MicroRNA-153 suppresses the osteogenic differentiation of human mesenchymal stem cells by targeting bone morphogenetic protein receptor type II

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Abstract. Elucidation of the molecular mechanisms governing the osteogenic differentiation of human mesenchymal stem cells (hMSCs) is of great importance for improving the treatment of bone-related diseases. MicroRNAs (miRNAs or miRs), a class of small non-coding RNAs, are critical in a number of biological processes, including the proliferation, differentiation and survival of cells and organisms. Emerging evidence indicates that miRNAs are essential in regulating osteoblastogenesis and bone formation. However, the role of miRNAs in osteoblast mechanotransduction remains to be defined. The present study aimed to examine the role of miR-153 in the osteogenesis of hMSCs and to investigate the impact of miR-153 on bone morphogenetic protein receptor type II (BMPR2) expression. The overexpression of miR-153 inhibited the osteogenic differentiation of hMSCs, whereas downregulation of miR-153 enhanced the process. Furthermore, bioinformatic analysis predicted that miR-153 is a potential regulator of BMPR2. The direct binding of miR-153 to the BMPR2 3'-untranslated region (3'-UTR) was demonstrated by a luciferase reporter assay using a construct containing the BMPR2 3'-UTR. In addition, knockdown of BMPR2 by RNA interference inhibited the osteogenic differentiation of hMSCs, with a similar effect to the upregulation of miR-153. In conclusion, the results suggest that miR-153 is a mechano-sensitive miRNA that regulates osteoblast differentiation by directly targeting BMPR2, and that therapeutic inhibition of miR-153 may be an efficient anabolic strategy for skeletal disorders caused by pathological mechanical loading.

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Introduction

Fracture healing is a physiological process of repair that proceeds in stages, each characterized by a different predominant tissue in the fracture gap. Chondrogenesis as well as osteogenesis in fracture healing and is downregulated in the later phase of osteogenesis, as osteoblasts mature to osteocytes. In addition, neurotrophins and their receptors may be of importance to osteoblasts and endothelial cells during fracture healing. Cell viability, osteoblast differentiation, and gene expression are altered in human osteoblasts from hypertrophic fracture non-unions. For fracture non-unions that do not heal after appropriate surgical intervention, the question arises as to what extent systemic cellular dysfunction is a pathogenic factor.

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a variety of unique mesenchymal cell types, such as osteoblasts, chondrocytes and adipocytes (1). Human MSCs (hMSCs) have been reported to be a population of self-renewing multipotent cells that may have clinical therapeutic potential (2,3). They can differentiate into several lineages, including osteoblasts, in response to stimulation by multiple environmental factors (4). Skeletal development and homeostasis are dependent on the activity of osteoblasts derived from MSCs. In addition, bone development is delicately regulated by homeostasis, which is maintained by the balance between osteoblasts and adipocytes (5). MSCs have emerged as key regulators of various biological and pathological processes, and disruption of this differentiation balance leads to various bone-related metabolic diseases.

MicroRNAs (miRNAs or miRs) are an abundant class of small (18-25 nucleotides) non-coding single-stranded RNAs found in diverse organisms. miRNAs have emerged as important post-transcriptional regulators of gene expression (6-8). They negatively regulate the translation of specific mRNAs by base pairing with partially or fully complementary sequences in target mRNAs (6-9). Although the biological functions of the majority of miRNAs are not yet fully understood, they may be essential in the regulation of various biological processes, including cell proliferation (10,11), apoptosis (12), cell differentiation (13) and tumor formation (14,15). An increasing number of miRNAs have been found to positively regulate osteoblast

differentiation and bone formation by targeting negative regulators of osteogenesis or negatively by targeting important osteogenic factors (16-19). The effect of miRNAs has also been investigated in the osteogenesis of hMSCs. A recent study demonstrated that miRNAs target the principal transcription factors and signaling molecules involved in osteoblast differentiation of MSCs and osteoblast functions (20). miRNAs may thus represent novel therapeutic targets for pharmacological control of bone cell functions and enhancing bone formation.

In the present study, it was investigated whether microRNA-153 (miR-153) was involved in the osteoblastic differentiation of hMSCs. The expression of miRNAs during osteogenic differentiation of hMSCs was assessed and miR-153 was found to be significantly downregulated. Furthermore, bioinformatics analysis was conducted using PicTar, TargetScan and microRNA.org to predict the miRNAs that bind to the bone morphogenetic protein receptor type II (BMPR2) 3'-untranslated region (3'-UTR). Among the candidates, miR-153 was selected for further investigation. The regulation of BMPR2 by miR-153 was verified by the construction of deletion mutants, a luciferase reporter assay, reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and western blot analysis. The potential role of miR-153 in osteoblast differentiation was also investigated using alkaline phosphatase (ALP) staining and Alizarin Red staining (ARS).

Materials and methods

hMSC isolation and culture. Nine bone marrow samples from young subjects (<30 years old) and seven bone marrow samples from older subjects (>60 years old) with slight or severe osteoporosis were obtained, from which hMSCs were isolated and identified by cell surface markers, as previously described (21). hMSCs were cultured in α-Minimum Essential Medium (α-MEM; HyClone, Salt Lake City, UT, USA), supplemented with 10% fetal bovine serum (FBS; Gibco Invitrogen, Carlsbad, CA, USA) and 1% penicillin and streptomycin (Gibco Invitrogen). The cells were incubated at 37˚C in a 95% humidity incubator with 5% CO_2. The study was approved by the Ethics Committee of Henan Hospital of Traditional Chinese Medicine. Informed consent was signed by the participants.

Osteogenic differentiation. hMSCs were plated at a cell density of $1x10^5$ cells in 12-well plates. At 80% confluence, medium was replaced with osteoblast-specific induction medium containing high-glucose Dulbecco's modified Eagle's medium (DMEM), 10% FBS, 5 mM β-glycerophosphate, 100 nM dexamethasone, and 50 μg/ml ascorbic acid (all purchased from Sigma-Aldrich, St. Louis, MO, USA) to induce differentiation. hMSCs were cultured in induction medium for 15 days. The induction medium was changed every 3 days.

RNA extraction RT-qPCR. Total RNA was isolated with TRIzol reagent according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA, USA). The purity and concentration of total RNA were determined in an Ultraviolet Spectrophotometer (Eppendorf, Hamburg, German). cDNA was synthesized from 1 μg total RNA using a Reverse Transcription kit (ReverTra Dash; Toyobo, Tokyo, Japan) according to the manufacturer's instructions. qPCR was conducted using an ABI 7500 Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA). The reaction (20 μl) contained the cDNA synthesized earlier, forward and reverse primers, and SYBR-Green PCR MasterMix (Applied Biosystems). The amplification conditions were 50˚C for 2 min, 95˚C for 10 min, followed by 40 cycles of denaturation at 95˚C for 15 sec, and annealing and extension at 60˚C for 1 min. Relative expression of mRNA or microRNA was evaluated using the $2^{-\Delta\Delta Ct}$ method and normalized to the expression of β-actin or U6, respectively. The primers for the genes are listed in Table I.

Western blot analysis. Cells were lysed in radioimmunoprecipitation assay buffer with protease inhibitors (Roche Diagnostics, Indianapolis, IN, USA). Protein concentrations were determined using a Pierce BCA Protein Assay kit (Thermo Fisher Scientific, Inc., Logan, UT, USA). Equal quantities of protein were loaded and separated on 10% SDS-PAGE, and then transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). Non-specific protein interactions were blocked by incubation with 3% fat-free milk in Tris-buffered saline buffer (containing 150 mM NaCl and 50 mM Tris-HCl, pH 7.6) at 4˚C for 1 h. The membranes were incubated with the following primary antibodies: Anti-BMPR2 antibody (1:1,000; Cell Signaling Technology, Beverly, MA, USA), anti-ALP antibody (1:100) or anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:2,000) antibody (both from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 4˚C overnight. Unbound antibody was removed by washing in TBS with Tween-20 (TBST) three times (10 min/wash). The membranes were incubated with horseradish peroxide-conjugated secondary antibodies (Zhongshan, Beijing, China) at 25˚C for 1 h, and followed by washing with TBST buffer three times (10 min/wash). The blots were developed with chemiluminescent ECL reagent (Millipore). GAPDH was used as an internal control.

ALP staining. The osteoblast phenotype was evaluated by determining ALP activity. ALP staining was performed using an ALP staining kit (Institute of Hematology, Chinese Academy of Medical Sciences, Beijing, China) followed by the accessory procedure. Cells seeded in 24-well plates were transfected with miR-153 inhibitor or mimics and the respective negative control (GenePharma, Inc., Shanghai, China) using Lipofectamine™ 2000 (Invitrogen). Prior to staining, the transfected cells were fixed in 10% paraformaldehyde for 10 min at 25˚C. After washing with phosphate-buffered saline (PBS), the cells were stained using 300 μg/ml BCIP/NBT solution (Thermo Fisher Scientific, Inc.) for 20 min at 25˚C. ALP-positive cells were stained blue/purple.

ARS mineralization assay. ARS was performed to detect calcification during late induction. Cells seeded in 24-well plates were transfected with miR-153 inhibitor or mimics and the respective negative control using Lipofectamine™2000. After cells had been fixed in 5% paraformaldehyde for 10 min, samples were evaluated by ARS staining. Briefly, cells were stained with 2% Alizarin Red (pH 7.2) for 15 min and then washed twice with PBS. Orange and red bodies were identified as calcium nodules.

Dual luciferase reporter genes construct. A 94-bp fragment of the BMPR2 3'-UTR containing the predicted binding site for

Table I. Primer sequences for reverse transcription-quantitative polymerase chain reaction.

Name	Forward (5'-3')	Reverse (5'-3')
ALP	GACAAGAAGCCCTTCACTGC	AGACTGCGCCTGGTAGTTGT
OC	TGCTTGTGACGAGCTATCAG	GAGGACAG GGAGGATCAAGT
COL1A1	GCAACAGTCGCTTCACCTACA	CAATGTCCAAGGGAGCCACAT
BMPR2	CATTCGCTCAAGCAGTTTAGTGGAC	GGTTCTGAAGCATTTCCTGGGC
U6	CGCTTCACGAATTTGCGTGTCAT	GCTTCGGCAGCACATATACTAAAAT
β-actin	AGATGTGGATCAGCAAGCAG	GCGCAAGTTAGGTTTTGTCA

ALP, alkaline phosphatase; OC, osteocalcin; COL1A1, collagen type I, BMPR2, bone morphogenetic protein receptor type II.

miR-153 was amplified by PCR from human genomic DNA. The sequence for the mutation of the miR-153 binding sites was introduced using the fast mutation kit (NEB, Ipswich, MB, Canada) according to the manufacturer's instructions. The constructed plasmids were termed BMPR2-WT (BMPR2-wild-type) and BMPR2-Mut (BMPR2-mutation), respectively. The fragment was purified and inserted into a pRL-TK vector (Promega Corporation, Madison, WI, USA) between *Xho*I and *Not*I cleavage sites.

Dual luciferase reporter assay. Luciferase assays were performed according to a previously published protocol. Cells were co-transfected with the wild-type BMPR2 3'-UTR (WT) or (Mut) and miR-153 mimic or the control mimic using Lipofectamine™2000 reagent according to the manufacturer's instructions. After transfection (48 h), cells were collected and luciferase activity was assayed for *Renilla* and Firefly luciferase activity using the Dual-Luciferase Reporter assay system (Promega Corportation), and each experiment was repeated in triplicate.

Statistical analysis. All data are presented as the mean ± standard deviation (n≥3). Differences between groups were analyzed via Student's t-test using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

miR-153 decreases during osteogenic differentiation. Initially the changes in miR-153 expression during osteogenic differentiation were examined using RT-qPCR. hMSCs were used as a cell model and osteogenic differentiation was induced. Several osteogenic factors, such as ALP, osteocalcin (OC) and collagen type I (COL1A1), were used as phenotypic marker genes of osteogenic differentiation. There was a marked increase in ALP, OC and COL1A1 mRNA levels in the induced cells, suggesting successful induction of osteogenic differentiation (Fig. 1A). It was also identified that the miR-153 levels were significantly lower in the induced cells compared with the non-induced cells (Fig. 1B). These data suggest that miR-153 may negatively regulate osteogenic differentiation.

miR-153 suppresses osteogenic differentiation of hMSCs. To further investigate the role of miR-153 in osteogenic differentiation, hMSCs were transfected with miR-153 inhibitor or mimics and the respective negative control; and then the capacity for osteogenesis was examined. The effect of the miR-153 mimic/inhibitor was determined by RT-qPCR. The expression of miR-153 was decreased after transfection of miR-153 inhibitor, and increased after transfection of miR-153 mimics (Fig. 2A). In addition, all of the osteogenic differentiation markers examined were significantly increased following transfection of the miR-153 inhibitors (Fig. 2B). By contrast, hMSCs transfected with miR-153 mimics showed decreased expression of ALP, OC and COL1A1 compared with those

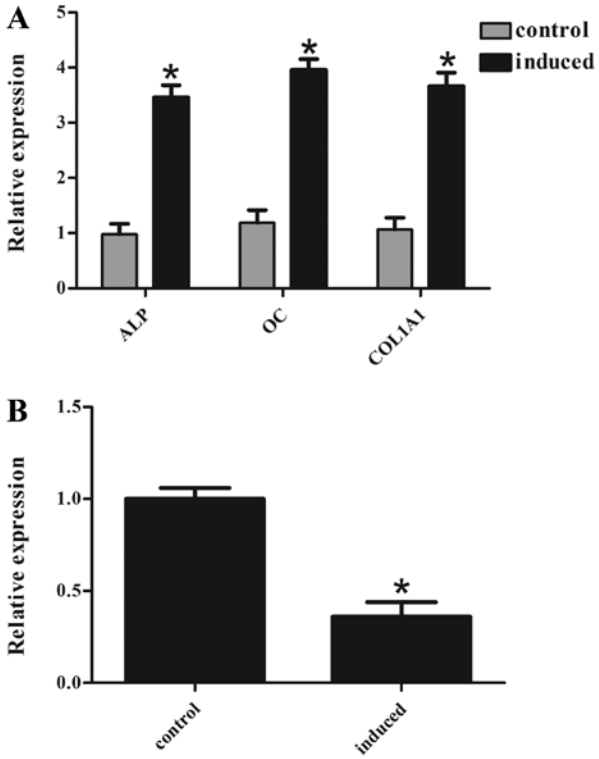

Figure 1. miR-153 was decreased during osteogenic differentiation. (A) Expression of ALP, OC and COL1A1 was determined by RT-qPCR. The data are normalized to glyceraldehyde 3-phosphate dehydrogenase. (B) The levels of miR-153 in induced and non-induced (control) human mesenchymal stem cells were determined using RT-qPCR. U6 was used as the internal control. The data were drawn from three independent experiments. *P<0.05, compared with the control. miR, microRNA; ALP, alkaline phosphatase; OC, osteocalcin; COL1A1, collagen type I; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

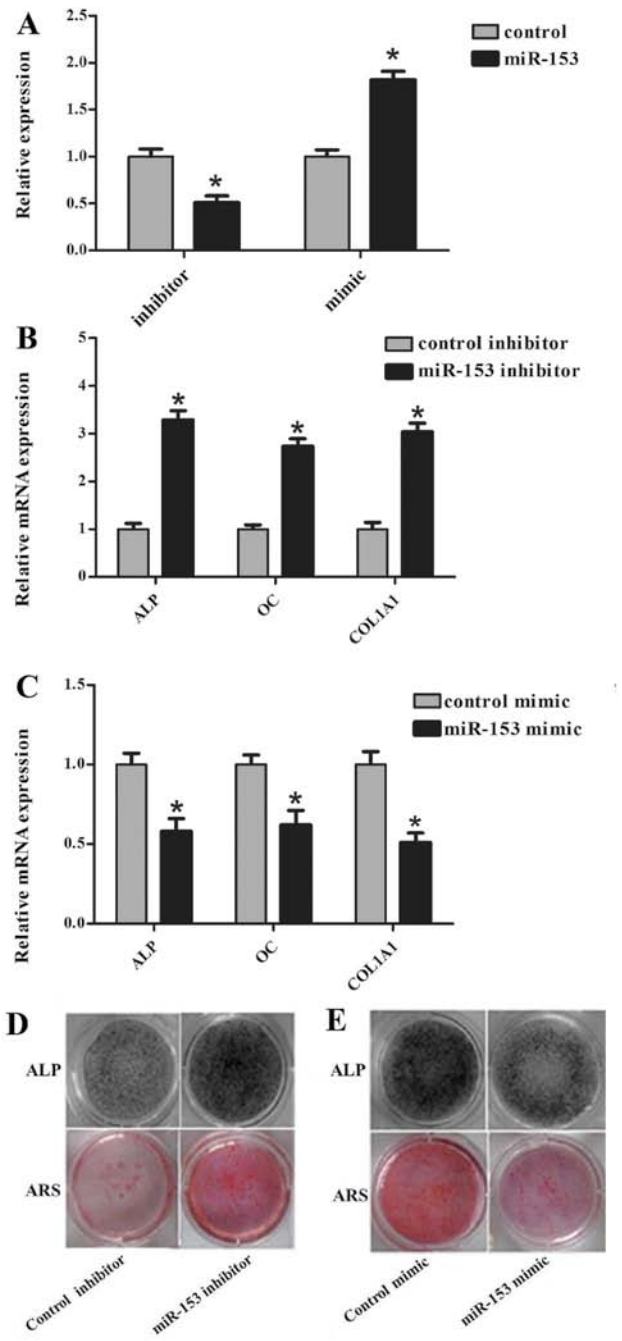

Figure 2. miR-153 suppresses osteogenic differentiation. (A) Cells were transfected with miR-153 inhibitor (100 nM) or the control for 36 h. Expression of ALP, OC and COL1A1 was determined by RT-qPCR. (B) Cells were transfected with miR-153 mimic (100 nM) or the negative control for 36 h. Expression of ALP, OC and COL1A1 was determined by RT-qPCR. Glyceraldehyde 3-phosphate dehydrogenase was used as the internal control. (C) Cells were transfected with miR-153 mimic or miR-153 inhibitor compared with their separate negative control. Expression of miR-153 was determined by RT-qPCR. U6 was used as the internal control. The data were obtained from three independent experiments. *P<0.05 compared with the control. (D and E) ALP staining (upper) at 6 days and ARS (lower) at 15 days showed ALP activity and calcification of osteogenic differentiation after transfection with miR-153 mimic or miR-153 inhibitor compared with their separate negative control. miR, microRNA; ALP, alkaline phosphatase; OC, osteocalcin; COL1A1, collagen type I; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; ARS, Alizarin Red staining.

transfected with the negative control (Fig. 2C). At 6 day of differentiation, ALP staining (Fig. 2D upper) was more intense in the cells transfected with the miR-153 inhibitor when compared with the cells transfected with control inhibitor. Furthermore, the staining of the cells transfected with miR-153 mimic was less intense than those transfected with the control mimic (Fig. 2E upper). ARS (Fig. 2D and E lower) on day 15 showed the same tendency at the matrix mineralization level. These results indicate that miR-153 could suppress osteogenic differentiation.

BMPR2 is a direct target of miR-153. It is well known that miRNAs act by suppressing the expression of their target genes. Among the predicted candidates, BMPR2 was identified, which is a kinase receptor of BMPs with an established role in osteogenesis (22,23). To understand the molecular mechanisms underlying the effects of BMPR2, bioinformatics analyses using miRNA target analysis tools PicTar (http://www.pictar.org/cgi-bin/PicTar_vertebrate.cgi), TargetScan (http://www.targetscan.org/), and microRNA.org (http://www.microrna.org/microrna/home.do) were performed to predict the putative miRNAs that bind to the BMPR2 3'-UTR. According to the analysis, the three programs all predicted that the binding sequence in the 3'-UTR of BMPR2 was a match for the miR-153 seed (Fig. 3A and B). The miR-153 target site in the 3'-UTR of BMPR2 is highly conserved among vertebrates (Fig. 3C). These data indicate that the translation of BMPR2 protein may be regulated by miR-153.

miR-153 inhibits BMPR2 expression. To confirm the targeting of BMPR2 by miR-153, a luciferase reporter assay was performed. The pRL-TK vector contains *Renilla* luciferase and firefly luciferase genes. Firefly luciferase was used as an internal control and *Renilla* luciferase was linked with 3'-UTR sequences as a reporter. The luciferase activity assay (Fig. 4A) showed that the luciferase activity in BMPR2-WT transfected cells was significantly decreased in miR-153 mimic co-transfected cells compared with that in control co-transfected cells. In addition, site-directed mutagenesis of the seed region abolished the inhibitory effect of miR-153 mimics and Mut luciferase activity did not change. These results indicate that miR-153 significantly suppressed the activity of WT but not Mut BMPR2 3'-UTR in hMSCs. Overexpression of miR-153 significantly suppressed the protein level of BMPR2, while inhibition of miR-153 elevated the protein level of BMPR2 in hMSCs (Fig. 4B and C). These results reveal that miR-153 could negatively regulate BMPR2 expression through a partially complementary binding site in the 3'-UTR of BMPR2.

miR-153 suppresses osteogenic differentiation by targeting BMPR2. Since miR-153 could suppress osteogenic differentiation and inhibit BMPR2 expression, it was further explored whether inhibition of BMPR2 by siRNA could also have a similar effect as miR-153 overexpression on osteogenic differentiation of cells. The effect of BMPR2 siRNA was confirmed by RT-qPCR (Fig. 5A) and western blot analysis (Fig. 5B and C). As expected, cells transfected with BMPR2 siRNA showed a similar effect to cells transfected with miR-153 mimic (Figs. 2B and 5D). Furthermore, co-transfection of miR-153 mimic with BMPR2 overexpression plasmid attenuated the effect of miR-153 on the osteogenic differentiation of cells (Fig. 5E). These results indicate that miR-153 regulates osteogenic differentiation of cells through BMPR2.

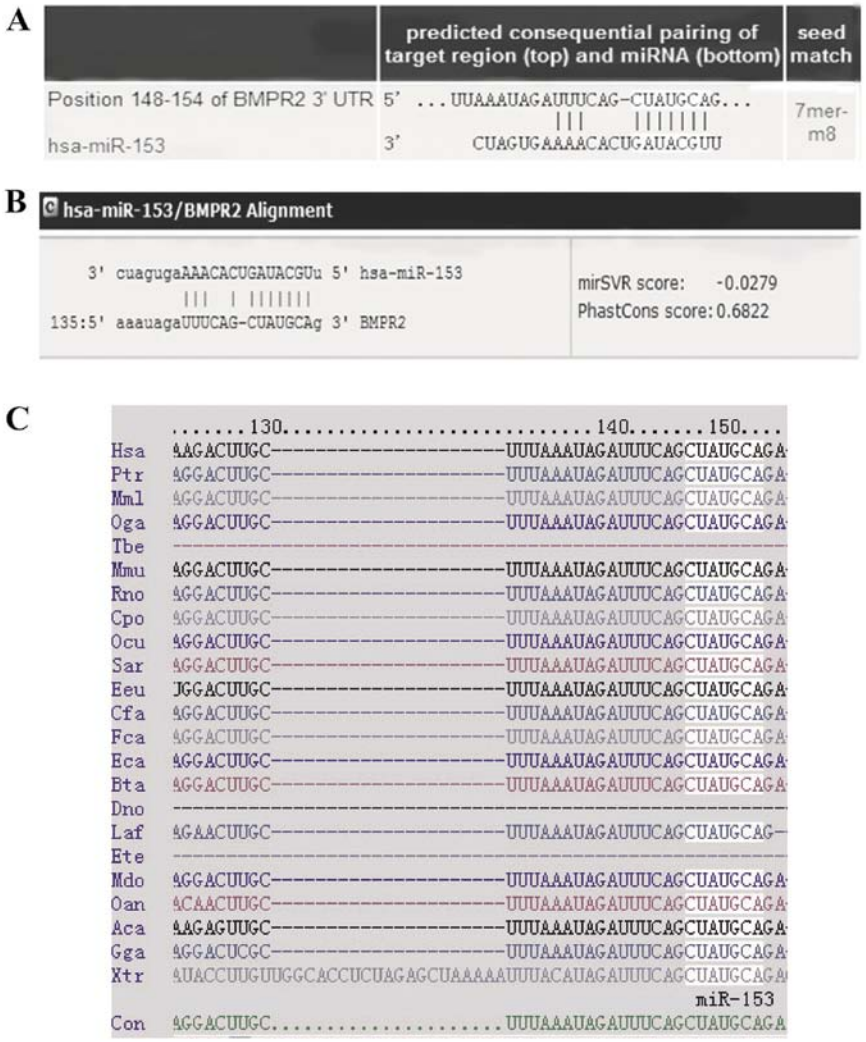

Figure 3. miR-153 targets the 3'-UTR of BMPR2 mRNA. (A) TargetScan and (B) microRNA.org were used to explore the potential target of miR-153. miR-153 is partially complementary to a region in the BMPR2 3'-UTR. The complementary miR-153 binding site found in the BMPR2 3'-UTR or mutant site was inserted downstream of the luciferase reporter plasmid pRL-TK. (C) The predicted, single binding site of miR-153 (unshaded) in the 3'-UTR of BMPR2 is conserved among vertebrates. miR, microRNA; UTR, untranslated region; BMPR2, bone morphogenetic protein receptor type II.

Discussion

Bone homeostasis involves a balance between bone formation and bone absorption by osteoblasts and osteoclasts respectively (24). A growing body of evidence has revealed that miRNAs are critical in normal biological processes and the pathogenesis of human diseases by post-transcriptionally regulating gene expression (25). A number of miRNAs have been emerging as important negative or positive regulators of post-transcriptional gene expression and are considered critical for osteogenesis (26). For example, miR-34 was reported to inhibit osteoblast differentiation by targeting Special AT-rich sequence-binding protein 2 (27). miR-100 suppressed osteoblast differentiation by inhibiting BMPR2 (28). miR-133 and miR-135 functionally inhibit osteoprogenitor differentiation by abolishing Runx2 and SMAD family member 5 (Smad5) pathways, which contributed to bone formation synergistically (29). Sp7 has recently been identified as a key regulator of osteoblast differentiation, and was reported to be regulated by miR-93, miR-125b, miR-214 and miR-637 (30-33). miRNAs are endogenous small noncoding RNAs that regulate the activities of target mRNAs and cellular processes. Although no miRNA has been reported to be important in the regulation of fracture healing, several miRNAs control key elements in tissue repair processes, such as inflammation, hypoxia response, angiogenesis, stem cell differentiation, osteogenesis, and chondrogenesis. In this study, miR-153 was identified as a suppressive regulator of osteogenic differentiation. It was demonstrated that miR-153 was downregulated during the osteogenic differentiation of cells. Overexpression of miR-153 inhibited osteogenic differentiation, whereas inhibition of miR-153 enhanced the osteogenic potential.

It has been reported that miR-153 is important in a number of types of cancer (34,37). However, its effects do not appear to be consistent. miR-153 suppresses tumor growth in glioblastoma (34), epithelial cancer (35) and leukemia (36). Conversely, in prostate cancer, miR-153 promotes cell proliferation via downregulation of the PTEN tumor suppressor gene (37). However, the role of miR-153 in the regulation of osteoblastic differentiation remains poorly understood. In the present study, a novel role of miR-153 in the osteogenesis of cells was identified. To investigate the molecular mechanism by which miR-153 regulates the osteogenic differentiation of cells, potential target genes

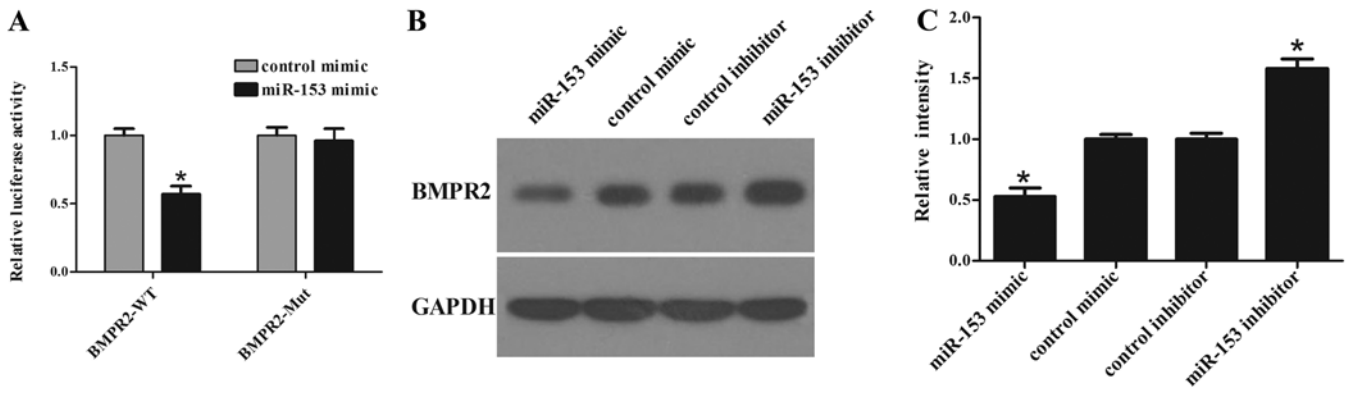

Figure 4. miR-153 inhibited BMPR2 expression. (A) BMPR2-WT or BMPR2-Mut was co-transfected in cells with miR-153 mimics or the control, and the luciferase activity assay was performed. Relative *Renilla* luciferase activity was normalized to that of firefly luciferase. Data represent mean ± SD of three independent experiments. *P<0.05 compared with cells transfected with control and BMPR2-WT. (B and C) Human mesenchymal stem cells were transfected with miR-153 mimic/inhibitor or the respective control, and then the protein expression of BMPR2 was analyzed by western blotting. The quantitative determination represents the mean ± SD of three independent experiments. *P<0.05 compared with the control. miR, microRNA; BMPR2, bone morphogenetic protein receptor type II; WT, wild type; Mut, mutant; SD, standard deviation.

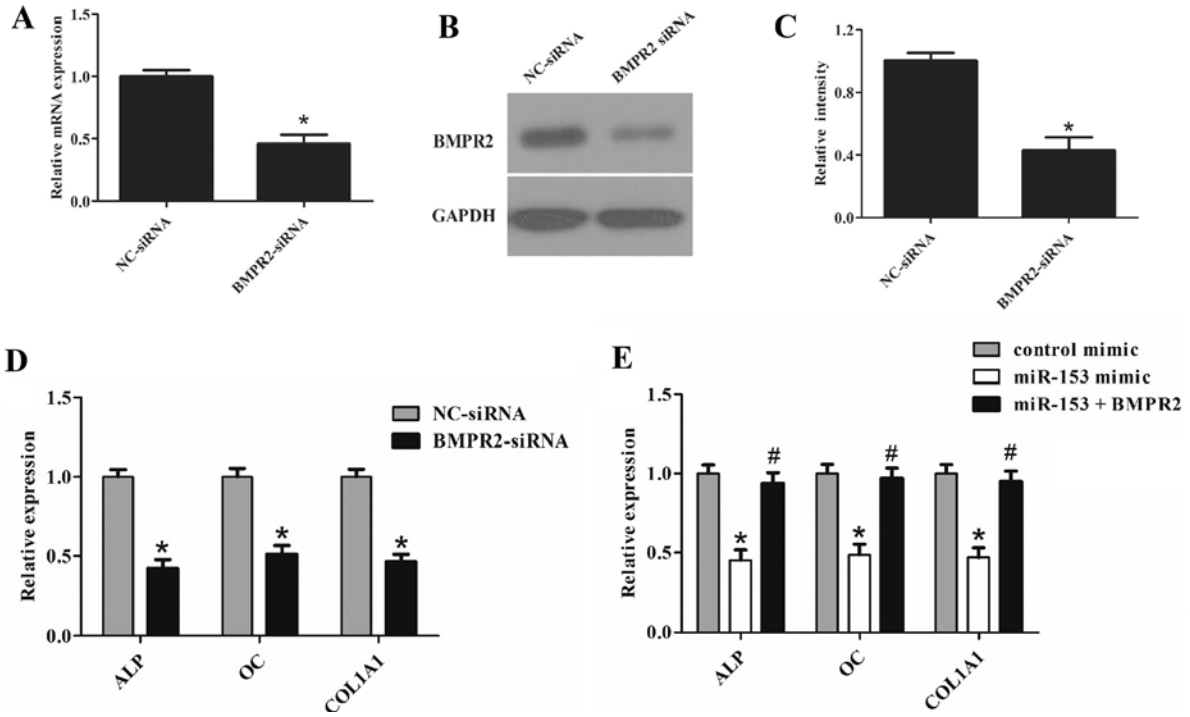

Figure 5. miR-153 suppressed osteogenic differentiation by targeting BMPR2. (A) Cells were transfected with BMPR2 siRNA or the control for 72 h. Expression of BMPR2 was determined by RT-qPCR. (B and C) Cells were transfected with BMPR2 siRNA or the control for 72 h. Western blot analysis was used to detect the protein level of BMPR2. (D) Cells were transfected with BMPR2 siRNA or the control for 72 h. Expression of ALP, OC and COL1A1 was determined by RT-qPCR. (E) Cells were co-transfected with miR-153 mimic (100 nM)/BMPR2-pcDNA3 for 48 h. Expression of ALP, OC and COL1A1 was determined by RT-qPCR. Glyceraldehyde 3-phosphate dehydrogenase was used as the internal control. The data were obtained from three independent experiments. *P<0.05, compared with the control; #P<0.05, compared with miR-153 mimic-transfected cells. miR, microRNA; BMPR2, bone morphogenetic protein receptor type II; siRNA, small interfering RNA; ALP, alkaline phosphatase; OC, osteocalcin; COL1A1, collagen type I; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

were analyzed that have an established function in promoting osteogenesis using the target prediction tools, PicTar, TargetScan and microRNA.org. The results revealed that BMPR2 may be a possible target with a 9-nt non-consecutive match site complementary to miR-153 in its 3'-UTR. It was demonstrated that overexpression of miR-153 resulted in the suppression of BMPR2 expression, whereas functional inhibition of miR-153 led to elevation of BMPR2, strongly suggesting that BMPR2 is regulated by miR-153. In addition, the dual luciferase reporter assay also identified BMPR2 as a direct target of miR-153. Supplementing BMPR2 could also partially reverse the suppressive effect of miR-153 on osteoblastic differentiation. These data suggest that miR-153 may negatively regulate osteogenic differentiation partially by targeting BMPR2.

BMP signaling has been reported to be important in the commitment of human adipose-derived mesenchymal stem cell (hASC) osteogenic differentiation (38) and BMPs have important clinical significance serving as strong osteoinductive factors for bone tissue repair and regeneration (39). BMP signaling is initiated by the binding of extracellular BMPR, resulting in BMPR2-mediated activation of the BMPR1, which, in turn, causes the phosphorylation and activation of intracellular Smad signaling molecules, and then activates osteoblast-essential genes (23). These studies indicated that BMPR2 may be a crucial factor in the pathway of osteogenic differentiation. BMP2 is key in skeletal development, repair and regeneration. In the present study, it was demonstrated that miR-153 negatively targets BMPR2 and suppresses osteogenic differentiation.

In conclusion, the data indicate that miR-153 is a novel regulator of BMPR2 and that it serves as a suppressor of osteogenic differentiation. Thus, BMPR2 and miR-153 may be potential therapeutic targets in the management of skeletal diseases.

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