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Low expression of miR-1 promotes osteogenic repair of bone marrow mesenchymal stem cells by targeting TLR1

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Abstract. – OBJECTIVE: Bone marrow mesenchymal stem cells (BMSCs) promote bone tissue repair. MiR-1 regulates myogenic and osteogenic differentiation of human adipose tissue stem cells. However, miR-1's effect on BMSCs osteogenesis is unclear.

MATERIALS AND METHODS: Rat BMSCs were isolated and divided into control group, miR-1 group, and si-miR-1 group respectively transfected with miR-1 plasmid and miR-1 siRNA followed by analysis of cell proliferation by MTT assay and Caspase 3 activity. The expression of osteogenic genes Runx2 and OPN was measured by Real Time-PCR. Healthy male Sprague-Dawley rats were separated into fracture group, NC group, and si-miR-1 group followed by analysis of bone mineral density, miR-1 level by Real Time-PCR, type I collagen, and BMP-2 by enzyme-linked immunosorbent assay (ELISA), and TLR1 expression by Western blot.

RESULTS: Transfection of miR-1 siRNA into BMSCs significantly downregulated miR-1 expression, promoted BMSCs cell proliferation, inhibited Caspase 3 activity, as well as promoted osteogenic genes Runx2 and OPN expression and decreased TLR1 expression ($p<0.05$). The upregulation of miR-1 expression significantly reversed the above changes. TLR1 is a target of miR-1. Downregulation of miR-1 expression in BMSCs of fractured rats significantly increased bone density and ALP activity, promoted type I collagen and BMP-2 expression, and decreased TLR1 expression ($p<0.05$).

CONCLUSIONS: The downregulation of miR-1 promotes BMSCs osteogenic differentiation via targeting TLR1, which promotes osteogenic differentiation and bone healing.

Key Words:
MiR-1, TLR1, BMSCs, Osteogenic differentiation.

Introduction

Tissue engineering combines biological principles and bioengineering techniques, and related technologies have been widely used in clinical practice. In particular, bone tissue engineering technology provides an advanced way for bone repair, bringing hope to patients with bone defects[1,2]. Tissue engineering technology has been widely concerned by the isolation and culture of seed cells, which lays a solid foundation for the repair, replacement and improvement of damaged tissues and even organs[3,4]. Therefore, selection of tissue engineering seed cells is particularly important. Bone mesenchymal stem cells (BMSCs) can promote bone tissue repair[5]. BMSCs are derived from bone marrow and are mainly found in bone marrow stromal cells, which can differentiate to specific cell types, such as osteoblasts, chondroblasts, neuroblasts, adipocytes, and myoblasts[6,7]. BMSCs secrete cytokines and construct a hematopoietic microenvironment which is important to maintain bone mass and bone health[8,9]. Treatment based on BMSCs has shown great potential in regenerative medicine and BMSCs are widely used in tissue engineering to repair damaged tissues[10,11]. However, in the treatment of orthopedic diseases, there are still some deficiencies in the ability of BMSCs differentiation into osteoblasts, thus limiting their clinical application[12]. Therefore, improving bone disease, increasing the survival of BMSCs, and inducing the differentiation of BMSCs into osteoblasts have become one of the research hotspots[11].

Non-coding RNA is involved in many biological processes. MicroRNAs, also known as miRNAs, small RNAs or microRNAs, are widely found in animals and plants. Small molecule short-stranded RNA regulates cell survival, proliferation, and differentiation[13,14]. Abnormal expression of miRNAs may be involved in the occurrence and development of diseases and may serve as a central regulator of proliferation and differentiation of BMSCs, and regulate BMSCs differentiation into specific lineages[15]. MiR-1 has been shown to be involved in myogenic and osteogenic differentiation of human

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adipose tissue stem cells and is not affected by osteogenic differentiation and adipogenic differentiation to promote skeletal muscle differentiation[16,17]. However, miR-1's role in BMSCs osteogenesis and the repair of bone injury has not been reported.

Materials and Methods

Animals

Ten female Sprague-Dawley rats, aged 9 weeks and weighted 250±20 g, were bought from our animal center and fed in SPF conditions including maintaining the temperature at (21±1)°C and maintaining relative humidity (50-70%) under constant temperature and constant humidity conditions, ensuring a 12/day cycle every 12 hours. Experiments were done in strict accordance with the experimental design and performed by experienced technicians to minimize animal suffering. This study was approved by the Ethics Committee of the hospital.

Reagents and Instruments

Sodium pentobarbital was purchased from Shanghai Zhaohui Pharmaceutical Co., Ltd (Shanghai, China). The miR-1 siRNA and pcDNA miR-1 plasmid were constructed by Shanghai Gemma Gene Company (Shanghai, China). Fetal bovine serum (FBS) and cyan chain double antibody were purchased from HyClone Corporation (San Angelo, TX, USA). Dimethyl sulfoxide (DMOS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) powder was purchased from Gibco (Grand Island, NY, USA); trypsin-ethylenediaminetetraacetic acid (EDTA) digest from Sigma-Aldrich (St. Louis, MO, USA). The Lipofectamine 2000 transfection reagent was purchased from Invitrogen (Carlsbad, CA, USA). Enhanced chemiluminescence (ECL) reagents were purchased from Amersham Biosciences (Little Chalfont, Buckinghamshire, UK), rabbit anti-mouse TLR1 antibody and secondary antibody were from Cell Signaling Technology (Danvers, MA, USA). The RNA extraction and the reverse transcription kit were from Axygen (Union City, CA, USA). The alkaline phosphatase (ALP) and Caspase 3 activity detection kit was from Wuhan Boster Bio Co., Ltd. (Wuhan, China). Type I collagen, BMP-2 enzyme-linked immunosorbent assay (ELISA) kit was purchased from American R&D Company (Minneapolis, MN, USA). Surgical microscopy equipment was purchased from Suzhou Medical Instrument Factory (Suzhou, China). The Hera cell CO_2 incubator was purchased from Thermo Fisher Scientific Company (Waltham, MA, USA).

BMSCs Isolation

Rats were sacrificed *via* cervical dislocation and immersed in 70% alcohol for 20-30 min. The skin was cut layer by layer to expose the femur and tibia which were separated and placed into a sterile plate, in which 3 ml of PBS was added to make a cell suspension followed by centrifugation to remove supernatant and fat. Then, an equal volume of lymphocyte separation solution was added and centrifuged followed by an addition of 5 ml of a suspension containing bone marrow mesenchymal cells into the mononuclear cell separation solution and centrifugation to isolate the BMSCs. BMSCs were then cultured in a medium containing 5 ml of α-MEM containing 10% FBS, 1% double antibody in a 37°C incubator with 5% CO_2. After 48 h, non-adherent cells were removed and cell culture was continued, and passage was performed until the cell colonies reached 80% of confluence. The 3-5 generation BMSCs cells were taken for testing.

Transfection of MiR-1 siRNA and pcDNA MiR-1 Plasmid into Bone Marrow Mesenchymal Stem Cells

The miR-1 siRNA and pcDNA miR-1 plasmids were transfected into BMSCs in logarithmic growth phase, respectively. The miR-1 siRNA sequence was: the upstream sequence 5'-AGCGCTGGAGAATCTTCA-3'; the downstream sequence 5'-GAGGAGACGGTCACTCA-3. The cell density was fused to 70-80% in a 6-well plate; the BMP-2 plasmid and the negative control liposome were separately added to 200 μl of serum-free medium and mixed well. The mixed Lipofectamine 2000 was mixed with the miR-1 siRNA and pcDNA miR-1 plasmid dilutions, and then, added to cultured cells. After 6 hours, the serum culture solution was replaced and cultured for 48 hours for experimental research.

Cell Grouping

BMSCs were divided into control group, miR-1 group, and si-miR-1 group transfected with miR-1 plasmid and miR-1 siRNA, respectively. In addition, BMSCs were randomly divided into two groups. BMSCs were inoculated into nano-hydroxyapatite/chitosan composite scaffold as control group and auto-oxygenated nano-bionic scaffold as experimental group. Nano-hydroxyapatite/chitosan composite scaffolds were inoculat-

ed with 2×10^6/mL BMSCs or BMSCs transfected with si-miR-1, and each scaffold was inoculated with 200 μL to examine the effects on cell proliferation and apoptosis. 6×10^6/mL BMSCs were used to inoculate nano-hydroxyapatite/chitosan composite scaffold or auto-oxygenated nano-bionic scaffold with 10% FBS Dulbecco's Modified Eagle's Medium (DMEM; containing 50 μg/mL ascorbic acid and 10 nmol/L β-sodium glycerate) to induce medium culture for 30 days.

Preparation and Grouping of Rat Fracture Models

Under aseptic conditions, 10% chloral hydrate was used for anesthesia, according to 0.3g/kg body weight. The left leg of the rat was depilated and sterilized. The inside of the left humeral platform was 1-2 mm, and the wire sawed the tibia. The rats in the fracture group were separated into fracture group, si-NC group, and si-miR-1 group, in which 1×10^5 bone marrow mesenchymal stem cells transfected with miR-1 negative control or si-miR-1 plasmid were injected into the injured site. 100,000 units of penicillin injection was performed to prevent infection for three consecutive days. At 6 weeks after operation, 2 ml of blood was collected from portal vein in each group, and centrifuged to obtain the serum which was stored at -20°C for use. Five rats were sacrificed, and the specimens were cut around the fracture site.

Observation of the Growth of Osteophytes

After the rats were sacrificed, the bone mineral density (BMD) of the fractures of the rats was measured by local bone densitometry. The rapid scan mode was applied, and the fracture line was used as the center point within 2.0 mm x 1.5 mm rectangular area.

Determination of ALP Content

The ALP content was determined according to the instructions of the ALP test kit. After centrifugation, the precipitate was collected by adding Triton-X100. After mixing, various OD values were measured at 520 nm, and the ALP content was calculated.

MTT Assay for the Proliferation of BMSCs in Each Group

BMSCs were treated as mentioned above. After 48 hours of cell treatment in each dose group, 20 μl of sterile MTT was added with 3 replicate wells in each group. After 4 hours of continuous culture, the supernatant was completely removed, 150 μl/well of DMSO was added for 10 min followed by measuring absorbance (A) value to calculate cell proliferation rate.

Caspase 3 Activity Detection

The changes in the Caspase 3 activity in each group of cells were examined according to the kit instructions.

Real Time-PCR

The bone tissues of each group were ground on ice, extracted with TRIzol reagent, and subjected to DNA reverse transcription synthesis according to the kit instructions. The primers were designed according to each gene sequence by PrimerPremier 6.0 and synthesized by Yingjun Biotechnology Co., Ltd. (Shanghai, China) (Table I). Real Time-PCR reaction conditions were as follows: 56°C 1 min, 92°C 30 s, 58°C 45 s, 72°C 35 s, for a total of 35 cycles. Data were collected using the PCR reactor software and GAPDH was used as a reference. According to the fluorescence quantification, the starting cycle number (CT) of all samples and standards was calculated. Based on the standard CT value, $2^{-\Delta Ct}$ method was applied to analyze gene expression

Western Blot

The bone tissues of each group were extracted and the protein was isolated using RIPA lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA) and quantified followed by separation on 10% Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE) for Western blot analysis using 1:1000 dilution of primary

Table I. Primer sequences.

Gene	Forward 5′-3′	Reverse 5′-3′
GAPDH	AGTACCAGTCTGTTGCTGG	TAATAGACCCGGATGTCTGGT
Runx2	CTTAGTGGTCTCTACTTGTT	TCACCCTCTCACAGCTTG
miR-1	CCCACCTCTTCTAGAATCT	TATTGGACCTCGCGGTAATT
OPN	AGTGGGGTCTCTAGCTTGTT	CTCCCAACACAGCTTG

antibody TLR1 monoclonal antibody. The membrane was developed after addition of enhanced chemiluminescence (ECL) for 1 min.

ELISA

Blood was taken from the abdominal aorta and centrifuged to collect the serum which was used to measure type I collagen and BMP-2 level by ELISA according to kit instructions.

Dual-Luciferase Report Assay

The predicted miR-1 sequence was inserted into the pmirGLO vector and the reporter and TLR1 mimics were co-transfected into BMSCs for 48 h followed by detecting the Luciferase activity.

Statistical Analysis

Statistical Product and Service Solution software (SPSS 16.0; Chicago, IL, USA) was applied for analyzing data which were displayed as mean ± standard deviation (SD) and assessed by one-way ANOVA. $p<0.05$ indicates a difference.

Results

Regulation of MiR-1 on MiR-1 Expression in BMSCs

MiR-1 siRNA significantly downregulated miR-1 expression in BMSCs ($p<0.05$). The transfection of pcDNA miR-1 plasmid into BMSCs significantly upregulated miR-1 expression ($p<0.05$) (Figure 1).

Effect of MiR-1 on the Proliferation of BMSCs Cells

The transfection of miR-1 siRNA to BMSCs downregulated miR-1 and significantly promoted the proliferation of BMSCs ($p<0.05$). The transfection of pcDNA miR-1 plasmid into BMSCs upregulated miR-1 expression and significantly inhibited BMSCs cell proliferation. ($p<0.05$) (Figure 2).

Effect of MiR-1 on the Activity of Caspase 3 in BMSCs Cells

Transfection of miR-1 siRNA downregulated miR-1 expression in BMSCs and significantly inhibited Caspase 3 activity ($p<0.05$). The transfection of pcDNA miR-1 plasmid into BMSCs upregulated miR-1 expression and significantly promoted Caspase 3 activity ($p<0.05$) (Figure 3).

Effect of MiR-1 on the Expression of Osteogenic Genes Runx2 and OPN in BMSCs

Transfection of miR-1 siRNA into BMSCs downregulated miR-1, which significantly promoted the expression of osteogenic genes Runx2 and OPN in BMSCs ($p<0.05$). Transfection of pcDNA miR-1 plasmid into BMSCs upregulated miR-1 expression and significantly inhibited the expression of osteogenic genes Runx2 and OPN in BMSCs (Figure 4).

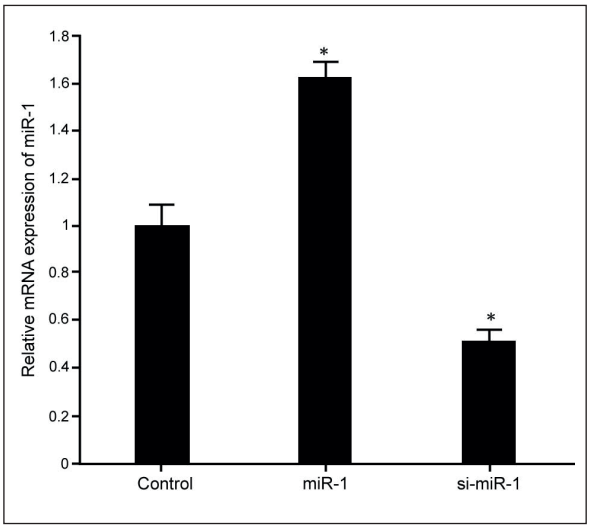

Figure 1. Regulation of the effect of miR-1 on the expression of miR-1 in BMSCs. Compared with the control group, *$p<0.05$.

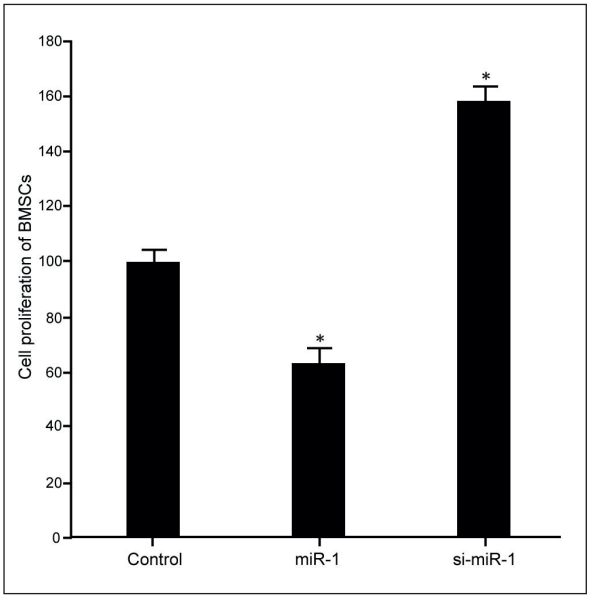

Figure 2. Regulation of the effect of miR-1 on the proliferation of BMSCs. Compared with the control group, *$p<0.05$.

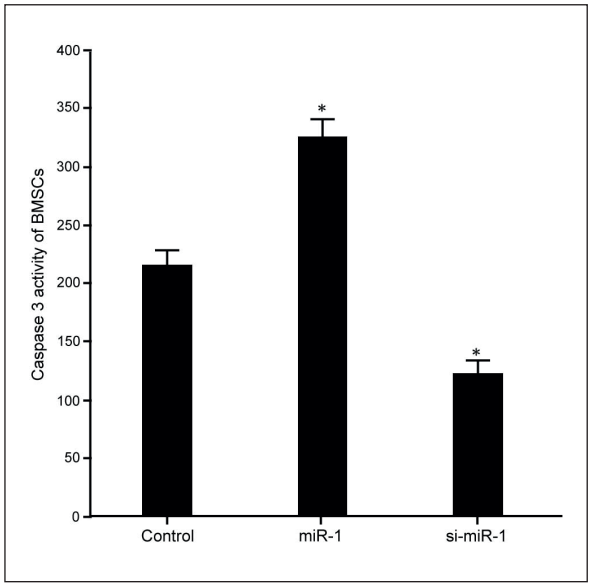

Figure 3. Regulation of the effect of miR-1 on Caspase 3 activity in BMSCs. Compared with the control group, *p<0.05.

Effect of MiR-1 on TLR1 level in BMSCs Cells

MiR-1 siRNA downregulated miR-1 expression in BMSCs and significantly inhibited TLR1 expression (p<0.05). The transfection of pcDNA miR-1 plasmid into BMSCs upregulated miR-1 expression and significantly promoted TLR1 expression (p<0.05) (Figure 5).

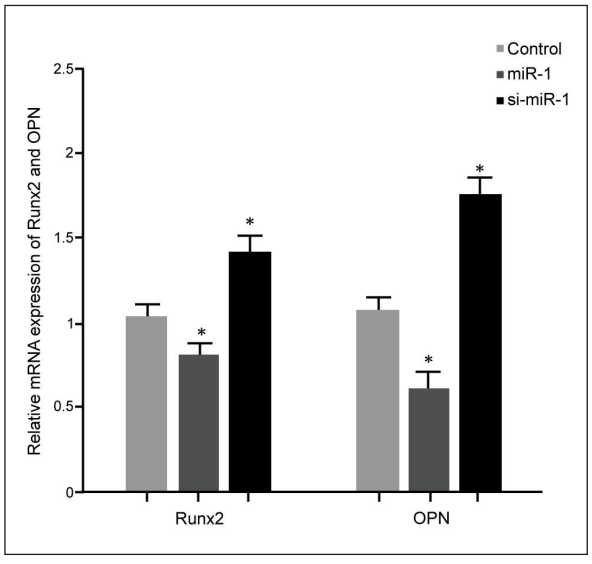

Figure 4. Effect of miR-1 on the expression of osteogenic genes Runx2 and OPN in BMSCs. Compared with the control group, *p<0.05.

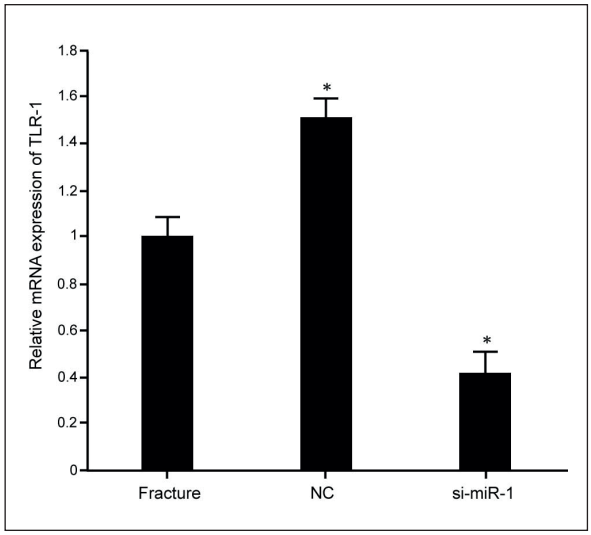

Figure 5. The effect of miR-1 on the expression of TLR1 in BMSCs cells. Compared with the control group, *p<0.05.

MiR-1 Targeting Gene Analysis in BMSCs

Luciferase reporter assay was performed to analyze miR-1 targeting genes in BMSCs by establishing miR-1 wild type (WT) and variant (Mut), respectively. The results showed that targeted reporter analysis predicted TLR1 to be a target of miR-1. TLR1 mimics inhibited miR-1 Luciferase activity (Figure 6).

Effect of MiR-1 Downregulation on Fractured Rats and Bone Mineral Density in Fractured Rats

At 6 weeks postoperatively, the bone mineral density of the bone tissue in each group was measured. The results showed that BMSCs transfected with miR-1 negative control or miR-1 siRNA significantly increased the serum bone mineral density (p<0.05), with more changes in the miR-1 siRNA BMSCs group than miR-1 negative control (p<0.05) (Figure 7).

Effect of Downregulation of MiR-1 Expression in BMSCs of Fractured Rats on ALP Activity in Bone Tissue of Fractured Rats

MiR-1 transfection significantly promoted ALP activity in bone tissue of fractured rat (p<0.05), while miR-1 siRNA-transfected BMSCs in fractured rats significantly increased ALP activity, compared to miR-1 negative control (p<0.05) (Figure 8).

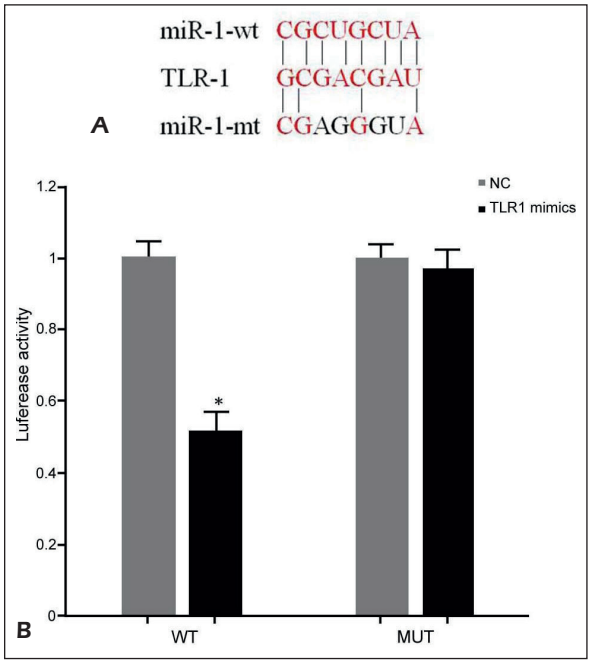

Figure 6. Targeted gene analysis of miR-1 in BMSCs. **A**, MiR-1 targeted predictive analysis in BMSCs. **B**, Luciferase report statistical analysis, compared with NC group, *p<0.05.

Effect of MiR-1 Downregulation in BMSCs of Fractured Rats on Type I Collagen and BMP-2 level in Fractured Rats

ELISA analysis showed that miR-1 transfection significantly upregulated type I collagen and BMP-2 in bone tissue of fractured rats (p<0.05) with more significant changes in miR-1 siRNA compared to NC (Figure 9).

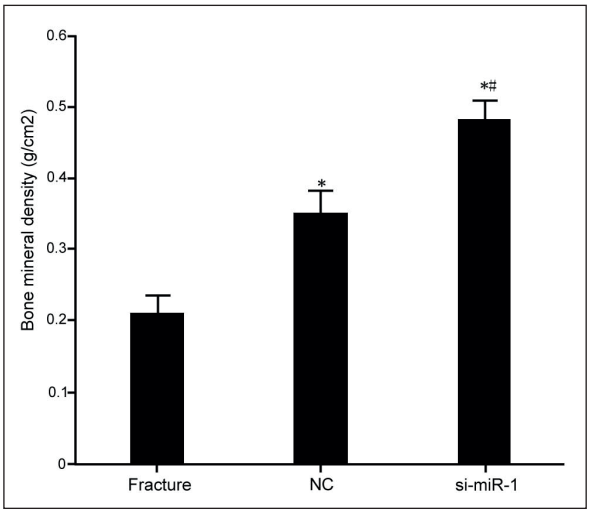

Figure 7. Downregulation of miR-1 expression in BMSCs of fractured rats on bone mineral density in fractured rats. Compared with the fracture group, *p<0.05; compared with the NC group, $^{\#}p$<0.05.

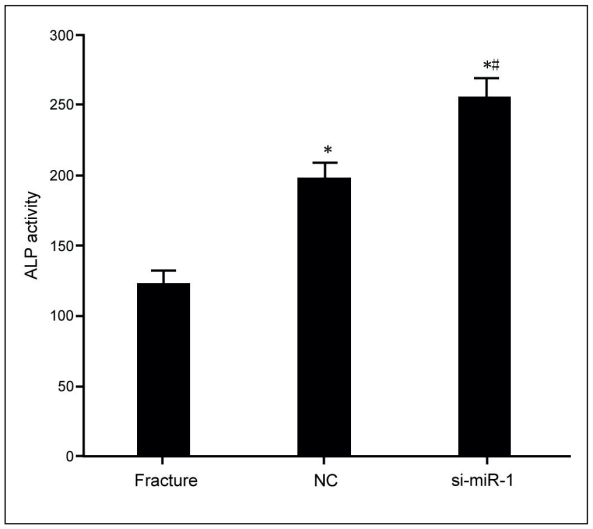

Figure 8. Effect of downregulation of miR-1 expression in BMSCs of fractured rats on ALP activity in bone tissue of fractured rats. Compared with the fracture group, *p<0.05; compared with the NC group, $^{\#}p$<0.05.

Effect of MiR-1 Downregulation in BMSCs of Fractured Rats on TLR1 Expression in Fractured Rats

Western blot analysis showed significantly upregulated TLR1 in the miR-1 negative control group (p<0.05). Downregulation of miR-1 expression in BMSCs of fractured rats significantly inhibited TLR1 expression in fractured rats (p<0.05) (Figure 10).

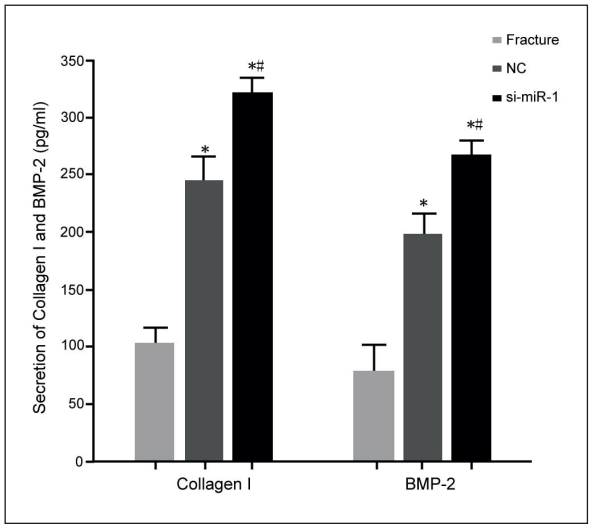

Figure 9. Downregulation of miR-1 expression in BMSCs of fractured rats on the expression of type I collagen and BMP-2 in fractured rats. Compared with the fracture group, *p<0.05; compared with the NC group, $^{\#}p$<0.05.

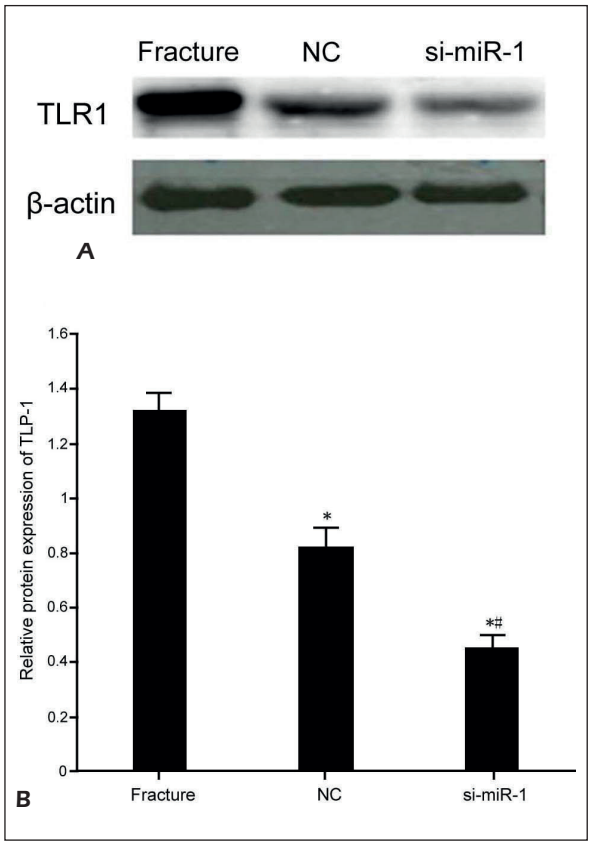

Figure 10. Downregulation of miR-1 expression in BMSCs of fractured rats and its effect on TLR1 expression in fractured rats. **A**, Western blot analysis down-regulated the expression of miR-1 in BMSCs of fractured rats on the expression of TLR1 in fractured rats. **B**, Effect of miR-1 down-regulation in BMSCs of fractured rats on the expression of TLR1 in fractured rats, compared with fractures group, $^{*}p<0.05$; compared with NC group, $^{\#}p<0.05$.

Discussion

BMSCs can differentiate in multiple directions and the direction of osteogenic differentiation is a key factor restricting bone growth and involves in bone development, regeneration, and repair[18]. Hong et al[19] have shown that BMSCs can provide intrinsic osteogenesis and thus supplementing a large amount of bone loss. MiRNAs have been shown to be involved in the development, progression, and prognosis of various diseases and can also be used to diagnose and treat diseases. Some MiRNAs regulate differentiation of mesenchymal stem cells and osteoblasts during bone differentiation and regeneration. The expression of MiRNAs is closely related to bone tissue hyperplasia, and MiR-1 may regulate osteogenic differentiation[17,20]. However, MiR-1's role in osteogenesis remains to be elucidated. This study demonstrates that transfection of miR-1 siRNA into BMSCs downregulates miR-1 expression, promotes BMSCs cell proliferation, inhibits Caspase 3 activity, and promotes osteogenic genes Runx2 and OPN expression, whereas transfection of miR-1 plasmid into BMSCs reverses these changes, inhibits BMSCs cell proliferation, promotes Caspase 3 activity, inhibits osteogenic genes Runx2 and OPN expression, suggesting that low miR-1 level can promote BMSCs cell proliferation, inhibit apoptosis, and promote BMSCs differentiation into osteoblasts.

The repair effect of low expression of miR-1 on BMSCs on bone injury in fractured rats was further analyzed. By transfecting miR-1 siRNA into BMSCs, miR-1 can be downregulated in bone fracture rats which increased BMD and ALP activity, and upregulated type I collagen and BMP-2 and promoted bone defect repair. Type I collagen and BMP-2 can induce irreversible differentiation of mesenchymal cells into chondrocytes and osteoblasts, upregulate osteocalcin and alkaline phosphatase, and promote the expression of most bone matrix proteins and the mineralization of extracellular matrices, leading to new bone formation[21,22]. Toll-Like receptors (TLRs) are important protein molecules in non-specific immunity. They are model recognition receptors and serve as a bridge between non-specific immunity and specific immunity. TLR1 is an important member of the TLR family. It not only regulates the immune response, but also inhibits the osteogenic potential, suggesting that inhibition of TLR1 expression is involved in stem cell osteogenesis regulation[23,24]. This study showed TLR2 to be target of miR-1. Transfection of miR-1 siRNA downregulated miR-1 in BMSCs and inhibited TLR1 expression. The expression of TLR1 was increased in the fracture group and miR-1 downregulation in BMSCs of the fractured rats significantly inhibited the expression of TLR1, which promoted the repair of fractures, indicating that miR-1 downregulation promotes BMSCs proliferation and the osteogenic activity by targeting TLR1, thereby promoting bone repair.

Conclusions

In summary, the downregulation of miR-1 promotes BMSCs osteogenic differentiation *via* targeting TLR1 expression, which can promote osteogenic differentiation and bone healing.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Lomonte ABV, Mendonca JA, Brandao GD, Castro ML. Multicenter, randomized, double-blind clinical trial to evaluate efficacy and safety of combined glucosamine sulfate and chondroitin sulfate capsules for treating knee osteoarthritis. Adv Rheumatol 2018; 58.
2) Salaffi F, Di Carlo M, Carotti M, Farah S, Ciapetti A, Gutierrez M. The impact of different rheumatic diseases on health-related quality of life: a comparison with a selected sample of healthy individuals using SF-36 questionnaire, EQ-5D and SF-6D utility values. Acta Biomed 2019; 89: 541-557.
3) Dumenci L, Perera RA, Keefe FJ, Ang DC, Slover J, Jensen MP, Riddle DL. Model-based pain and function outcome trajectory types for patients undergoing knee arthroplasty: a secondary analysis from a randomized clinical trial. Osteoarthritis Cartilage 2019; 27: 878-884.
4) Ackland DC, Robinson D, Redhead M, Lee PVS, Moskaljuk A, Dimitroulis G. A personalized 3D-printed prosthetic joint replacement for the human temporomandibular joint: from implant design to implantation. J Mech Behav Biomed 2017; 69: 404-411.
5) Ji QB, Xu XJ, Kang L, Xu YM, Xiao JB, Goodman SB, Zhu X, Li WC, Liu J, Gao X, Yan ZF, Zheng YX, Wang Z, Maloney WJ, Ye QN, Wang Y. Hematopoietic PBX-interacting protein mediates cartilage degeneration during the pathogenesis of osteoarthritis. Nat Commun 2019; 10.
6) Parry E, Ogollah R, Peat G. Significant pain variability in persons with, or at high risk of, knee osteoarthritis: preliminary investigation based on secondary analysis of cohort data. BMC Musculoskelet Disord 2017; 18: 80.
7) Barre FP, Flinders B, Garcia JP, Jansen I, Huizing LR, Porta T, Creemers LB, Heeren RM, Cillero-Pastor B. Derivatization strategies for the detection of triamcinolone acetonide in cartilage by using matrix-assisted laser desorption/ionization mass spectrometry imaging. Anal Chem 2016; 88: 12051-12059.
8) Park YB, Ha CW, Lee CH, Yoon YC, Park YG. Cartilage regeneration in osteoarthritic patients by a composite of allogeneic umbilical cord blood-derived mesenchymal stem cells and hyaluronate hydrogel: results from a clinical trial for safety and proof-of-concept with 7 years of extended follow-up. Stem Cell Transl Med 2017; 6: 613-621.
9) Huang X, Chen X, Chen H, Xu D, Lin C, Peng B. Rho/rho-associated protein kinase signaling pathway-mediated downregulation of runt-related transcription factor 2 expression promotes the differentiation of dental pulp stem cells into odontoblasts. Exp Ther Med 2018; 15: 4457-4464.
10) Pan J, Lu X, Yang G, Han Y, Tong X, Wang Y. Lumbar disc degeneration was not related to spine and hip bone mineral densities in chinese: facet joint osteoarthritis may confound the association. Arch Osteoporos 2017; 12: 20.
11) Gebraad A, Kornilov R, Kaur S, Miettinen S, Haimi S, Peltoniemi H, Mannerstrom B, Seppanen-Kaijansinkko R. Monocyte-derived extracellular vesicles stimulate cytokine secretion and gene expression of matrix metalloproteinases by mesenchymal stem/stromal cells. FEBS J 2018; 285: 2337-2359.
12) Gu H, Chen J, Song Y, Shao H. Gastric adenocarcinoma predictive long intergenic non-coding RNA promotes tumor occurrence and progression in non-small cell lung cancer via regulation of the miR-661/eEF2K signaling pathway. Cell Physiol Biochem 2018; 51: 2136-2147.
13) Yan Y, Qin D, Hu B, Zhang C, Liu S, Wu D, Huang W, Huang X, Wang L, Chen X, Zhang L. Deletion of miR-126a promotes hepatic aging and inflammation in a mouse model of cholestasis. Mol Ther Nucleic Acids 2019; 16: 494-504.
14) Zhang X, Sai B, Wang F, Wang L, Wang Y, Zheng L, Li G, Tang J, Xiang J. Hypoxic BMSC-derived exosomal miRNAs promote metastasis of lung cancer cells via STAT3-induced EMT. Mol Cancer 2019; 18: 40.
15) Chen QX, Zhou R, Zhang YC, Zhu SG, Xiao CC, Gong J, Li K, Tang H, Sun C, Zhang J. Bone marrow mesenchymal stromal cells attenuate liver allograft rejection may via upregulation PD-L1 expression through downregulation of miR-17-5p. Transpl Immunol 2018; 51: 21-29.
16) Novello C, Pazzaglia L, Cingolani C, Conti A, Quattrini I, Manara MC, Tognon M, Picci P, Benassi MS. MiRNA expression profile in human osteosarcoma: role of miR-1 and miR-133b in proliferation and cell cycle control. Int J Oncol 2013; 42: 667-675.
17) Lee YE, Hong CY, Lin YL, Chen RM. MicroRNA-1 participates in nitric oxide-induced apoptotic insults to MC3T3-E1 cells by targeting heat-shock protein-70. Int J Biol Sci 2015; 11: 246-255.
18) Nakajima N, Takahashi T, Kitamura R, Isodono K, Asada S, Ueyama T, Matsubara F, Oh H. MicroRNA-1 facilitates skeletal myogenic differentiation without affecting osteoblastic and adipogenic differentiation. Biochem Bioph Res Co 2006; 350: 1006-1012.
19) Hong RH, Xie JJ, Zhang FG, Pan HS, Guo CJ. Identification of association between rs1057317 polymorphism in TLR4 3'-untranslated region and the susceptibility to osteoporosis. J Cell Biochem 2019; 120: 13765-13774.
20) Sotor J, Martynkien T, Schunemann PG, Mergo P, Rutkowski L, Sobon G. All-fiber mid-infrared source tunable from 6 to 9 μm based on difference frequency generation in OP-GaP crystal. Opt Express 2018; 26: 11756-11763.

21) Mazzone AL, Baker RA, McNicholas K, Woodman RJ, Michael MZ, Gleadle JM. Circulating and Urinary miR-210 and miR-16 Increase during cardiac surgery using cardiopulmonary bypass - a pilot study. J Extra Corpor Technol 2018; 50: 19-29.

22) Dohare P, Cheng B, Ahmed E, Yadala V, Singla P, Thomas S, Kayton R, Ungvari Z, Ballabh P. Glycogen synthase kinase-3β inhibition enhances myelination in preterm newborns with intraventricular hemorrhage, but not recombinant Wnt3A. Neurobiol Dis 2018; 118: 22-39.

23) Quan LL, Wang Y, Liang JL, Qiu T, Wang HY, Zhang Y, Zhang Y, Hui Q, Tao K. Screening for genes, transcription factors and mirnas associated with the myogenic and osteogenic differentiation of human adipose tissue-derived stem cells. Int J Mol Med 2016; 38: 1839-1849.

24) Abdi J, Mutis T, Garssen J, Redegeld FA. Toll-like receptor (TLR)-1/2 triggering of multiple myeloma cells modulates their adhesion to bone marrow stromal cells and enhances bortezomib-induced apoptosis. PLoS One 2014; 9.