European Review for Medical and Pharmacological Sciences 2018; 22: 5804-5814

MiR-203 is essential for the shift from osteogenic differentiation to adipogenic differentiation of mesenchymal stem cells in postmenopausal osteoporosis

L. QIAO[1], D. LIU[2], C.-G. LI[3], Y.-J. WANG[3]

[1]Department of Arthropodidae, Shanghai Guanghua Hospitals of Traditional Chinese and Western Medicine, Shanghai, China
[2]Department of Rehabilitation Medicine, Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China
[3]Institute of Spinal Disease, Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China

Liang Qiao and *Dan Liu* contributed equally to this work

Abstract. – OBJECTIVE: The purpose of this study was to investigate how miR-203 promotes osteogenic differentiation of bone marrow mesenchymal cells (BMSCs) by regulating its target gene DKK1, thereby inhibiting the occurrence of osteoporosis.

PATIENTS AND METHODS: A total of 60 cases with postmenopausal osteoporosis and 40 cases of normal individuals were recruited. The expression of miR-203 in serum of all cases was detected by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). The capacity of osteogenesis and adipogenic differentiation of MSCs was determined by alizarin red staining and oil red staining, respectively. Transfection of miR-203 mimics and miR-203 inhibitor were mediated by Liposomes, and then the MSCs were induced osteogenic and adipogenic differentiation. MiR-203 mimic was co-transfected with wild-type or mutant DKK1 for luciferase reporter gene detection. In the osteoporosis model of rats, the tibia was taken for micro-CT examination of bone mineral density (BMD) and bone volume/structural parameters (BV/TV), while the femur was taken for the measurement of absorption parameters (Ob.S)./BS) and the number of osteoclasts per circumference of bone (N.Oc/B.Pm).

RESULTS: The expression level of miR-203 was significantly lower in patients with postmenopausal osteoporosis than that in normal individuals. The osteogenic capacity of BMSCs in these patients was reduced, while their adipogenic capacity was enhanced. MiR-203 promoted the expression of osteogenic genes and inhibited that of adipogenic genes. Knockdown of miR-203 decreased the level of osteogenic related genes but increased that of adipogenic related genes, while overexpression of miR-203 led to the opposite results. Furthermore, miR-203 inhibited the protein expression of DKK1. In addition, bone density and bone volume/structural parameters were lower in ovariectomized rats than those in normal rats. Meanwhile, bone resorption parameters and the number of osteoclasts per bone circumference in ovariectomized rats were higher than those in normal rats.

CONCLUSIONS: MiR-203 can promote osteogenic differentiation of mesenchymal stem cells by downregulating the gene expression of DKK1.

Key Words:
Osteoporosis, miR-203, DKK1, Bone marrow mesenchymal stem cells.

Introduction

Osteoporosis is a kind of bone metabolic disease with very high risk and morbidity. It seriously threatens the health of patients with osteoporosis in the elderly, especially in postmenopausal women[1]. Postmenopausal osteoporosis, as the most common primary osteoporosis, is a degenerative bone disease caused by a decline of ovarian function in elderly women[2]. The main manifestations are characterized by the deterioration of the microstructure of bone tissue, the decreasing proportion of bone mineral components and bone matrix, the thinning of bone, and the re-

Corresponding Author: Yongjun Wang, MD; e-mail: yjwang8888@126.com

duction of trabecular bone number[3]. According to statistics, about 40% of postmenopausal women suffer from osteoporosis[3]. Osteoporotic fracture has become one of the most important diseases causing death and disability in middle-aged and old women[4]. Therefore, osteoporosis has been listed as one of the three major age-related diseases in China.

MiRNAs are single-stranded, short-sequence non-coding RNAs. MiRNAs can regulate the expression of multiple genes by specifically binding to the 3' non-coding region of their target mRNAs and thus participate in multiple biological processes[5]. In recent years, studies have demonstrated that various miRNAs exert an important regulatory effect on the occurrence and development of bone metabolism abnormalities and osteoporosis. Reported in the literature, miR-125b can specifically bind to ERBB2 receptor tyrosine kinase, and then inhibit the differentiation of MSCs to osteoblasts[6]. MiR-133 and miR-135 are able to inhibit osteogenic differentiation of MSCs by inhibiting the expression of RUNX2 and SMAD5 during bone formation[7]. Additionally, in the late stages of differentiation of MSCs into osteoblast cell lines, miR-26a negatively regulates the differentiation of osteoblasts by binding to the SMAD1 transcription factor and leads to the down-regulation of certain osteogenic genes, such as alkaline phosphatase, type I collagen, osteocalcin, ancient bridge protein, etc.[8].

MiR-203 is the first skin-specific miRNA discovered in recent years. It can act as a carcinogen or tumor suppressor to come together with target genes to participate in tumor cell proliferation, differentiation, invasion, metastasis, and apoptosis[9]. Recent investigations[10-14] have shown that miR-203 as a tumor suppressor is widely involved in the pathogenesis of many tumors including colorectal cancer, gastric cancer, breast cancer, melanoma, and liver cancer. In summary, the research of miRNAs on osteoporosis is hot, but the role of miR-203 in the disease has not been clarified.

DKK1 is an important molecule in embryonic as well as adult skeletal development, health, and disease. Preclinical data suggest that DKK1 is involved in the osteoporosis, which is associated with deficiency of glucocorticoids and estrogen[15]. DKK1 has become a promising drug target for the treatment of osteoporosis or cancer-induced bone loss, and bone and joint diseases. Therefore, it is of great significance to study how miR-205 inhibits osteoporosis by regulating the DKK1 gene to promote osteogenesis of MSCs.

Patients and Methods

Research Object and Sample Collection

A total of 60 patients with clinically confirmed postmenopausal osteoporosis were selected to collect clinical data, and 40 normal subjects were used as controls. Fasting blood of all subjects were collected in the morning. 8 mL venous blood of each patient was taken and allowed to stand for 30 min, and the upper serum (non-hemolytic state) was collected by centrifugation at 4°C 3000 g/min for 10 min, and centrifuged at 13500 g/min for 4 min at 4°C. The serum was split in Eppendorf (EP) tube with 200 uL per tube and placed in a -80°C refrigerator for storage. This study was approved by the Ethics Committee of Shanghai Guanghua Hospitals of Traditional Chinese and Western Medicine. Signed written informed consents were obtained from all participants.

RNA Extraction and Quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR) Detection

Total RNA was extracted from serum using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) as described. The reverse transcription reaction was performed on samples using the Applied Microsystems miRNA Reverse Transcription Kit (TaKaRa, Otsu, Shiga, Japan), followed by fluorescence quantitative PCR detection to investigate the mRNA levels of miR-203, osteogenic related genes (ALP, Bglap, Runx2), and adipogenic related genes (PPARγ, LPL).

MSCs Extraction and Culture

Human BMSCs were obtained from bone marrow of postmenopausal osteoporosis patients and normal subjects. After collecting the bone marrow, it was washed twice with phosphate-buffered saline (PBS) and resuspended in cell culture medium, including 10 mL of fetal bovine serum, 2 mL of glutamine, and 1 ml of double antibody per 100 mL of a-MEM. MSCs were seeded in a cell culture flask and cultured in a 37°C, 5% CO_2 incubator. After 24 h, the culture solution in the flask was aspirated and fresh solution was added, and the culture fluid was changed every 2 days. When the cell confluence reached 90%, they were passaged after digestion with 0.05% trypsin-EDTA (ethylene diamine tetraacetic acid) (Thermo Fisher Scientific, Waltham, MA, USA).

Construction of Rat Osteoporosis Model

Eight-week-old female C57BL/6J rats were divided into ovaxy (OVX) and sham control groups. 1% pentobarbital sodium was intraperitoneally injected into the rats, and then they were fixed and sterilized. Their skin and muscle were incised on both sides of the dorsal spinal midline. Ovaries on both sides of the OVX group and the surrounding adipose tissue were removed. Their muscle and skin were then sutured. Only peri-ovarian adipose tissue was excised in rats of sham group. MSCs were then isolated from control and ovariectomized rats. Rat bone marrow cells in femurs and iliac bones were collected and rinsed with PBS. The single cell suspension was seeded into a 10 cm diameter petri dish containing MSCs culture medium and placed in a cell incubator at 37°C with 5% CO_2.

Osteogenic and Adipogenic Differentiation of MSCs

The third generation of well-grown MSCs was seeded in a 6-well cell culture plates. When the cells were adherently grown to a cell density of 80-90%, osteogenic or adipogenic inducer was added to each induced well. Osteogenic induction medium included α-MEM (minimum Eagle's medium) basal medium with 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA), 10 mM β-sodium glycerophosphate, 0.1 μM dexamethasone, 0.2 mM vitamin C. Adipogenic medium included high sugar MEM medium with 10% FBS, 1% L-glutamine, 1% double antibody, 10 mM 3-isobutyl-1-methylxanthine, 10 mM indomethacin, 10 nM dexamethasone.

Alizarin Red Staining

After 14 days of osteogenic induction, the cells were fixed with 4% paraformaldehyde for 20 minutes, then washed with PBS for 3 times, drained, stained with 40 mM alizarin red for 10 minutes. After washed with alizarin red and PBS twice, they were photographed under a phase contrast microscope. After staining, a 100 mmol/L cetylpyridinium chloride solution was added to the 6-well plate, and the absorbance was measured using a spectrophotometer.

Oil Red Staining

After adipogenic induction for 14 days, oil red staining was performed. After staining, the cell culture medium was discarded and the cells were washed 3 times with PBS. 4% paraformaldehyde was used to fix the cells at room temperature for 20 minutes. Oil red stain was added and dyed for 30 minutes. After the oil red dye was discarded, cells were photographed with a phase contrast microscope.

Liposome Transfection

The third generation of bone marrow mesenchymal stem cells in logarithmic growth phase was used for transfection. 50 μL of L-DMEM (low-dulbecco's modified eagle medium) medium (Gibco, Rockville, MD, USA) were added to the EP tube, then 2.5 μL stock solution of miR-203 mimics and miR-203 inhibitor and miR-NC were added, respectively. The mixture reacted at room temperature for 5 minutes as A solution. 50 μL of L-DMEM medium and 20 μL of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) were added to the EP tube, mixed and allowed to react at room temperature for 5 minutes as B solution. Subsequently, solution B was added to solution A to mix thoroughly, and allowed to stand at room temperature for 20 minutes. Next, the mixed solution was added to each well in a 24-well plate. The plates were incubated in a 37°C, 5% $C0^2$, and 95% humidity incubator. Subsequent experimental tests were performed after 24 hours of transfection.

Luciferase Detection

Sample Luciferase activity assay was performed using Promega's Dual-Luciferase Reporter Assay System (E1910) and according to its product description (Promega, Madison, WI, USA). Twenty-four hours after the above transfection, the old medium was aspirated and the cells were washed twice with PBS. 100 μL of PLB (Passive Lysis Buffer) were added to each well and cell lysate was collected after shaking for 15 minutes at room temperature. After 20 μL of cell lysate were added to the luminescent plate, the background value was read for 2 s using a GloMax bioluminescence detector. 100 μL of LARII working solution per sample were added, mixed quickly, and read 2 s. After reading, 100 μL of Stop & Glo & Reagent were added to each sample, mixed quickly and put in the luminometer to read for 2 s. The data were saved and the results were analyzed. The F-value was firefly luciferase, the R-value was Renilla luciferase, and the activity fold value was (R/F) sample/(R/F) control.

Western Blot

The cells transfected with miR-203 mimics were collected and lysate was added to obtain the total protein solution. Sodium dodecyl sulphate (SDS) sample buffer was added to protein solution in a 100°C water bath to eliminate protein cross-linking. Each group of protein samples was subjected to polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) and blocked with skimmed milk powder for 1 h. The primary antibodies were used to incubate the protein bands cut from PVDF membrane overnight at 4°C and the secondary antibody was used to incubate at room temperature for 1 h before exposure using enhanced electrochemiluminescence (ECL) chemiluminescence (Thermo Fisher Scientific, Waltham, MA, USA). The relative protein expression level was reflected by the target protein/internal reference GAPDH (gray value).

Micro-CT Measurement

Rats were sacrificed using dislocation of cervical spinal cord. The rat iliac bone was taken and fixed in a solution of 40 g/L paraformaldehyde for 48 h. Micro-CT scanning was performed in the prepared tibia specimen by Latheta LCT-200, the conditions of which included a source voltage of 55 kV, a source current of 131 μA, and 300 ms exposure time and 10 μm resolution. The VGStudio MAX V2.2 3D reconstruction software was used to reconstruct the three-dimensional images of the tibial Micro-CT scans, and the acquired data were analyzed.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis on the data. Measured data were expressed as mean±standard deviation ($\bar{x} \pm s$). Differences between groups were compared using *t*-test analysis. $p < 0.05$ was considered statistically significant. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Results

MiR-203 Shows Low Expression in Postmenopausal Osteoporosis Patients

Totally, 60 serum samples of postmenopausal osteoporosis patients and 40 normal subject were collected. The results of qRT-PCR indicated that the expression of miR-203 in serum of postmenopausal osteoporosis patients was significantly lower than that of normal subjects, and the difference was statistically significant ($p < 0.001$) (Figure 1A). Additionally, there was no significant difference in age and height between the two groups ($p > 0.05$), but body weight, BMI, lumbar spine BMD, total hip BMD, and femoral neck BMD of the two groups were significantly different ($p < 0.05$). These results suggested that miR-203 might play a role in osteoporosis.

The Osteogenic Capacity of MSCs in Postmenopausal Patients with Osteoporosis is Reduced, and the Adipogenic Capacity is Enhanced

We performed osteogenic and adipogenic differentiation in MSCs obtained from postmenopausal osteoporosis patients and normal subjects, and stained them with alizarin red and oil red on 14th day. Osteoblastic differentiation capacity of MSCs derived from postmenopausal osteoporosis patients was weak, resulting in the formation of mineralized nodules. The adipogenic differentiation ability of the MSCs was enhanced, resulting in the formation of lipid droplets (Figure 2A). At the same time, qRT-PCR was used to detect the expression levels of osteogenesis-related genes (ALP, Bglap, Runx2) and adipogenesis-related

Table I. Clinical characteristics of participants.

	Osteoporosis (n = 60)	Control (n = 40)	*p*-value
Age, year	63.4 ± 2.4	59.3 ± 3.2	0.059
Height, cm	153.5 ± 4.1	155.1 ± 5.2	0.088
Weight, kg	53.7 ± 4.3	50.1 ± 1.3	0.045
BMI, kg/m^2	22.8 ± 1.2	24.2 ± 1.5	0.023
Lumbar spine BMD, g/cm^2	0.68 ± 0.09	0.78 ± 0.08	0.031
Total hip BMD, g/cm^2	0.71 ± 0.07	0.82 ± 0.06	0.025
Femoral neck BMD, g/cm^2	0.59 ± 0.07	0.67 ± 0.08	0.018

Abbreviations: BMI, body mass index; BMD, bone mineral density.

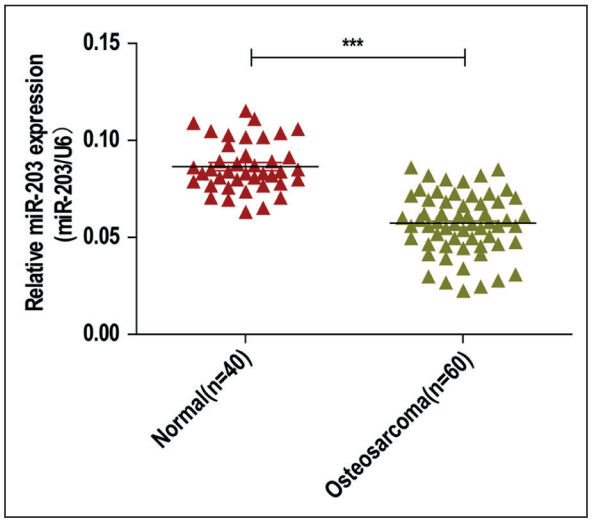

Figure 1. The expression of MiR-203 was significantly low in postmenopausal osteoporosis patients. QRT-PCR detection showed that miR-203 in serum (n=60) of postmenopausal osteoporosis patients was significantly lower than that of normal human. U6 RNA was used as a standardized indicator.

genes (PPARγ, LPL) in the corresponding group. The analysis showed that the levels of osteogenic related gene in MSCs from postmenopausal osteoporosis patients were lower than that from the normal group, while levels of lipid-associated genes were higher, which was consistent with staining results (Figure 2B-C). Rat osteoporosis model was further used to verify the above results. MSCs were isolated from control and ovariectomized rats to induce osteogenic and adipogenic differentiation. The cells were cultured for 14 days in osteogenic inducing medium and stained with alizarin red. Results showed that the osteogenic differentiation ability of MSCs derived from ovariectomized rats was lower than that from normal rats, and less mineralized nodules were formed. After MSCs were cultured in adipogenic medium for 14 days, results of oil red staining indicated that adipogenic differentiation capacity of MSCs derived from ovariectomized rats was stronger than that from normal rats, resulting in increased lipid droplet formation (Figure 2D). QRT-PCR was used to detect the expression levels of osteogenic related genes (ALP, Bglap, Runx2) and adipogenic related genes (PPARγ, LPL) in the corresponding group. The osteogenic related gene expression of MSCs in ovariectomized rats was lower than that in the control group, while lipid-related gene expression was higher, and the results were consistent with staining results (Figure 2E-F). The above results indicated that the culture method of BMSCs was correct, and the rat osteoporosis model was successfully constructed and could be used in the following experiments. At the same time, the characteristics of osteogenic and adipogenic differentiation of MSCs in osteoporotic patients and rat models of osteoporosis were demonstrated.

MiR-203 Promotes the Expression of Osteogenic Genes but Inhibits that of Adipogenic Genes

MSCs were obtained from normal humans for different days of osteogenic and adipogenic induction, and miR-203 level was detected by qRT-PCR. The results of qRT-PCR showed that miR-203 was increased during osteogenic induction but decreased during adipogenic induction (Figure 3A-B). MiR-203 oligonucleotides were used to inhibit miR-203, and osteoblasts and adipogenic differentiation were performed. The level of bone-related genes (ALP, Bglap, Runx2) in the miR-203-inhibitory group was reduced (Figure 3C), while that of adipogenic-related genes (PPARγ, LPL) (Figure 3D) was increased. MiR-203 mimic was transfected into MSCs to over-express miR-203 and osteogenesis and adipogenic differentiation were induced. The results of qRT-PCR indicated that the level of bone-related genes (ALP, Bglap, Runx2) in miR-203 mimic group was higher than that in control group. (Figure 3E), while the expression of adipogenesis-related genes (PPARγ, LPL) in the former group was reduced (Figure 3F). These results indicated that miR-203 could promote the expression of osteogenic genes but inhibit that of adipogenic genes.

The Luciferase Reporter Assay Verifies that DKK1 is the Target Gene of miR-203

To demonstrate that miR-203 directly targets DKK1 and inhibits its expression, we performed a luciferase gene assay. After 24 hours of transfection, we observed that the expression level of DKK1 (Luciferase activity) was significantly lower in the group of co-transfecting miR-203 mimic and wild-type DKK1 than in the control group ($p < 0.01$). However, after co-transfection of miR-203 mimic and mutant DKK1, no significant difference was found in the expression of DKK1 compared with the control group, indicating that the expression of Lucifer in gene was inhibited when miR-203 co-existed with the 3′UTR

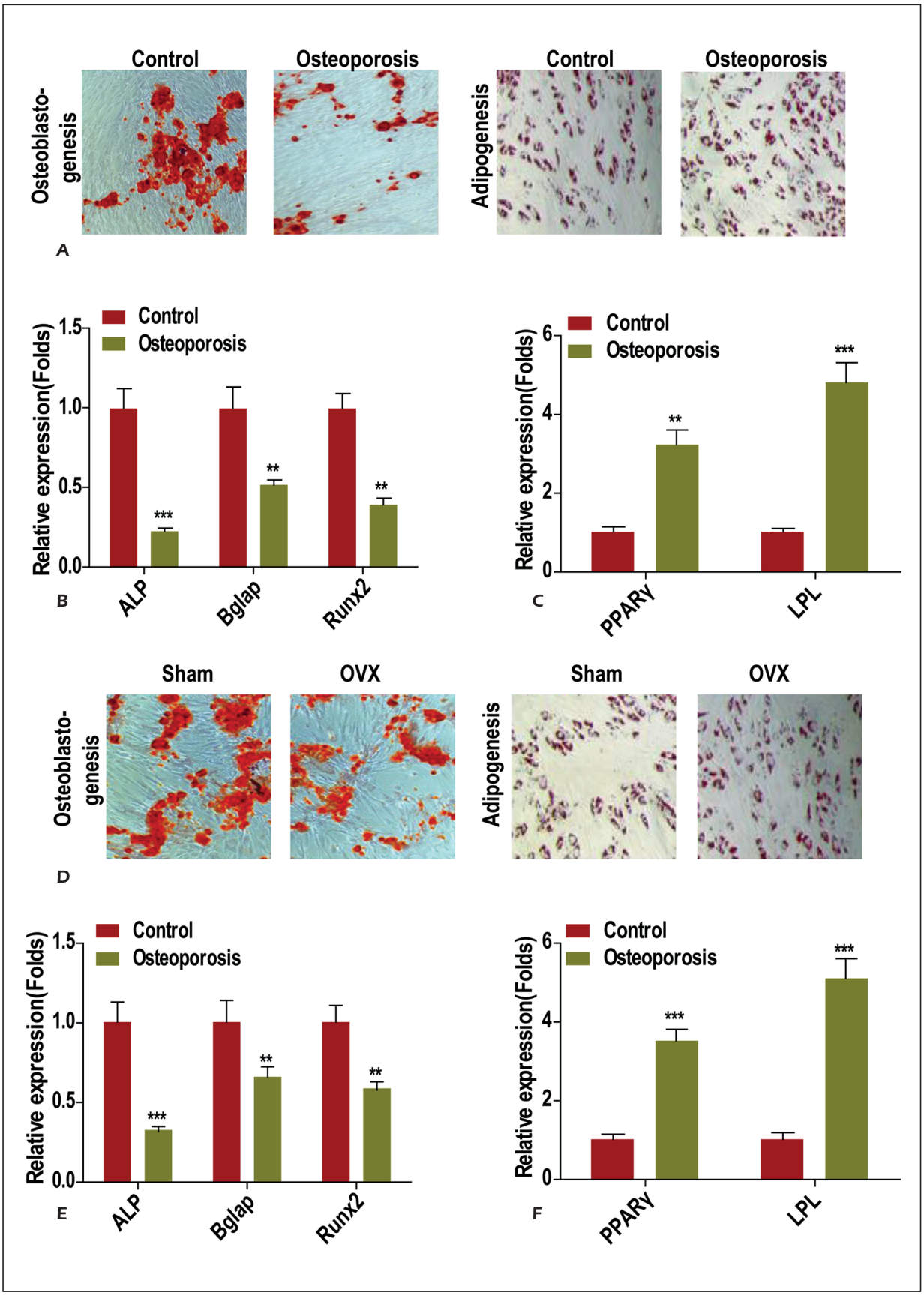
Control
Osteoporosis
Osteoblasto-genesis
Adipogenesis
A
Relative expression(Folds)
Control
Osteoporosis
ALP
Bglap
Runx2
B
PPARγ
LPL
C
Sham
OVX
D
E
F

Figure 2. Osteogenic capacity of MSCs derived from postmenopausal osteoporosis patients was decreased and adipogenic capacity was increased. MSCs were obtained from postmenopausal osteoporosis patients (n=3) and normal individuals (n=3) and cultured for 3-8 generations for experiments. Induction of osteogenic and adipogenic differentiation was performed. ***A,*** MSCs were cultured for 14 days in osteogenic induction medium and stained with alizarin red. MSCs derived from postmenopausal osteoporosis patients had weak osteogenic differentiation ability and formed few mineralized nodules. MSCs were cultured for 14 days in adipogenic induction medium and stained with oil red. Adipogenic differentiation ability of MSCs derived from postmenopausal osteoporosis patients increased and lipid droplet formation increased. ***B-C,*** QRT-PCR was used to detect the expression levels of osteogenic related genes (ALP, Bglap, Runx2) and adipogenic related genes (PPARγ, LPL) in the corresponding groups. The results were consistent with the staining results. ***D-F,*** MSCs were isolated from control and ovariectomized rats to induce osteogenic and adipogenic differentiation. ***D,*** MSCs were cultured in osteogenic inducing medium for 14 days and stained with alizarin red. The osteogenic differentiation ability of MSCs derived from ovariectomized rats was lower than that from normal mice and mineralized nodules were formed. MSCs were cultured for 14 days in adipogenic inducing medium and stained with oil red. Adipogenic differentiation of MSCs derived from ovariectomized rats was stronger than that from normal mice, resulting in increased lipid droplet formation. ***E-F,*** Q-PCR was used to detect the expression levels of osteogenic related genes (ALP, Bglap, Runx2) and adipogenic related genes (PPARγ, LPL) in the corresponding groups. The results were consistent with the staining results.

region of DKK1. It was initially confirmed that DKK1 is the target gene of miR-203 (Figure 4A). Western blot assay was used to detect DKK1 expression after transfection of miR-203 mimic in MSCs extracted from normal people. The results of Western blot showed that the expression level of DKK1 in cells decreased (Figure 4B), indicating that DDK1 protein levels decreased after over-expression of miR-203. The rat osteoporosis model was further used to verify the above results. The control and the ovariectomized rats were injected with mut antagomiR-203 (Control) and antagomir-203 (miR-203 Inhibitor), respectively. After six weeks, the rat tibia was taken for micro-CT. The bone mineral density (BMD) and bone volume/structural parameters BV/TV were measured in each group. The test results showed that the parameters of the antagomir-203 group were lower than those of the control group, and the parameters of the ovariectomized rats group were lower than the normal rats (Figure 4C-D). Ob.S/BS (the femur bone resorption parameter), N.Oc/B.Pm (the number of osteoclasts per bone circumference), and the parameters of ovariectomized rat group with antagomir-203 injected were higher than those of normal rats (Figure 4E-F). These results indicated that in the rat osteoporosis model, BMD and BV/TV in OVX group with inhibition of miR-203 expression are lower than those of Sham group.

Discussion

Postmenopausal osteoporosis (OPM) is the most common type of osteoporosis. It is systemic chronic bone disease without obvious clinical symptoms caused by post-menopausal ovarian dysfunction and decreased levels of estrogen in the body[16]. Studies have shown that the worldwide occurrence of OPM fractures increases by 18% every 5 years[17]. In recent years, the most fundamental reason for the occurrence of OPM is that the abnormal differentiation of BMSCs can result in a decrease in the number of osteoblasts and an increase in the number of adipocytes[18]. However, the differentiation of BMSCs is unbalanced. The specific regulatory mechanism is not yet clear. Additionally, it is known that the process of osteogenesis and differentiation of MSCs is closely related to occurrence and development of many diseases, which is also a breakthrough point in the field of treatment of orthopedic diseases and tissue engineering repair. Many studies have confirmed that miRNA can alter the osteogenic differentiation ability of MSCs by regulating the level of target genes. Almeida et al[19] found that miR-195 may bind to VEGF to regulate the osteogenic differentiation, proliferation and vascularization of MSCs. In MSCs, artificially up-regulating the expression of miR-195 or miIM97 can inhibit the osteogenic differentiation and colonization of MSCs. On the contrary, down-regulation of miR-195 relied on alkaline phosphatase activity in MSCs. Su et al[20] showed that miR-26a targets GSK3-beta and SMAD1 genes to regulate Wnt/BMP signal transduction pathways, which can significantly affect the osteogenic differentiation of MSCs and adipose stem cells (ADSCs). Taken together, miRNA plays a crucial role in osteoporosis, which is worth exploring.

MicroRNA (miRNA) is a class of non-coding small RNA, which is thought to be a gene expression regulator. MiRNA can specifically

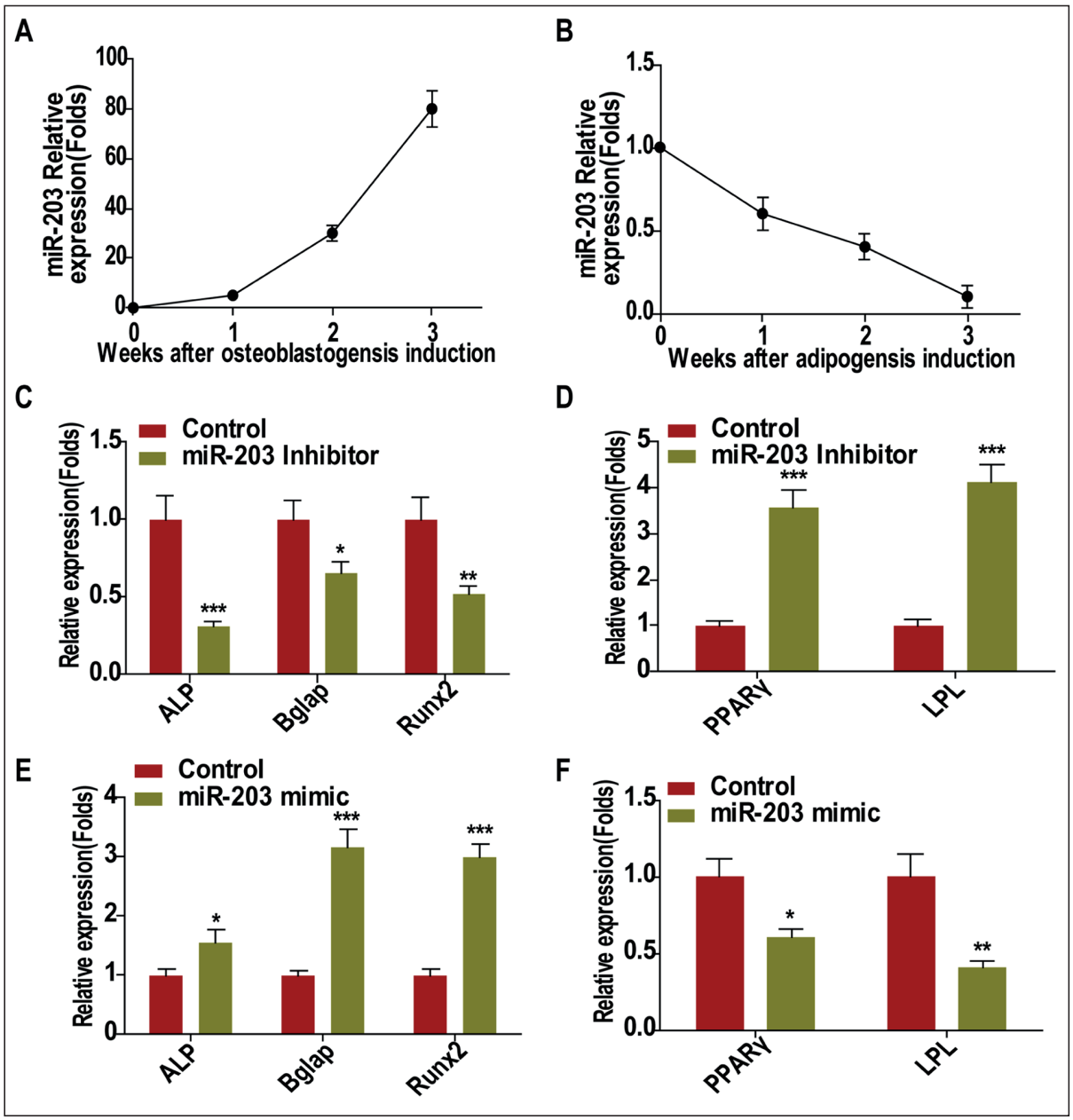

Figure 3. MiR-203 plays a critical role in the differentiation of bone marrow mesenchymal stem cells. MSCs were obtained from postmenopausal osteoporosis patients (n=3) and normal individuals (n=3) and cultured for 3-8 generations for experiments. The cells were induced osteogenic and adipogenic differentiation. ***A-B,*** After osteogenesis (***A***) and adipogenesis (***B***) induction of different days in MSCs obtained from normal humans, qRT-PCR indicated that miR-203 expression level in cells increased during osteoinduction, decreased during adipogenic induction. ***C-D,*** After inhibition of miR-203 with miR-203 oligonucleotides, osteogenic and adipogenic differentiation were performed. Expression of bone-related genes (ALP, Bglap, Runx2) decreased in the miR-203-inhibitory group compared to the control group (***C***), while adipogenic-related adipogenic-related genes (PPARγ, LPL) increased (***D***). The miR-203 mimics were transfected. ***E-F,*** MiR-203 mimic was transfected into MSCs to overexpress miR-203, and osteogenic and adipogenic differentiation were performed. The expression of bone-related genes (ALP, Bglap, Runx2) in miR-203 mimic group was elevated (***E***), while expression of adipogenic-related adipogenic-related genes (PPARγ, LPL) decreased (***F***).

inhibit gene expression at transcriptional level or promote degradation of target mRNA through binding to the 3' non-coding region of the target mRNA. Current research demonstrated that miR-203 has low expression in esophageal, cervical, colon, prostate, and hematopoietic tumors[21-23]. Meanwhile, studies have reported that low expression of miR-203 is involved in the tumor

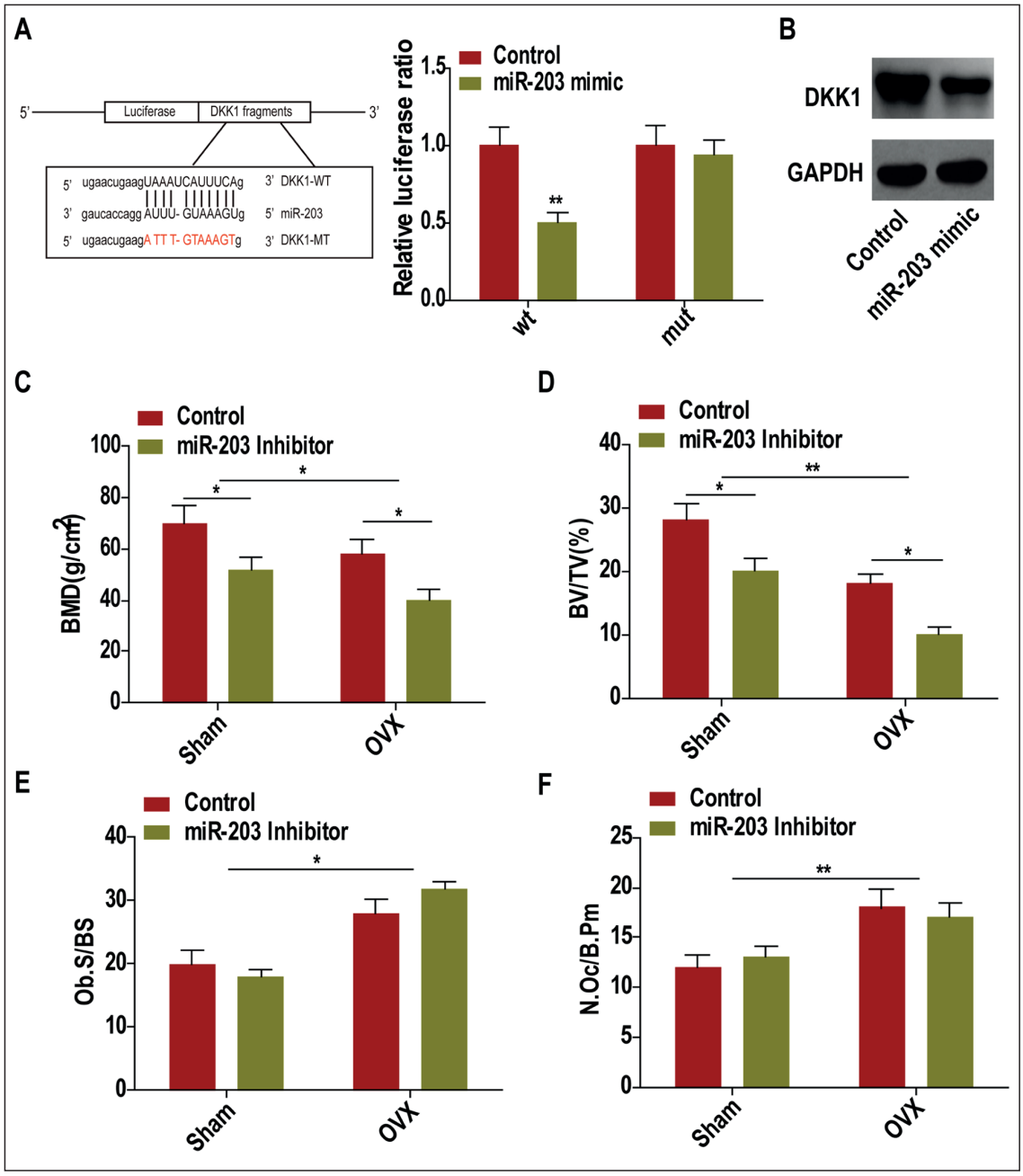

Figure 4. DKK1 is the target gene of miR-203. ***A,*** Binding site of DKK1 and miR-203 was shown, luciferase activity was measured 24 h after transfection. ***B,*** After transfection of miR-203 mimics in MSCs extracted from normal humans, the expression level of DKK1 in the cells was decreased. ***C-F,*** Control group and ovariectomized rats were injected with mut antagomiR-203 (Control) and antagomir-203 (miR-203 Inhibitor) respectively. After six weeks, the tibias of rats were taken for micro-CT scan to detect bone mineral density (BMD) of each group (***C***) and Bone volume/structural parameters (BV/TV) (***D***). The parameters of the antagomir-203 group were lower than those of the control group and the ovariectomized rats. The parameters of the femur bone resorption parameters (Ob.S/BS) (***E***) and the number of osteoclasts per bone circumference (N.Oc/B.Pm) (***F***) were lower in the ovariectomized rats injected with antagomir-203 than those in normal rats.

cell formation of chronic myelogenous leukemia (CML), acute lymphocytic leukemia (ALL), and head and neck squamous cell carcinoma[24,25]. Furuta et al[26] reported that miRNA-203 down-regulated its target genes including CDK6, SET, GAP1, vimentin, SMYD3, and ABCE1 in HCC. It is certain that miRNA-203 causes different tumors to occur through different target genes.

However, the mechanism of the role of miR-203 in osteoporosis has not been reported yet, so exploring the mechanism has a certain value. In this study, we investigated the role of miR-203 in postmenopausal osteoporosis and found that miR-203 showed low expression in patients with osteoporosis, which was consistent with the results in rat osteoporosis model.

DKK1 is an inhibitor of the classical Wnt signaling pathway and plays a vital role in occurrence and development of bone disease[27]. Studies have shown that DKK1 expression is closely related to apoptosis of osteochondral cartilage cells and rheumatoid arthritis disease[28]. Meanwhile, DKK1 overexpression is associated with osteoblastic bone metastases caused by prostate cancer cells and multiple osteolytic damage induced by multiple myeloma[29,30]. In this report, luciferase reporter gene assay was used to verify that DKK1 is a downstream target gene of miR-203. Furthermore, the results of Western blot displayed that the level of DKK1-related protein was decreased after miR-203 overexpression. These results suggested that miR-203 may be involved in osteoporosis by regulating DKK1.

Conclusions

We showed that miR-203 is significantly low in postmenopausal osteoporosis patients. miR-203 can promote the differentiation of MSCs into osteoblasts mainly by down-regulating its target gene DKK1, which suggests a new basis for exploring the pathogenesis of postmenopausal osteoporosis.

Acknowledgements

This work was supported by the National Nature Science Foundation (81574001). Major Diseases of Shanghai's Joint Research Project (2013ZYJB0701). Three year plan of Shanghai TCM (ZY3-CCCX-2-1002). National Clinical Research Base of TCM Project (JDZX2015075). Preventive Treatment and Health Protection Project of Shanghai (ZY3-FWMS-1-1001-KYJS-07).

Conflict of Interest

The Authors declare that they have no conflict of interests.

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