Original Article
Deletion of p16 prevents estrogen deficiency-induced osteoporosis by inhibiting oxidative stress and osteocyte senescence

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Abstract: To investigate whether p16 deletion can prevent osteoporosis caused by estrogen deficiency, we first confirmed that p16 protein expression levels were significantly up-regulated in bony tissue of ovariectomized (OVX) wild-type mice. Eight-week-old wild-type and p16$^{-/-}$ mice were then sham-operated or bilateral OVX. After 12 weeks, the bone phenotypes of all models were analyzed by radiography, micro-computed tomography, histology, immuno-histochemistry, and molecular biology. The results showed that p16 deficiency could rescue OVX-induced osteoporosis by significantly increased bone mineral density, trabecular bone volume, total collagen positive area, osteoblast number, type I collagen positive area, fibroblast colony-forming unit (CFU-f) and alkaline phosphatase-positive CFU-f with up-regulation of the mRNA expression levels of *Alp, Runx2, type I collagen* and *osteocalcin*, and significantly reduced osteoclast surface and the ratio of *RANKL/OPG* mRNA expression level. Furthermore, we also demonstrated that p16 deletion inhibited OVX-induced oxidative stress and bone cell senescence, such as a significant decrease in reactive oxygen species levels, up-regulation of superoxide dismutase 1 and 2 protein expression levels, and reduction of the percentage of β-galactosidase-positive osteocytes and p21 protein expression levels in bony tissue. Our results indicate that p16 deletion can prevent estrogen deficiency-induced osteoporosis by inhibiting oxidative stress, osteocyte senescence and osteoclastic bone resorption, stimulating osteogenesis and osteoblastic bone formation. Therefore, this study provides new insights into the potential of p16 as a novel therapeutic target for estrogen deficiency-induced osteoporosis.

Keywords: Estrogen deficiency, osteoporosis, p16, oxidative stress, osteocyte senescence

Introduction

Osteoporosis is characterized by low bone mass, microarchitectural disruption, and skeletal fragility, resulting in decreased bone strength and an increased risk of fracture [1]. Osteoporosis has become a global health problem and research hotspot of many countries all over the world in the past decade. The bone loss triggered by declining serum sex steroids in postmenopausal humans has been extensively investigated [2]. As the most common type and with the highest incidence rate, postmenopausal osteoporosis leading bone loss resulted mainly from stimulation of bone resorption via increased osteoclast formation or increased osteoclast lifespan coupled with insufficient osteoblastic bone formation [3]. Currently, clinical treatment of postmenopausal osteoporosis mainly includes hormone replacement therapy, calcium, vitamin D, bisphosphonate, selective estrogen receptor modulator, and parathyroid hormone [4]. However, the therapeutic effects are not ideal. Exploring the mechanism of postmenopausal osteoporosis is a primary task for scientific researchers so that it can develop drugs that prevent and treat postmenopausal osteoporosis.

Accumulating studies have shown that the oxidative stress induced by reactive oxygen species (ROS) is increased with aging, which is implicated in the pathophysiology of post-menopausal osteoporosis [5-10]. Studies have shown that estrogen protects bone by acting as an antioxidant and resists oxidative stress [11]. Besides, studies have suggested that the transition from "estrogen-centered" pathogenesis to oxidative stress is also involved in the mechanism of osteoporosis [2]. Oxidative stress is an important cause of cellular senescence. Cellular senescence is the process by which a cell enters a permanent cell cycle block, and senescent cells display a senescence-associated secretory phenotype (SASP) [12]. Our recent study has demonstrated that estrogen deficiency-induced bone loss was not only associated with increased oxidative stress and osteoclastic bone resorption but also associated with increased osteocyte senescence, as shown in increased the percentages of p16 positive osteocytes and p16 protein expression levels in bony tissue [13].

P16 is one of the products of the *INK4/ARF* locus and was molecularly cloned by virtue of its interaction with cyclin-dependent kinase 4 (CDK4). It was soon realized that p16 controls the G1 phase of the cell cycle, is a key regulator of cell senescence, and is frequently inactivated in cancer and also is regarded as related to many age-related pathologies, including myocardial infarction, type 2 diabetes, and osteoporosis [14]. The cell cycle-dependent kinase inhibitor p16 is not only a recognized indicator of cellular senescence, but it also acts as a critical effector of cellular senescence [15]. During the development of physiological aging and aging-related diseases, the expression level of p16 is gradually increased [16]. Recent studies have shown that p16-positive cells in different tissues contribute to the development and progression of aging-related lesions, resulting in a shortened healthy life span, while the elimination of p16-positive senescent cells can delay the development and progression of senescence-related lesions in different tissues. Deletion of p16-positive senescent cells not only prolongs the lifespan of premature aging mice but also extends the lifespan of natural aging mice [17, 18]. However, it remains unknown whether p16 deletion can rescue estrogen deficiency-induced osteoporosis by

inhibiting oxidative stress and osteocyte senescence, suppressing osteoclastic bone resorption, stimulating osteogenesis of bone marrow mesenchymal stem cells (BM-MSCs) and osteoblastic bone formation.

In this study, we confirmed that p16 protein expression level was upregulated significantly in wild-type ovariectomized (WT-OVX) mice compared with WT-sham mice. Then 8-week-old WT and p16$^{-/-}$ mice were sham-operated or bilaterally ovariectomized (OVX). After 12 weeks, bone phenotypes of all models were analyzed by radiography, micro-computed tomography (micro-CT), histology immunohistochemistry, and molecular biology to determine whether deletion of p16 could rescue estrogen deficiency-induced osteoporosis by inhibiting oxidative stress and osteocyte senescence.

Materials and methods

Mice and genotyping

Male and female p16$^{+/-}$ mice of the FVB N2 background were mated to produce offspring heterozygous at both loci, which were then mated to generate p16$^{-/-}$ and wild-type (WT) pups. Their genotypes were analyzed, as described previously [19]. All animal experiments were carried out in compliance with, and approval by approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

Ovariectomy

Eight-week-old wild-type and p16$^{-/-}$ mice were sham-operated or bilaterally ovariectomized (OVX) according to previously established methods [20]. After 12 weeks, the success of ovariectomy was confirmed by X-ray images of the coccygeal vertebra that were taken with a Faxitron and shown significantly lower bone mineral density (data not shown).

Radiographs and micro-computed tomography

The vertebral bodies were removed, and all soft tissues were dissected for contact radiography or micro-computed tomography (micro-CT) as described [21]. The measurements of bone mineral density (BMD), trabecular volume relative to tissue volume were measured as previously described [22].

Histology

All animals were sacrificed by cervical dislocation after inhaled anesthesia with ether. Vertebrae were removed and dissected free of soft tissue, fixed with 4% paraformaldehyde, decalcified with EDTA glycerol solution, and embedded in paraffin. Sections were stained with H&E or histochemically for total collagen or tartrate-resistant acid phosphatase (TRAP) as previously described [21].

Immunohistochemical staining

Immunohistochemical staining for type I collagen and β-galactosidase (β-gal) was performed using the avidin-biotin-peroxidase complex technique with affinity-purified rabbit anti-mouse β-gal (Santa Cruz, CA, USA) and p21 (Santa 192 Cruz, CA, USA) as described previously [13].

Fibroblast colony-forming unit assay

Bone marrow cells were flushed out from femurs and tibias. For fibroblast colony-forming unit (CFU-f) and alkaline phosphatase (ALP)-expressing CFU-f assays, total bone marrow cells were cultured in six-well-plates at 1×10^6 cells/well in 2 mL of a modified essential medium (α-MEM) containing 10% fetal calf serum (FBS) (Hyclone Laboratories, Logan, UT, USA), 50 mg/mL ascorbic acid and 10 mM β-glycerophosphate for 10 days. At the end of the culture period, cells were washed with PBS, fixed with PLP fixative, and stained with methyl blue or cytochemical for ALP. Following staining, the quantitation of total colony (CFU-f) area and of ALP-positive (CFU-fap) area was determined using computer-assisted image analysis.

Quantitative real-time RT-PCR

Total RNA was isolated from mouse bone tissue or bone marrow mesenchymal stem cells (BM-MSCs) with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Real-time RT-PCR was performed as described previously [13].

Western blot analysis

For examination of protein expression level, proteins were extracted from the vertebral bodies, quantitated with a protein assay kit (Bio-Rad, Mississauga, Ontario, Canada). Protein samples (15 μg) were fractionated by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blotted with primary antibodies against SOD1 (Abcam), SOD2 (Novus Biological), p16 (Santa Cruz, MA), p21 (Santa Cruz, MA) and β-actin (Bioworld Technology, St. Louis Park, MN, USA) were used as a loading control. Immunoblotting was carried out, as described previously [23].

Reactive oxygen species (ROS) examination

Bone marrow cells were filtered and incubated with 5 mM diacetyldichlorofluorescein (DCFDA) (Invitrogen) and 10% FBS for 30 mins at 37°C. Then cells were analyzed with a FACS Calibur flow cytometer (Becton Dickinson, Heidelberg, Germany) for the mean fluorescent intensity (MFI) and relative fluorescent intensity to MFI of shame-WT mice.

Statistical analysis

All the described data represent at least three separate experiments (n>5/group) and are expressed as the Mean ± SEM. Comparisons between the two groups were analyzed using a two-tailed unpaired Student's *t*-test. Comparisons between more than two groups were performed using one-way ANOVA followed by Bonferroni post-hoc multiple comparisons. The significant level was P<0.05.

Results

Effect of p16 deletion on OVX-induced bone loss

Our recent study showed that OVX induced the upregulation of p16 expression levels in wild-type (WT) mice. In this study, we confirmed that the protein expression levels were upregulated dramatically in WT-OVX mice relative to WT-sham mice (**Figure 1A** and **1B**). P16^{-/-} mice and their WT littermates were surgically OVX or sham-operated to determine whether p16 deletion could rescue OVX-induced bone loss. After 12 weeks, bone phenotypes of all models were analyzed by radiography, micro-CT, and total collagen staining. The results showed that bone mineral density, trabecular bone volume, and total collagen positive area were decreased significantly in WT-OVX mice and increased
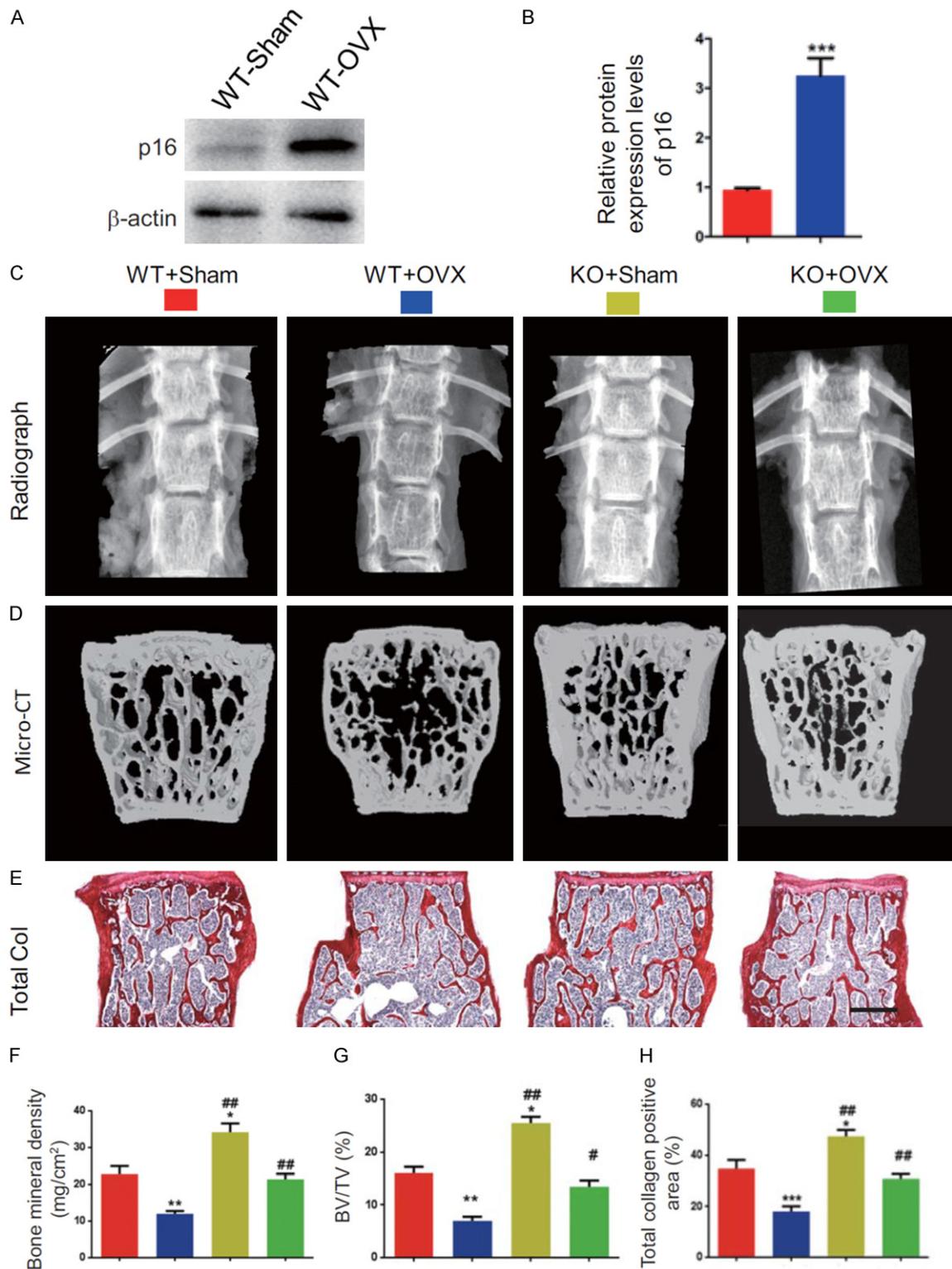
Figure 1. p16 deletion rescues OVX-induced bone loss. (A) The protein expression levels of p16 were detected in bony tissue from WT-Sham and WT-OVX mice by Western blotting. β-actin was used as the loading control. (B) p16 protein levels were assessed by densitometric analysis calculated as a ratio relative to β-actin protein levels and expressed relative to levels of WT-Sham mice. (C) The representative radiographs and (D) micro-CT images of three-dimensional reconstructed images of the vertebral bodies from WT-Sham, WT-OVX, p16 knockout sham (KO-Sham) and p16 knockout OVX (KO-OVX) mice. (E) Representative micrographs of paraffin-embedded sections of vertebral

bodies stained histochemically for total collagen. Scale bars represent 400 µm. (F) Bone mineral density. (G) Trabecular bone volume/total volume (BV/TV). (H) Total collagen positive areas. Data represented as mean ± SEM of determinations in 5 mice of each group. *: P<0.05; **: P<0.01; ***: P<0.001, compared with WT-Sham mice. #: P<0.05 and ##: P<0.01, compared with the same operational WT mice.
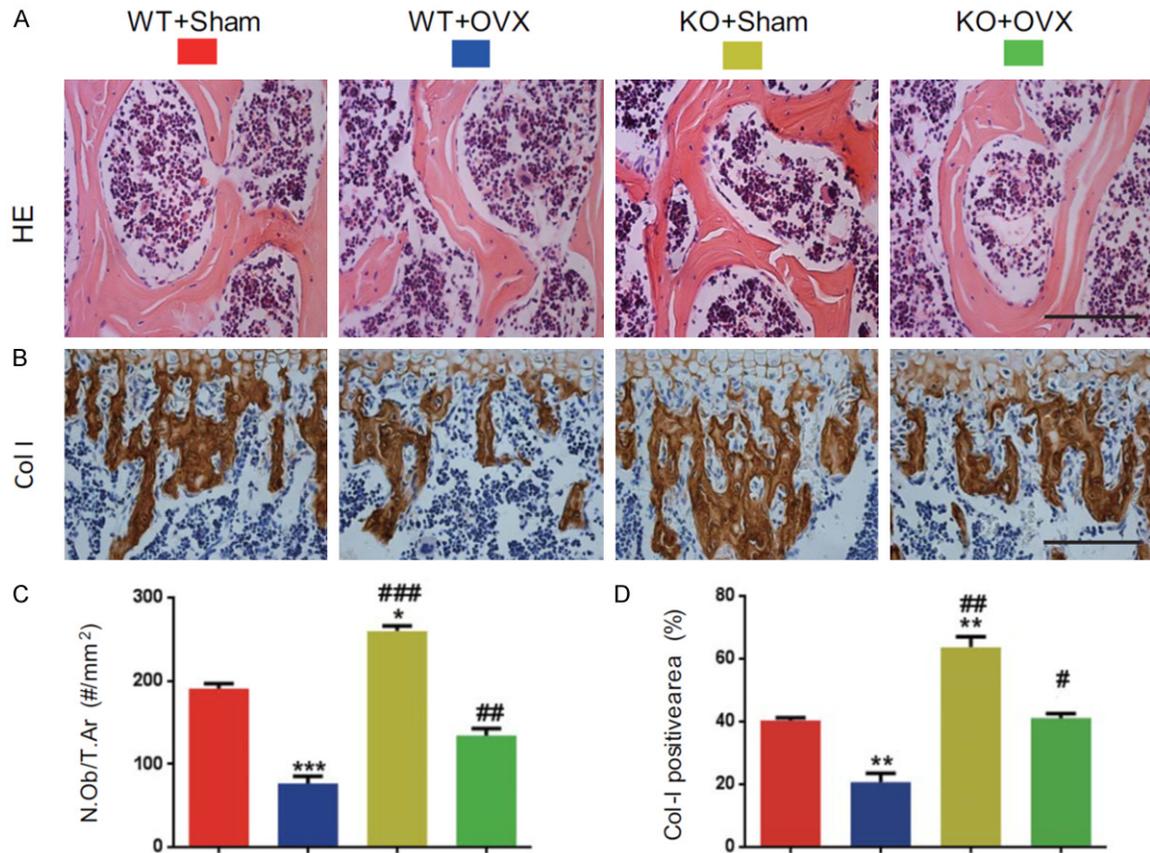
Figure 2. p16 deletion increased osteoblastic bone formation in OVX mice. (A) Representative micrographs of paraffin-embedded sections of vertebral bodies stained with H&E and (B) immunostaining for type I. Scale bars represent 50 µm in (A and B). (C) Osteoblast number relative to tissue area (N.Ob/T. Ar, #/mm^2). (D) Type I collagen (Col-I) positive areas were measured by computer-assisted image analysis. Data represented as mean ± SEM of determinations in 5 mice of each group. **: P<0.01; P<0.05; ***: P<0.001, compared with WT-Sham mice. ##: P<0.01 and ###: P<0.001, compared with the same operational WT mice.

substantially in p16$^{-/-}$-sham (KO-sham) mice. However, they were normalized in p16$^{-/-}$-OVX (KO-OVX) mice compared with WT-sham mice (**Figure 1C-H**). These parameters were markedly increased in KO-sham or KO-OVX mice compared with WT-sham or WT-OVX mice (**Figure 1C-H**). These results demonstrated that p16 deletion could rescue OVX-induced bone loss.

Effect of p16 deletion on osteoblastic bone formation in OVX mice

To determine whether rescued OVX-induced bone loss by p16 deletion is associated with increased osteoblastic bone formation, osteoblast number and collagen deposition in the bone matrix were examined by histology, immunohistochemical staining for type I collagen and computer-assisted image analyses. The results showed that osteoblast number and type I collagen positive area were decreased significantly in WT-OVX mice and increased considerably in KO-sham mice, however, they were normalized in KO-OVX mice compared with WT-sham mice (**Figure 2A-D**). These parameters were increased significantly in KO-sham or KO-OVX mice compared with WT-sham or WT-OVX mice (**Figure 2A-D**). These

results implied that p16 deletion could rescue OVX-induced bone loss through increasing osteoblastic bone formation.

Effect of p16 deletion on osteogenesis of bone marrow mesenchymal stem cells in OVX mice

To determine whether rescued OVX-induced bone loss by p16 deletion is associated with enhanced osteogenesis of bone marrow mesenchymal stem cells (BM-MSCs), CFU-f assays were performed by ex vivo bone marrow cell cultures. Results revealed that CFU-f and CFU-fap areas were decreased significantly in WT-OVX mice and increased significantly in KO-sham mice; however, they were normalized in KO-OVX mice compared with WT-sham mice (**Figure 3A-D**). These parameters were increased considerably in KO-sham or KO-OVX mice compared with WT-sham or WT-OVX mice (**Figure 3A-D**). We also examined the effect of p16 deletion on osteogenic gene expression using real-time RT-PCR. Results showed that mRNA expression levels of *Alp, Runx2, type I collagen,* and *osteocalcin* were down-regulated significantly in WT-OVX mice and upregulated significantly in KO-sham mice. However, they were normalized in KO-OVX mice compared with WT-sham mice (**Figure 3E-H**). They were markedly upregulated in KO-sham or KO-OVX mice compared with WT-sham or WT-OVX mice (**Figure 3E-H**). These results suggest that p16 deletion could rescue OVX-induced osteogenic defect through stimulating osteogenesis of BM-MSCs.

Effect of p16 deletion on osteoclastic bone resorption in OVX mice

To determine whether rescued OVX-induced bone loss by p16 deletion is associated with decreased osteoclastic bone resorption, osteoclast surface and the ratio of *RANKL/OPG* mRNA expression levels were examined using histochemical staining for TRAP and real-time RT-PCR. Results showed that osteoclast surface and the ratio of *RANKL/OPG* mRNA expression levels were increased dramatically in WT-OVX mice and reduced significantly in KO-sham mice, however, they were normalized in KO-OVX mice compared with WT-sham mice (**Figure 4A-C**). These parameters were decreased significantly in KO-sham or KO-OVX mice compared with WT-sham or WT-OVX mice (**Figure 4A-C**). These results implied that p16

deletion could rescue OVX-induced bone loss through inhibiting osteoclastic bone resorption.

Effect of p16 deletion on redox balance in OVX mice

To determine whether the estrogen deficiency-induced osteoporosis rescued by p16 deletion is associated with reduced oxidative stress induced by OVX, we examined the alterations of ROS levels and expression levels of antioxidant enzymes in bony tissue. Results showed that ROS levels increased dramatically in WT-OVX mice and reduced significantly in KO-sham mice, however, they were normalized in KO-OVX mice compared with WT-sham mice and were decreased significantly in KO-sham or KO-OVX mice compared with WT-sham or WT-OVX mice (**Figure 5A**, **5B**). The protein expression levels of superoxide dismutase (SOD) 1 and 2 were down-regulated significantly in WT-OVX mice and upregulated clearly in KO-sham mice, however, they were normalized in KO-OVX mice compared with WT-sham mice (**Figure 5C-E**). The protein expression levels of SOD1 and SOD2 were upregulated significantly in KO-sham or KO-OVX mice compared with WT-sham or WT-OVX mice (**Figure 5C-E**). These results demonstrated that p16 deletion could inhibit oxidative stress in OVX mice by inhibiting the expression levels of antioxidant enzymes.

Effect of p16 deletion on osteocyte senescence in OVX mice

To determine whether the estrogen deficiency-induced osteoporosis rescued by p16 deletion is associated with reduced osteocyte senescence induced by OVX, we examined the alterations of osteocyte senescence by immunostaining for β-galactosidase (β-gal) and Western blot for p21 expression. Results showed that the percentages of β-gal positive osteocytes and p21 protein expression levels in bony tissue were increased dramatically in WT-OVX mice and reduced significantly in KO-sham mice, however, they were normalized in KO-OVX mice compared with WT-sham mice. They were decreased significantly in KO-sham or KO-OVX mice compared with WT-sham or WT-OVX mice (**Figure 6A-D**). These results demonstrated that p16 deletion could inhibit osteocyte senescence induced by OVX.

Deletion of p16 prevents estrogen deficiency-induced osteoporosis
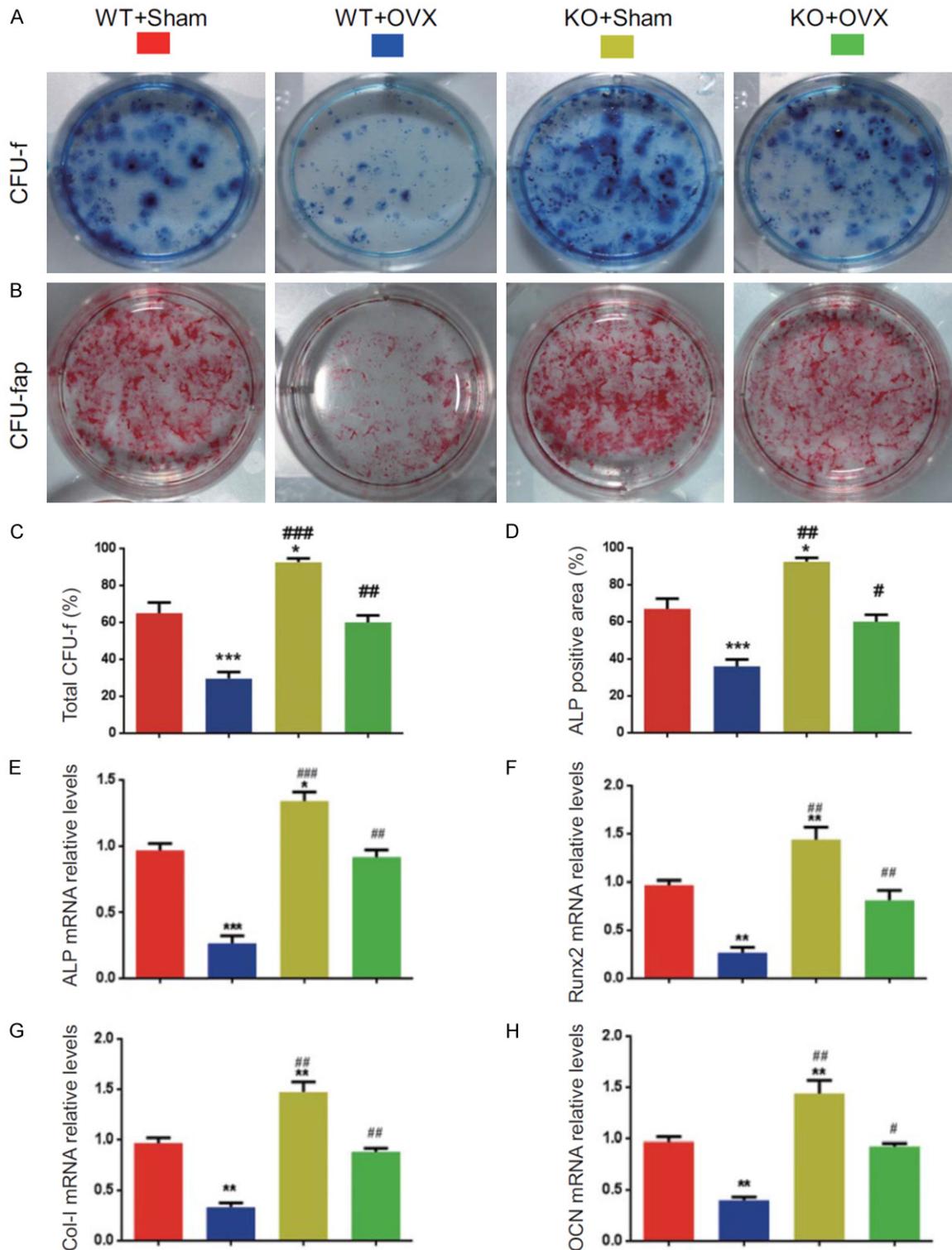
Figure 3. p16 deletion stimulated osteogenesis of BM-MSCs in OVX mice. Primary bone marrow cells from 6-month-old WT-Sham, WT-OVX, KO-Sham, and KO-OVX mice were cultured ex vivo in osteogenic differentiation medium for 18 days, and resulting cultures were stained with (A) methylene blue for the total number of colonies (CFU-F) and (B) cytochemically for ALP to show CFU-Fap. (C) Total CFU-f-positive areas and (D) ALP-positive areas relative to the culture dish area. Real-time RT-PCR analysis of bone marrow MSC extracts for the expression of (E) *Alp*, (F) *Runx2*, (G) *Col-I,* and (H) *osteocalcin* (*OCN*). Messenger RNA expression assessed by real-time RT-PCR is expressed as a ratio to Gapdh expression level and expressed relative to WT-Sham mice. Data represented as mean ± SEM of determinations in 5 mice of each group. *: P<0.05; **: P<0.01; ***: P<0.001, compared with WT-Sham mice. #: P<0.05; ##: P<0.01 and ###: P<0.001, compared with the same operational WT mice.
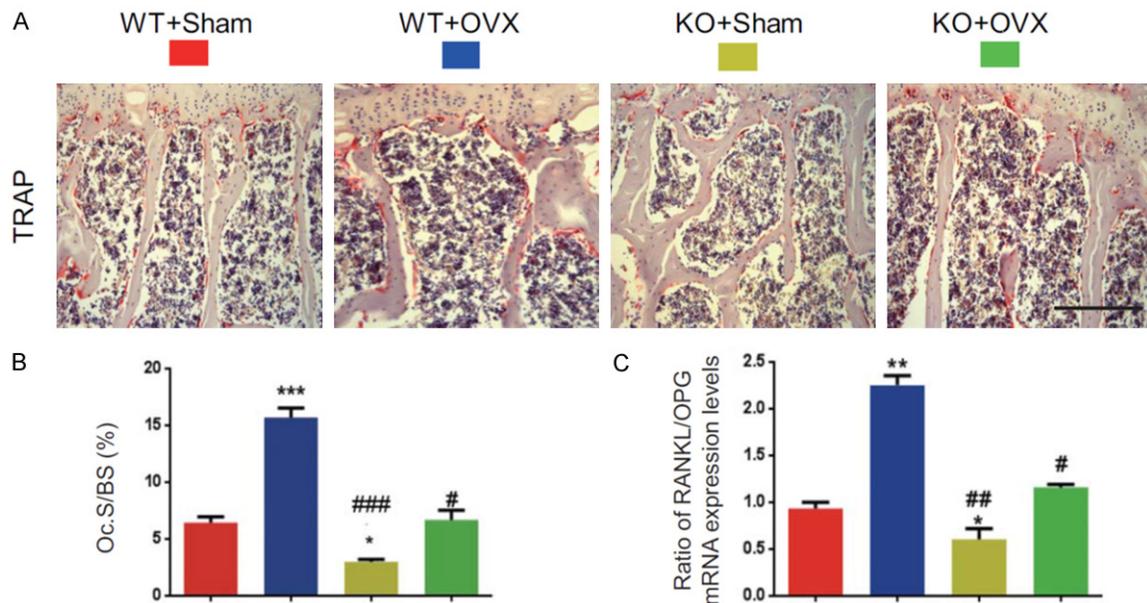
Figure 4. p16 deletion inhibited osteoclastic bone resorption in OVX mice. (A) Representative micrographs of paraffin-embedded sections of vertebral bodies stained histochemically for TRAP. Scale bars represent 50 μm in (A). (B) Osteoclast surface/bone surface (Oc.S/BS, %). (C) Real-time RT-PCR analysis of vertebral extracts for the expression of *NANKL* and *OPG* and expressed the ratio of *NANKL/OPG* mRNA expression levels. Data represented as mean ± SEM of determinations in 5 mice of each group. *: P<0.05; **: P<0.01; ***: P<0.001, compared with WT-Sham mice. #: P<0.05; ##: P<0.01 and ###: P<0.001, compared with the same operational WT mice.

Discussion

In the present study, we employed OVX mouse model, which is a well-established and widely used animal model in the study of postmenopausal osteoporosis [24-26] to assess the effect of p16 deletion on the estrogen deficiency-induced osteoporosis. We first confirmed that OVX resulted in bone loss with reduced BMD, trabecular bone volume, and total collagen positive areas. Simultaneously, we demonstrated that p16 deletion rescued bone loss induced by OVX.

Although the significant consequence of estrogen deficiency is an increase in bone resorption, estrogen is also essential for maintaining bone formation at the cellular level [27]. Thus, we evaluated the effects of OVX and p16 deletion on bone turnover parameters. Our current study demonstrated that OVX induced not only osteoclastic bone resorption, as shown by increased TRAP-positive osteoclast surface and *RANKL/OPG* ratio but also reduced osteoblastic bone formation and osteogenesis of BM-MSCs as shown decreased osteoblast number, type I collagen positive areas, CFU-f

and ALP positive CFU-f formation. In contrast, all parameters for osteoclastic bone resorption and osteoblastic bone formation and osteogenesis were normalized by p16 deletion. Our results indicate that p16 deletion can prevent estrogen deficiency-induced osteoporosis by inhibiting osteoclastic bone resorption, stimulating osteoblastic bone formation, and osteogenesis of BM-MSCs.

Next, we asked whether the estrogen deficiency-induced osteoporosis prevented by p16 deletion is associated with reduced oxidative stress induced by OVX. Recent studies have powerfully shown that estrogen deficiency accelerates bone aging as it dramatically diminishes the defense mechanisms against oxidative stress [2, 28]. Research has demonstrated that BMI-1 works as a direct downstream target of estrogen signaling. In that study, 17β-estradiol treatment upregulated BMI-1 expression levels in ERα-positive MCF-7 cells but not in ERα-negative MDA-MB-231 cells, and ERα upregulated BMI-1 expression at a transcriptional level by directly binding to BMI-1 core promoter [29]. Our recent studies have shown that estrogen deficiency down-regulates
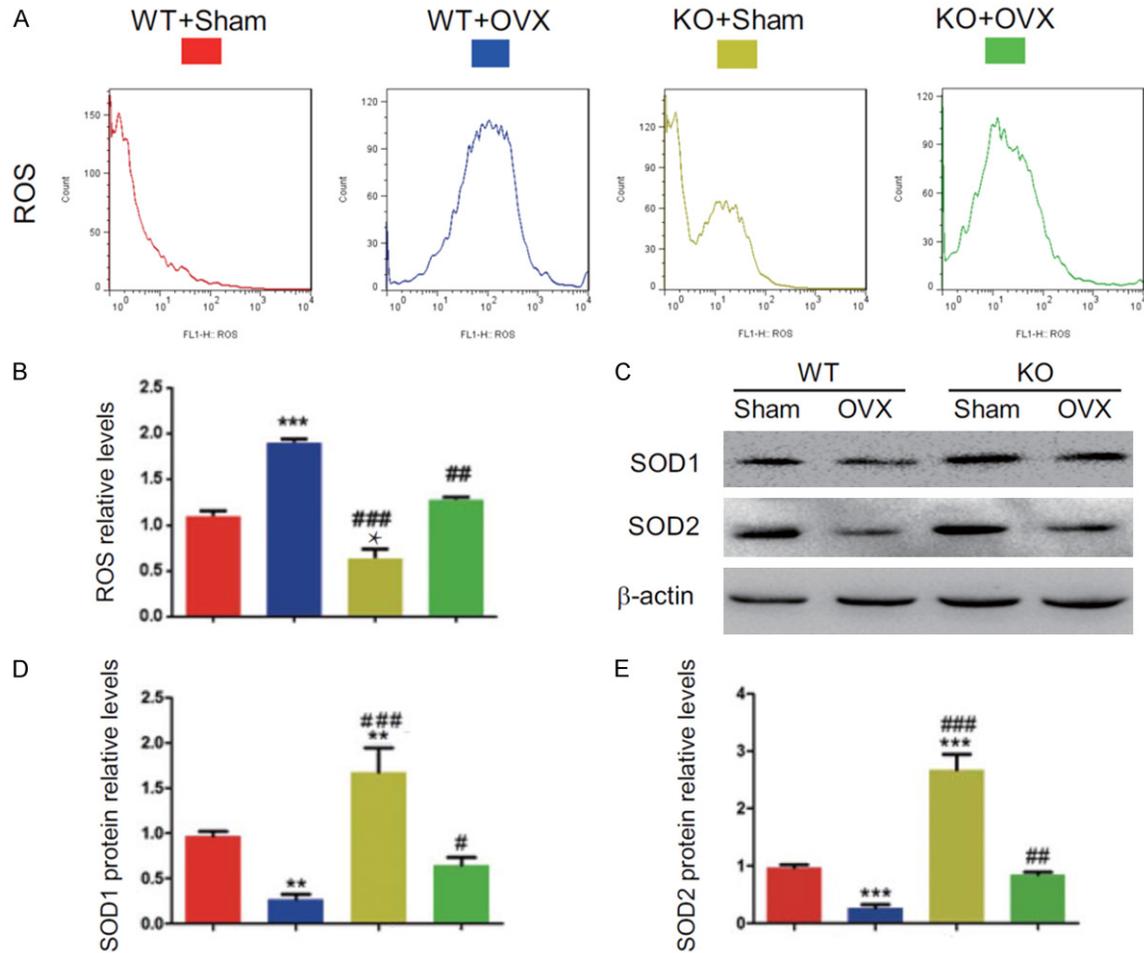
Figure 5. p16 deletion reduced oxidative stress in OVX mice. (A) Representative graphs of flow cytometry analysis for ROS levels in the bone marrow and (B) ROS relative levels. (C) The protein expression levels of SOD1 and SOD2 were detected by Western blotting. β-actin was used as the loading control. (D) SOD1 and (E) SOD2 protein levels were assessed by densitometric analysis calculated as a ratio relative to β-actin protein levels and expressed relative to levels of WT-Sham mice. Data represented as mean ± SEM of determinations in 5 mice of each group. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$, compared with WT-Sham mice. ##: $P < 0.01$ and ###: $P < 0.001$, compared with the same operational WT mice.

Bmi1, increases ROS, T cell activation, and RANKL production in T cells, thereby enhancing osteoclastogenesis and accelerating bone loss. Our previous studies have also shown that estrogen deficiency causes oxidative stress in mouse bone tissue with reduced antioxidant enzyme levels and activity, and impairs osteogenesis and osteoblastic bone formation, while the use of antioxidant N-acetyl-l-cysteine can significantly improve osteogenesis and osteoblastic bone formation in OVX mice [20]. The previous study has shown that oxidative stress increases p16 expression, which in turn, accelerates senescence in human dental pulp stem cells cultured under ambient oxygen ten-

sion [30]. Previous studies suggest that the relationship between p16 levels and oxidative stress may be highly dependent on the circumstances and cell system utilized. For example, it was reported that over-expression of p16 increases cellular ROS levels in a human diploid fibroblast line (TIG-3), and p16 knockdown decreases ROS in a conditionally immortalized human fibroblast line (SVts8) [31]. In contrast, another study has presented evidence that p16 deficiency leads to dysregulation of intracellular ROS in multiple cell types [32]. Our current study confirmed that estrogen deficiency could induce oxidative stress, including increased ROS levels and reduced antioxidase levels

Figure 6. p16 deletion inhibited osteocyte senescence in OVX mice. (A) Representative micrographs of paraffin-embedded sections of vertebral bodies immunostained for β-gal. Scale bars represent 50 μm in (A). (B) The percentage of β-gal positive cells. (C) The protein expression levels of p21 were detected by Western blotting. β-actin was used as the loading control. (D) p21 protein levels were assessed by densitometric analysis calculated as a ratio relative to β-actin protein levels and expressed relative to levels of WT-Sham mice. Data represented as mean ± SEM of determinations in 5 mice of each group. *: $P<0.05$; ***: $P<0.001$, compared with WT-Sham mice. ##: $P<0.01$ and ###: $P<0.001$, compared with the same operational WT mice.

including down-regulating SOD1 and SOD2 protein expression levels in bony tissue, whereas p16 deletion could reduce oxidative stress by increasing antioxidase levels in OVX mice. Results from previous and this study, therefore, support that estrogen plays a role in stimulating osteoblastic bone formation and inhibiting osteoclastic bone resorption in vivo, at least partially through defense against oxidative stress and inactivation of p16 signaling.

We recently reported that p16 deletion could partly prevent aging resulting from $1,25(OH)_2D_3$ deficiency by enhancing cell proliferative ability and reducing cell senescence [23]. In the current study, we found that OVX could activate p16 signaling, whereas p16 deletion rescues OVX-induced osteoporosis by stimulating osteoblastic bone formation, inhibiting osteoclastic bone resorption, and osteocyte senescence. Studies have used the "suicide" transgenic

INK-ATTAC to eliminate relatively small senescent cells, which permits inducible elimination of senescent cells expressing p16, prolongs health span, and prevents the development of multiple age-related morbidities in both progeroid and normal chronologically-aged mice [17, 18]. In old mice with established bone loss, activation of the INK-ATTAC caspase 8 in senescent cells or treatment with senolytics or the JAKi for 2-4 months resulted in higher bone mass and strength and better bone microarchitecture compared to vehicle-treated mice [33], suggesting that targeting cellular senescence could prevent age-related bone loss in mice. Our findings indicate that estrogen could prevent bone loss by targeting the p16 cellular senescence pathway.

In conclusion, this study demonstrated that p16 deletion rescued estrogen deficiency-induced osteoporosis by inhibiting oxidative

stress and osteocyte senescence, suppressing osteoclastic bone resorption, stimulating osteogenesis of BM-MSCs, and osteoblastic bone formation. Our results from this study, therefore, provide new insight indicating that p16 possesses excellent potential as a novel class of therapeutic target for osteoporosis induced by estrogen deficiency.

Acknowledgements

Disclosure of conflict of interest

None.

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