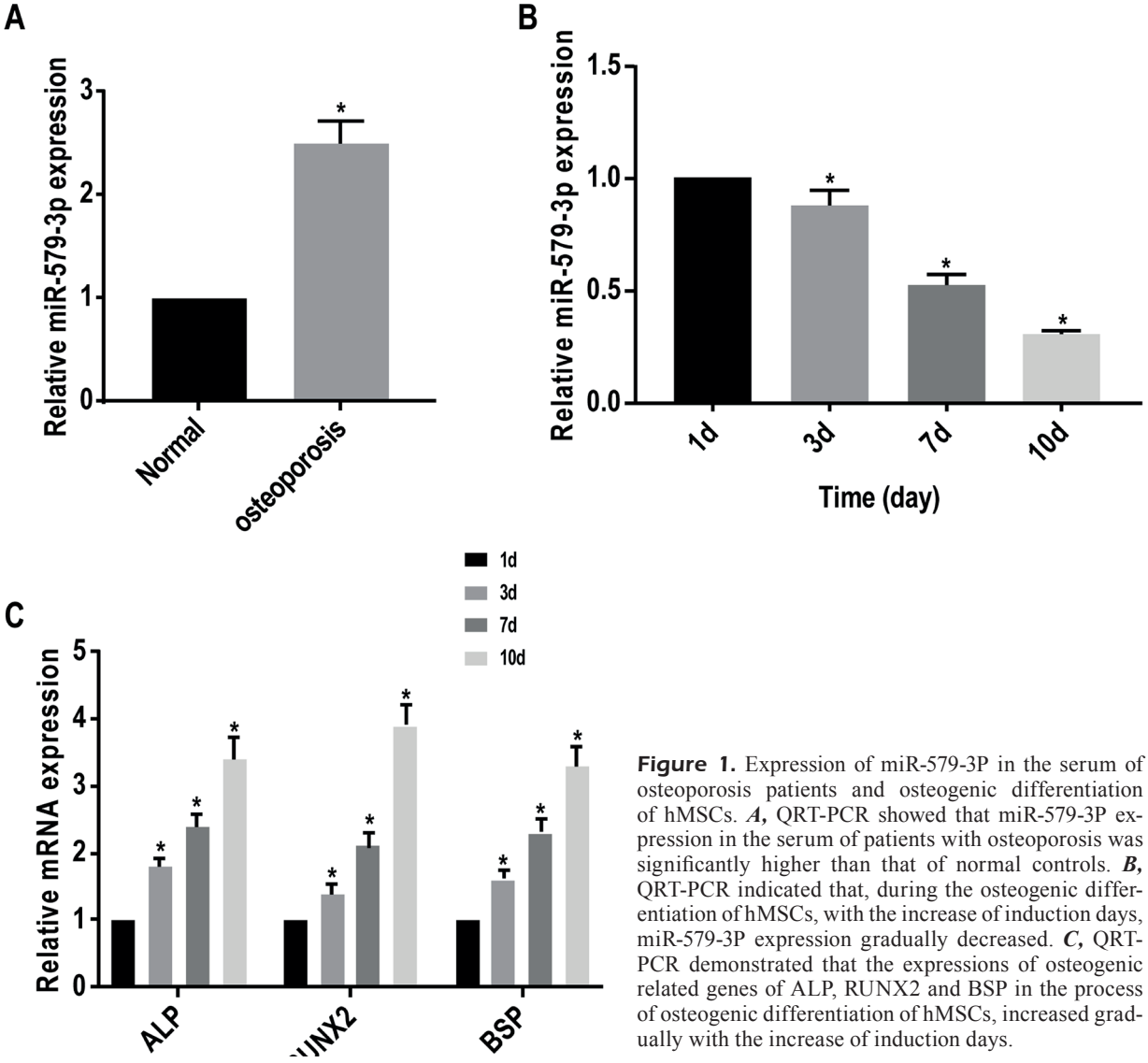

Figure 1. Expression of miR-579-3P in the serum of osteoporosis patients and osteogenic differentiation of hMSCs. ***A,*** QRT-PCR showed that miR-579-3P expression in the serum of patients with osteoporosis was significantly higher than that of normal controls. ***B,*** QRT-PCR indicated that, during the osteogenic differentiation of hMSCs, with the increase of induction days, miR-579-3P expression gradually decreased. ***C,*** QRT-PCR demonstrated that the expressions of osteogenic related genes of ALP, RUNX2 and BSP in the process of osteogenic differentiation of hMSCs, increased gradually with the increase of induction days.

mRNA and protein expression levels of osteogenic-related genes, such as ALP, RUNX2 and BSP, decreased significantly. However, knocking down microRNA-579-3P could markedly elevate the expressions of the above genes (Figure 2A, 2B). Meanwhile, overexpression of microRNA-579-3P significantly decreased ALP activity, which was upregulated after knockdown of microRNA-579-3P (Figure 2C). After overexpression of microRNA-579-3P, ALP staining showed significant decrease in ALP content. Meanwhile, alizarin red staining detected a significant decrease in mineralized nodule formation. On the other hand, knocking down of microRNA-579-3P could remarkably reverse these phenotypes (Figure 2D). These results indicated that high expression of could inhibit osteogenic differentiation of hMSCs, while microRNA-579-3P low expression could promote osteogenic differentiation. Our findings suggested that microRNA-579-3P had a regulatory effect on the development of osteoporosis.

MicroRNA-579-3P Targeted Binding to Sirt1

Bioinformatics predicted that microRNA-579-3P had a binding site with Sirt1 (Figure 3A). Luciferase reporter gene assay showed that overexpression of microRNA-579-3P significantly quenched wild-type Sirt1 fluorescence (Figure 3B), indicating that microRNA-579-3P could bind to Sirt1 in a targeted manner. To verify whether there was a regulatory relationship between microRNA-579-3P and Sirt1, we overexpressed microRNA-579-3P *in vitro*. Next, we detected the mRNA and protein expression levels of Sirt1 by qRT-PCR and Western blot. The results indicated that the expressions of Sirt1 were significantly reduced. After knocking down microRNA-579-3P, opposite results were observed (Figure 3C,D). These results indicated that microRNA-579-3P had the ability to target bind to Sirt1 and regulate its expression.

MicroRNA-579-3P Regulated Osteogenic Differentiation of hMSCs via Sirt1

To further verify the regulatory relationship between microRNA-579-3P and Sirt1 during osteogenic differentiation of hMSCs, we divided the cells into three groups, including: control group, microRNA-579-3P overexpression group, and microRNA-579-3P + Sirt1 overexpression group. The results showed that overexpression of Sirt1 significantly reversed the decrease in the mRNA expression levels of ALP, RUNX2 and BSP caused by microRNA-579-3P overexpression (Figure 4A, B). Besides, decreased ALP content and decreased mineralization forming ability were observed after Sirt1 overexpression (Figure 4C). These results suggested that microRNA-579-3P could inhibit osteogenic differentiation of hMSCs via Sirt1.

Discussion

Osteoporosis is a severe bone metabolic disease with high risk and incidence. It seriously threatens the health of patients in middle-aged and elderly people, especially in menopausal women[14]. With the aging of the population, osteoporosis has become a worldwide concern, resulting in an average of 8.9 million fractures per year[15]. Meanwhile, it may place a heavy burden on the medical system. Osteoblasts can synthesize and secrete bone matrix, as well as promote mineralization of bone matrix. They are a kind of cells that play an important role in bone formation[16]. MSC is the most important source of osteoblasts. With the increase of age, the number of MSCs gradually reduces, which is accompanied by cell aging. This may eventually result in decreased sources of osteoblasts, decrease activity and affect the normal balance of bone metabolism. Furthermore, it may cause a variety of bone diseases[17] such as osteoporosis, rheumatoid arthritis, etc. Therefore, how to promote the directional induction of MSCs into osteoblasts is a key issue in bone tissue engineering. MiRNAs are a class of non-coding small RNAs discovered in recent years. They can degrade mRNA of target genes and play vital roles in post-transcriptional regulation. Previously Li et al[18] have shown that miRNAs are important supplements to traditional regulatory mechanisms, which also have one-to-many and many-to-one characteristics. Dicer is an enzyme necessary for the maturation of miRNAs. The lack of Dicer will result in the formation of defects in all miRNAs. *In vivo* studies have found that conditional knockout of Dicer in bone precursor cells leads to death in mice after birth. Meanwhile, gene-deficient mice have significant skeletal malformations and bone and cartilage formation disorders. Conditional knockout of Dicer in osteoblasts results in delayed bone formation after birth, while bone remodeling is accelerated 4-8 months after birth[19,20]. With the deepening of research

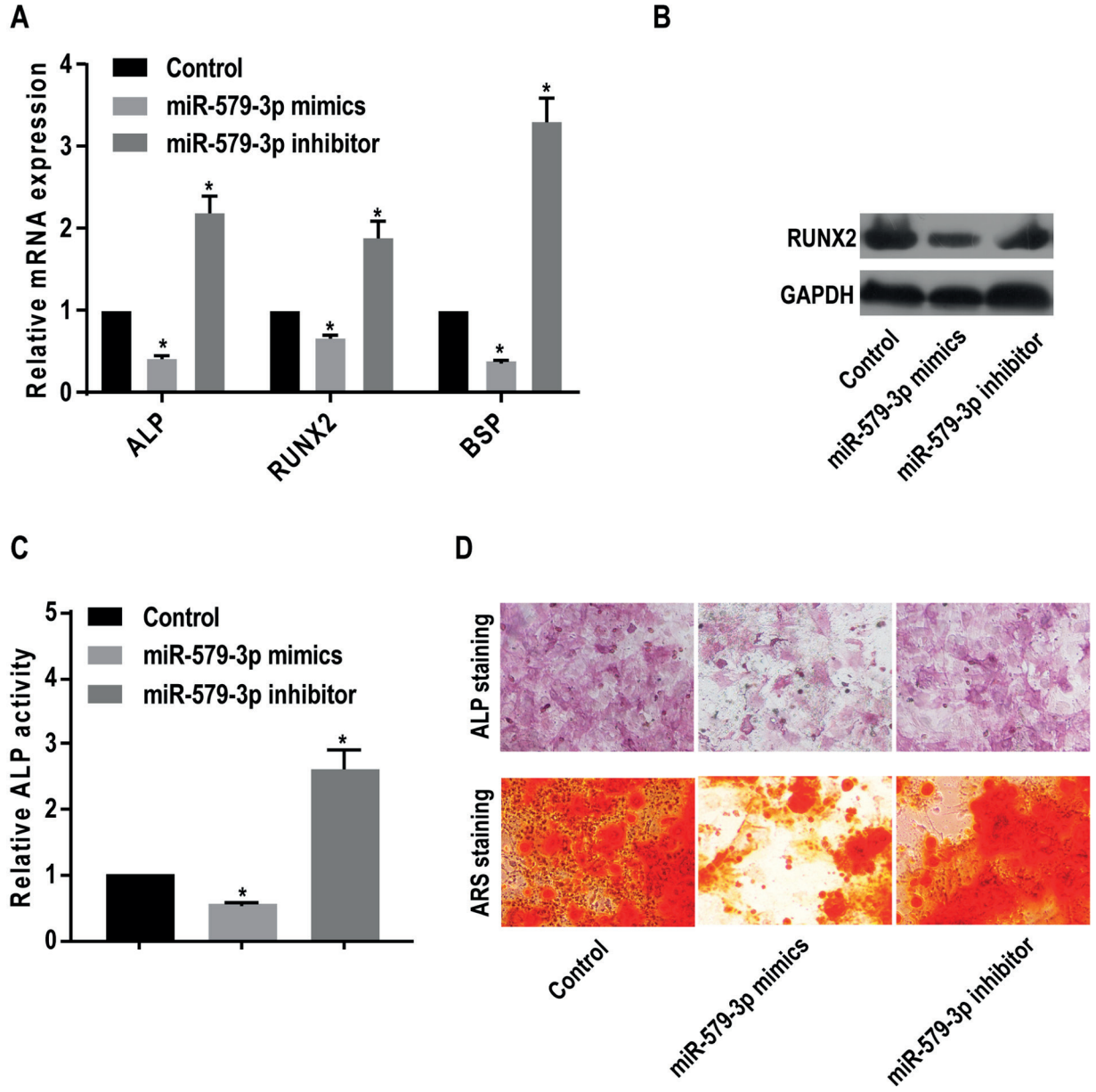

Figure 2. High expression of miR-579-3P inhibited osteogenic differentiation of hMSCs. ***A,*** After overexpression of miR-579-3P in hMSCs cells, the expression levels of ALP, RUNX2 and BSP decreased significantly, and the expressions of the above genes increased obviously after knockdown of miR-579-3P. ***B,*** After overexpression of miR-579-3P in hMSCs cells, the protein level of RUNX2 decreased significantly, while the expression of RUNX2 increased significantly after knockdown of miR-579-3P. ***C,*** After overexpression of miR-579-3P in hMSCs cells, ALP activity assay kit detected a significant decrease in ALP activity. ***D,*** After overexpression of miR-579-3P in hMSCs cells, ALP staining showed a significant decrease in ALP content. Meanwhile, alizarin red staining detected a significant decrease in mineralized nodule formation. After knocking down miR-579-3P, the staining results were reversed.

on miRNAs, their roles in the differentiation of stem cells into osteoblasts have also been carried out[21]. For example, overexpression of miR-346 promotes the differentiation of hMSCs into osteoblasts[22]. In this study, we found that microRNA-579-3P had a significant inhibitory effect on osteogenic differentiation of hMSCs. SIRT1 is an important regulator of Wnt signaling pathway, which promotes the expression of downstream differentiation-related factors by de-acetylating β-catenin[23]. It has been reported that SIRT1 regulates osteo-

genesis and adipogenic transcription factors. *In vitro* cultured MSCs, *via* stimulating SIRT1, can alter its lineage, up-regulate osteogenic differentiation and myogenic differentiation, as well as down-regulate adipogenic differentiation[24,25]. The involvement of miRNAs in the regulation of osteogenic differentiation is a complex process, in which multiple factors and multiple signaling pathways are co-regulated including BMP pathway[26] and classical Wnt pathway[27]. Lv et al[28] have found that miR133 inhibits MSCs differentiation into osteoblasts by inhibiting Runx2, which is a key transcription factor in osteogenic differentiation. In the present study, we found that microRNA-579-3P could bind to SIRT1 and regulate its expression. Overexpression of microRNA-579-3P significantly inhibited osteogenic differentiation of hMSCs, whereas overexpression of SIRT1 reversed the inhibitory outcomes. This suggested that the regulation of microRNA-579-3P on osteogenic differenti-

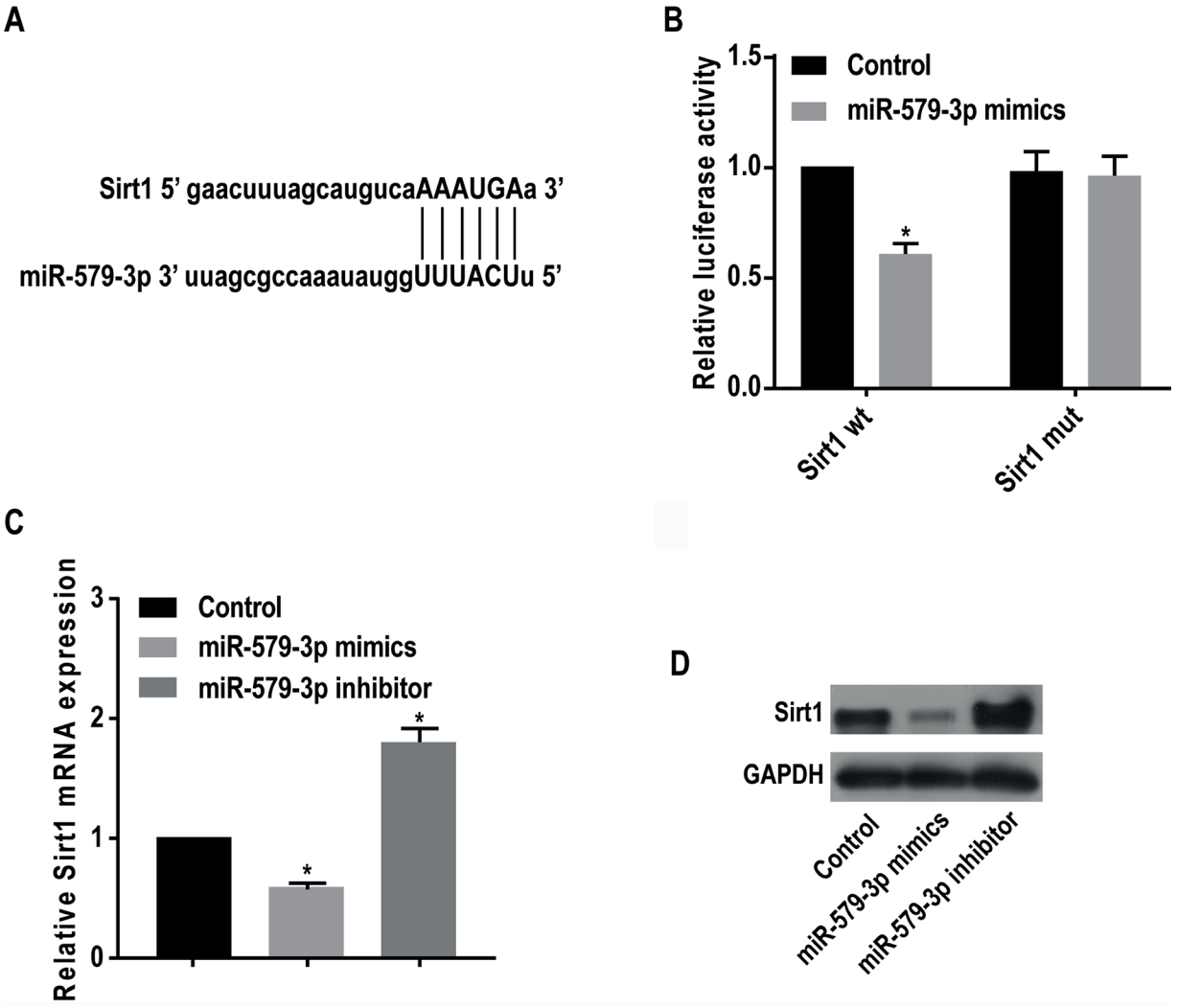

Figure 3. MiR-579-3P could target bind to Sirt1. ***A,*** Binding site of miR-579-3P to Sirt1. ***B,*** Luciferase reporter gene assay showed that overexpression of miR-579-3P significantly quenched wild-type Sirt1 fluorescence. ***C,*** QRT-PCR detection showed that after overexpression of miR-579-3P, the mRNA expression level of Sirt1 significantly decreased. After knocking down miR-579-3P, the expression level of Sirt1 mRNA significantly increased. ***D,*** Western blot indicated that after overexpression of miR-579-3P, the protein expression level of Sirt1 significantly decreased. After knocking down miR-579-3P, the protein expression level of SIRT1 significantly increased.

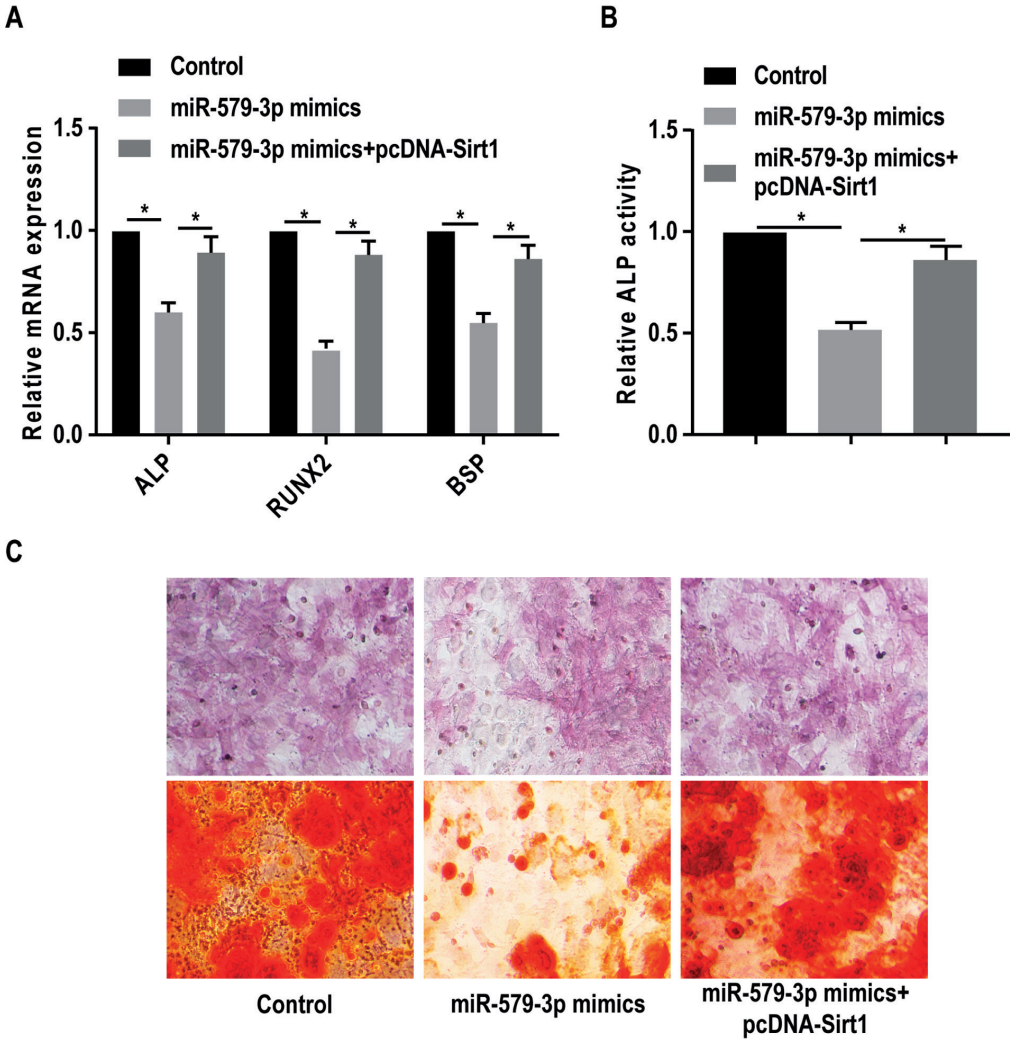

Figure 4. MiR-579-3P regulated osteogenic differentiation of hMSCs *via* Sirt1**.** Overexpression of miR-579-3P in hMSCs and overexpression of Sirt1 reversed the decrease in the mRNA expression levels of ***A,*** ALP, RUNX2 and OCN caused by overexpression of miR-579-3P; ***B,*** decreased ALP activity; ***C,*** decreased ALP content and mineralization ability.

ation of hMSCs was dependent on SIRT1. Our study first explored the relationship between microRNA-579-3P and osteoporosis. In addition, our findings might provide new ideas and directions for the diagnosis and treatment of clinical osteoporosis.

Conclusions

We demonstrated that microRNA-579-3P is highly expressed in the serum of patients with osteoporosis. Meanwhile, it can target bind to SIRT1, thereby inhibiting osteogenic differentiation of hMSCs and promoting the development of osteoporosis.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Wright NC, Looker AC, Saag KG, Curtis JR, Delzell ES, Randall S, Dawson-Hughes B. The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. J Bone Miner Res 2014; 29: 2520-2526.
2) Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, Lindsay R. Clinician's guide to prevention and treatment of osteoporosis. Osteoporos Int 2014; 25: 2359-2381.

3) Oh SH, Kim JW, Kim Y, Lee MN, Kook MS, Choi EY, Im SY, Koh JT. The extracellular matrix protein Edil3 stimulates osteoblast differentiation through the integrin alpha5beta1/ERK/Runx2 pathway. PLoS One 2017; 12: e188749.

4) Boyce BF, Rosenberg E, de Papp AE, Duong LT. The osteoclast, bone remodelling and treatment of metabolic bone disease. Eur J Clin Invest 2012; 42: 1332-1341.

5) Di Bella C, Aldini NN, Lucarelli E, Dozza B, Frisoni T, Martini L, Fini M, Donati D. Osteogenic protein-1 associated with mesenchymal stem cells promote bone allograft integration. Tissue Eng Part A 2010; 16: 2967-2976.

6) Seok H, Ham J, Jang ES, Chi SW. MicroRNA target recognition: insights from transcriptome-wide non-canonical interactions. Mol Cells 2016; 39: 375-381.

7) Zhang SY, Gao F, Peng CG, Zheng CJ, Wu MF. MiR-485-5p promotes osteoporosis via targeting Osterix. Eur Rev Med Pharmacol Sci 2018; 22: 4792-4799.

8) Fattore L, Mancini R, Acunzo M, Romano G, Lagana A, Pisanu ME, Malpicci D, Madonna G, Mallardo D, Capone M, Fulciniti F, Mazzucchelli L, Botti G, Croce CM, Ascierto PA, Ciliberto G. MiR-579-3p controls melanoma progression and resistance to target therapy. Proc Natl Acad Sci U S A 2016; 113: E5005-E5013.

9) Satoh A, Brace CS, Ben-Josef G, West T, Wozniak DF, Holtzman DM, Herzog ED, Imai S. SIRT1 promotes the central adaptive response to diet restriction through activation of the dorsomedial and lateral nuclei of the hypothalamus. J Neurosci 2010; 30: 10220-10232.

10) Correia M, Perestrelo T, Rodrigues AS, Ribeiro MF, Pereira SL, Sousa MI, Ramalho-Santos J. Sirtuins in metabolism, stemness and differentiation. Biochim Biophys Acta Gen Subj 2017; 1861: 3444-3455.

11) Yuan HF, Zhai C, Yan XL, Zhao DD, Wang JX, Zeng Q, Chen L, Nan X, He LJ, Li ST, Yue W, Pei XT. SIRT1 is required for long-term growth of human mesenchymal stem cells. J Mol Med (Berl) 2012; 90: 389-400.

12) Chen H, Liu X, Chen H, Cao J, Zhang L, Hu X, Wang J. Role of SIRT1 and AMPK in mesenchymal stem cells differentiation. Ageing Res Rev 2014; 13: 55-64.

13) Yoon DS, Choi Y, Lee JW. Cellular localization of NRF2 determines the self-renewal and osteogenic differentiation potential of human MSCs via the P53-SIRT1 axis. Cell Death Dis 2016; 7: e2093.

14) [No authors listed]. Prevention and management of osteoporosis. World Health Organ Tech Rep Ser 2003; 921: 1-164.

15) Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int 2006; 17: 1726-1733.

16) Khan AA, Rios LP, Sandor GK, Khan N, Peters E, Rahman MO, Clokie CM, Dore E, Dubois S. Bisphosphonate-associated osteonecrosis of the jaw in Ontario: a survey of oral and maxillofacial surgeons. J Rheumatol 2011; 38: 1396-1402.

17) Deng P, Chen QM, Hong C, Wang CY. Histone methyltransferases and demethylases: regulators in balancing osteogenic and adipogenic differentiation of mesenchymal stem cells. Int J Oral Sci 2015; 7: 197-204.

18) Li Z, Hassan MQ, Volinia S, van Wijnen AJ, Stein JL, Croce CM, Lian JB, Stein GS. A microRNA signature for a BMP2-induced osteoblast lineage commitment program. Proc Natl Acad Sci U S A 2008; 105: 13906-13911.

19) Hassan MQ, Gordon JA, Beloti MM, Croce CM, van Wijnen AJ, Stein JL, Stein GS, Lian JB. A network connecting Runx2, SATB2, and the miR-23a~27a~24-2 cluster regulates the osteoblast differentiation program. Proc Natl Acad Sci U S A 2010; 107: 19879-19884.

20) White NM, Khella HW, Grigull J, Adzovic S, Youssef YM, Honey RJ, Stewart R, Pace KT, Bjarnason GA, Jewett MA, Evans AJ, Gabril M, Yousef GM. miRNA profiling in metastatic renal cell carcinoma reveals a tumour-suppressor effect for miR-215. Br J Cancer 2011; 105: 1741-1749.

21) Sekar D, Saravanan S, Karikalan K, Thirugnanasambantham K, Lalitha P, Islam VI. Role of microRNA 21 in mesenchymal stem cell (MSC) differentiation: a powerful biomarker in MSCs derived cells. Curr Pharm Biotechnol 2015; 16: 43-48.

22) Wang Q, Cai J, Cai XH, Chen L. miR-346 regulates osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by targeting the Wnt/beta-catenin pathway. PLoS One 2013; 8: e72266.

23) Simic P, Zainabadi K, Bell E, Sykes DB, Saez B, Lotinun S, Baron R, Scadden D, Schipani E, Guarente L. SIRT1 regulates differentiation of mesenchymal stem cells by deacetylating beta-catenin. EMBO Mol Med 2013; 5: 430-440.

24) Chen H, Liu X, Chen H, Cao J, Zhang L, Hu X, Wang J. Role of SIRT1 and AMPK in mesenchymal stem cells differentiation. Ageing Res Rev 2014; 13: 55-64.

25) Backesjo CM, Li Y, Lindgren U, Haldosen LA. Activation of Sirt1 decreases adipocyte formation during osteoblast differentiation of mesenchymal stem cells. J Bone Miner Res 2006; 21: 993-1002.

26) Saito T, Ogawa M, Hata Y, Bessho K. Acceleration effect of human recombinant bone morphogenetic protein-2 on differentiation of human pulp cells into odontoblasts. J Endod 2004; 30: 205-208.

27) Macsai CE, Foster BK, Xian CJ. Roles of Wnt signalling in bone growth, remodelling, skeletal disorders and fracture repair. J Cell Physiol 2008; 215: 578-587.

28) Lv H, Sun Y, Zhang Y. MiR-133 is involved in estrogen deficiency-induced osteoporosis through modulating osteogenic differentiation of mesenchymal stem cells. Med Sci Monit 2015; 21: 1527-1534.