

miR-27b attenuates dexamethasone-inhibited proliferation and osteoblastic differentiation in MC3T3-E1 cells by targeting PPAR γ 2

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Abstract. Osteoporosis is a metabolic bone illness characterized by low bone density and a high risk of fracture. It is estimated that there are >60 million individuals in China suffering from this disease, which highlights an urgent requirement for the development of novel and safe drugs for the long-term treatment of osteoporosis. MicroRNAs (miRNAs/miRs) have previously been identified as critical regulators in the progression of osteoporosis. As an intronic miRNA, miR-27b enhances the osteoblastic differentiation of stem cells from the bone marrow and the maxillary sinus membrane. However, the mechanism underlying miR-27b in osteoporosis remains to be elucidated. In the present study, MC3T3-E1 pre-osteoblasts were treated with dexamethasone (DEX) to establish an *in vitro* model of osteoporosis. The results of the present study demonstrated that DEX treatment markedly inhibited the viability of MC3T3-E1 cells, and downregulated the expression level of miR-27b. The results of reverse transcription-quantitative PCR, western blotting and dual-luciferase assays revealed that miR-27b directly regulated and suppressed the expression of peroxisome proliferator-activated receptor γ 2 (PPAR γ 2) in MC3T3-E1 cells. Furthermore, overexpression of miR-27b by transfection

of cells with miR-27b mimic attenuated DEX-mediated inhibition of cell viability, alkaline phosphatase (ALP) activity and the expression levels of bone morphogenetic protein-2 (BMP2), runt-related protein 2 (Runx2) and osteocalcin (OCN). The results of the present study indicated that miR-27b alleviated DEX-inhibited proliferation and osteoblastic differentiation. Moreover, miR-27b knockdown repressed MC3T3-E1 cell viability, ALP activity and protein levels of BMP2, Runx2 and OCN. However, these effects were abrogated by small interfering RNA-mediated PPAR γ 2 silencing. In conclusion, the results of the present study demonstrated that miR-27b attenuated DEX-inhibited proliferation and osteoblastic differentiation in MC3T3-E1 pre-osteoblasts by targeting PPAR γ 2.

Introduction

Bone metabolism, including bone formation and resorption, is a continuous physiological process that regulates bone growth and remodeling (1). Mechanistically, bone formation is initialized by osteoblasts by synthesizing and secreting the main organic components of bone matrix, collagen and mucopolysaccharide (2). By contrast, osteoclasts trigger bone resorption by releasing proteinases to dissolve bone mineral and degrade bone matrix proteins (3). In healthy bone remodeling, bone formation and resorption are maintained in a dynamic balance. However, as the human body ages, the rate of bone formation decreases, disrupting the aforementioned balance, thus leading to the development of metabolic bone diseases such as osteoporosis (4).

Patients with osteoporosis exhibit decreased bone density and a high risk of fracture (5). In China, osteoporosis is a serious public health concern due to an increasing aging population (6). Previous studies have estimated that there are >60 million individuals in China suffering with osteoporosis (7,8). A number of medicines have been used to treat osteoporosis in the clinic; however, a number of potential side effects have been associated with these medicines that may impair the health of patients with osteoporosis during long-term treatment (9). Thus, further investigation into the development of novel, safe therapeutic strategies for the treatment of osteoporosis is required.

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Abbreviations: DEX, dexamethasone; ALP, alkaline phosphatase; BMP2, bone morphogenetic protein-2; Runx2, runt-related protein 2; OCN, osteocalcin; PPAR γ 2, peroxisome proliferator-activated receptor γ 2; miR-27b, microRNA-27b

Key words: miR-27b, proliferation, osteoblastic differentiation, PPAR γ 2

MicroRNAs (miRNAs/miRs) have been identified as critical regulators in the development of osteoporosis. Notably, miR-483-5p was found to be markedly upregulated and promoted osteoclast differentiation in patients with osteoporosis (10). Another study also investigated the role of miR-449b-5p in osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) and found that miR-449b-5p could aggravate osteoporosis by inhibiting osteogenic differentiation through targeting of *Satb2* (11). miR-27b is an intragenic miRNA located in the *C9orf3* gene on chromosome 9 (12). miR-27b is involved in a number of biological processes, such as cell differentiation, proliferation and apoptosis, by inhibiting the expression of target genes post-transcriptionally (13-15). Previous studies revealed that miR-27b expression levels were abnormally downregulated during the formation of osteoclasts (16,17). Moreover, miR-27b regulated the osteogenesis of stem cells from bone marrow and the maxillary sinus membrane (18,19). Thus, we hypothesize that miR-27b may be implicated in the pathological process of osteoporosis.

Peroxisome proliferator-activated receptor γ (PPAR γ) is a ligand-activated type II nuclear receptor that is mainly distributed in adipose tissue (20). As a critical transcription factor, PPAR γ is implicated in a number of metabolic processes, such as fatty acid and glucose metabolism (21,22). In bone metabolism, PPAR γ inhibits osteoblast formation and enhances osteoclastogenesis (23,24). A previous study demonstrated that PPAR γ 2, an isoform of PPAR γ , is negatively regulated by miR-27b in chondrocytes (25). Thus, miR-27b may play a key role in the development of osteoporosis by targeting PPAR γ 2.

An increasing number of studies have suggested that osteoblast dysfunction disrupts the balance between bone resorption and bone formation by inhibiting osteoblast differentiation and proliferation, and enhancing osteoblast apoptosis in glucocorticoid-induced osteoporosis (26,27). Exposure to dexamethasone (DEX) induced the apoptosis of osteoblasts and inhibited MC3T3-E1 cell proliferation (28). In the present study, MC3T3-E1 pre-osteoblasts were treated with DEX to induce osteoporosis *in vitro*. The aim of the present study was to determine the function of the miR-27b/PPAR γ axis in DEX-induced proliferation and osteoblastic differentiation in MC3T3-E1 cells.

Materials and methods

Cell culture and DEX treatment. Mouse MC3T3-E1 pre-osteoblasts obtained from American Type Culture Collection (cat. no. CRL-2593) were cultured in DMEM (cat. no. SH30243.01; HyClone; Cytiva) containing 10% FBS (cat. no. 16000e044; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (cat. no. P1400; Beijing Solarbio Science & Technology Co., Ltd.) under 5% CO₂ at 37°C. Osteogenic differentiation was induced as described in a previous study (29). MC3T3-E1 pre-osteoblasts were cultured in an osteogenic differentiation medium consisting of DMEM, 10% FBS, 4 mM glycerophosphate (cat. no. G9891; Sigma-Aldrich; Merck KGaA) and 25 μ g/ml ascorbic acid (cat. no. A4403; Sigma-Aldrich; Merck KGaA) until cells reached 70% confluence. Cells were treated with 1 μ M DEX (cat. no. D4902; Sigma-Aldrich; Merck KGaA) to induce osteoporosis *in vitro* and DMSO (Beijing Solarbio Science & Technology Co., Ltd.) was used as a control.

Cell transfection. For overexpression or knockdown of miR-27b-3p, the miR-27b-3p mimic (5'-UUCACAGUGGCU AAGUUCUGC-3'), miR-27b-3p inhibitor (5'-GCAGAACUUA GCCACUGUGAA-3') and their corresponding negative controls (NCs) (NC-mimic, 5'-UGAUACUGUAGACUCGUC AGC-3'; and NC-inhibitor, 5'-CAGUACUUUUGUGUAGUA CAA-3') were synthesized by Guangzhou RiboBio Co., Ltd. Three PPAR γ 2 small interfering (si)RNAs were designed to silence PPAR γ 2 expression and their sequences were as follows: siPPAR γ 2-1, 5'-CGCAUCCUUUGACAUCAATT-3'; siPPAR γ 2-2, 5'-CAAUGGUUGCUGAUUACAATT-3'; and siPPAR γ 2-3, 5'-GGGCGAUCUUGACAGGAAATT-3'. A scrambled siRNA was used as the corresponding NC (siNC, 5'-UUCUCCGAACGUGUCACGUTT-3'). MC3T3-E1 cells were trypsinized and suspended at a density of 1x10⁶ cells/ml. A total of 2 ml cell suspension was inoculated into six-well plates overnight at 37°C in a 5% CO₂ incubator. When MC3T3-E1 cells reached a 60-70% confluence, cells were transfected with 5 μ l mimic, inhibitor or siRNAs using Lipofectamine[®] 2000 (cat. no. 11668-019; Invitrogen; Thermo Fisher Scientific, Inc.) for 4-6 h at 37°C. Following 24 h of transfection, serum-free transfer solution was replaced with complete medium, (DMEM with 10% FBS) and cells were cultured for a further 48 h.

Cell Counting Kit-8 (CCK-8) assay. MC3T3-E1 cells were re-suspended in PBS at a density of 2x10⁴ cells/ml for 1 min at 37°C. A total of 100 μ l suspension was added into a 96-well plate and cultured overnight at 37°C. Following 0, 12, 24 or 48 h of treatment as aforementioned, cells were treated with 100 μ l CCK-8 solution (Signalway Antibody LLC) for 1 h. Cell viability was assessed by detecting the OD value at 460 nm.

Alizarin red S (ARS) staining. Following the induced osteogenic differentiation of MC3T3-E1 cells, osteogenic differentiation medium was discarded and the cells were washed three times with PBS. Cells were fixed with 4% paraformaldehyde for 30 min at 37°C in the dark, and subsequently stained with 1% ARS (Sigma-Aldrich; Merck KGaA) for 3-5 min at room temperature. Calcification nodules were observed, and images were captured using an inverted light microscope (magnification, x100).

Alkaline phosphatase (ALP) staining. Following the initiation of osteogenic induction, ALP staining was performed according to the manufacturer's protocol. Briefly, MC3T3-E1 cells were fixed in 4% formalin for 10 min at 25°C and washed three times with PBS. ALP staining was performed using a staining kit (cat. no. G1480; Beijing Solarbio Science & Technology Co., Ltd.) according to the manufacturer's protocol. Images of stained cells were captured using an inverted light microscope (magnification, x400).

Reverse transcription-quantitative (RT-q)PCR. RNA samples were extracted from MC3T3-E1 cells using TRIzol[®] (Thermo Fisher Scientific, Inc.) and reverse transcribed into cDNA using the RevertAid First Strand cDNA Synthesis kit (cat. no. K1621; Fermentas; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols, and subsequently

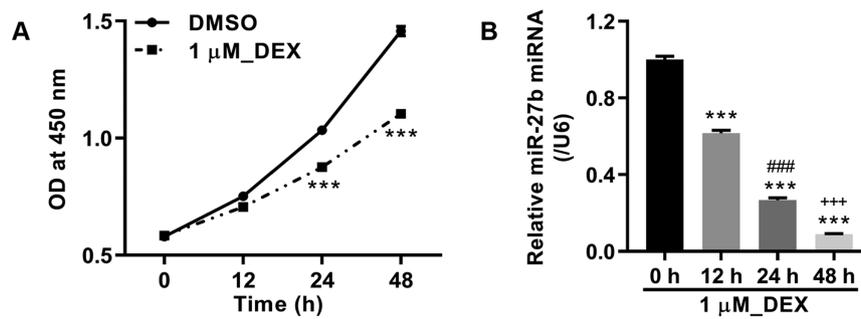


Figure 1. DEX treatment significantly downregulates cell viability and miR-27b expression levels in MC3T3-E1 pre-osteoblasts. (A) Cell viability and (B) miR-27b expression levels were detected at 0, 12, 24 and 48 h following DEX treatment. ***P<0.001 vs. DMSO or 0 h; ###P<0.001 vs. 12 h; +++P<0.001 vs. 24 h. OD, optical density; DEX, dexamethasone; miR/miRNA, microRNA.

amplified using the SYBR Green qPCR Master mix (cat. no. K0223; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The following thermocycling conditions were used for qPCR: 95°C for 10 min; followed by 40 cycles at 95°C for 15 sec and 60°C for 45 sec; final extension at 95°C for 15 sec, 60°C for 1 min, 95°C for 15 sec and 60°C for 15 sec. U6 and GAPDH were used as internal controls, and the relative levels of miR-27b and PPAR γ -2 mRNA were calculated using the $2^{-\Delta\Delta C_q}$ method (10). The primer sequences were as follows: miR-27b-3p forward, 5'-GCGCGTTCACAGTGGC TAAG-3' and reverse, 5'-AGTGCAGGGTCCGAGGTATT-3'; U6 forward, 5'-GCTTCGGCAGCAC-3' and reverse, 5'-GGAA CGCTTCACG-3; PPAR γ 2 forward, 5'-TGCGATCAAAGTAG AAC-3' and reverse, 5'-AAGCCTGATGCTTTATCC-3'; and GAPDH forward, 5'-CTGCCAGAACATCATCC-3' and reverse, 5'-CTCAGATGCCTGCTTAC-3'.

Western blotting. Target proteins were extracted from MC3T3-E1 cells using RIPA lysis buffer (Jrdun Biotechnology) and the protein concentration was determined by a bicinchoninic acid assay kit (Thermo Fisher Scientific, Inc.). The isolated proteins (25 μ g/lane) were separated by electrophoresis in 10% SDS-polyacrylamide gels, and transferred onto a PVDF membrane. Membranes were subsequently blocked with 5% non-fat milk overnight at 4°C, and incubated with the following primary antibodies: Anti-PPAR γ 2 (1:500; cat. no. ab45036; Abcam), anti-bone morphogenetic protein-2 (BMP2; 1:1,000; cat. no. orb334018; Biorbyt, Ltd.), anti-runt-related protein 2 (Runx2; 1:1,000; cat. no. ab23981; Abcam), anti-osteocalcin (OCN; 1:1,000; cat. no. ab93876; Abcam) and anti-GAPDH (1:2,000; cat. no. 5174; CST Biological Reagents Co., Ltd.) overnight at 4°C. Following primary incubation, membranes were incubated with the horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Beyotime Institute of Biotechnology; cat. nos. A0208 and A0216; both 1:1,000) at 37°C for 1 h. Signal quantification was performed by an enhanced chemiluminescence system (Bio-Rad Laboratories, Inc.). The bands were quantified by densitometry with ImageJ software (version 1.51; National Institutes of Health).

Dual-luciferase reporter assay. Bioinformatics software TargetScan 7.2 (targetscan.org/vert_72/) was used to predict target genes of miR-27b, and the results revealed the binding

sites between miR-27b and PPAR γ 2. Wild-type (wt) or mutant (mut) PPAR γ 2-3'untranslated regions (UTRs) were cloned into a pGL3-Promoter plasmid containing the firefly luciferase gene (Promega Corporation). The reconstructed pGL3-Promoter was introduced into the MC3T3-E1 pre-osteoblasts along with the pRL-TK-*Renilla* reporter (Promega Corporation) using Lipofectamine[®] 3000 reagent (Thermo Fisher Scientific, Inc.) following manufacturer's protocol for 4-6 h at 37°C. Following 6 h of transfection, cells were treated with the miR-27b mimic. After 24 h, luciferase activity was assessed using a Dual-Promoter Luciferase Assay kit (cat. no. E1910; Promega Corporation).

Biochemical detection. Following treatment aforementioned, the supernatant of MC3T3-E1 pre-osteoblasts was obtained by centrifugation at 800 x g for 10 min at 4°C, and ALP activity was determined using an ALP kit (cat. no. A059-2; Nanjing Jiancheng Bioengineering Institute). The supernatant and kit solution were mixed and incubated in a water bath for 15 min at 37°C, according to the manufacturer's protocol. The absorbance value was measured at 520 nm.

Statistical analysis. Quantitative analysis was conducted using GraphPad Prism 7.0 (GraphPad Software, Inc.) and each experiment was repeated three independent times. Data are presented as the mean \pm standard deviation. The difference between groups was analyzed using an unpaired t-test, two-way ANOVA followed by Bonferroni's multiple comparisons test or one-way ANOVA followed by Tukey's multiple comparisons test. P<0.05 was considered to indicate a statistically significant difference.

Results

DEX treatment significantly reduces cell viability and miR-27b expression levels in MC3T3-E1 pre-osteoblasts. MC3T3-E1 pre-osteoblasts were cultured with 1 μ M DEX, and cell viability was detected at 0, 12, 24 and 48 h after treatment. The results demonstrated that DEX treatment markedly inhibited the viability of MC3T3-E1 cells at 24 and 48 h compared with DMSO (Fig. 1A). In addition, the miR-27b level was also measured, and DEX treatment significantly downregulated the expression level of miR-27b at 12, 24 and 48 h compared with 0 h (Fig. 1B).

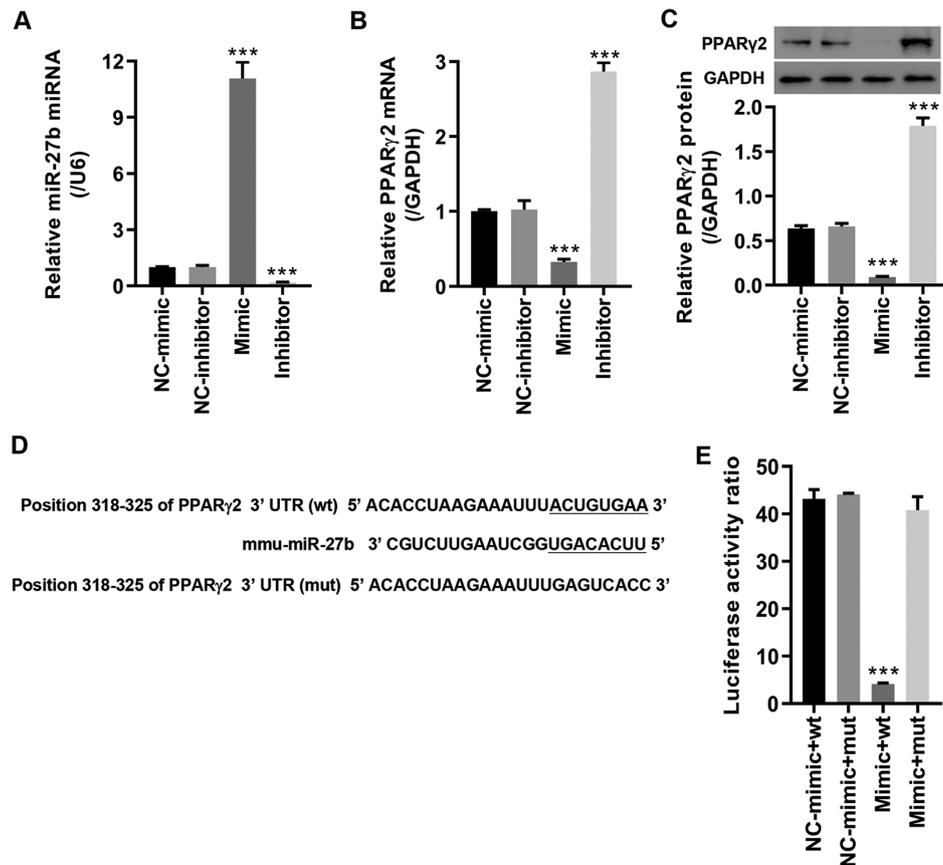


Figure 2. miR-27b negatively regulates the expression of PPAR γ 2. (A) miR-27b and (B) PPAR γ 2 mRNA expression levels were measured in MC3T3-E1 pre-osteoblasts by reverse transcription-quantitative PCR following transfection with the miR-27b mimic or inhibitor. (C) PPAR γ 2 protein levels were detected by western blot analysis. (D) The potential binding sequence of PPAR γ 2 wt and mut vectors with miR-27b. (E) MC3T3-E1 pre-osteoblasts were transfected with the miR-27b mimic and luciferase vector containing wt or mut PPAR γ 2-3'UTRs. Relative luciferase activity was assessed by the dual-luciferase assay. *** $P < 0.001$ vs. NC-mimic, NC-inhibitor or NC-mimic + wt. miR, microRNA; NC, negative control; PPAR γ 2, peroxisome proliferator-activated receptor γ 2; wt, wild-type; mut, mutant; UTR, untranslated region.

miR-27b directly regulates PPAR γ 2. The results of the TargetScan bioinformatics analysis revealed that PPAR γ 2 was predicted to be a potential target of miR-27b. To verify this interaction, the miR-27b mimic and inhibitor were transfected into MC3T3-E1 pre-osteoblasts. The results indicated that the expression level of miR-27b was markedly upregulated by the miR-27b mimic and significantly downregulated by the miR-27b inhibitor compared with its corresponding NC (Fig. 2A). Furthermore, PPAR γ 2 mRNA and protein expression was repressed by the miR-27b mimic and significantly enhanced by the miR-27b inhibitor compared with its corresponding NC (Fig. 2B and C). MC3T3-E1 pre-osteoblasts were co-transfected with the miR-27b mimic and the luciferase vector containing wt or mut PPAR γ 2-3'UTR (Fig. 2D). As demonstrated in Fig. 2E, miR-27b overexpression significantly downregulated the luciferase activity following transfection with PPAR γ 2-3'UTR wt compared with NC-mimic; however, no significant difference was observed in the luciferase activity following co-transfection with the PPAR γ 2-3'UTR mut and the miR-27b mimic. Collectively, these results suggested that miR-27b directly regulated and repressed the expression level PPAR γ 2.

miR-27b overexpression attenuates DEX-inhibited proliferation and osteoblastic differentiation in MC3T3-E1

pre-osteoblasts. The potential functions of miR-27b were observed following transfection of the miR-27b mimic in DEX-treated MC3T3-E1 cells. The results of the present study demonstrated that miR-27b overexpression partly reversed DEX-inhibited miR-27b expression and cell proliferation (Fig. 3A and B). The ARS and ALP staining of M3T3-E1 cells revealed that DEX markedly inhibited osteoblastic differentiation compared with DMSO plus NC-mimic group, while DEX-mediated effects were abrogated by the miR-27b mimic (Fig. 3C and D). In addition, the ALP activity, and the expression levels of BMP2, Runx2 and OCN were investigated as hallmarks of osteoblastic differentiation. The results of the present study demonstrated that DEX treatment significantly decreased the ALP activity and protein expression levels of BMP2, Runx2 and OCN, but increased PPAR γ 2 protein expression levels compared with DMSO plus NC-mimic group. Furthermore, DEX-mediated effects were abrogated by the miR-27b mimic (Fig. 3E-G). These results suggested that miR-27b overexpression attenuated DEX-inhibited osteoblastic differentiation.

Inhibition of miR-27b suppresses proliferation and osteoblastic differentiation in MC3T3-E1 pre-osteoblasts by upregulation of PPAR γ 2. The transfection efficiency of siRNAs targeting PPAR γ 2 in MC3T3-E1 cells was demonstrated by

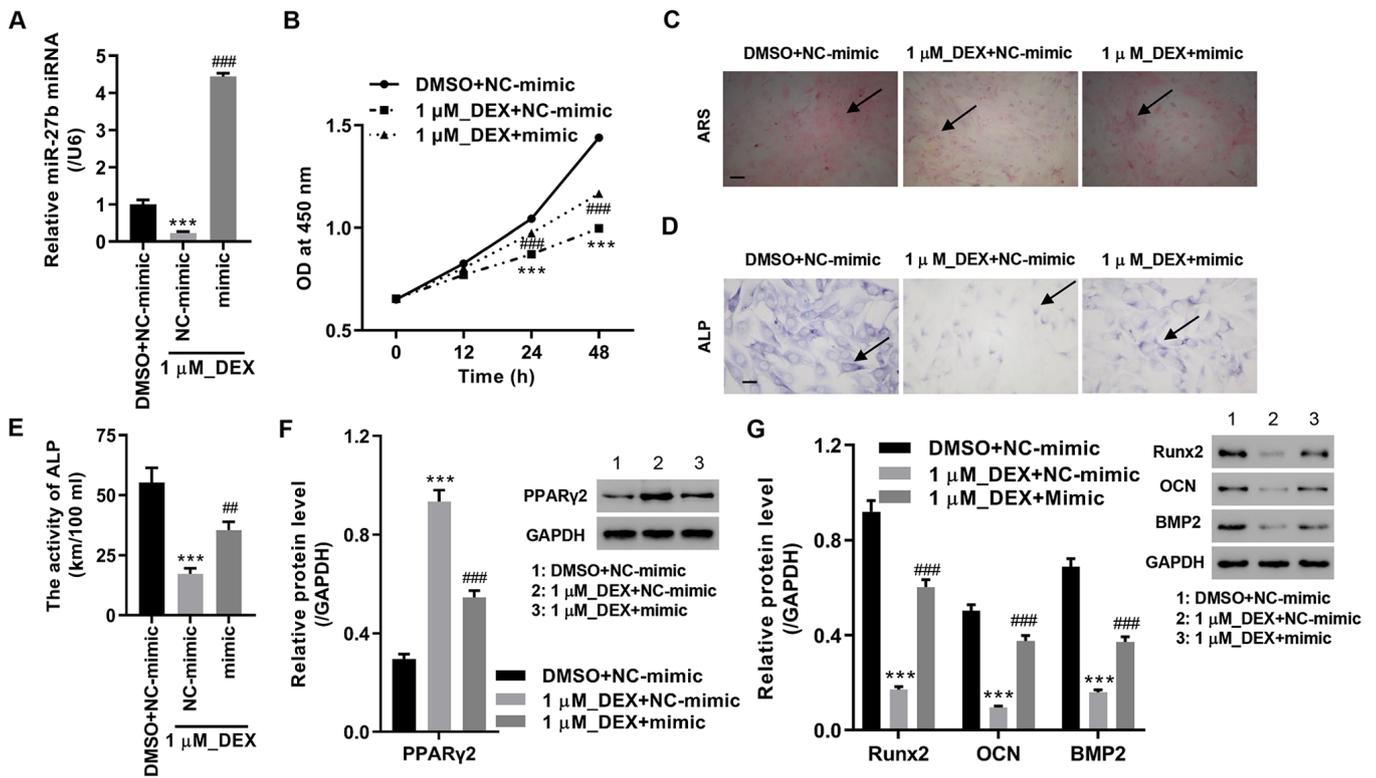


Figure 3. miR-27b mimic attenuates DEX-inhibited proliferation and osteoblastic differentiation in MC3T3-E1 pre-osteoblasts. miR-27b mimic and 1 μ M DEX were used to treat MC3T3-E1 pre-osteoblasts. (A) miR-27b expression levels were measured using reverse transcription-quantitative PCR. (B) Cell viability, (C) ARS staining (scale bar, 100 μ m), (D) ALP staining (scale bar, 25 μ m) and (E) ALP activity were measured. Expression levels of (F) PPAR γ 2, (G) Runx2, OCN and BMP2 were assessed in MC3T3-E1 pre-osteoblasts. ***P<0.001 vs. DMSO + NC-mimic; **P<0.01 and ###P<0.001 vs. 1 μ M_DEX + NC-mimic. miR, microRNA; NC, negative control; DEX, dexamethasone; ARS, Alizarin red S; ALP, alkaline phosphatase; PPAR γ 2, peroxisome proliferator-activated receptor γ 2; BMP2, bone morphogenetic protein-2; Runx2, runt-related protein 2; OCN, osteocalcin.

RT-qPCR and western blot analysis, with the lowest mRNA and protein expression detected in cells transfected with siPPAR γ 2-2. siPPAR γ 2-2 was therefore selected for subsequent analyses (Fig. 4A and B). To investigate the potential regulation of PPAR γ 2 by miR-27b, MC3T3-E1 pre-osteoblasts were co-transfected with the miR-27b inhibitor and PPAR γ 2 siRNA. The results of the present study indicated that miR-27b knockdown significantly increased PPAR γ 2 expression, and decreased cell viability, osteoblastic differentiation, ALP activity and the expression level of BMP2, Runx2 and OCN. However, these effects were abrogated by siPPAR γ 2-2 transfection (Fig. 4C-H). Thus, miR-27b knockdown inhibited proliferation and osteoblastic differentiation in MC3T3-E1 pre-osteoblasts by the upregulation of PPAR γ 2.

Discussion

miRNAs are a type of non-coding RNA that function by inhibiting the expression of downstream target genes (30). A previous study has demonstrated that miRNAs are critical regulators during the formation, viability and death of osteoblasts and osteoclasts (31). A number of miRNAs, such as miR-7b-5p and miR-19a-3p, alleviate the progression of osteoporosis (32,33). Thus, a number of miRNAs may act as novel targets for the development of safe and effective osteoporosis treatment options.

Individuals develop osteoporosis due to decreased viability and function of osteoblasts caused by glucocorticoid

treatment (26). In the present study, DEX treatment significantly decreased cell viability, ALP activity and osteoblastic differentiation of mouse MC3T3-E1 pre-osteoblasts, indicating the successful establishment of an osteoporosis model induced by DEX. miR-27b is an intragenic miRNA involved in a number of diseases. For example, miR-27b suppresses cancer cell proliferation and enhances apoptosis in neuroblastoma, bladder and gastric cancer (15,34,35). In cardiac disease, adenoviral vector encoding sense miR-27b overexpression causes cardiac hypertrophy and fibrosis (36,37). In osteoarthritis, miR-27b decreases the degradation of the extracellular matrix in chondrocytes (38). However, the exact pathological mechanisms underlying miR-27b in osteoporosis remain to be elucidated. The results of previous studies demonstrated that miR-27b inhibited osteogenesis in maxillary sinus membrane stem cells, and promoted osteoblastic differentiation in BMSCs (18,19). These results suggested that the effect of miR-27b on osteogenesis depends on the cell type. The results of the present study revealed that miR-27b knockdown repressed proliferation and osteoblastic differentiation in MC3T3-E1 pre-osteoblasts, which is consistent with the findings by Seenprachawong *et al* (18) that miR-27b promotes osteogenesis in human MSCs. Moreover, miR-27b overexpression attenuated DEX-inhibited proliferation and osteoblastic differentiation, highlighting the potential protective role of miR-27b in osteoporosis. However, the decreased cell viability and osteoblastic differentiation in MC3T3-E1 pre-osteoblasts induced by DEX were not reversed by miR-27b. Previous

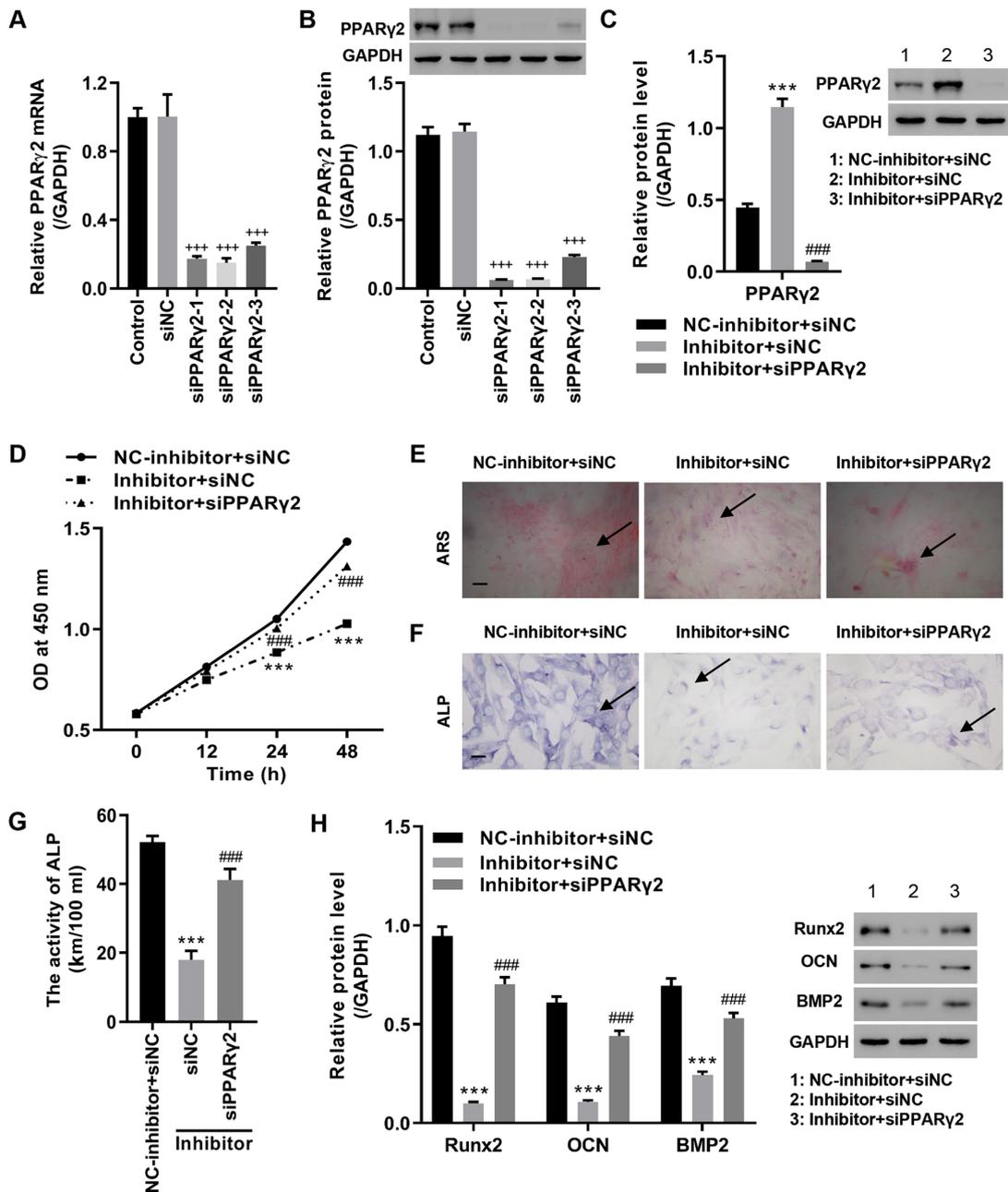


Figure 4. miR-27b inhibitor represses proliferation and osteoblastic differentiation in MC3T3-E1 pre-osteoblasts by regulating PPAR γ 2. Cells were transfected with siPPAR γ 2, and after 48 h, PPAR γ 2 (A) mRNA and (B) protein levels were measured. MC3T3-E1 pre-osteoblasts were also co-transfected with miR-27b inhibitor and siPPAR γ 2. (C) PPAR γ 2 expression was measured by western blot analysis. (D) Cell viability, (E) ARS staining (scale bar, 100 μ m), (F) ALP staining (scale bar, 25 μ m) and (G) ALP activity were measured. (H) Expression levels of Runx2, OCN and BMP2 were measured in MC3T3-E1 pre-osteoblasts. ^{***}P vs. siNC; ^{***}P<0.001 vs. NC-inhibitor + siNC; ^{###}P<0.001 vs. Inhibitor + siNC. PPAR γ 2, peroxisome proliferator-activated receptor γ 2; si, small interfering; NC, negative control; ARS, Alizarin red S; ALP, alkaline phosphatase; BMP2, bone morphogenetic protein-2; Runx2, runt-related protein 2; OCN, osteocalcin; miR, microRNA.

studies have reported that a number of other miRNAs, such as miR-365 (39), miR-199a (40), let-7f-5p (41) and miR-216a (42), play roles in the function of DEX in osteoporosis.

PPAR γ , a member of the nuclear receptor family, regulates the transcription of target genes by binding to the specific PPAR response element (43). Previous studies have reported that PPAR γ is directly regulated by miR-27b in a number of cell lines, including adipocytes, neuroblastoma cells and BMSCs (18,34,44). An isoform of PPAR γ , PPAR γ 2, is the target of miR-27b in chondrocytes (25). Consistent with the findings of previous studies, the results of the present study revealed

the regulatory effect of miR-27b on PPAR γ 2 in MC3T3-E1 cells. A previous study has revealed that PPAR γ 2 inhibits osteoblastogenesis and enhances adipogenesis (45). The results of the present study revealed that miR-27b enhanced proliferation and osteoblastic differentiation in MC3T3-E1 cells by targeting PPAR γ 2, highlighting the importance of PPAR γ 2 in the formation of osteoblasts.

Previous studies indicated that the expression level of miR-27b was significantly downregulated during the differentiation of Raw264.7 pre-osteoclasts into osteoclasts (16,17), implying the potential involvement of miR-27b in osteoclastic

formation. In addition, PPAR γ enhanced osteoclastic differentiation and activity (46,47). The results of previous studies have revealed that a number of miRNAs, such as miR-20a and miR-27a, regulate osteoclastic formation by targeting PPAR γ (46,48). Thus, we hypothesize that miR-27b may also function in osteoclastogenesis by regulating PPAR γ . The results of a previous study indicated that miR-27b enhanced osteogenesis in human BMSCs by the specific downregulation of PPAR γ (18). Furthermore, inhibition of PPAR γ ameliorated DEX-induced osteoporosis in a mouse model (49), highlighting the role of miR-27b and PPAR γ in osteoporosis. However, the molecular mechanism underlying the increased expression of PPAR γ induced by DEX remains to be elucidated. To the best of our knowledge, the present study is the first to demonstrate the increased level of miR-27b induced by DEX, and the function of the miR-27b/PPAR γ axis in DEX-induced proliferation and osteoblastic differentiation in MC3T3-E1 cells.

In conclusion, the results of the present study demonstrated that miR-27b alleviated DEX-inhibited proliferation and differentiation in MC3T3-E1 pre-osteoblasts. Therefore, miR-27b may act as a potential target for the treatment of osteoporosis. Further *in vitro* experiments and clinical practice are required to explore the potential role of miR-27b in osteoporosis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SL and HL designed this study. TY and MW performed the experiments. AH, HJ and MM analyzed and interpreted the data. TY and SL confirm the authenticity of all the raw data. HL, TY and SL wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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