MicroRNA-410 participates in the pathological process of postmenopausal osteoporosis by downregulating bone morphogenetic protein-2

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Abstract. The present study aimed to investigate bone morphogenetic protein (BMP)-2 and microRNA (miR)-410 expression and the mechanism of regulation in serum and CD^{14+} peripheral blood mononuclear cells (PBMCs) from postmenopausal osteoporosis patients and model mice. A total of 26 patients with postmenopausal osteoporosis were included in the experimental group and 29 age-matched healthy subjects were included in the control group. A total of 60 mice were divided into sham and ovariectomized (OVX) groups. Following surgery, 28 mice remained in the sham and 25 mice remained in OVX group. BMP-2 protein expression in serum and CD^{14+} PBMCs from patients and model mice was determined using ELISA and western blotting, respectively. Reverse transcription-quantitative polymerase chain reaction assays were performed to determine miR-410 and BMP-2 mRNA levels in serum and CD^{14+} PBMCs from patients and model mice. Dual luciferase reporter assays were used to identify direct interactions between miR-410 and BMP-2 mRNA. Compared with the control group, BMP-2 mRNA and protein expression in serum and CD^{14+} PBMCs from patients with postmenopausal osteoporosis and model mice were significantly decreased. miR-410 levels in serum and CD^{14+} PBMCs from patients with postmenopausal osteoporosis and model mice were significantly increased when compared with the control group. Dual luciferase reporter assays revealed that BMP-2 was a target gene of miR-410. The current study demonstrated that decreased BMP-2 expression in serum and CD^{14+} PBMCs from patients with postmenopausal osteoporosis was associated with the upregulation of miR-410. These results suggest that miR-410 may participate in the pathological process of postmenopausal osteoporosis by downregulating BMP-2.

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Introduction

Osteoporosis affects >40% of postmenopausal females (1) and is characterized by damage to the bone microstructure, a decrease in bone mineral and matrix composition, a decrease in bone density and an increase in bone fragility and fracture risks (2). A survey performed in 2008 revealed that ~54.1 million Chinese females were diagnosed with osteoporosis, while 113 million Chinese females exhibited a reduction in bone mass (3). This has led to severe health threats and a social and economic burden (3). Postmenopausal osteoporosis is caused by imbalance of bone absorption and formation (4). Despite the availability and clinical use of various drugs that effectively target bone resorption, treatments that promote bone formation have yet to be identified (5).

CD^{14+} peripheral blood mononuclear cells (PBMCs) are precursors of osteoclasts and are associated with the pathogenesis of osteoporosis (6,7). Under the joint action of macrophage colony stimulating factor and receptor activator of nuclear factor-κB (RANK) ligand (L), CD^{14+} PBMCs that are differentiated from CD^{14-} PBMCs differentiate into osteoclasts during cultivation, suggesting that CD^{14+} PBMCs are precursor cells of osteoclasts (8). CD^{14+} PBMCs express RANK, which is activated following binding to RANKL and mediates osteoclast differentiation (9).

To date, a number of active bone morphogenetic proteins (BMPs) have been discovered (10,11). BMP-2 has been reported to be a member of transforming growth factor-β supergene family (12), and serves important roles in osteogenesis (13), fracture healing (14) and bone formation (15). BMP-2 further serves important roles in the process of ossification, by stimulating the differentiation of pluripotent stromal stem cells into osteoblasts and enhancing the functions of osteoblasts (16-18). There are various approaches to regulate BMP-2 expression, including the use of microRNAs (miRs), which has been widely studied. miR-98 (19), miR-203 and miR-320 (20) have been reported to regulate BMP-2 expression. Additional miRs may exert regulatory effects on BMP-2. A previous study reported that miR-410 enhances the stem cell characteristics of cells (21); however, whether miR-410 regulates BMP-2 expression is currently unclear. Therefore, the aim of

the present study was to investigate the mechanisms underlying the pathogenesis of postmenopausal osteoporosis, and to understand the role of miR-410 and BMP-2 in the disease.

Materials and methods

Patients. A total of 26 female patients with postmenopausal osteoporosis that received treatment at Changhai Hospital (Shanghai, China) between October 2012 and March 2017 were included in the present study. The age range was 50-59 years and the mean age was 55.6±4.8 years. In addition, 29 aged-matched healthy female subjects were recruited into the normal control group at the same hospital within the same date range (age range, 50-59 years; mean age, 55.1±4.6 years). Subjects in the control group and patients with postmenopausal osteoporosis had similar serum levels of estrogen, vitamin D and parathyroid hormone. Osteoporosis and normal bone mass were determined according to the standards of the World Health Organization (22-24). The inclusion and exclusion criteria were the same as these standards. Fasting peripheral blood was collected from all subjects in the morning on the day of diagnosis and stored at -20˚C. To obtain serum samples, blood was centrifuged at 400 x g and 4˚C for 10 min and serum was transferred into fresh tubes (100 μl/tube). All procedures were approved by the Ethics Committee of Changhai Hospital. Written informed consent was obtained from all patients or their families.

Animals. A total of 60 female C57BL/6 mice (age, 5 weeks) were purchased from Chongqing TengXin Biotech Company (Chongqing, China). The weight of the mice ranged between 18 and 22 g. Mice were maintained in individual cages in a room with 50-65% humidity, at 26˚C with a 12-h light/dark cycle. One week prior to experiments, mice had free access to food and water to acclimate to the environment. Access to food and water was not changed during the experiments. The Reduction, Replacement and Refinement animal welfare principle was followed during the experiments (25). Mice were randomly divided into sham operation group (sham; n=30) and ovariectomized model group (OVX; n=30). Mice in the OVX group were anesthetized by intraperitoneal injection of 5% chloral hydrate at a dosage of 400 mg/kg animal body weight. Both sides of the ovaries were extirpated. Mice in the sham group were treated using the same surgical protocols but without ovarian extirpation. At 3 months following surgery, mice were anesthetized by intraperitoneal injection of 5% chloral hydrate at 400 mg/kg body weight and underwent distal femur scanning using microcomputed tomography (80 kV, 500 μA; SkyScan; Bruker Corporation, Billerica, MA, USA) along the long axis of femur (360˚ scanning angle; 10.44 μm resolution) to test for postmenopausal osteoporosis symptoms (data not shown). Peripheral blood was collected from mice in the sham group (n=28) and OVX group (n=25) during operation, and serum was obtained by centrifugation at 400 x g and 4˚C for 10 min. Mice that did not develop postmenopausal osteoporosis were excluded from the study. Serum was transferred into tubes (100 μl/tube). All animal experiments were conducted according to the Ethical Guidelines of Changhai Hospital. The present study was approved by the Ethics Committee of Changhai Hospital.

Cells. CD^{14+} PBMCs were separated by gradient centrifugation from both human and mice (26) and Ficoll-Paque according to the manufacturer's instructions (cat. no. 17-1440-03; GE Healthcare, Chicago, IL, USA). Cells were cultured in α-minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 ng/ml streptomycin (all reagents from Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37˚C and 5% CO_2. Following 2 h, non-adherent cells were removed and adherent cells were resuspended in fresh complete α-MEM medium. Using a monocyte isolation kit (cat. no., 130-117-337; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), CD^{14+} PBMCs were isolated from the cell suspension according to the manufacturer's protocol. CD^{14+} PBMCs ($3x10^5$/well) were cultured in 24-well plates according to a previously published method (27).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Samples (200 μl serum or $3x10^6$ PBMCs) were lysed using 1 ml TRIzol reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was extracted using the phenol chloroform method. The concentration and quality of RNA was measured spectrophotometrically (Nanodrop ND2000; NanoDrop; Thermo Fisher Scientific, Inc., Pittsburgh, PA, USA), and OD260/A280 and A260/A230 ratios were used for evaluation of quality. cDNA was obtained by RT using 1 μg RNA, and stored at -20˚C. RT of the extracted RNA was achieved using the TIANScript II cDNA First Strand Synthesis kit (Tiangen Biotech Co., Ltd., Beijing, China).

The SuperReal PreMix (SYBR Green) RT-qPCR kit (Tiangen Biotech Co., Ltd.) was used to detect the expression of human BMP-2 using GAPDH as internal standard, and mouse BMP-2 expression using β-actin as internal reference. The primer sequences were as follows: Human BMP-2 forward, 5'-CCTATATGCTCGACCTGTAC-3', and reverse, 5'-CCCACTCATTTCTGAAAGTTC-3'; GAPDH forward, 5'-GCACAGTCAAGGCTGAGAAT-3', and reverse, 5'-TGAAGACGCCAGTAGACTCC-3'; mouse BMP-2 forward, 5'-TGTGAGGATTAGCAGGTCTT-3', and reverse, 5'-GTTAGTGGAGTTCAGGTGGT-3'; β-actin forward, 5'-CTCTTTTCCAGCCTTCCTTCT-3', and reverse, 5'-TGGAAGGTGGACAGTGAGG-3'. Reaction mixtures (20 μl) consisted of qPCR-mix (10 μl), forward primer (0.5 μl; 10 μmol/μl), reverse primer (0.5 μl; 10 μmol/μl), cDNA (2 μl) and ddH_2O (7 μl). Thermocycling conditions were as follows: Initial denaturation at 95˚C for 30 sec followed by 39 cycles of denaturation at 95˚C for 5 sec and elongation at 60˚C for 20 sec (iQ5; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The $2^{-\Delta\Delta Cq}$ method (28) was used to calculate the relative expression of human or mouse BMP-2 mRNA vs. GAPDH or β-actin, respectively. Each sample was analyzed in triplicate.

miR-410 expression was determined using the miRcute miRNA qPCR detection kit (Tiangen Biotech Co., Ltd.) using U6 as an internal reference. Primer sequences for human samples were as follows: Human miR-410 forward, 5'-GTCAGCGCAATATAACACAG-3'; human U6 forward, 5'-GTCAGCGCGTGCTCGCTTCG-3', and the human universal reverse primer, 5'-GTGCAGGGTCCGAGGT-3' (provided in the kit). The same aforementioned reaction mixtures were used. Thermocycling conditions were as follows: Initial

denaturation at 95˚C for 5 min followed by 40 cycles of denaturation at 95˚C for 10 sec, annealing at 60˚C for 20 sec and extension at 72˚C for 10 sec (iQ5; Bio-Rad Laboratories, Inc.). Primer sequences for murine samples were as follows: Mouse miR-410 forward, 5'-AGGTTGTCTGTGATGAGTTCG-3'; mouse U6 forward, 5'-CTCGCTTCGGCAGCACATATA CT-3' and the mouse universal reverse primer, 5'-ACGCTT CACGAATTTGCGTGTC-3' (provided in the kit). Reaction mixtures were prepared as described above. Thermocycling conditions were as follows: Initial denaturation at 95˚C for 5 min followed by 40 cycles of denaturation at 95˚C for 15 sec, annealing at 60˚C for 15 sec and extension at 72˚C for 10 sec (iQ5; Bio-Rad Laboratories, Inc.). The $2^{-\Delta\Delta Cq}$ method was used to calculate human or mouse miR-410 expression relative to U6. Each sample was analyzed in triplicate.

Western blotting. PBMCs in each group were lysed using prechilled radioimmunoprecipitation assay lysis buffer (600 μl; 50 mM Tris-base, 1 mM EDTA, 150 mM NaCl, 0.1% SDS, 1% TritonX-100, 1% sodium deoxycholate; Beyotime Institute of Biotechnology, Haimen, China). Following lysis for 30 min on ice, the mixture was centrifuged at 12,000 x g for 10 min at 4˚C. The protein concentration of the supernatant was determined using a bicinchoninic acid protein concentration determination kit (cat. no., RTP7102; Real-Times (Beijing) Biotechnology Co., Ltd., Beijing, China). Protein samples (50 μg) were mixed with SDS loading buffer (5X) and denatured in a boiling water bath for 10 min. Samples were then separated on 10% SDS-PAGE gels. Proteins were transferred to polyvinylidene difluoride membranes (100 V, 1 h) in an ice box and blocked with 5% skimmed milk at room temperature for 1 h. Membranes were incubated with rabbit anti-human or rabbit anti-mouse BMP-2 polyclonal primary antibodies (dilution, 1:1,000; cat. no. ab14933; Abcam, Cambridge, USA) and rabbit anti-human or rabbit anti-mouse β-actin primary antibody (dilution 1:5,000; cat. no. ab8227; Abcam) at 4˚C overnight. Following washing with PBS containing Tween 20 (concentration, 0.1%; five washes for 5 min each time), membranes were incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (dilution, 1:3,000; cat. no. ab6721; Abcam) for 1 h at room temperature prior to washing with PBS containing Tween 20 (5 washes for 5 min each time). Membranes were developed with an enhanced chemiluminescence detection kit (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Image lab 3.0 (Bio-Rad Laboratories, Inc.) was used to analyze the results. BMP-2 protein levels were quantified relative to β-actin.

ELISA. Human and mouse BMP-2 ELISA kits (ab119581 and ab119582, respectively; Abcam) were used to determine concentrations of human and mouse BMP-2 in serum samples. In 96-well plates, kit standards (50 μl) and samples (10 μl serum and 40 μl kit diluent) were added to the wells; empty wells served as blanks. Horseradish peroxidase-labeled conjugate (100 μl) was added to the wells prior to sealing and the plate was incubated at 37˚C for 1 h. The samples were washed five times using a washing reagent supplied in the kit, substrates A (50 μl) and B (50 μl) were added to each well and plates were incubated at 37˚C for 15 min. Stop solution (50 μl) was added to each well and the absorbance was measured at 450 nm within 15 min of adding the stop solution.

Bioinformatics. To investigate the regulatory mechanisms of BMP-2, miRanda (http://www.microrna.org/microrna/home.do), TargetScan (http://www.targetscan.org), PITA (http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html), RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/) and PicTar (http://pictar.mdc-berlin.de/) were used to predict miR targets that may regulate BMP-2.

Dual luciferase reporter assay. Wild-type (WT; UUAUAU) and mutant miR-410 seed regions (AAUAUA) in the 3'-untranslated region (UTR) of BMP-2 were synthesized *in vitro*. Spe-1 and HindIII restriction sites were created at the ends and constructs were cloned into the pMIR-REPORT luciferase reporter plasmid (Ambion; Thermo Fisher Scientific, Inc.). Plasmids (0.8 μg) with WT or mutant 3'-UTR DNA sequences were co-transfected with agomiR-410 (100 nM; Sangon Biotech Co., Ltd., Shanghai, China) into 293T cells Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) using Lipofectamine® 2000 transfection reagent (Thermo Fisher Scientific, Inc.). Negative control (NC) group was transfected with agomiR-410 and empty plasmid. Following cultivation at 37˚C for 24 h, cells were lysed using the dual luciferase reporter assay kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol, and fluorescence intensity was measured using a GloMax 20/20 luminometer (Promega Corporation). *Renilla* fluorescence activity was used as internal reference.

Statistical analysis. Results were analyzed using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA). All data is presented as the mean ± standard deviation. Data were tested for normality. Multigroup comparisons were analyzed using one-way ANOVA. In case of homogeneity of variance, the least significant difference and Student-Newman-Keuls test were used; in case of heterogeneity of variance, Tamhane's T2 or Dunnett's test was used. Comparisons between two groups were analyzed using a Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patients with postmenopausal osteoporosis exhibit reduced BMP-2 and elevated miR-410 expression. To measure miR-410 and BMP-2 mRNA and protein levels in serum samples from patients with postmenopausal osteoporosis, RT-qPCR and ELISA tests were employed, respectively. RT-qPCR analysis revealed that miR-410 levels in the serum of patients with postmenopausal osteoporosis were significantly increased when compared with the healthy control group ($P<0.01$; Fig. 1A), while BMP-2 mRNA levels were significantly decreased compared with the control group ($P<0.01$; Fig. 1B). In addition, BMP-2 protein levels in the serum were significantly decreased when compared with the control group ($P<0.05$; Fig. 1C). These results suggest that reduced BMP-2 and elevated miR-410 expression in serum may be associated with postmenopausal osteoporosis.

Patients with postmenopausal osteoporosis exhibit decreased BMP-2 and increased miR-410 expression in CD^{14+} PBMCs. To determine miR-410 and BMP-2 mRNA and protein

levels in CD^{14+} PBMCs from patients with postmenopausal osteoporosis, RT-qPCR and western blotting analyses were performed, respectively. The RT-qPCR results demonstrated that miR-410 levels in CD^{14+} PBMCs from patients with postmenopausal osteoporosis were significantly increased when compared with the control group (P<0.01; Fig. 2A), while BMP-2 mRNA levels in CD^{14+} PBMCs were significantly decreased compared with the control group (P<0.01; Fig. 2B). In addition, BMP-2 protein levels in CD^{14+} PBMCs from patients with postmenopausal osteoporosis were significantly decreased compared with the control group (P<0.05; Fig. 2C). The results provide further evidence that decreased BMP-2 and increased miR-410 expression in CD^{14+} PBMCs may be associated with postmenopausal osteoporosis.

Reduced BMP-2 and increased miR-410 expression is observed in serum of mouse model with postmenopausal osteoporosis. To examine miR-410 and BMP-2 mRNA and protein levels in serum from mice in the sham (n=28) and OVX groups (n=25), RT-qPCR and ELISA assays were performed, respectively. The RT-qPCR results revealed that miR-410 levels in serum samples from the OVX group were significantly increased when compared with the sham group (P<0.05; Fig. 3A), while BMP-2 mRNA levels in serum samples from the OVX group were significantly decreased compared with the sham group (P<0.01; Fig. 3B). In addition, BMP-2 protein levels in serum from the OVX group were significantly decreased compared with the sham group (P<0.05; Fig. 3C). These results confirmed that reduced BMP-2 and increased miR-410 expression may be associated with postmenopausal osteoporosis *in vivo*.

Decreased BMP-2 and increased miR-410 expression is observed in CD^{14+} PBMCs derived from a mouse model of postmenopausal osteoporosis. To analyze miR-410 and BMP-2 mRNA and protein levels in CD^{14+} PBMCs from mice, RT-qPCR and western blotting analyses were performed, respectively. The RT-qPCR results demonstrated that miR-410 levels in CD^{14+} PBMCs from the OVX group were significantly increased when compared with the sham group (P<0.01; Fig. 4A), while BMP-2 mRNA levels in CD^{14+} PBMCs from OVX group were significantly decreased compared with the sham group (P<0.05; Fig. 4B). In addition, BMP-2 protein levels in CD^{14+} PBMCs from the OVX group were significantly decreased when compared with the sham group (P<0.05; Fig. 4C). The results indicated that CD^{14+} PBMCs derived from a mouse model of postmenopausal osteoporosis exhibited decreased BMP-2 and elevated miR-410 expression levels.

miR-410 binds to the 3'-UTR seed region of BMP-2 and regulates its expression. Bioinformatics analysis revealed that miR-410 was a potential regulator of BMP-2 (Fig. 5). To identify interactions between miR-410 and the 3'-UTR of human and mouse BMP-2 mRNA, dual luciferase reporter assays were performed. The level of fluorescence generated by cells co-transfected with miR-410 mimics and pMIR-REPORT-WT luciferase reporter plasmids was significantly decreased when compared with the negative control group (P<0.01; Fig. 6). By contrast, the level of fluorescence generated by cells co-transfected with miR-410 mimics and pMIR-REPORT-mutant luciferase reporter plasmids was not significantly altered when compared with the negative control group (P>0.05; Fig. 6). These results suggest that miR-410 binds to the 3'-UTR seed region of BMP-2 mRNA and regulates its expression.

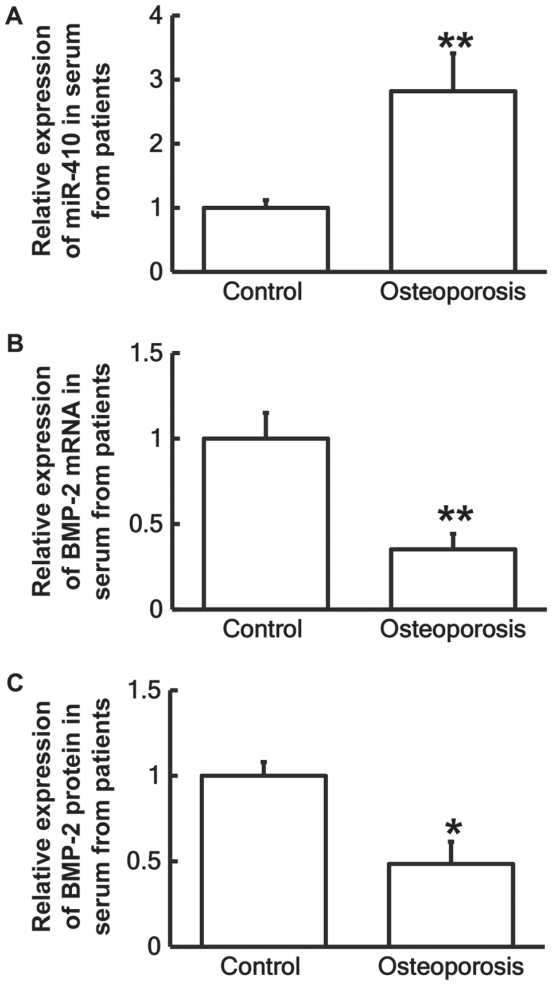

Figure 1. BMP-2 and miR-410 expression in serum samples from patients with postmenopausal osteoporosis. Levels of (A) miR-410 and (B) BMP-2 mRNA, and the protein expression levels of (C) BMP-2 in the serum from controls and patients with postmenopausal osteoporosis. Reverse transcription-quantitative polymerase chain reaction analysis was performed to measure miRNA and mRNA expression, and ELISA was used to determine BMP-2 protein expression levels. *P<0.05 and **P<0.01 vs. control group. miR, microRNA; BMP-2, bone morphogenetic protein-2; control, healthy subjects.

Discussion

It is generally accepted that the underlying cause of postmenopausal osteoporosis is an imbalance between bone formation and bone resorption induced by estrogen deficiency, which leads to bone remodeling disorders (29). Treatment of postmenopausal osteoporosis is focused on recovery and maintenance of a balance between bone remodeling and bone resorption (30,31). Understanding the molecular mechanisms underlying the disease is beneficial for clinical prevention, diagnosis and treatment.

BMP, a factor that induces osteogenesis, promotes the differentiation of mesenchymal cells into bone, cartilage, ligament, tendon and nerve tissues (32). BMP-2 has been demonstrated to transform murine myoblasts into osteoblast

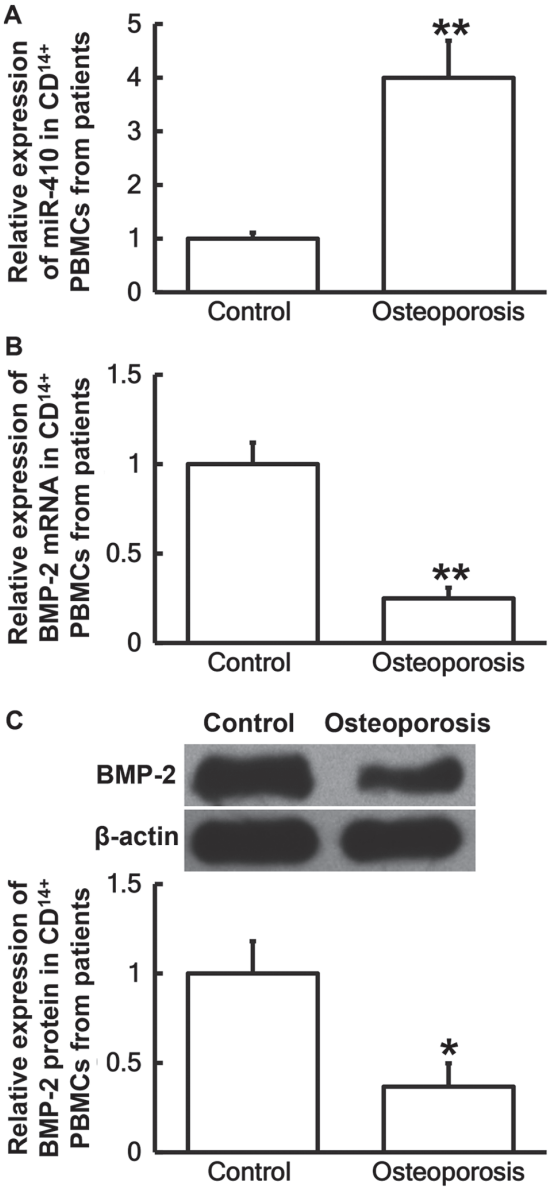

Figure 2. BMP-2 and miR-410 expression in CD^{14+} PBMCs from patients with postmenopausal osteoporosis. (A) miR-410, (B) BMP-2 mRNA and (C) BMP-2 protein expression in CD^{14+} PBMCs from controls and patients with postmenopausal osteoporosis. Reverse transcription-quantitative polymerase chain reaction analysis was performed to measure miRNA and mRNA expression levels in PBMCs and western blotting was employed to determine BMP-2 protein levels. *P<0.05 and **P<0.01 vs. control group. miR, microRNA; BMP-2, bone morphogenetic protein-2; PBMC, peripheral blood mononuclear cells; controls, healthy subjects.

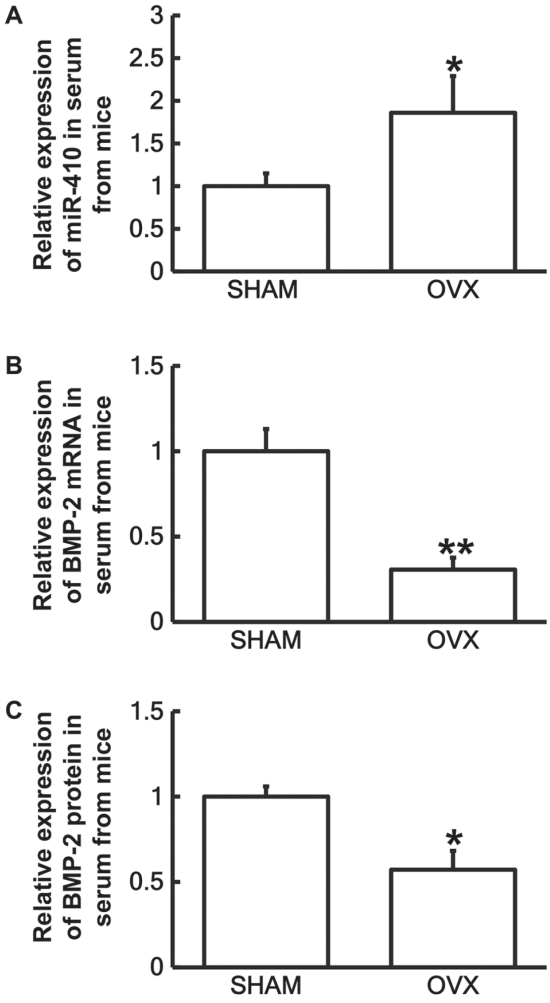

Figure 3. BMP-2 and miR-410 expression in serum from a mouse model of postmenopausal osteoporosis. (A) miR-410, (B) BMP-2 mRNA and (C) BMP-2 protein expression in serum from mice in the sham and OVX groups. Reverse transcription-quantitative polymerase chain reaction analysis was performed to measure miRNA and mRNA expression in serum, and ELISA was used to determine BMP-2 protein levels. *P<0.05 and **P<0.01 vs. sham group. miR, microRNA; BMP-2, bone morphogenetic protein-2; OVX, ovariectomized.

cells (33,34). It is thought that BMP-2 levels in osteoblasts may reflect bone formation ability (35,36). In the present study, BMP-2 levels in serum samples and CD^{14+} PBMCs derived from patients with postmenopausal osteoporosis were observed to be significantly lower when compared with healthy individuals, suggesting that reduced BMP-2 expression may be associated with postmenopausal osteoporosis. Similarly, in a mouse model of postmenopausal osteoporosis, BMP-2 expression in serum and CD^{14+} PBMCs from mice in the OVX group was downregulated when compared with the sham group. These results indicated that BMP-2 may be closely associated with postmenopausal osteoporosis.

miRs are important post-transcriptional regulators. It has been reported that miRs are widely associated with the regulation of cartilage development, osteocyte proliferation and osteoporosis (37,38). The authors of the present study hypothesized an association between BMP-2 and postmenopausal osteoporosis, and miRs that may regulate BMP-2 were investigated in the present study. Previous studies have identified a number of miRs as biomarkers for different diseases (39,40). In the current study, bioinformatics tools were utilized to identify upstream genes predicted to regulate BMP-2, which resulted in the identification of miR-410 as a potential upstream regulator. To date, there are a limited number of reports that have investigated the functional role of miR-410 in human disease. Wheeler *et al* (41) reported that miR-410 and miR-431 are expressed in the central nervous system. Goodarzi *et al* (42) demonstrated that miR-410 serves an important regulatory role in the pathological process of male alopecia. Hennessy *et al* (43) discovered that miR-410 serves an important role in the regulation of insulin secretion.

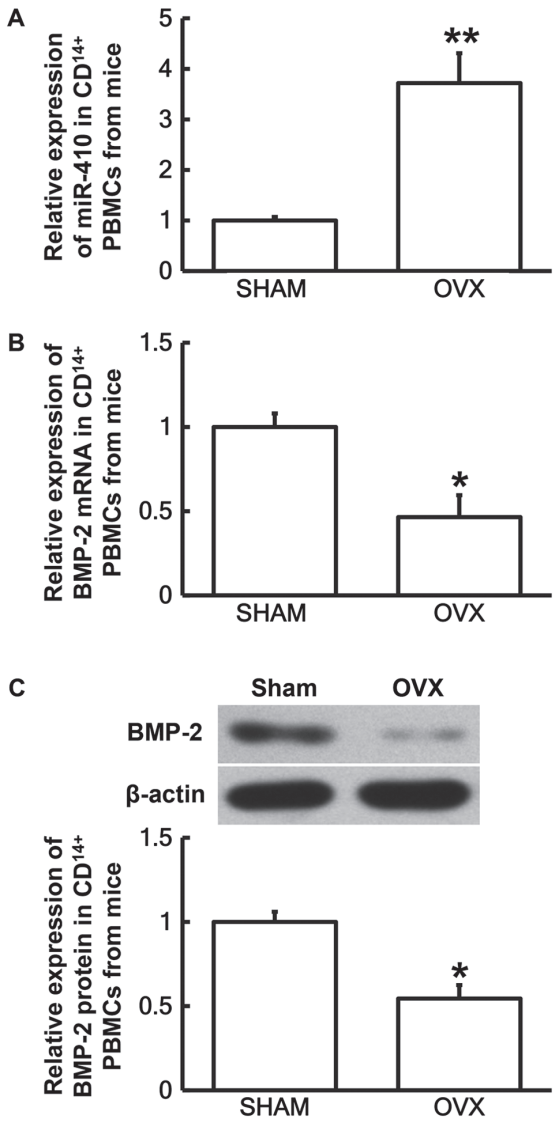

Figure 4. BMP-2 and miR-410 expression in CD^{14+} PBMCs from a mouse model with postmenopausal osteoporosis. (A) miR-410, (B) BMP-2 mRNA and (C) BMP-2 protein expression in CD^{14+} PBMCs from mice in the sham and OVX groups. Reverse transcription-quantitative polymerase chain reaction analysis was performed to measure miRNA and mRNA expression levels in PBMCs, and western blotting analysis was employed to detect BMP-2 protein levels. *P<0.05 and **P<0.01 vs. sham group. miR, microRNA; BMP-2, bone morphogenetic protein-2; OVX, ovariectomized; PBMC, peripheral blood mononuclear cells.

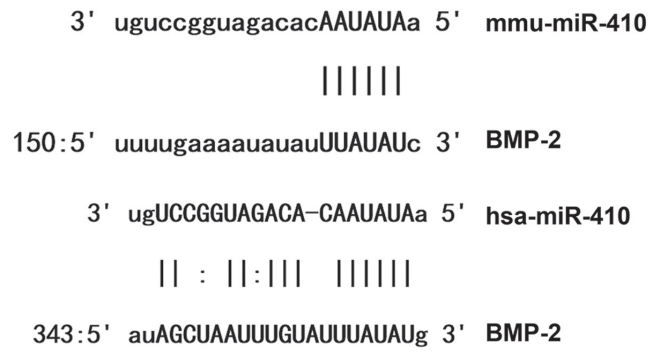

Figure 5. Interactions between miR-410 and BMP-2 mRNA in mouse and human cells. Bioinformatics tools predicted miR-410 as potential regulator of BMP-2. miR, microRNA; BMP-2, bone morphogenetic protein-2; mmu, *Mus musculus*; has, *Homo sapiens*.

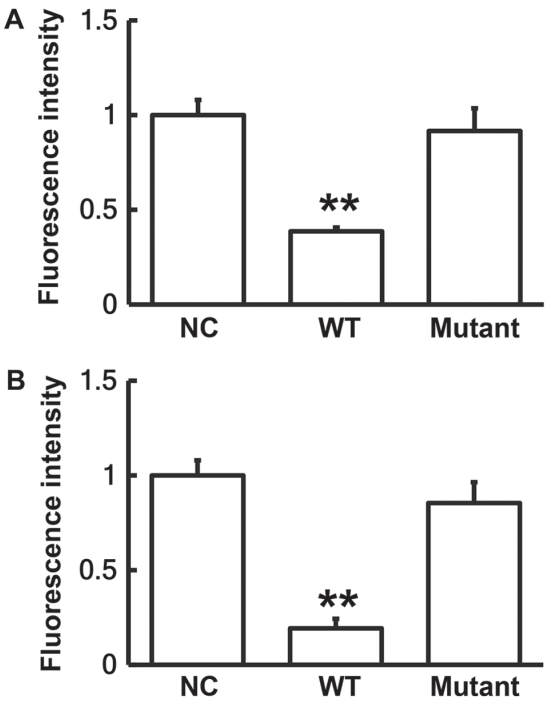

Figure 6. Interactions between miR-410 and BMP-2 in (A) human and (B) mouse. The fluorescence intensity of cells transfected with WT and mutant seed regions of miR-410 in the 3'-untranslated region of BMP-2 was determined using dual luciferase reporter assays. *Renilla* fluorescence activity served as internal reference. Data are presented as relative intensity. **P<0.01 vs. NC. miR, microRNA; BMP-2, bone morphogenetic protein-2; WT, wild-type; NC, negative control.

A previous study demonstrated that miR-410 expression was decreased in endothelial cells with Hantaan virus-induced alterations in cell permeability (44). In addition, miR-410 has been demonstrated to serve important regulatory roles in the occurrence and development of prostate, breast and colon cancer (45-47). In the present study, miR-410 expression in serum and CD^{14+} PBMCs from patients with postmenopausal osteoporosis was elevated when compared with normal healthy controls. Considering that BMP-2 expression in serum and CD^{14+} PBMCs was decreased, it was hypothesized that upregulation of miR-410 may underlie the observed downregulation of BMP-2 in patients with postmenopausal osteoporosis. Similar results were observed in the mouse model of postmenopausal osteoporosis, which indicates that an association between miR-410 and BMP-2 may exists across different species. Dual luciferase reporter assays revealed that miR-410 bound to the 3'-UTR of BMP-2 and regulated its expression.

The present study was limited by the small number of samples included and the lack of genetic diversity. Future studies may include an increased number of samples from multiple ethnic groups. In conclusion, the present study demonstrated that enhanced miR-410 expression in serum and CD^{14+} PBMCs from patients with postmenopausal osteoporosis targeted BMP-2 and may downregulate its mRNA expression thus leading to decreased BMP-2 protein levels. The association between miR-410 and BMP-2 may therefore serve a biological role in the occurrence and development of postmenopausal osteoporosis. The present study provided a

novel insight into the mechanisms underlying postmenopausal osteoporosis, and provided a theoretical basis for the diagnosis, prevention and treatment of the disease.

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Availability of data and materials

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

Authors' contributions

The final version of the manuscript has been read and approved by all authors, and each author believes that the manuscript represents honest work. HZ, WD, FJ and DW collaborated to design the study. HZ and WD were responsible for performing experiments. HZ, WD, FJ and DW analyzed the data. All authors collaborated to interpret results and develop the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Changhai Hospital. Written informed consent was obtained from all patients or their families.

Patient consent for publication

Written informed consent for publication of any associated data and accompanying images wsa obtained from all patients or their parents, guardians or next of kin.

Competing interests

The authors declare that they have no competing interests.

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