# MicroRNA-148a inhibition protects against ovariectomy-induced osteoporosis through PI3K/AKT signaling by estrogen receptor α

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**Abstract.** The present study aimed to investigate the effect of microRNA-148a downregulation on osteoporosis by using an ovariectomized rat model. Reverse transcription-quantitative polymerase chain reaction was used to analyze microRNA-148a expression levels, MTT and flow cytometry assays were used to examine cytotoxicity and apoptosis, respectively. The gap-associated proteins were quantified using western blotting. The expression of microRNA-148a was significantly increased in osteoporosis rat following ovariectomy. Overexpression of microRNA-148a significantly promoted apoptosis and inhibited cell growth, whereas downregulation of microRNA-148a significantly reduced apoptosis and increased cell growth. Overexpression of microRNA-148a significantly reduced estrogen receptor a (ERα) protein expression and suppressed phosphoinositide-3-kinase regulatory subunit 1 (PI3K) and phosphorylated-protein kinase B (AKT) protein expression in osteoblasts *in vitro*. The inhibition of ERα increased the microRNA-148a effect on apoptosis in osteoblasts *in vitro*. Subsequently, LY294002, an PI3K inhibitor, significantly increased the effect of microRNA-148a on apoptosis in osteoblasts *in vitro*. The findings of the present study revealed that anti-microRNA-148a protected cells against ovariectomy-induced osteoporosis through ERα by PI3K/AKT signaling.

## Introduction

Osteoporosis is a metabolic bone disease that is characterized by lower bone mass and bone microstructure and leads to the increase of osteopsathyrosis and increased fractures (1). National Institutes of Health defines osteoporosis as a bone disease which the damages bone strength, leads to degeneration of bone tissue microstructure and an increased risk of fractures (2). Osteoporosis may be divided into two types, primary and secondary: Primary osteoporosis occurs in aging patients and is associated with the reduction of hormone secretion and accounts for ~90% of cases (1), whereas secondary osteoporosis accounts for 10% of cases (3). Ovariectomy-induced osteoporosis (OIO) is the most common type of osteoporosis. In postmenopausal osteoporosis, bone formation and bone resorption is imbalanced, and occurs due to reduced estrogen levels following menopause (3,4). Clinical characteristics of postmenopausal osteoporosis include chronic pain in lumbar spinal cord and limbs, humpback, shortened height and fractures in skeleton, vertebra and forearm (5).

The common factors contributing to osteoporosis pathogenesis may be divided as follows: i) Internal secretion; ii) nutritional iii) physical; iv) immune; and v) genetic factors. The reduction of estrogen levels in internal secretion is the primary factor for the occurrence of osteoporosis following menopause (6). Various drugs have various disadvantages, reduced calcium absorption or they may lead to the increase of osteopsathyrosis, particularly in estrogen replacement therapy (7). Therefore, there is a great potential to investigate the possible targets of anti-bone absorption drugs. The osteoprotegerin/receptor activator of nuclear factor κB (RANK)/RANK ligand (RANKL) system is the primary determinant of bone mass and is a promising target (7,8).

Osteoporosis is a bone metabolism obstructive disease of the whole body, which is characterized by damaging bone tissue microstructure, thickening the bone cortex, reducing the number of bone trabecula and increasing bone fragility. Osteoblasts and osteoclasts regulate bone formation and bone resorption, respectively (9). The dynamic balance between them maintains normal bone mass (10). As the regulatory factors of bone metabolism, phosphoinositide-3-kinase regulatory subunit 1 (PI3K) and protein kinase B (AKT) may promote osteoblast precursors to differentiate into mature osteoblasts and advocate bone formation (11).

MicroRNAs (miRNAs) are a class of single-strand non-coding RNAs with short sequence. They are extensively distributed in animals and plants, where they have important roles (12). miRNA is a small regulatory molecule at the post-transcription level (13). It may specifically bind to the 3'-untranslated region of target mRNA; therefore, it may regulate the expression of multiple genes. Therefore, it is involved in multiple biological processes, including cell development, differentiation, apoptosis and tumor genesis (13). Intracellular miRNAs expression may be changed to some extent under disease conditions. Changes in miRNA expression may lead to regulatory functional changes at the post-transcription level (14). Therefore, they

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may induce a series of biological functional changes in cells. A recent study revealed that miRNAs may regulate biological characteristics of multiple immune cells (13). They may have an important influence on the regulation of the immune function of body. Therefore, to investigate the effect and mechanism of microRNA-148a protection against ovariectomy-induced osteoporosis the present study used *in vitro* and *in vivo* models.

## Materials and methods

*Experimental design and diet*. Female Sprague-Dawley rats (6-weeks old; weight, 180-230 g, n=20) were purchased from Animal Experiment Center of Tianjin Medical University and were housed in polycarbonate cages at (22±2˚C) with 55±5% relative humidity, 12-h light/dark cycle with free access to food and water for an adaptation period of 3 days. The experimental protocol was approved by the Animal Care and Use Review Committee of Tianjin Hospital (Tianjin, China). Rats were anesthetized with 2% of isoflurane (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and ovaries were removed bilaterally. Rats were randomly divided into two treatment groups: i) Sham-operated group (control); and ii) ovariectomized rats (model). Rats were anesthetized with 2% of isoflurane (Sinopharm Chemical Reagent Co., Ltd.), and peripheral blood was gathered from caudal vein.

*Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis*. Total RNA was prepared from blood samples using an RNeasy Mini kit (Qiagen, Inc., Valencia, CA, USA). Total RNA (1 $\mu$g) was reverse-transcribed at 37˚C for 30 min and 85˚C for 1 min to synthesize complementary cDNA using the SuperScript III First-Strand Synthesis System (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) using the following primers: miR-148a RT-primer, 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACT GGATACGACAACAAAGTT-3' and U6 RT-primer, 5'-CGC TTCACGAATTTGCGTGTCAT-3'. qPCR was performed using the SYBR Premix Ex Tag kit (Takara Bio, Inc., Otsu, Japan) by an ABI 7500 Sequencing Detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The reaction conditions were: 95˚C for 10 min, followed by 40 cycles of 95˚C for 30 sec and 60˚C for 30 sec. The following primers used for qPCR were: MicroRNA-148a forward, 5'-GCTAGT GTTCTGAGACACTCCG-3' and reverse, 5'-GTGCAGGGT CCGAGGT-3'; and U6 forward, 5'-GCTTCGGCAGCACAT ATACTAAAAT-3' and reverse, 5'-CGCTTCACGAATTTG CGTGTCAT-3'. The relative expression was analyzed by the $2^{-\Delta\Delta Cq}$ method (15).

*H&E staining*. The caput femoris (n=6/group) was collected and fixed with 10% paraformaldehyde for 72 h at room temperature. Formalin-fixed paraffin-embedded sections of caput femoris were sectioned at 10 $\mu$M and stained with H&E for 30 min at room temperature. Tissue samples were observed using an Olympus fluorescent photomicroscope (Olympus Corporation, Tokyo, Japan).

*Cell culture and transfection*. MC3T3-E1 cells were obtained from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and maintained in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and at 37˚C with 5% $CO_2$. MC3T3-E1 cells treated with RANKL (50 ng/ml; R&D Systems, Inc., Minneapolis, MN, USA) for 4 days. MC3T3-E1 cells ($1x10^5$ cell/well) were transfected with 100 nM microRNA-148a (5'-GAGGCAAAGTTCTGAGA-3' and 5'-AGAACTTTGTCTC-3'), 100 nM anti-microRNA-148a (5'-CTCCGTTTCAAGACTCTGGC-3' and 5'-TCTTGAAAC AGAGGAGA-3'), and negative mimics (5'-CCCCCCCCC-3') using Lipofectamine® 3000 (Thermo Fisher Scientific, Inc.). MC3T3-E1 cells were treated with RANKL (50 ng/ml; R&D Systems, Inc.) for 3 days, 6 h after transfection. ERα inhibitor, AZD9496 (0.1 nM; MedChemExpress, Shanghai, China) was added to transfected cells for 24 h at 37˚C.

*Cytotoxicity assay*. Following transfection at 24, 48 and 72 h the cells were seeded onto 96-well plates at a density of $3x10^4$ cells/well. The cells were stained with MTT solution for 4 h and dimethyl sulfoxide was added into the wells for 20 min. The absorbance was detected using a FlexStation 3 Multi-Mode microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) at 492 nm.

*Apoptosis assay*. Cells were seeded onto 6-well plates at a density of $1x10^6$ cells/well 48 h after transfection. The cells were then stained with Annexin-V and propidium iodide assay kit (BD Biosciences, Franklin Lakes, NJ, USA) for 15 min. The apoptotic rate was quantified using C6 flow cytometer (BD Biosciences) and analyzed using FlowJo 7.6.1 (FlowJo; Tree Star, Inc., Ashland, OR, USA).

*Western blotting and caspase-3/9 activity levels*. Protein was extracted with RIPA lysis buffer (Beyotime Institute of Biotechnology, Jiangsu, China). Total protein was quantified using Enhanced BCA Protein Assay kit (Beyotime Institute of Biotechnology). Equal quantity (50 $\mu$g) of total protein was resolved by 10% SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (EMD Millipore, Bedford, MA, USA). The membranes were blocked with 5% non-fat milk in tris-buffered saline with 0.1% Tween-20 for 1 h at 37˚C and incubated with various primary antibodies: Bcl2-associated X (Bax; 1:500; sc-6236; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), PI3K (1:500; sc-7174; Santa Cruz Biotechnology, Inc.), estrogen receptor a (ERα; sc-544, 1:500; Santa Cruz Biotechnology, Inc.), phosphorylated (p)-AKT (cat. nos. sc-293125 and sc-7985-R, 1:500; Santa Cruz Biotechnology, Inc.) and GAPDH (cat. no. sc-25778; 1:2,000; Santa Cruz Biotechnology, Inc.) at 4˚C overnight and subsequently incubated with an anti-mouse immunoglobulin G (IgG) antibody conjugated to horseradish peroxidase (cat. no. AAT-16440; 1:5,000; Amyjet Scientific Inc.,) for 1 h at 37˚C. The membranes were visualized using enhanced chemiluminescence kits (GE Healthcare, Chicago, IL, USA) and assessed by densitometry using MacBiophotonics ImageJ version 1.41a (https://imagej.nih.gov/ij/).

Equal quantity (50 $\mu$g) of total protein was used to quantify caspase-3/9 activity levels using commercial kits (C1116 and C1158; Beyotime Institute of Biotechnology). The absorbance was detected using a FlexStation 3 Multi-Mode microplate reader at 405 nm.

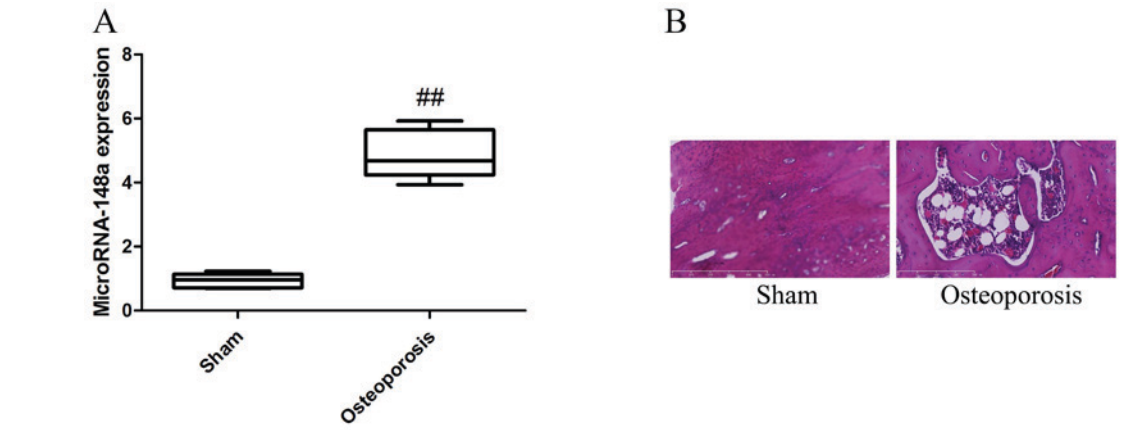


Figure 1. (A) Expression of microRNA-148a and (B) hematoxylin and eosin staining (magnification, x40) in osteoporosis rat model following ovariectomy. ##P<0.01 vs. sham group.

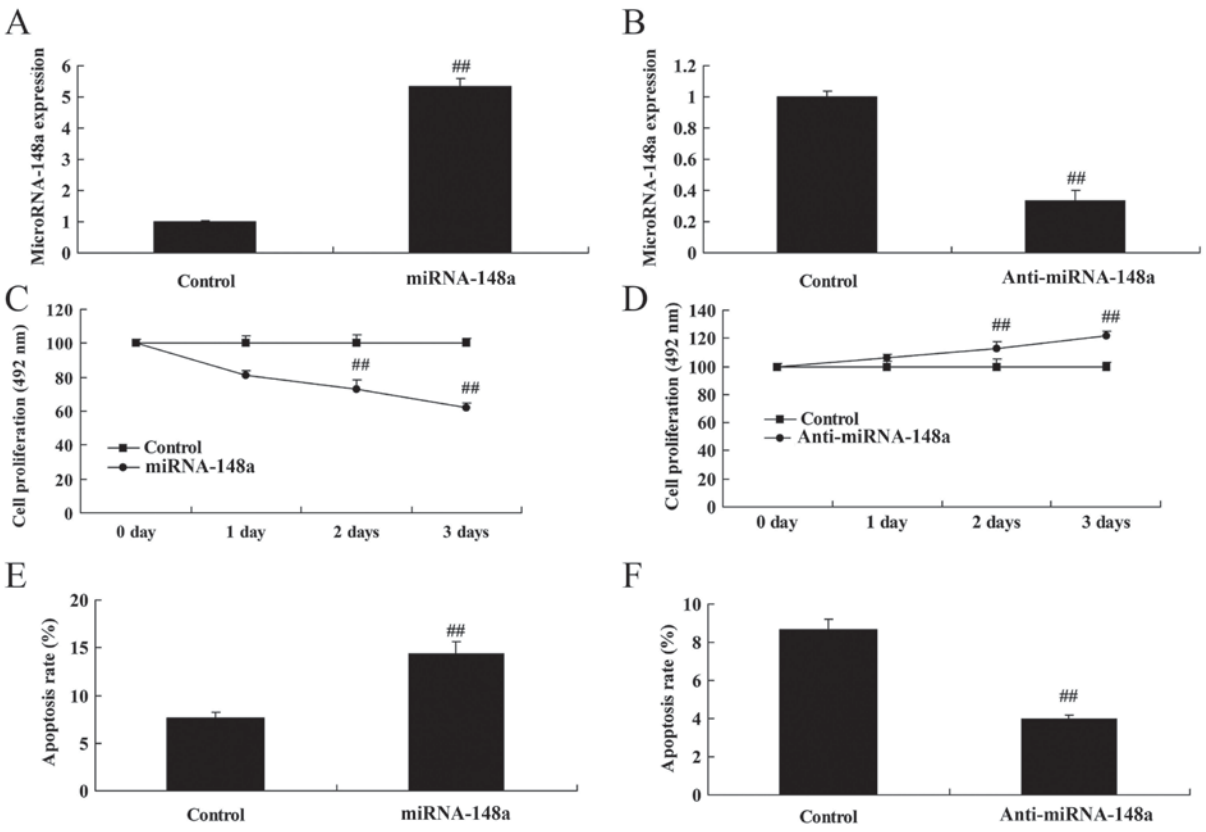


Figure 2. Effect of miRNA-148a on osteoblasts *in vitro*. The effects of miRNA-148a expression was (A) upregulated and (B) downregulated using transfection. Cell proliferation and apoptosis were determined in bone cells treated with (C and D) miRNA-148a and (E and F) anti-148a. miRNA-148a, overexpressing group; Anti-miRNA-148a, downregulating group. ##P<0.01 vs. control group. miRNA, microRNA.

*Luciferase reporter assay*. mRNA of ERα 3'UTR was inserted downstream of the luciferase reporter gene in a pMIR-REPORT vector (Thermo Fisher Scientific, Inc.). HEK293 cells (1x$10^5$ cell) were co-transfected with miR-148a mimic and ERα-3'UTR (Promega Corporation, Madison, WI, USA) using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.). The luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega Corporation) after 48 h of incubation at 37˚C with 5% $CO_2$. The ratio of *Renilla* luciferase of firefly luciferase was calculated for each well.

*Statistical analysis*. Data are expressed as the mean ± standard deviation. Statistical significance was determined using Student's t-test or one-way analysis of variance which was followed by Tukey's Honest Significant Difference as a post hoc test. P<0.05 was considered to indicate a statistically significant difference.

## Results

*Expression of microRNA-148a in osteoporosis rats following ovariectomy*. RT-qPCR was used to analyze the changes of

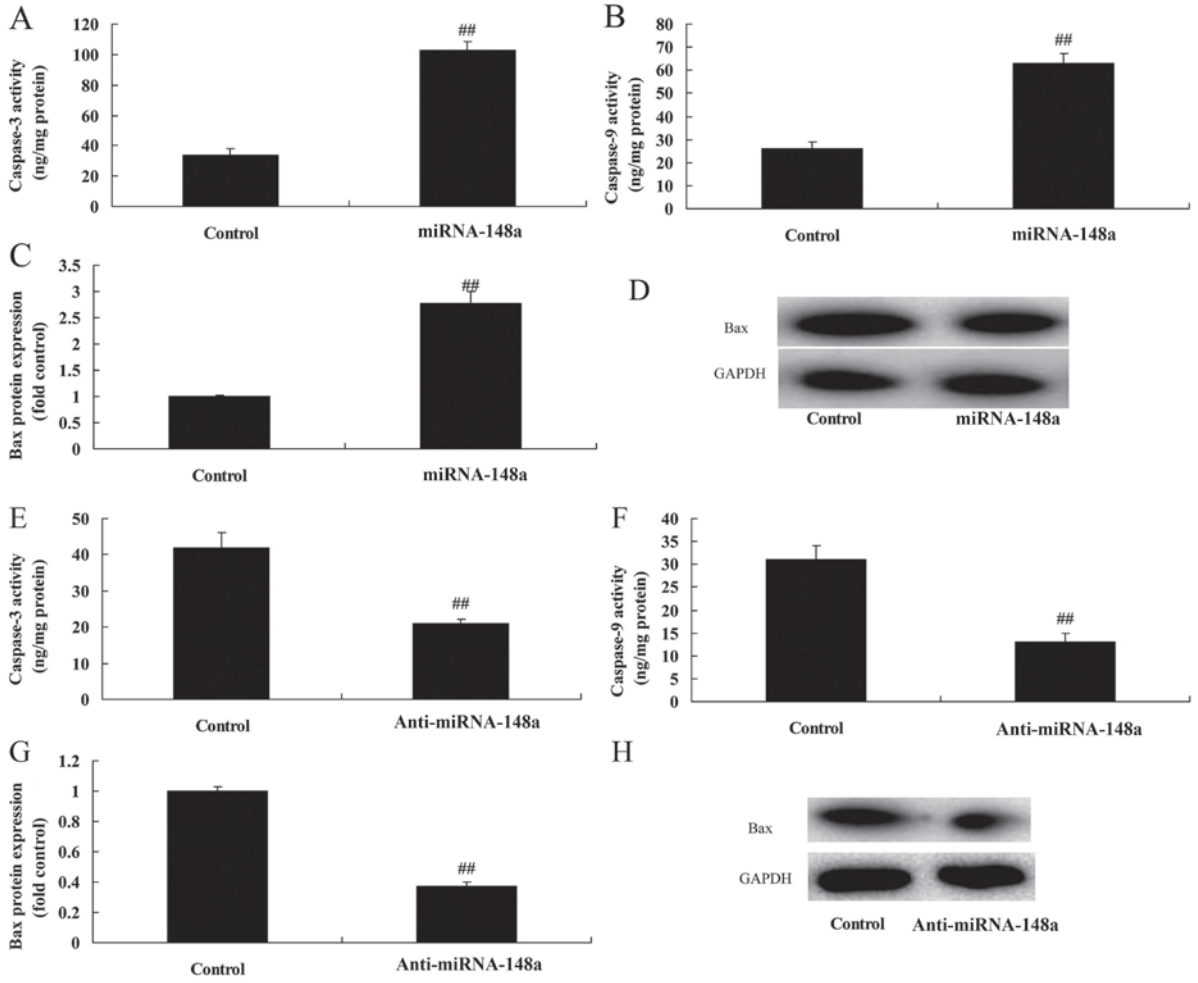


Figure 3. Effect of miRNA-148a expression on Bax protein expression and caspase-3/9 activity *in vitro*. (A) Caspase-3 and (B) caspase-9 activity and (C and D) Bax protein expression were determined in the miRNA-148a group. (E) Caspase-3 and (F) caspase-9 activity and (G and H) Bax protein expression were determined in the Anti-miRNA-148a group. miRNA-148a, overexpressing group; Anti-miRNA-148a, downregulating group. $^{\#\#}$P<0.01 vs. control group. miRNA, microRNA; Bax, BCL2-associated X.

microRNAs in osteoporosis rats following ovariectomy and it was determined that microRNA-148a expression level was significantly upregulated in the osteoporosis rats following ovariectomy when compared the sham group (Fig. 1).

*Effects of microRNA-148a on bone cell growth in vitro*. The function of microRNA-148a on bone cell growth in osteoblasts *in vitro* was investigated using microRNA-148a and anti-microRNA-148a mimics to upregulate and downregulate microRNA-148a expression in osteoblasts *in vitro* (Fig. 2). Overexpression of microRNA-148a inhibited bone cell growth and induced apoptosis in osteoblasts *in vitro* (Fig. 2C and E). However, downregulation of microRNA-148a promoted bone cell growth and reduced apoptosis in osteoblasts *in vitro* (Fig. 2D and F). Additionally, overexpression of microRNA-148a induced Bax protein expression and caspase-3/9 activity in osteoblasts *in vitro* (Fig. 3A-D). Downregulation of microRNA-148a reduced Bax protein expression and caspase-3/9 activity in osteoblasts *in vitro* (Fig. 3E-H).

*Effects of microRNA-148a on bone cell growth in vitro through PI3K/AKT signaling by ERa*. The present study investigated the effect of microRNA-148a on bone cell growth *in vitro*, and the effect of ovariectomy-induced osteoporosis association with ERa. Bioinformatics and luciferase reporter assays revealed that ERα was a target gene of microRNA-148a (Fig. 4A) and overexpression of microRNA-148a reduced and reduced ERa, PI3K and p-Akt protein expression levels in osteoblasts *in vitro* (Fig. 4B-E). However, the downregulation of microRNA-148a upregulated ERα, PI3K and p-Akt protein expression levels in osteoblasts *in vitro* (Fig. 4F-I).

*Inhibition of ERα increases the effect of microRNA-148a on apoptosis in osteoblasts in vitro*. In order to investigate the underlying effect of microRNA-148a on osteoporosis, the present study used ERα inhibitor, 0.1 nM of AZD9496, to reduce reduced ERα expression in osteoblasts *in vitro*. As presented in Fig. 5A-D, the inhibition of ERα (BHPI, inhibitor used) reduced ERα, PI3K and p-Akt protein expression in osteoblasts *in vitro* following microRNA-148a transfection when compared with the microRNA-148a only group. The inhibition of ERα increased the effect of the microRNA-148a the inhibition of osteoblast growth and activation of apoptosis in osteoblasts *in vitro* when compared with the microRNA-148a only group (Fig. 5E and F). Additionally, the inhibition of ERα increased Bax protein expression and caspase-3/9 activity in osteoblasts *in vitro* (Fig. 6).

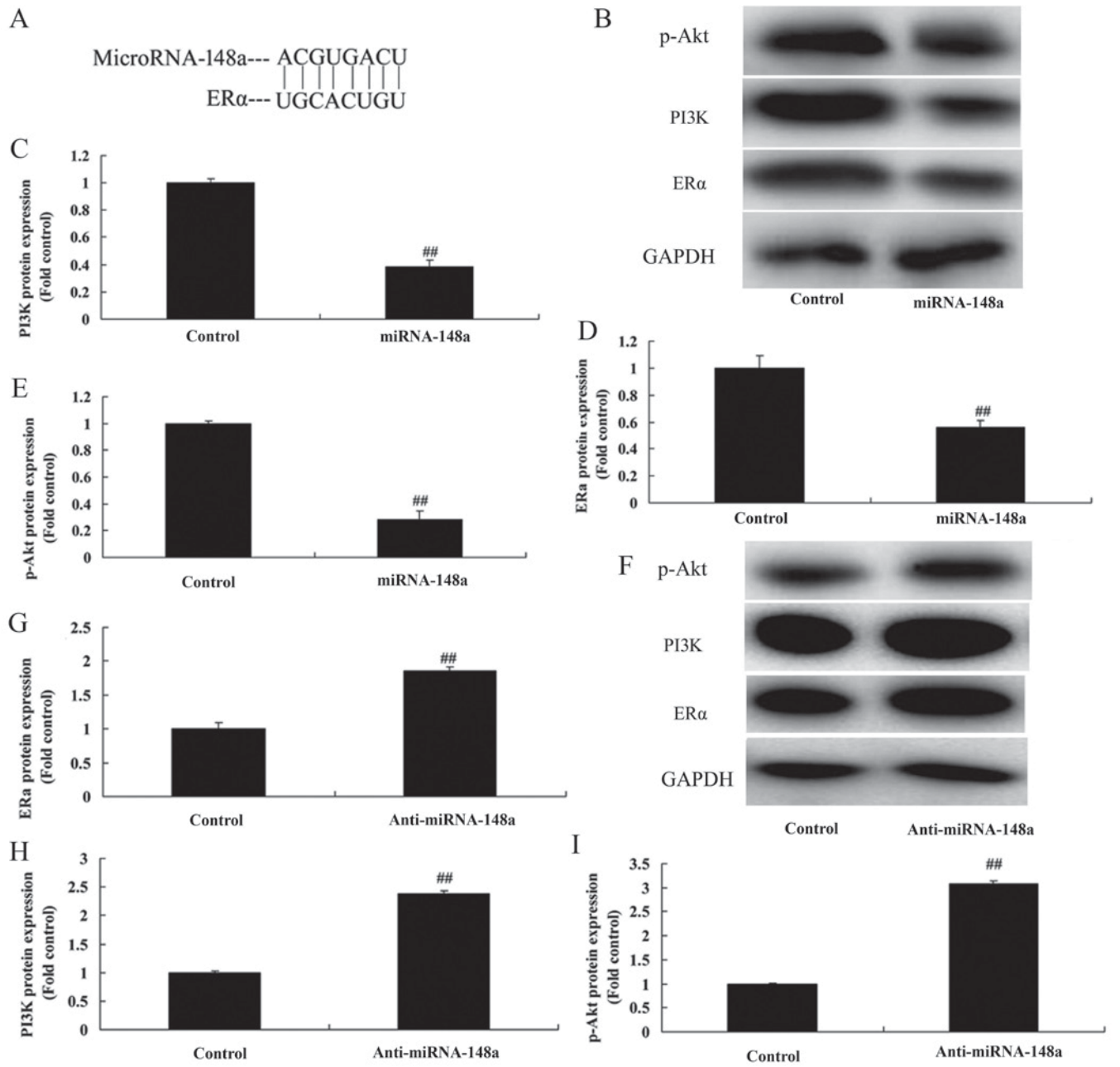


Figure 4. Effect of miRNA-148a expression on osteoblast cell growth *in vitro* PI3K/AKT signaling. (A) Bioinformatics and luciferase reporter assays revealed that ERα may be a target gene of miRNA-148a. (B) Western blotting was used to quantify ERα, PI3K and p-AKT protein expression in the miRNA-148a group. Quantification of (C) PI3K, (D) ERα and (E) p-Akt protein expression levels in the miRNA-148 a group. (F) Western blotting was used to quantify ERα, PI3K and p-AKT protein expression in the miRNA-148a group. Quantification of (G) ERα, (H) PI3K and (I) p-Akt protein expression levels in the anti-miRNA-148 a group. miRNA-148a, overexpressing group; Anti-miRNA-148a, downregulating group. $^{\#\#}$P<0.01 vs. control group. miRNA, microRNA; p, phosphorylated; AKT, protein kinase B; PI3K, phosphoinositide 3-kinase; ERα, estrogen receptor α.

*Inhibition of PI3K reduces the effect of microRNA-148a on osteoblast apoptosis in vitro*. In order to investigate whether the effect of microRNA-148a on osteoblast apoptosis *in vitro* by PI3K/AKT signaling the present study used an PI3K inhibitor to reduce PI3K/AKT signaling in osteoblasts *in vitro*. As presented in Fig. 7A-C, the PI3K inhibitor reduced PI3K/AKT signaling in osteoblasts *in vitro* following microRNA-148a transfection when compared with microRNA-148a only group. Subsequently, the inhibition of PI3K reduced the effect of microRNA-148a on the inhibition of osteoblast proliferation and increased the apoptotic rate in osteoblasts *in vitro* when compared with the microRNA-148a only group (Fig. 7D and E). The inhibition of PI3K also increased Bax protein expression levels and caspase-3, caspase-9 activity in osteoblasts *in vitro* (Fig. 7F-I).

## Discussion

Patients with osteoporosis worldwide have exceeded 200 million, including 80% of patients with postmenopausal osteoporosis and fractures in 50% of postmenopausal women are associated with osteoporosis (7). In addition, a previous study revealed the that annual bone loss rate of women after 5-10 years of menopause is 2-4% and which is evidently higher

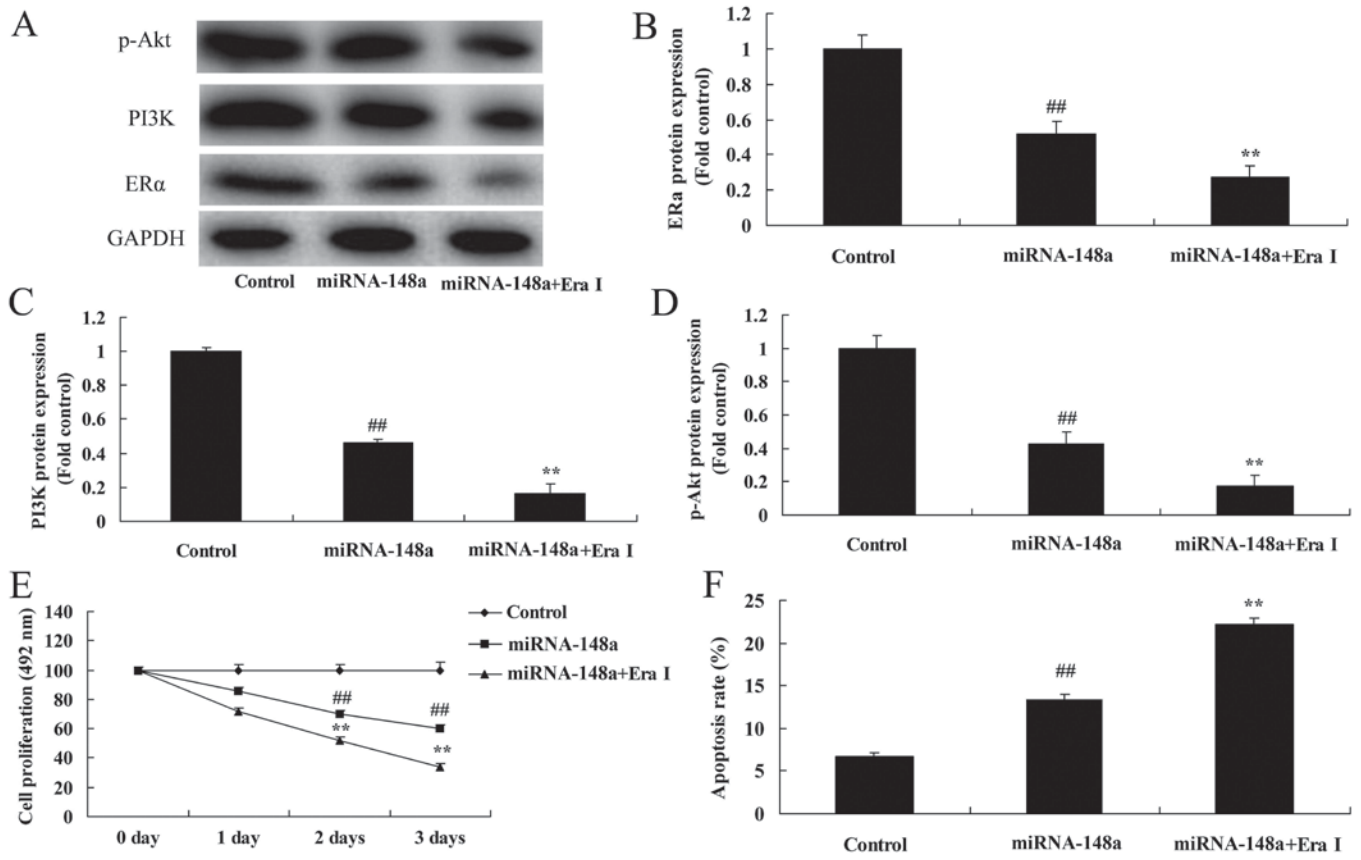


Figure 5. Inhibition of ERα increased the pro-apoptotic effect of miRNA-148a on osteoblasts *in vitro*. (A) Western blotting analysis was used to determine (B) ERα, (C) PI3K and (D) p-AKT protein expression levels and (E) cell proliferation and (F) apoptosis. miRNA-148a, overexpressing group; Anti-miRNA-148a, downregulating group. $^{\#\#}$P<0.01 vs. control group. $^{**}$P<0.01 vs. miRNA-148a group. miRNA, microRNA.

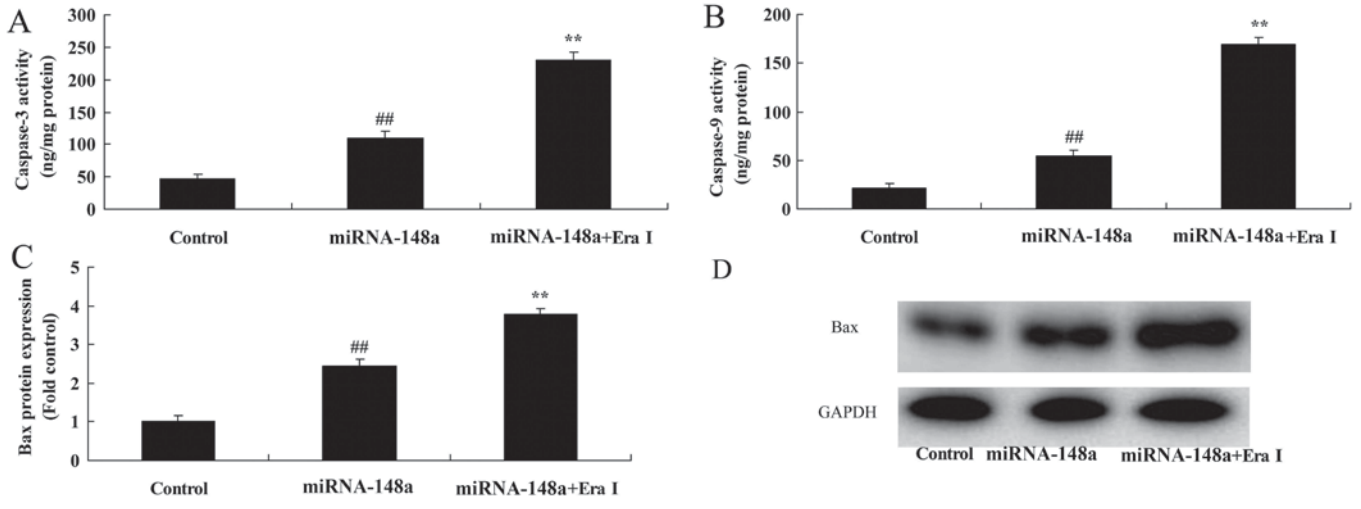


Figure 6. Inhibition of ERα increased the effect of microRNA-148a on caspase-3 and -9 activity and Bax protein expression on osteoblasts *in vitro*. (A) Caspase-3 and (B) caspase-9 activity and (C) quantification of Bax protein expression using (D) western blotting. miRNA-148a, overexpressing group; miRNA-148a+ERα I, overexpressing and ERα inhibitor group. $^{\#\#}$P<0.01 vs. control group. $^{**}$P<0.01 vs. miRNA-148a group. miRNA, microRNA; p, phosphorylated; AKT, protein kinase B; PI3K, phosphoinositide 3-kinase; ERα, estrogen receptor α.

than 1% estimated in men (6). OIO is a common disease which frequently occurs in the elderly. The commonest one is postmenopausal osteoporosis (16). As the global population is aging the morbidity of osteoporosis is growing at an alarming rate. Therefore, prevention of osteoporosis may become an important research topic in the future (17). To the best of our knowledge, the present study is the first to demonstrate that microRNA-148a expression was upregulated in osteoporosis rats following ovariectomy.

Thousands of miRNAs have been previously discovered in various organisms with increasingly intensive research on miRNA (12). miRNAs regulate various activities in

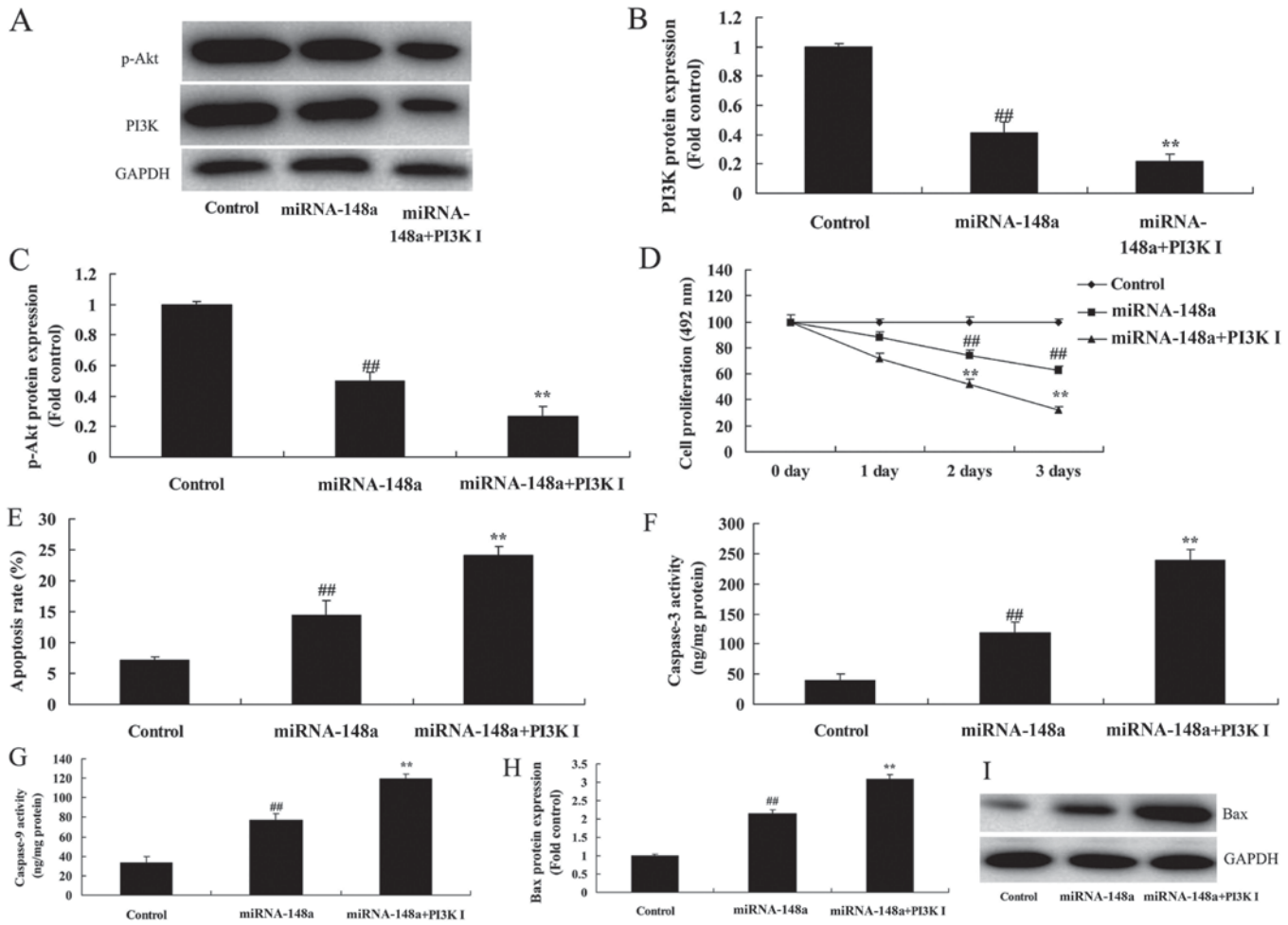


Figure 7. Inhibition of PI3K reduced the effect of miRNA-148a on osteoblast apoptosis *in vitro*. (A) Western blotting was used to determine (B) PI3K and (C) p-AKT protein expression levels. (D) Cell proliferation and (E) apoptosis were also quantified. (F) Caspase-3 and (G) caspase-9 activity was determined. (H) Bax protein expression was determined using (I) western blotting analysis. miRNA-148a, overexpressing group; mIiR-148a + PI3K I, overexpressing and PI3K inhibitor group. $^{\#\#}$P<0.01 vs. control group. $^{**}$P<0.01 vs. miRNA-148a group. miRNA, microRNA; p, phosphorylated; AKT, protein kinase B; PI3K, phosphoinositide 3-kinase.

organisms, such as growth, development and aging (18). It has been estimated that ~50% human genes are regulated by miRNAs. This is currently verified by using bioinformatics and experiments where one miRNA may specifically bind to multiple genes (18). Additionally, one gene may also be regulated by multiple miRNAs, thus exerting the regulatory function (19). The presents study determined that overexpression of microRNA-148a inhibited osteoblast proliferation and induced apoptosis of osteoblasts *in vitro*.

Although instruments and methods used for detecting the degree of osteoporosis quantitatively domestically and overseas are constantly improving constantly, there is a lack of effective measures in terms of prevention, additionally drug treatments are very limited (20). Currently, ERα remains to be the primary target for the prevention of bone loss in postmenopausal women (21). A previous study revealed that ERa may prevent the occurrence of postmenopausal osteoporosis (22). The present *in vitro* study revealed that overexpression of microRNA-148a reduced ERα, PI3K and p-AKT protein expression levels in osteoblasts *in vitro*. Ma *et al* previously reported that microRNA-148a suppresses estrogen induced viability and migration through ERα expression in breast cancer cells (23). Zhang *et al* suggested that microRNA-148a promotes cancer cell growth by targeting PI3K/Akt protein expression in osteosarcoma (24).

PI3K in the signaling pathway required for osteoblast differentiation. PI3K stimulates mesenchymal stem cells (MSCs) to differentiate into the osteoblasts and promote bone formation (25). In MSCs, activating PI3K/AKT signal pathway may reduce bone morphogenetic protein-induced alkaline phosphatase (ALP) and osteopontin expression, in order to impact osteoprogenitor cells and reduce osteoblasts (10). PI3K kinase-specific inhibitor prevents the PI3K signaling pathway from excitation. A previous study used ALP staining to observe changes of preosteoblastic cell MC3T3E1 differentiation and revealed that blocking the excitation of the signal pathway may limit ALP activity. Based on the findings of the current study it is possible that the inhibition of ERα or PI3K may significantly increase the effect of microRNA-148a on apoptosis of osteoblasts *in vitro*.

In conclusion, the present study demonstrated that microRNA-148a significantly increased apoptosis in ovariectomized rats via PI3K/AKT signaling. Therefore, microRNA-148a/PI3K/AKT signaling pathway is a promising candidate for the

development of future therapeutic agents against osteoporosis in postmenopausal women.

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**Availability of data and materials**

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

**Authors' contributions**

YX designed the experiment, analyzed the data and wrote the manuscript. YX, BL and JL performed the experiment.

**Ethics approval and consent to participate**

The experimental protocol was approved by the Animal Care and Use Review Committee of Tianjin Hospital (Tianjin, China).

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Krantz E, Trimpou P and Landin-Wilhelmsen K: Effect of growth hormone treatment on fractures and quality of life in postmenopausal osteoporosis: A 10-year follow-up study. J Clin Endocrinol Metab 100: 3251-3259, 2015.
2. Hansen KE, Nabak AC, Johnson RE, Marvdashti S, Keuler NS, Shafer MM and Abrams SA: Isotope concentrations from 24-h urine and 3-h serum samples can be used to measure intestinal magnesium absorption in postmenopausal women. J Nutr 144: 533-537, 2014.
3. Sugimoto T, Nakamura T, Nakamura Y, Isogai Y and Shiraki M: Profile of changes in bone turnover markers during once-weekly teriparatide administration for 24 weeks in postmenopausal women with osteoporosis. Osteoporos Int 25: 1173-1180, 2014.
4. Bonnick S, De Villiers T, Odio A, Palacios S, Chapurlat R, DaSilva C, Scott BB, Le Bailly De Tilleghem C, Leung AT and Gurner D: Effects of odanacatib on BMD and safety in the treatment of osteoporosis in postmenopausal women previously treated with alendronate: A randomized placebo-controlled trial. J Clin Endocrinol Metab 98: 4727-4735, 2013.
5. Liu HF, He HC, Yang L, Yang ZY, Yao K, Wu YC, Yang XB and He CQ: Pulsed electromagnetic fields for postmenopausal osteoporosis and concomitant lumbar osteoarthritis in southwest China using proximal femur bone mineral density as the primary endpoint: Study protocol for a randomized controlled trial. Trials 16: 265, 2015.
6. Pérez Ruixo JJ, Zheng J and Mandema JW: Similar relationship between the time course of bone mineral density improvement and vertebral fracture risk reduction with denosumab treatment in postmenopausal osteoporosis and prostate cancer patients on androgen deprivation therapy. J Clin Pharmacol 54: 503-512, 2014.
7. Stuss M, Rieske P, Cegłowska A, Stêpień-Kłos W, Liberski PP, Brzeziańska E and Sewerynek E: Assessment of OPG/RANK/RANKL gene expression levels in peripheral blood mononuclear cells (PBMC) after treatment with strontium ranelate and ibandronate in patients with postmenopausal osteoporosis. J Clin Endocrinol Metab 98: E1007-E1011, 2013.
8. Bai P, Sun Y, Jin J, Hou J, Li R, Zhang Q and Wang Y: Disturbance of the OPG/RANK/RANKL pathway and systemic inflammation in COPD patients with emphysema and osteoporosis. Respir Res 12: 157, 2011.
9. Baek JM, Kim JY, Yoon KH, Oh J and Lee MS: Ebselen is a potential anti-osteoporosis agent by suppressing receptor activator of nuclear factor kappa-B ligand-induced osteoclast differentiation in vitro and lipopolysaccharide-induced inflammatory bone destruction in vivo. Int J Biol Sci 12: 478-488, 2016.
10. Li H, Li T, Fan J, Li T, Fan L, Wang S, Weng X, Han Q and Zhao RC: miR-216a rescues dexamethasone suppression of osteogenesis, promotes osteoblast differentiation and enhances bone formation, by regulating c-Cbl-mediated PI3K/AKT pathway. Cell Death Differ 22: 1935-1945, 2015.
11. Yuan FL, Xu RS, Jiang DL, He XL, Su Q, Jin C and Li X: Leonurine hydrochloride inhibits osteoclastogenesis and prevents osteoporosis associated with estrogen deficiency by inhibiting the NF-κB and PI3K/Akt signaling pathways. Bone 75: 128-137, 2015.
12. Hao L, Fu J, Tian Y and Wu J: Systematic analysis of lncRNAs, miRNAs and mRNAs for the identification of biomarkers for osteoporosis in the mandible of ovariectomized mice. Int J Mol Med 40: 689-702, 2017.
13. Chen J, Qiu M, Dou C, Cao Z and Dong S: MicroRNAs in bone balance and osteoporosis. Drug Dev Res 76: 235-245, 2015.
14. Li H, Wang Z, Fu Q and Zhang J: Plasma miRNA levels correlate with sensitivity to bone mineral density in postmenopausal osteoporosis patients. Biomarkers 19: 553-556, 2014.
15. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
16. Brixen K, Chapurlat R, Cheung AM, Keaveny TM, Fuerst T, Engelke K, Recker R, Dardzinski B, Verbruggen N, Ather S, *et al*: Bone density, turnover, and estimated strength in postmenopausal women treated with odanacatib: A randomized trial. J Clin Endocrinol Metab 98: 571-580, 2013.
17. Binkley N, Bolognese M, Sidorowicz-Bialynicka A, Vally T, Trout R, Miller C, Buben CE, Gilligan JP and Krause DS; Oral Calcitonin in Postmenopausal Osteoporosis (ORACAL) Investigators: A phase 3 trial of the efficacy and safety of oral recombinant calcitonin: The Oral Calcitonin in Postmenopausal Osteoporosis (ORACAL) trial. J Bone Miner Res 27: 1821-1829, 2012.
18. Ma Y, Shan Z, Ma J, Wang Q, Chu J, Xu P, Qin A and Fan S: Validation of downregulated microRNAs during osteoclast formation and osteoporosis progression. Mol Med Rep 13: 2273-2280, 2016.
19. Jiang ZS and Hao ZH: An insertion/deletion polymorphism within the 3'-untranslated region of COL1A2 confers susceptibility to osteoporosis. Mol Med Rep 14: 4415-4421, 2016.
20. Zhang D, Maalouf NM, Adams-Huet B, Moe OW and Sakhaee K: Effects of sex and postmenopausal estrogen use on serum phosphorus levels: A cross-sectional study of the National Health and Nutrition Examination Survey (NHANES) 2003-2006. Am J Kidney Dis 63: 198-205, 2014.
21. Martynetz FA, Pessole Biondo-Simões Mde L, Martynetz JR, Martynetz TD, Zimerman E and Neto HM: Tomografic and tensiometric assessment on femurs from oophorectomized rats subjected to hormone replacement therapy. Rev Bras Ortop 45: 40-45, 2015.
22. Sanders S and Geraci SA: Osteoporosis in postmenopausal women: Considerations in prevention and treatment: (women's health series). South Med J 106: 698-706, 2013.
23. Ma F, Feng Y, Li W, Li Z, Liu T and Li L: miR-148a suppresses estrogen-induced viability and migration of breast cancer cells via inhibition of estrogen receptor α expression. Exp Ther Med 13: 2515-2522, 2017.
24. Zhang H, Wang Y, Xu T, Li C, Wu J, He Q, Wang G, Ding C, Liu K, Tang H and Ji F: Increased expression of microRNA-148a in osteosarcoma promotes cancer cell growth by targeting PTEN. Oncol Lett 12: 3208-3214, 2016.
25. Ge P, Cui Y, Liu F, Luan J, Zhou X and Han J: L-carnitine affects osteoblast differentiation in NIH3T3 fibroblasts by the IGF-1/PI3K/Akt signalling pathway. Biosci Trends 9: 42-48, 2015.