Int. J. Morphol.,
42(1):216-224, 2024.

Resveratrol Alleviates Osteoporosis by Promoting Osteogenic Differentiation of Bone Marrow Mesenchymal Stem Cells via SITR1/PI3K/AKT Pathway

El Resveratrol Alivia la Osteoporosis al Promover la Diferenciación Osteogénica de las Células Madre Mesenquimales de la Médula Ósea a Través de la Vía SITR1/PI3K/AKT

Xu Han[1]; Guo-feng Jia[2] & Feng Zhu[3]

HAN, X.; JIA, G. F. & ZHU, F. Resveratrol alleviates osteoporosis by promoting osteogenic differentiation of bone marrow mesenchymal stem cells via SITR1/PI3K/AKT pathway. *Int. J. Morphol., 42(1)*:216-224, 2024.

SUMMARY: Senile osteoporosis is mainly caused by reduced osteoblast differentiation and has become the leading cause of fractures in the elderly worldwide. Natural organics are emerging as a potential option for the prevention and treatment of osteoporosis. This study was designed to study the effect of resveratrol on osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) in osteoporosis mice. A mouse model of osteoporosis was established by subcutaneous injection of dexamethasone and treated with resveratrol administered by gavage. *In vivo* and *in vitro*, we used western blot to detect protein expression, and evaluated osteogenic differentiation of BMSCs by detecting the expression of osteogenic differentiation related proteins, calcium deposition, ALP activity and osteocalcin content. Resveratrol treatment significantly increased the body weight of mice, the level of serum Ca^{2+}, 25(OH)D and osteocalcin, ration of bone weight, bone volume/total volume, trabecular thickness, trabecular number, trabecular spacing and cortical thickness in osteoporosis mice. In BMSCs of osteoporosis mice, resveratrol treatment significantly increased the expression of Runx2, osterix (OSX) and osteocalcin (OCN) protein, the level of calcium deposition, ALP activity and osteocalcin content. In addition, resveratrol treatment also significantly increased the expression of SIRT1, p-PI3K / PI3K and p-AKT / AKT in BMSCs of osteoporosis mice. In vitro, resveratrol increased the expression of SIRT1, p-PI3K / PI3K and p-AKT / AKT, Runx2, OSX and OCN protein, the level of calcium deposition, ALP activity and osteocalcin content in BMSCs in a concentration-dependent manner, while SIRT1 knockdown significantly reversed the effect of resveratrol. Resveratrol can attenuate osteoporosis by promoting osteogenic differentiation of bone marrow mesenchymal stem cells, and the mechanism may be related to the regulation of SIRT1/PI3K/AKT pathway.

KEY WORDS: Resveratrol; Osteoporosis; Osteogenic differentiation; Bone marrow mesenchymal stem cells; SIRT1.

INTRODUCTION

Osteoporosis (OP) is a systemic metabolic disease characterized by decreased bone tissue mass, increased bone resorption, degeneration of bone microstructure, and increased bone fragility (Baccaro *et al*., 2015; Kanis *et al*., 2019). Osteoporosis is prone to osteoporotic fractures due to the decrease in bone strength, while the disability and mortality rate of osteoporotic fractures and their complications increase significantly, bringing great economic burden to families and society, and seriously affecting the quality of life (Kanis *et al*., 2020; Cheng *et al*., 2021). The causes of osteoporosis are complex, including decreased estrogen levels, aging due to aging, and excessive use of glucocorticoids (Wang *et al*., 2021; Wu *et al*., 2022). Although the pathogenesis of osteoporosis varies, they are mainly manifested as a breakdown of bone reconstitution balance due to decreased bone formation and increased bone resorption (Chen *et al*., 2018; Kim *et al*., 2020). Therefore, promoting bone formation and inhibiting bone resorption to replace lost bone tissue are key to the treatment of osteoporosis.

As the main source of osteoblasts, bone marrow mesenchymal stem cells (BMSCs) have weakened osteogenic differentiation and enhanced lipogenic differentiation ability, which is one of the important causes of osteoporosis (Zhao *et al*., 2018a; Yang *et al*., 2019). Bone

[1] Department of Orthopedics, Affiliated Wuxi Fifth Hospital of Jiangnan University (The Fifth People's Hospital of Wuxi), Wuxi, Jiangsu 214100, China.
[2] Department of Orthopedics, Suzhou Kowloon Hospital Shanghai Jiao Tong University School of Medicine. Suzhou, Jiangsu 215000, China.
[3] Department of Orthopedics, The 904th Hospital of Joint Logistic Support Force of PLA. Wuxi, Jiangsu 214100, China.

Received: 2023-09-10 Accepted: 2023-10-26

marrow mesenchymal stem cells are the only way to renew bone tissue in the body, so regulating the osteogenic differentiation of bone marrow mesenchymal stem cells will help the treatment of osteoporosis (Qi *et al*., 2017; Chen *et al*., 2021a). Resveratrol, a non-flavonoid polyphenol organic compound, is an antitoxin produced by many plants when stimulated. Recently, resveratrol has been found to promote osteogenic differentiation of mesenchymal stem cells, including synergistic BMP9 effects (Wang *et al*., 2022), regulation of miR-193a/SIRT7 axis (Song *et al*., 2022), and regulation of Wnt/ b-Catenin pathway (Zhao *et al*., 2018b) and inhibition of endogenous reactive oxygen species production (Zhou *et al*., 2019). Study showed that SIRT1 has been found to be very important for bone growth and development in mice. SIRT1 conditional knockout mice not only greatly affect long bone development (Cohen-Kfir *et al*., 2011; Iyer *et al*., 2014), such as reduced bone mineral density, long bone length margin and reduced calcium nodules, but also accelerate the course of osteoarthritis in mice (Jeong *et al*., 2013; Matsuzaki *et al*., 2014). However, it is still unknown whether resveratrol can promote osteogenic differentiation of BMSCs by activating SIRT1, ultimately achieving the function of preventing or treating osteoporosis.

In this study, we first evaluated the effect of resveratrol treatment on osteoporosis mice and evaluated the effect of resveratrol treatment on osteogenic differentiation of BMSCs in osteoporosis mice. To verify the mechanism, we established SIRT1-knocked BMSCs *in vitro* and then investigated the effect of resveratrol on osteogenic differentiation of BMSCs.

MATERIAL AND METHOD

Experimental animals and administration: There are 30 male C57BL/6 mice (6 weeks old, 18-20 g) purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd, and they were kept in an environment with a temperature of 25 degrees Celsius and 50 % humidity for 1 week. Then, 30 mice were randomly divided into 3 groups (10 mice in each group), namely, control group, OP group and RES group. Mice in OP group and RES group were subcutaneously injected with 40 mg/kg dexamethasone once every day for 4 weeks to establish osteoporosis mice. And mice in control group were give equal amounts of normal saline during the construction of a mouse model of osteoporosis. For resveratrol (R5010, sigma) treatment, osteoporosis mice in RES group were treated with resveratrol (40 mg/kg body weight, Sigma-Aldrich, China) was performed intraperitoneally once every day for 8 weeks, and mice in control group and OP group were given equal amounts of normal saline.

Detection of serum Ca2+, 25(OH)D, osteocalcin and DPD. At the 4th and 8th weeks of resveratrol treatment, we collected mouse blood through the tail vein to isolate serum, and measured serum 25(OH)D (ab213966, abcam) and osteocalcin (ml026391, mlbio) levels using ELISA kit, and detected serum Ca2+using biochemical automatic analyzer (PUZS-600A/B, PERLONG). At the same time, we collected the urinary of mice, and determined deoxypyridinoline (DPD) using an ELISA kit (ml001904, mlbio).

Bone microstructure analysis. At the 8th week of resveratrol treatment, we firstly measured bone mineral density (BMD) of mice proximal tibia using an X-ray absorptiometry (NORLAND XR-46, Nanjing Norland International Trading Co., Ltd). And then a micro-CT (SKYSCAN 1276, BRUKER) was used to scan the proximal metaphysis of the tibia in mice and select the target area to reconstruct the three-dimensional bone result map for analyzing bone volume/total volume (BV/TV), trabecular thickness (Tb Th), trabecular number (Tb N), trabecular spacing (Tb sp) and cortical thickness.

Western blot analysis. After different treatments, BMSCs are collected and then added to the RIPA lysis buffer to extract the total protein. Next, the total protein from BMSCs was analyzed using a 10 % SDS-PAGE (PN203, New Cell & Molecular Biotech Co., Ltd). After transferring, PVDF membranes (LC2002, ThermoFisher) was first blocked with 5 % skimmed milk powder, and then was probed with primary antibodies against Runx2 (1:1000, ab192256, ABCAM), Osteocalcin (OCN, 1:500, ab93876, ABCAM), osterix (OSX, 1:500, ab183910, ABCAM), SIRT1 (1:500, 8469, Cell Signaling Technology), p-PI3K (1:200, 17366, Cell Signaling Technology), PI3K (1:1000, 4292, Cell Signaling Technology), p-AKT (1:500, 4060, Cell Signaling Technology), AKT (1:1000, 9272, Cell Signaling Technology). Proteins were visualized with ECL solution (WBKLS0100, Beijing Xinjingke Biotechnologies Co., Ltd?China), followed by densitometry analysis using Imag J 3.0 (IBM, USA) and b-actin was loading as control.

Calcium deposition, ALP activity and osteocalcin content. On one hand, BMSCs from osteoporosis mice were directly lysed for detection, including used a mouse OC/BGP (Osteocalcin) ELISA Kit (D721126, Sangon Biotech) to analyze the content of osteocalcin, and used an ALP activity assay kit (ab267583, ABCAM) to detect ALP activity, and use the Calcium Assay Kit (S1063S, Beyotime) to measure calcium deposition. On the other hand, normal BMSCs were treated in osteogenic induction medium (OIM, PD-003, Procell) with resveratrol at different concentrations (0, 5, 15 and 25 mg/mL) cultivate for 21 days. And then we collected BMSCs to detect calcium deposition, ALP activity and osteocalcin content using the corresponding reagent kit.

Isolation, identification, and administration of BMSCs. After the mouse was sacrificed by cervical dislocation, we collected the bone marrow cavity rinse, filtered using a strainer to prepare a single-cell suspension (FSTR100, Betotime), and seed the cell suspension in a 100 mm cell culture dish (CCD06-100A, BIOLAND). BMSCs were cultured in DMEM medium with 10 % fetal bovine serum (10091148, ThermoFisher scientific), and third generation BMSCs were used for identification by detecting cell surface markers using flow cytometry, such as CD29 (ab193591, abcam), CD90 (ab226, abcam), CD34 (ab187568, abcam) and CD45 (ab305209) using corresponding antibodies. Resveratrol was treated with BMSCs at concentrations of 0, 5, 15 and 25 mg/mL for 1, 3, 7 and 14 days.

Establishment of SIRT1 knockdown BMSCs. About 4 × 106 BMSCs were seeded into 10 cm cell culture dishes. 24 h later, lentiviral particles targeting SIRT1 (si-SIRT1) were added according to the instructions (sc-40987, Santa Cruz Biotechnology), and negative control lentiviral particles (si-NC) were added into BMSCs as control. 24 h after lentivirus infection, we changed the medium of BMSCs. 72 h after lentivirus infection, we collected BMSCs to verify successful establishment of SIRT1 knockdown BMSCs cell lines via detecting SIRT1 protein expression by western blotting.

Statistical analysis. SPSS20.0 software (IBM, USA) was used to analyze data in this study. Differences between two groups were compared usingunpaired t test, and difference between multiple groups were compared using one-way ANOVA with Tukey test as post hoc test. And $P<0.05$ indicated significant difference.

RESULTS

Resveratrol alleviates deterioration of bone microstructure in osteoporosis mice. At the 4th and 8th week of resveratrol treatment, we measured the body weight and bone metabolism related indicators of each group of mice, and found that the body weight (Fig. 1A), serum Ca2+ (Fig. 1B), serum 25 (OH) D (Fig. 1C), serum osteocalcin (Fig. 1D) in the osteoporosis (OP) group were all lower than that in control group and resveratrol treatment (RES) group, but the level of DPD in urine in OP group was significantly higher than that in control group and RES group (Fig. 1E). At the same time, we measured the weight of tibia and femur at 8th week of resveratrol treatment, and results showed that compared to control group, the weight of tibia and femur / body weight in OP group were significantly reduced, while the weight of tibia and femur / body weight in RES group were significantly higher than that in OP group (Fig. 1F).

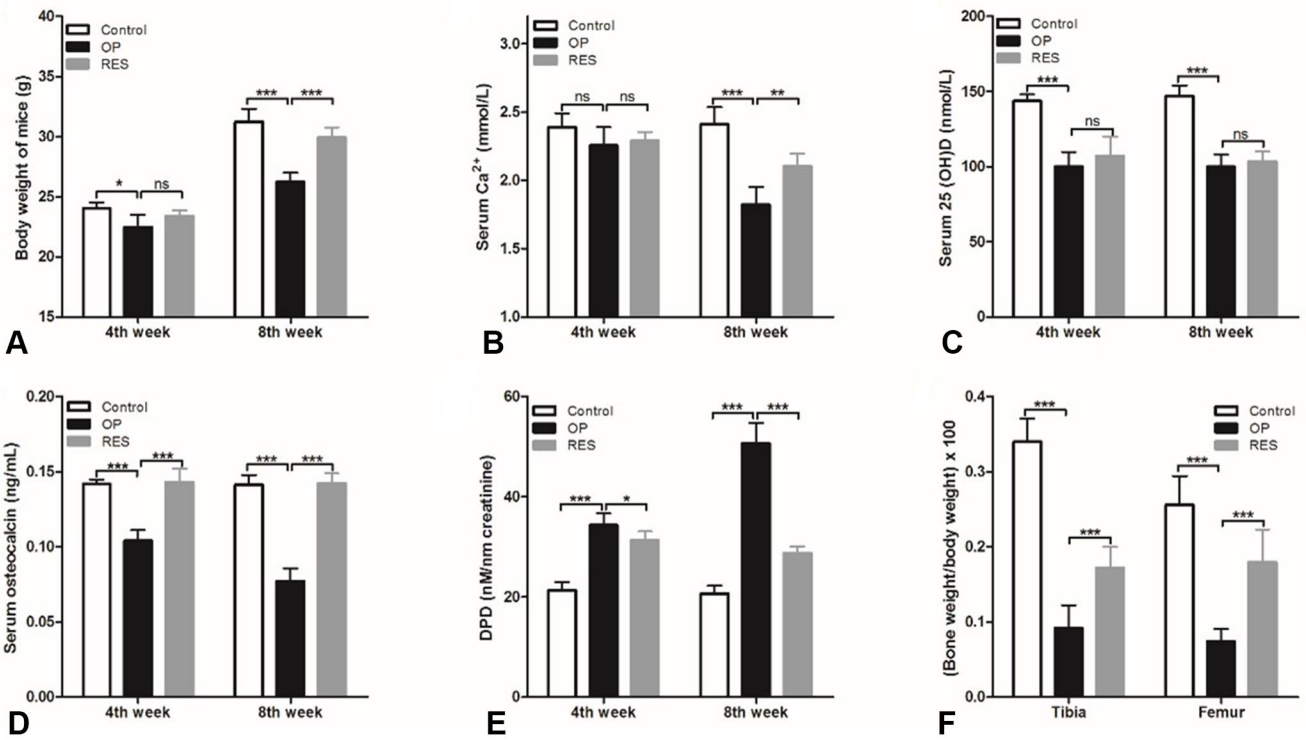

Fig. 1. Effect of resveratrol on the general data of osteoporosis mice. At weeks 4 and 8 of resveratrol treatment, we measured the level of the body weight (A), serum Ca2+ (B), serum 25 (OH) D (C), serum osteocalcin (D) and urinary DPD (E) in osteoporosis mice and detected the weight of tibia and femur at 8th weeks (F). 5 mice in each group, and data were expressed as (mean ± standard deviation). ns $P>0.05$, * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

At the 8th week of resveratrol treatment, we measured the BMD of mice in each group and found that the BMD of mice in OP group were significantly lower than that in control group and RES group (Fig. 2A). At the same time, we also used the micro-CT to determine BV/TV (Fig. 2B), Tb Th (Fig. 2C), Tb N (Fig. 2D), Tb sp (Fig. 2E) and cortical thickness (Fig. 2F), and found that the BV/TV, Tb Th, Tb N and cortical thickness of mice in OP group were significantly lower than that in control group and RES group, while the Tb sp of mice in OP group were significantly lower than that in control group and RES group.

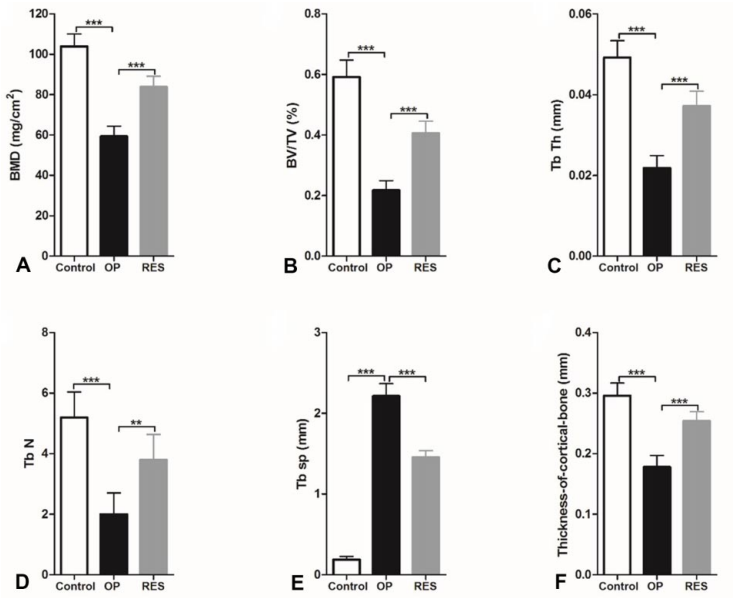

Fig. 2. Resveratrol treatment attenuates deterioration of bone microstructure in osteoporosis mice. After 8 weeks of resveratrol treatment, we used a dual energy X-ray absorptiometry to measure Bone mineral density (BMD) (A), and micro-CT to measure bone volume/total volume (BV/TV) (B), trabecular thickness (Tb Th) (C), trabecular number (Tb N) (D), trabecular spacing (Tb sp) (E) and cortical thickness (F) in osteoporosis mice. 5 mice in each group, and data were expressed as (mean ± standard deviation). **P<0.01, ***P<0.001.

Resveratrol promotes osteogenic differentiation of BMSCs in osteoporosis mice. Firstly, we determined the expression of osteogenic differentiation related proteins in BMSCs at the 8th week of resveratrol treatment, such as Runx2, OSX and OCN (Fig. 3A), and we found that the expression of Runx2 (Fig. 3B), OSX (Figure 3C) and OCN (Fig. 3D) protein in BMSCs of mice in OP group were all significantly lower than that in control group and RES group. In addition, we cultured BMSCs of mice in each group in OIM medium for 21 days, and then collected the cell culture medium of BMSCs and BMSCs to analyze the osteogenic differentiation indicators. Results suggested that the level of calcium deposition (Fig. 3E), ALP activity (Fig. 3F) and osteocalcin content (Fig. 3G) in OP group during osteogenic differentiation induction of BMSCs were all significantly lower than that in control group and RES group.

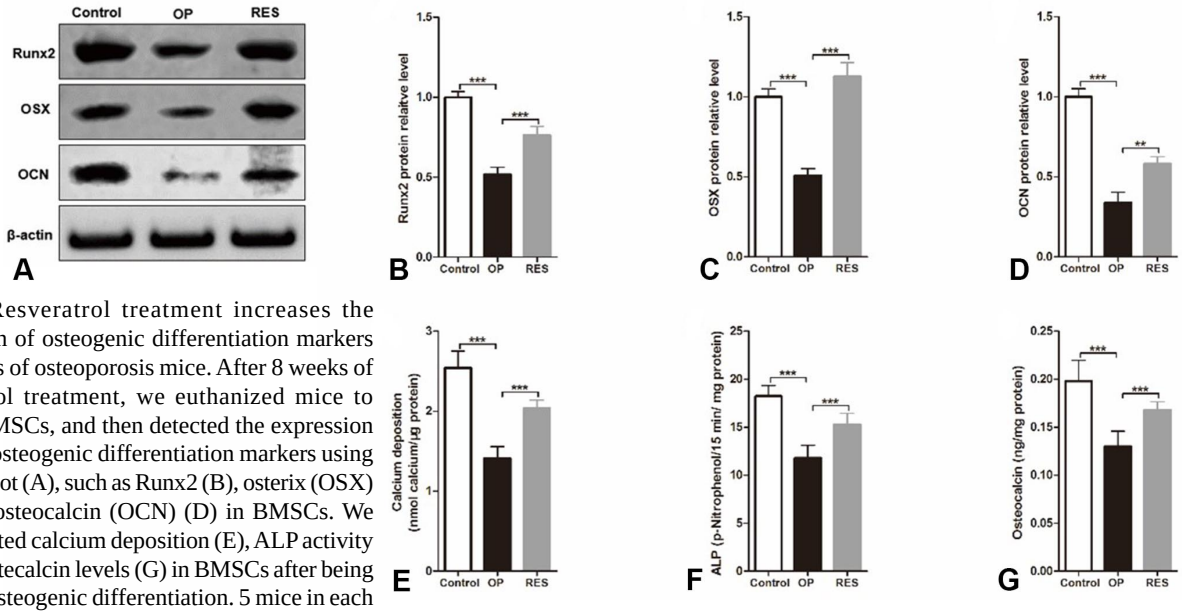

Fig. 3. Resveratrol treatment increases the expression of osteogenic differentiation markers in BMSCs of osteoporosis mice. After 8 weeks of resveratrol treatment, we euthanized mice to isolate BMSCs, and then detected the expression levels of osteogenic differentiation markers using western blot (A), such as Runx2 (B), osterix (OSX) (C), and osteocalcin (OCN) (D) in BMSCs. We also detected calcium deposition (E), ALP activity (F) and ostecalcin levels (G) in BMSCs after being induced osteogenic differentiation. 5 mice in each group, and data were expressed as (mean ± standard deviation). ***P<0.001.

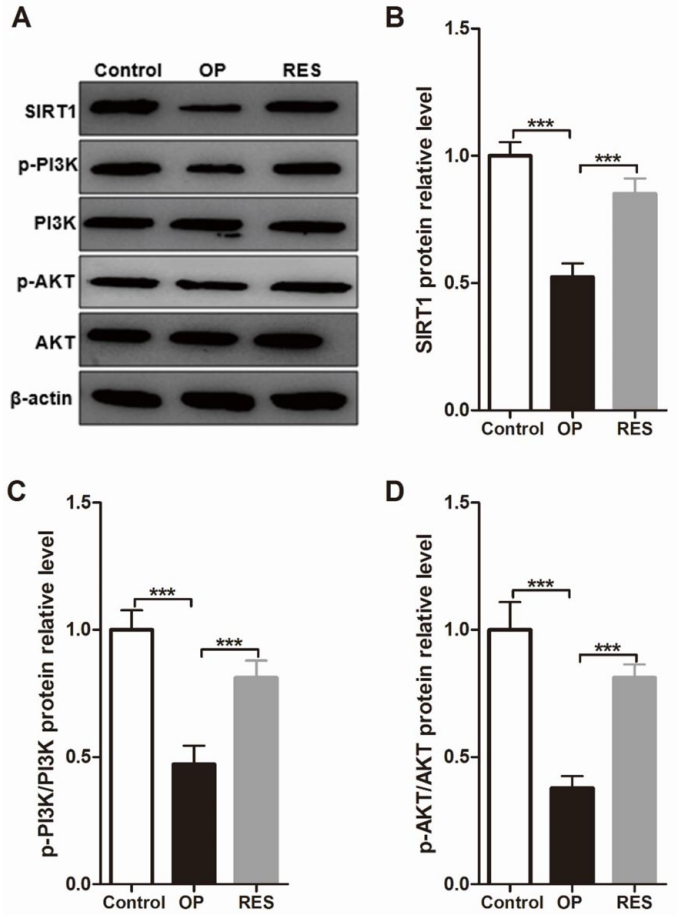

Fig. 4. Resveratrol treatment activates the SIRT1/PI3K/AKT pathway in BMSCs of osteoporotic mice. After 8 weeks of resveratrol treatment, we euthanized mice to isolate BMSCs, and then detected the expression of key proteins in SIRT1/PI3K/AKT pathway using western blot (A), such as SIRT1 (B), p-PI3K and PI3K (C), p-AKT and AKT (D). 5 mice in each group, and data were expressed as (mean ± standard deviation). ***P<0.001.

Resveratrol activates SIRT1/PI3K/AKT pathway in BMSCs of osteoporosis mice. SIRT1 has been shown to be associated with bone regeneration in mice [17, 18]. Therefore, we evaluated resveratrol for changes in the SIRT1 protein and its downstream PI3K/AKT pathway in BMSCs of osteoporosis mice using western blot (Fig. 4A), and the grayscale analysis results of protein bands showed that the expression of SIRT1 (Fig. 4B), p-PI3K / PI3K (Fig. 4C) and p-AKT / AKT (Fig. 4D) protein in OP group all significantly lower than that in control group and RES group.

Resveratrol promotes osteogenic differentiation of BMSCs *in vitro*. To investigate the molecular mechanism by which resveratrol promotes osteogenic differentiation of BMSCs, we firstly prepared BMSCs from mice and harvested the third generation BMSCs for analysis using flow cytometry (Fig. 5A). Results showed that a total of 99.24 % cells were positive for CD29 and 97.83 % cells were positive for CD90, while only 0.98 % cells were positive for CD34 and 1.03 % were positive for CD45 (Fig. 5B). And then we cultured BMSCs in OIM medium with different concentration of resveratrol (0, 5, 15 and 25 mg/mL) for different days (1, 3, 7 and 14 days) to detect the activity of BMSCs using CCK-8 kit. We found that resveratrol increased cellular activity of BMSCs in a concentration-dependent manner (Fig. 5C).

Next, we analyzed the expression of osteogenic differentiation related proteins and the level of osteogenic differentiation indicators in BMSCs *in vitro*, and results showed that resveratrol increased the expression of Runx2, OSX and OCN protein in BMSCs in a

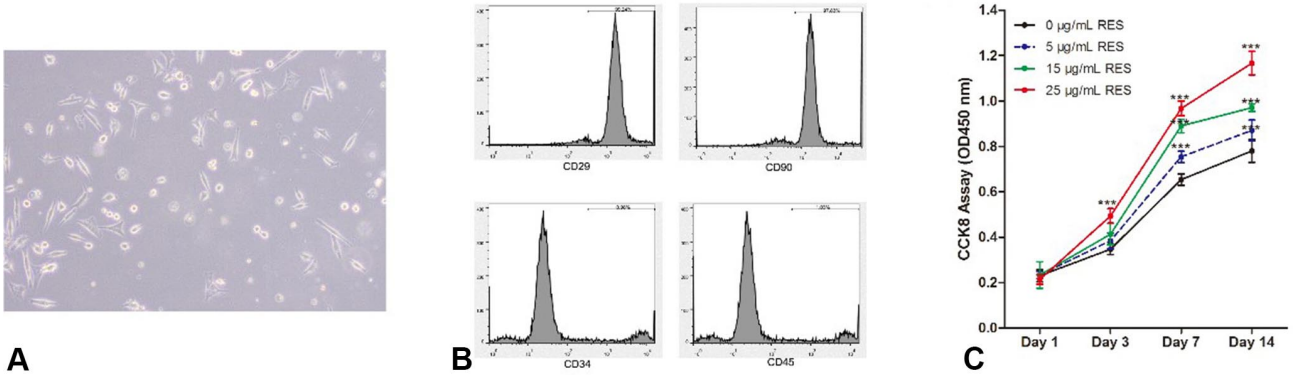

Fig. 5. Morphology and identity of mouse bone marrow stromal cells. (A) The morphology of third generation BMSCs under a microscope (100X). (B) Flow cytometry analysis of BMSCs surface markers, including CD29, CD90, CD34 and CD45. (C) CCK-8 kit was used to detect cell viability after incubating BMSCs at different concentrations of resveratrol for different time in osteogenic induction medium (OIM) medium. 3 independent replicates per test and data were expressed as (mean ± standard deviation). Compared with 0 mg/mL RES, ***P<0.001.

concentration-dependent manner (Fig. 6A-D). Moreover, results of osteogenic differentiation indicators indicated that resveratrol also increased the levels of calcium deposition (Fig. 6E), ALP activity (Fig. 6F) and osteocalcin content (Fig. 6G) in BMSCs in a concentration-dependent manner.

Resveratrol promotes osteogenic differentiation of BMSCs by activating SIRT1-mediated PI3K/AKT pathway. To verify that resveratrol promotes osteogenic differentiation of BMSCs in osteoporosis mice via SIRT1, we established SIRT1-knockdown BMSCs cell lines *in vitro*, and western blotting verified the successful establishment of SIRT1-knockdown BMSCs cell lines (Fig. 7A). Next, we used western blotting to assess SIRT/PI3K/AKT pathway in different BMSCs after being stimulated with resveratrol in OIM medium (Fig. 7B) and found that resveratrol significantly increased the expression of SIRT1 (Fig. 7C), p-PI3K/PI3K (Fi. 7D) and p-AKT/AKT (Fig. 7E) proteins in BMSCs, while si-SIRT1 significantly reversed the effect of resveratrol. In addition, we also found that the level of calcium deposition (Fig. 7F), ALP activity (Fig. 7G) and osteocalcin content (Fig. 7H) in RES group were significantly higher than that in Control group and RES + si-SIRT1 group.

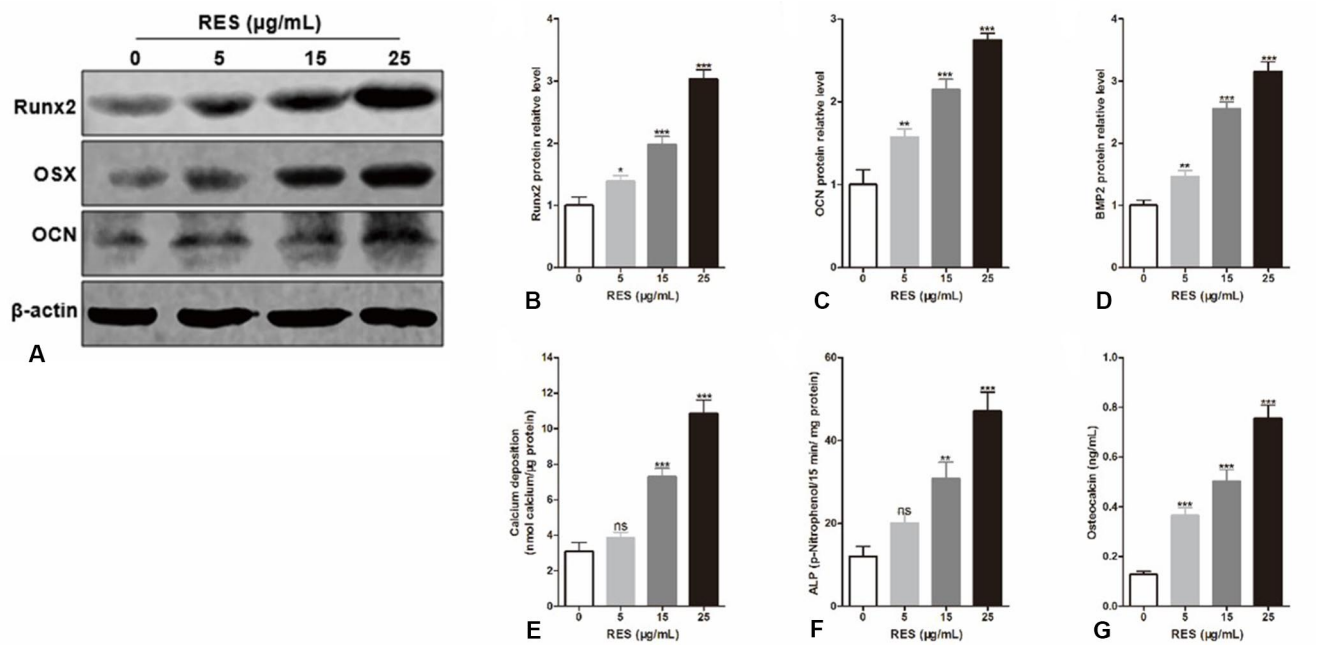

Fig. 6. Resveratrol promotes osteogenic differentiation of BMSCs *in vitro*. After 21 days of incubation of BMSCs with different concentrations of resveratrol in OIM medium, we collected BMSCs to detect he expression of osteogenic differentiation markers using western blot (A), such as Runx2 (B), OSX (C), and OCN (D), and to measure the level of calcium deposition (E), ALP activity (F) and ostecalcin levels (G). 3 independent replicates per test and data were expressed as (mean ± standard deviation). Compared with 0 mg/mL RES, ns P>0.05, * P<0.05, ** P<0.01 and ***P<0.001.

DISCUSSION

Bones are organs with active metabolism, and constantly synthesize new bone through osteoblasts to replace damaged or old bone absorbed by osteoclasts, so that the body's skeletal system maintains normal function (Chen *et al*., 2018; Kim *et al*., 2020). However, the delicate balance between bone synthesis and bone breakdown and absorption disappears with aging or pathological destruction, leading to bone loss and osteoporosis (Hu *et al*., 2022; Phromnoi *et al*., 2022). Epidemiological statistics show that 20 % of men and 50 % of women will experience a decrease in bone density after the age of 50, and OP is one of the main causes of fractures in elderly patients (Lorentzon, 2019; Imamudeen *et al*., 2022). In China, there were about 2.69 million new cases of major osteoporotic fractures (hip, vertebral body, and wrist) in 2015, and about 4.83 million new cases are expected in 2035 and about 5.99 million new cases in 2050 (Wang *et al*., 2021; Wu *et al*., 2022). At present, most of the drug mechanisms of action for the treatment of osteoporosis are by inhibiting bone resorption and promoting bone formation, the former including calcitonin, bisphosphonates, estrogens, etc., and the latter including parathyroid hormone and its analogues, but its long-term application also brings many toxic side effects (Slupski *et al*., 2021; Reid & Billington, 2022). Therefore, the demand for

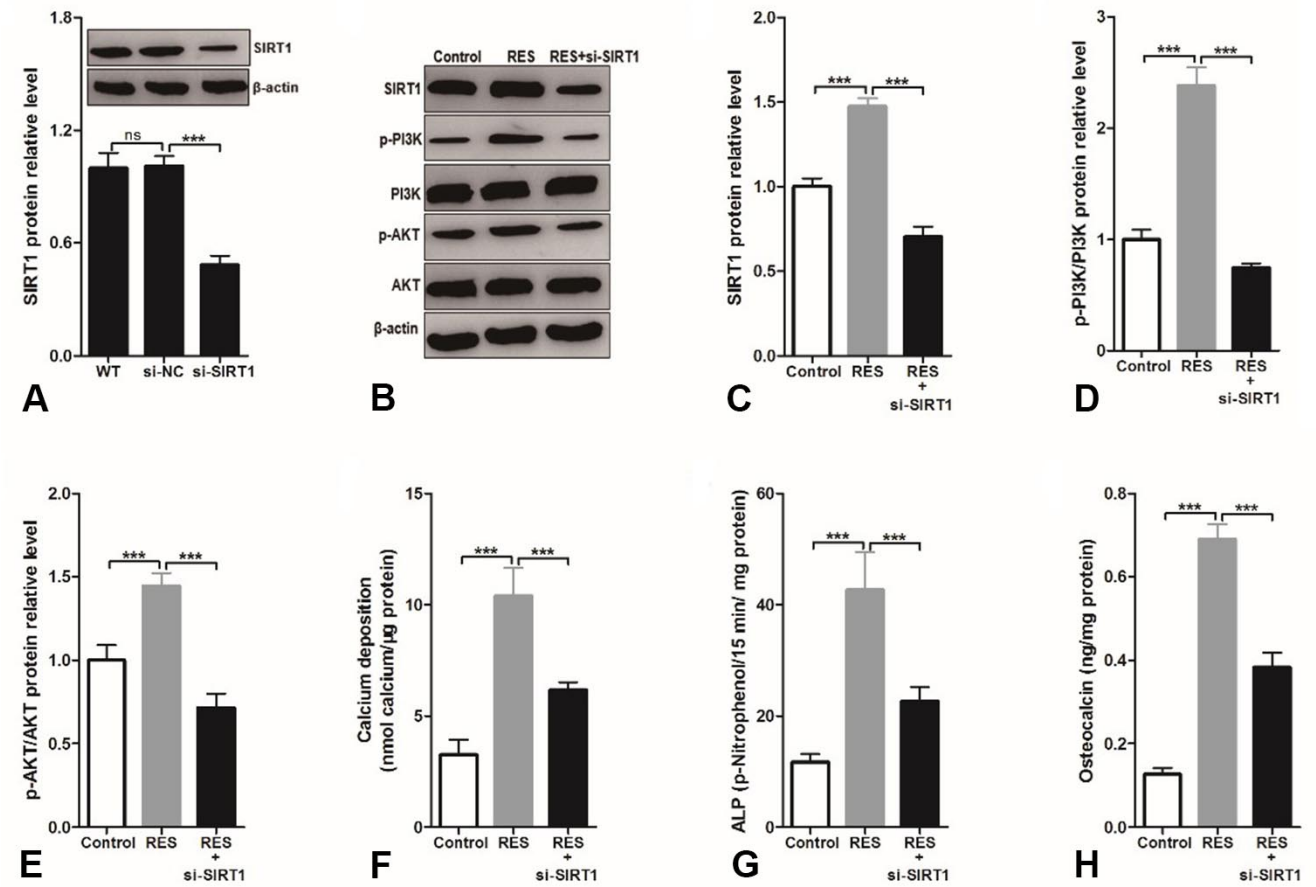

Fig. 7. Resveratrol promotes osteogenic differentiation of BMSCs by activating the SIRT1 mediated PI3K/AKT pathway. (A) Western blot verification successful construction of SIRT1 knockdown BMSCs. (B-E) After 21 days of incubation of BMSCs with resveratrol in OIM medium, we detected the expression of key proteins in SIRT1/PI3K/AKT pathway using western blot (B), such as SIRT1 (C), p-PI3K and PI3K (D), p-AKT and AKT (E). (F-G) After 21 days of incubation of BMSCs with resveratrol in OIM medium, we collected BMSCs to measure the level of calcium deposition (F), ALP activity (G) and ostecalcin levels (H). 3 independent replicates per test and data was expressed as (mean ± standard deviation). ***P<0.001.

drugs that play a safer and more reliable effect in the treatment of osteoporosis is becoming more and more obvious.

Resveratrol, also known as 3,4',5-trihydroxystilbene, is a classic natural polyphenol plant antitoxin, widely present in many plants and traditional Chinese medicine, with enhance autoimmunity, anti-inflammatory, antibacterial, antioxidant, cardiovascular protection, anti-tumor, and other effects (Tian & Liu, 2020; Zhou *et al*., 2021; Meng *et al*., 2021). Recently, resveratrol has increasingly attracted attention in the treatment of osteoporosis due to its multi-target, immediate availability, low cost, and low toxicity (Wang *et al*., 2022; Song *et al*., 2022). Study showed that osteoporosis caused by osteoclast and osteoblast imbalance is associated with osteogenic differentiation of BMSCs (Jiang *et al*., 2021; He *et al*., 2022). In this study, we treated osteoporosis mice by intraperitoneal injection of resveratrol, and found that resveratrol treatment could not only improve the content of bone metabolic indicators, but also help to alleviate the deterioration of bone microstructure in osteoporosis mice. Importantly, we also found that resveratrol treatment promoted the osteogenic differentiation of BMSCs in osteoporosis mice.

SIRT1 is the most widely studied member of the sirtuins family and has been shown to play an important role in the development of neurodegenerative diseases, diabetes, tumors, inflammation, and aging (Jiao & Gong, 2020; Iside *et al*., 2020; Yang *et al*., 2022). Previous studies have shown that SIRT1 conditional knockout not only reduces bone mineral density in mice, resulting in shorter long bone length and cortical bone thickness, but also leads to a decrease in bone calcium nodules and inhibits the formation of sclerosteostin (Cohen-Kfir *et al*., 2011; Iyer *et al*., 2014; Chen *et al*., 2021b). At the same time, the activation of SIRT1 is also thought to promote osteogenic differentiation of

mesenchymal stem cells, such as Lu *et al.* (2022), found that SRT2104, an activator of SIRT1, can promote osteogenic differentiation and angiogenesis of mesenchymal stem cells by activating the BMP/Smad and BMP/MAPK signaling pathways mediated by SIRT1. And Xu *et al.* (2021) found that thrombin-activated platelet-rich plasma enhanced osteogenic differentiation of human periodontal ligament stem cells by activating SIRT1-mediated autophagy. Our study also found that resveratrol treatment significantly increased the expression of SIRT1 in BMSCs in osteoporosis mice and knocking out SIRT1 *in vitro* significantly reversed the role of resveratrol in promoting bone differentiation of BMSCs. Therefore, our results indicated that resveratrol promoted the osteogenic differentiation of BMSCs by promoting SIRT1 expression, thereby weakening the disease progression in osteoporosis mice.

PI3K-AKT signaling pathway is one of the important signal transduction pathways in subcells, which can play a key role in inhibiting apoptosis and promoting proliferation and differentiation in cells by affecting the activation state of a variety of downstream effector molecules and play an important role in the occurrence and development of various human diseases (Yu & Cui, 2016; Xu *et al*., 2020).

In the present study, we found that resveratrol treatment could activate PI3K-AKT pathway in BMSCs of osteoporosis mice, while SIRT1 knockdown attenuated the function of resveratrol on the activation of the PI3K-AKT pathway in BMSCs, suggesting that resveratrol promotes osteogenic differentiation of BMSCs through the SIRT1-mediated PI3K/AKT pathway.

CONCLUSION

All in all, our results suggested that resveratrol can attenuate hormone-induced osteoporosis by promoting osteogenic differentiation of bone marrow mesenchymal stem cells, and the mechanism may be related to the regulation of SIRT1/PI3K/AKT pathway.

HAN, X.; JIA, G. F. & ZHU, F. El resveratrol alivia la osteoporosis al promover la diferenciación osteogénica de las células madre mesenquimales de la médula ósea a través de la vía SITR1/PI3K/AKT. *Int. J. Morphol., 42(1)*:216-224, 2024.

RESUMEN: La osteoporosis senil es causada principalmente por una diferenciación reducida de osteoblastos y se ha convertido en la principal causa de fracturas en las personas mayores en todo el mundo. Los productos orgánicos naturales están surgiendo como una opción potencial para la prevención y el tratamiento de la osteoporosis. Este estudio fue diseñado para estudiar el efecto del resveratrol en la diferenciación osteogénica de las células madre mesenquimales de la médula ósea (BMSC) en ratones con osteoporosis. Se estableció un modelo de osteoporosis en ratones mediante inyección subcutánea de dexametasona y se trató con resveratrol administrado por sonda. *In vivo* e *in vitro*, utilizamos Western blot para detectar la expresión de proteínas y evaluamos la diferenciación osteogénica de BMSC detectando la expresión de proteínas relacionadas con la diferenciación osteogénica, la deposición de calcio, la actividad de ALP y el contenido de osteocalcina. El tratamiento con resveratrol aumentó significativamente el peso corporal de los ratones, el nivel sérico de Ca^{2+}, 25(OH)D y osteocalcina, la proporción de peso óseo, el volumen óseo/volumen total, el espesor trabecular, el número trabecular, el espaciado trabecular y el espesor cortical en ratones con osteoporosis. En BMSC de ratones con osteoporosis, el tratamiento con resveratrol aumentó significativamente la expresión de las proteínas Runx2, osterix (OSX) y osteocalcina (OCN), el nivel de deposición de calcio, la actividad de ALP y el contenido de osteocalcina. Además, el tratamiento con resveratrol también aumentó significativamente la expresión de SIRT1, p-PI3K/PI3K y p-AKT/AKT en BMSC de ratones con osteoporosis. *In vitro*, el resveratrol aumentó la expresión de las proteínas SIRT1, p-PI3K/PI3K y p-AKT/AKT, Runx2, OSX y OCN, el nivel de deposición de calcio, la actividad de ALP y el contenido de osteocalcina en BMSC de manera dependiente de la concentración, mientras que La caída de SIRT1 revirtió significativamente el efecto del resveratrol. El resveratrol puede atenuar la osteoporosis al promover la diferenciación osteogénica de las células madre mesenquimales de la médula ósea, y el mecanismo puede estar relacionado con la regulación de la vía SIRT1/PI3K/AKT.

PALABRAS CLAVE: Resveratrol; Osteoporosis; Diferenciación osteogénica; Células madre mesenquimales de médula ósea; SIRT1.

REFERENCES

Baccaro, L. F.; Conde, D. M.; Costa-Paiva, L. & Pinto-Neto, A. M. The epidemiology and management of postmenopausal osteoporosis: a viewpoint from Brazil. *Clin. Interv. Aging., 10*:583-91, 2015.

Chen, L.; Shi, X.; Xie, J.; Weng, S. J.; Xie, Z. J.; Tang, J. H.; Yan, D. Y.; Wang, B. Z.; Fang, K. H.; Hong, C. X.; *et al.* Apelin-13 induces mitophagy in bone marrow mesenchymal stem cells to suppress intracellular oxidative stress and ameliorate osteoporosis by activation of AMPK signaling pathway. *Free Radic. Biol. Med., 163*:356-68, 2021a.

Chen, X.; Wang, Z.; Duan, N.; Zhu, G.; Schwarz, E. M. & Xie, C. Osteoblast-osteoclast interactions. *Connect. Tissue Res., 59(2)*:99-107, 2018.

Chen, Y.; Zhou, F.; Liu, H.; Li, J.; Che, H.; Shen, J. & Luo, E. SIRT1, a promising regulator of bone homeostasis. *Life Sci., 269*:119041, 2021b.

Cheng, X.; Zhao, K.; Zha, X.; Du, X.; Li, Y.; Chen, S.; Wu, Y.; Li, S.; Lu, Y.; Zhang, Y.; *et al*. Opportunistic screening using low-dose CT and the prevalence of osteoporosis in china: a nationwide, multicenter study. *J. Bone Miner. Res., 36(3)*:427-35, 2021.

Cohen-Kfir, E.; Artsi, H.; Levin, A.; Abramowitz, E.; Bajayo, A.; Gurt, I.; Zhong, L.; D'Urso, A.; Toiber, D.; Mostoslavsky, R.; *et al*. Sirt1 is a regulator of bone mass and a repressor of Sost encoding for sclerostin, a bone formation inhibitor. *Endocrinology, 152(12)*:4514-24, 2011.

He, M.; Lei, H.; He, X.; Liu, Y.; Wang, A.; Ren, Z.; Liu, X.; Yan, G.; Wang, W.; Wang, Y., *et al*. METTL14 regulates osteogenesis of bone marrow mesenchymal stem cells via inducing autophagy through m6A/IGF2BPs/Beclin-1 signal axis. *Stem Cells Transl. Med., 11(9)*:987-1001, 2022.

Hu, L.; Xie, X.; Xue, H.; Wang, T.; Panayi, A. C.; Lin, Z.; Xiong, Y.; Cao, F.; Yan, C.; Chen, L.; *et al*. MiR-1224-5p modulates osteogenesis by coordinating osteoblast/osteoclast differentiation via the Rap1 signaling target ADCY2. *Exp. Mol. Med., 54(7)*:961-72, 2022.

Imamudeen, N.; Basheer, A.; Iqbal, A.M.; Manjila, N.; Haroon, N.N. & Manjila, S. Management of osteoporosis and spinal fractures: contemporary guidelines and evolving paradigms. *Clin. Med. Res., 20(2)*:95-106, 2022.

Iside, C.; Scafuro, M.; Nebbioso, A. & Altucci, L. SIRT1 activation by natural phytochemicals: an overview. *Front. Pharmacol., 11*:1225, 2020.

Iyer, S.; Han, L.; Bartell, S. M.; Kim, H. N.; Gubrij, I.; de Cabo, R.; O'Brien, C. A.; Manolagas, S. C. & Almeida, M. Sirtuin1 (Sirt1) promotes cortical bone formation by preventing b-catenin sequestration by FoxO transcription factors in osteoblast progenitors. *J. Biol. Chem., 289(35)*:24069-78, 2014.

Jeong, J. K.; Moon, M. H.; Lee, Y. J.; Seol, J. W. & Park, S. Y. Autophagy induced by the class III histone deacetylase Sirt1 prevents prion peptide neurotoxicity. *Neurobiol. Aging, 34(1)*:146-56, 2013.

Jiang, Y.; Zhang, P.; Zhang, X.; Lv, L. & Zhou, Y. Advances in mesenchymal stem cell transplantation for the treatment of osteoporosis. *Cell Prolif., 54(1)*:e12956, 2021.

Jiao, F. & Gong, Z. The beneficial roles of SIRT1 in neuroinflammation-related diseases. *Oxid. Med. Cell. Longev., 2020*:6782872, 2020.

Kanis, J. A.; Cooper, C.; Rizzoli, R. & Reginster, J. Y. Executive summary of the European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Calcif. Tissue Int., 104(3)*:235-8, 2019.

Kanis, J. A.; Harvey, N. C.; McCloskey, E., Bruyère, O.; Veronese, N.; Lorentzon, M.; Cooper, C.; Rizzoli, R.; Adib, G.; Al-Daghri, N.; *et al*. Algorithm for the management of patients at low, high and very high risk of osteoporotic fractures. *Osteoporos. Int., 31(1)*:1-12, 2020.

Kim, J. M.; Lin, C.; Stavre, Z.; Greenblatt, M. B. & Shim, J. H. Osteoblast-osteoclast communication and bone homeostasis. *Cells, 9(9)*:2073, 2020.

Lorentzon, M. Treating osteoporosis to prevent fractures: current concepts and future developments. *J. Intern. Med., 285(4)*:381-94, 2019.

Lu, Y.; Ma, Z. X.; Deng, R.; Jiang, H. T.; Chu, L. & Deng, Z. L. The SIRT1 activator SRT2104 promotes BMP9-induced osteogenic and angiogenic differentiation in mesenchymal stem cells. *Mech. Ageing Dev., 207*:111724, 2022.

Matsuzaki, T.; Matsushita, T.; Takayama, K.; Matsumoto, T.; Nishida, K.; Kuroda, R. & Kurosaka, M. Disruption of Sirt1 in chondrocytes causes accelerated progression of osteoarthritis under mechanical stress and during ageing in mice. *Ann. Rheum. Dis., 73(7)*:1397-404, 2014.

Meng, T.; Xiao, D.; Muhammed, A.; Deng, J.; Chen, L. & He, J. Anti-inflammatory action and mechanisms of resveratrol. *Molecules, 26(1)*:229, 2021.

Phromnoi, K.; Yodkeeree, S.; Pintha, K.; Mapoung, S.; Suttajit, M.; Saenjum, C. & Dejkriengkraikul, P. Anti-osteoporosis effect of perilla frutescens leaf hexane fraction through regulating osteoclast and osteoblast differentiation. *Molecules, 27(3)*:824, 2022.

Qi, M.; Zhang, L.; Ma, Y.; Shuai, Y.; Li, L.; Luo, K.; Liu, W. & Jin, Y. Autophagy maintains the function of bone marrow mesenchymal stem cells to prevent estrogen deficiency-induced osteoporosis. *Theranostics, 7(18)*:4498-516, 2017.

Reid, I. R. & Billington, E. O. Drug therapy for osteoporosis in older adults. *Lancet, 399(10329)*:1080-92, 2022.

Slupski, W.; Jawien, P. & Nowak, B. Botanicals in postmenopausal osteoporosis. *Nutrients, 13(5)*:1609, 2021.

Song, C. Y.; Guo, Y.; Chen, F. Y. & Liu, W. G. Resveratrol promotes osteogenic differentiation of bone marrow-derived mesenchymal stem cells through miR-193a/SIRT7 axis. *Calcif. Tissue Int., 110(1)*:117-30, 2022.

Tian, B. & Liu, J. Resveratrol: a review of plant sources, synthesis, stability, modification and food application. *J. Sci. Food Agric., 100(4)*:1392-404, 2020.

Wang, L.; Yu, W.; Yin, X.; Cui, L.; Tang, S.; Jiang, N.; Cui, L.; Zhao, N.; Lin, Q.; Chen, L.; *et al*. Prevalence of osteoporosis and fracture in China: the China osteoporosis prevalence study. *JAMA Netw. Open, 4(8)*:e2121106, 2021.

Wang, Y.; Xia, C.; Chen, Y.; Jiang, T.; Hu, Y. & Gao, Y. Resveratrol synergistically promotes BMP9-induced osteogenic differentiation of mesenchymal stem cells. *Stem Cells Int., 2022*:8124085, 2022.

Wu, X. Y.; Li, H.; Shen, Y.; Tan, L. H.; Yuan, L. Q.; Dai, R. C.; Zhang, H.; Peng, Y. Q.; Xie, Z. J. & Sheng, Z. F. Effect of body surface area on severe osteoporotic fractures: a study of osteoporosis in Changsha China. *Front. Endocrinol. (Lausanne), 13*:927344, 2022.

Xu, F.; Na, L.; Li, Y. & Chen, L. Roles of the PI3K/AKT/mTOR signalling pathways in neurodegenerative diseases and tumours. *Cell Biosci., 10(1)*:54, 2020.

Xu, Y.; Wang, X.; Liu, W. & Lu, W. Thrombin-activated platelet-rich plasma enhances osteogenic differentiation of human periodontal ligament stem cells by activating SIRT1-mediated autophagy. *Eur. J. Med. Res., 26(1)*:105, 2021.

Yang, X.; Yang, J.; Lei, P. & Wen, T. LncRNA MALAT1 shuttled by bone marrow-derived mesenchymal stem cells-secreted exosomes alleviates osteoporosis through mediating microRNA-34c/SATB2 axis. *Aging (Albany NY), 11(20)*:8777-91, 2019.

Yang, Y.; Liu, Y.; Wang, Y.; Chao, Y.; Zhang, J.; Jia, Y.; Tie, J. & Hu, D. Regulation of SIRT1 and Its Roles in Inflammation. *Front. Immunol., 13*:831168, 2022.

Yu, J. S. & Cui, W. Proliferation, survival and metabolism: the role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination. *Development, 143(17)*:3050-60, 2016.

Zhao, P.; Xiao, L.; Peng, J.; Qian, Y. Q. & Huang, C. C. Exosomes derived from bone marrow mesenchymal stem cells improve osteoporosis through promoting osteoblast proliferation via MAPK pathway. *Eur. Rev. Med. Pharmacol. Sci., 22(12)*:3962-70, 2018a.

Zhao, X. E.; Yang, Z.; Zhang, H.; Yao, G.; Liu, J.; Wei, Q. & Ma, B. Resveratrol promotes osteogenic differentiation of canine bone marrow mesenchymal stem cells through Wnt/beta-catenin signaling pathway. *Cell. Reprogram., 20(6)*:371-81, 2018b.

Zhou, D. D.; Luo, M.; Huang, S. Y.; Saimaiti, A.; Shang, A.; Gan, R. Y. & Li, H. B. Effects and mechanisms of resveratrol on aging and age-related diseases. *Oxid. Med. Cell. Longev., 2021*:9932218, 2021.

Zhou, T.; Yan, Y.; Zhao, C.; Xu, Y.; Wang, Q. & Xu, N. Resveratrol improves osteogenic differentiation of senescent bone mesenchymal stem cells through inhibiting endogenous reactive oxygen species production via AMPK activation. *Redox Rep., 24(1)*:62-9, 2019.

Corresponding author:
Feng Zhu
Department of Orthopedics
The 904th Hospital of Joint Logistic Support Force of PLA
Wuxi
Jiangsu 214100
CHINA

E-mail: 13961787144@163.com