# Eldecalcitol Plays a Role in Postmenopausal Osteoporosis through Mir-151a-3p/Socs5 Pathway

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**Objective: This study set out to explore the specific mechanism of Eldecalcitol in postmenopausal osteoporosis (PMOP) and its relationship with miR-151a-3p/SOCS5 pathway. Methods: Forty-five rats were randomly and equally divided into sham operation group (SOG), model control group (MCG) and Eldecalcitol group (EG). miR-151a-3p, SOCS5 and bone mineral density (BMD) levels in each group were detected. MC3T3-E1 cells were modeled and divided into control group (CG), model group (MG) and EG. miR-151a-3p-inhibitor and pcDNA3.1-SOCS were transfected into model cells. miR-151A-3P, SOCS5, RANKL and OPG levels as well as cell activity of cells in each group were observed. Results: Eldecalcitol intervention in rats can reduce BMD reduction caused by PMOP, reduce the miR-151a-3p level and increase the SOCS5 level. Cell experiments found that Eldecalcitol intervention can improve cell activity, inhibit the miR-151a-3p level and promote the SOCS5 expression, all of which can improve bone resorption of model cells, increase cell activity, inhibit the RANKL level and promote the OPG level. Conclusion: Eldecalcitol plays a role in PMOP by inhibiting miR-151a-3p and promoting the SOCS5 level.**

**Keywords: Eldecalcitol, miR-151a-3p, SOCS5, postmenopausal osteoporosis (PMOP)**

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## INTRODUCTION

Osteoporosis is a common chronic metabolic bone disease, and patients are prone to fracture, which is a frequent public health problem all over the world [1]. In osteoporosis patients, the microstructure of bone tissue will be damaged and the bone mass will be reduced. Patients often have their osteoblast function inhibited, and osteoclasts will be activated and their activity will be enhanced, resulting in increased bone fragility and fracture susceptibility of patients [2, 3]. In China, with the aging of society and the change of lifestyle, the morbidity of osteoporosis is gradually increasing. At present, the total reported morbidity is about 27.96%, and the prevalence rate of women is 25.41%, which is significantly higher than that of men (15.33%) [4, 5]. This is often because women after menopause lack the estrogen, which will accelerate the bone transfer and net bone loss of women, thus causing PMOP to become the most typical form of osteoporosis. Therefore, PMOP is a disease requiring attention [6,7].

Eldecalcitol is an active vitamin D3 analogue, which can inhibit bone resorption by osteoclasts, reduce bone metastasis, improve bone mass and bone strength, and form new bone [8]. Vitamin D3 preparation can improve osteoporosis by increasing BMD and bone strength, while Eldecalcitol has better effect on improving the two than other vitamin D3 preparations, and its safety has no difference [9]. In many studies, Eldecalcitol has been recorded to have a good efficacy on PMOP [10, 11], but the specific mechanism of its action is still not fully understood.

JAK2/STAT3 is a signaling pathway that can affect the formation and function of osteoclasts [12, 13]. Zhu et al. [14] found that hypoxia inducible factor 1α could enhance osteoclast differentiation mediated by osteoclasts through activating the JAK2/STAT3 pathway. Fu et al. [15] found that miR-151a-3p activated JAK2/STAT3 signaling pathway by targeting SOCS5, thus reducing BMD of ovariectomized osteoporosis rats and promoting

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the development of osteoporosis. miR-151A-3P/SOCS5 is also expected to become a potential therapeutic approach for PMOP. However, it'sunclear whether the regulation of miR-151A-3P/SOCS5 pathway is involved in the treatment mechanism of Eldecalcitol.

Hence, this study hopes to provide the clinical basis and direction by observing the effect of Eldecalcitol on miR-151A-3P/SOCS5 pathway and the mechanism of its role in PMOP.

## DATA AND METHODS

### Culture of Animal Models

Forty-five healthy female SD rats, 10-12 weeks old, 200-250 g, license key: SCXK (Shanghai) 2017-0011, were randomized into 3 groups (MCG, SCG and EG), 15 rats in each group. The rats were given free food and drinking water, 12h/12h light and dark cycle, and maintained at 21±2℃ for 7 days to adapt to the laboratory environment. The research was approved by the Animal Ethics Committee and the experimental process followed the principles of animal protection and use. On the 8th day, the rats in the MCG and the EG were subjected to modeling operation. Modeling method [16]: The rats were anesthetized with 1-2% isoflurane, ventilated by tracheal intubation, and subjected to midline longitudinal incision. Then, bilateral ovaries were carefully exposed and excised. After that, they were sutured and repaired. The SOG was subjected to the same surgical procedures, but the ovaries were not excised. Rats were fed for 4 weeks after operation, and Eldecalcitol was orally administered to the EG at a dose of 25 ng/kg/day, while the other two groups were orally administered with the same amount of normal saline, and they were killed 4 weeks after intervention. They were killed under deep anesthesia, bilateral femurs were taken out and BMD was detected by dual-energy X-ray BMD instrument.After 5 mL venous blood was collected and centrifuged (1500 ×g, 4℃, 10 min), serum was separated out and stored at -80℃.

### Cell Culture

MC3T3-E1 cells were purchased from BNCC284107. The cells were placed in DMEM medium (10% calf serum, 1% penicillin/streptomycin) and cultured in incubator (37℃, 5%$CO_2$). They were divided into CG, MG and EG. Altogether 50 mg/L ascorbic acid and 10 mmβ glycerophosphate were added to the cells of the MG and the EG to induce osteogenic differentiation, and $10^{-7}$ M Eldecalcitol was treated 24 h in the EG. miR-151a-3p-Inhibitor was transfected into MC3T3-E1 cells for 48 h, the miR-151A-3P expression was inhibited by Lipofectamine 2000, and the cDNA of SOCS5 was cloned into expression vector pcDNA3.1 to construct over-expression vector of pcDNA3.1-SOCS5 [17].

### Kits and Instruments

Main kits and instruments were as follows: DMEM, PBS, fetal bovine serum, Penicillin-Streptomycin (Gibco, USA, 10566024, 10010010023, 2640044, 15070063), RIPA reagent, BCA protein reagent, ECL kit, trypsin, Lipofectamine™ 2000 Transfection Reagent (Thermo Fisher Scientific , USA, 89900, 23250, 32209, 90059, 11668019), TransScript Green miRNA Two-Step qRT-PCR SuperMix, TransScript II Green Two-Step qRT-PCR SuperMix (TransGen Biotech, Beijing, China,AQ202-01, AQ301-01), PcDNA3.1(Invitrogen, Carlsbad, California, USA), dual-luciferase reporter gene detection kit (Solarbio, D0010, Beijing, China), isoflurane (Abbott, Shanghai, China), Eldecalcitol (MedChemexpress, HY-A0020, USA), PCR instrument (ABI, 7500, USA), dual-energy X-ray BMD instrument detection (MEDILINK, MEDIX-90, France), microplate reader (BioTek, PerkinElmer, USA). All primers were designed and synthesized by Shanghai GenePharma Co., Ltd.

### RT-qPCR Analysis

The total RNA was extracted from the obtained cells and serum by Trizol reagent, and thenthe purity, concentration and integrity were detected by ultraviolet spectrophotometer and agarose gel electrophoresis. miR-151a-3p and SOCS5 reverse transcription are strictly carried out in the light of the instructions of the kit. Amplification conditions were as below: PCR reaction conditions: pre-denaturation at 94℃ for 30 s, denaturation at 94℃ for 5 s, annealing extension at 60℃ for 30 s, a total of 40 cycles. Each sample was supplied with 3 repeated wells, and the experiment was conducted 3 times. U6 and GAPDH were used as internal parameters, and the data were analyzed via $2^{-\Delta\Delta ct}$.

### Western Blot Test

The total protein was extracted from cultured cells by RIPA lysis, and its concentration was determined by BCA kit. We conducted 12% SDS-PAGE electrophoresis separation, transfered membrane to PVDF membrane after ionization, and then placed the transfer membrane in 5% defatted milk for sealing. Afterwards, we carried out immune reaction, incubated the membrane with 1: 1000 primary antibody at 4℃ all night long, washed the membrane to remove the primary antibody, and supplemented 1: 1000 horseradish peroxidase labeled goat anti-rabbit secondary antibody; it was then incubated 1 h at 37℃, rinsed 3 times with PBS, for 5 min each time. The protein bands on the membrane developed in a dark room

using the enhanced chemiluminescence reagent (ECL), and the excess liquid was absorbed with a filter paper. The luminescent protein bands were scanned and the gray value was analyzed via Quantity One. The relative expression level of each protein = the gray value of the target protein band / the gray value of the βActin protein band.

### CCK-8 Detection

Cell viability was detected by CCK-8 method. Cells were adjusted to $4*10^6$, inoculated on 96-well plates, and then cultured for 48 h. There were 3 duplicated wells in each group. Altogether 10 μL CCK-8 reagent was supplemented to each well, and the culture was terminated after one-hourincubation at 37℃. The absorbance (A) value of each well at 450 nm wavelength was measured with an enzyme reader within 15 min.

### Luciferase Reporter Gene Assay

Targetscan7.2 was used to predict the target gene downstream of miR-151a-3p. Lipofectamine™ 2000 kit was used to co-transfect WT or Mut-SOCS5 3'UTR plasmid with miR-151a-3p inhibitor or negative control (NC) into MC3T3-E1 cells. Forty-eight hours after transfection, luciferase activity was detected via dual-luciferase reporter gene detection kit.

### Statistical Methods

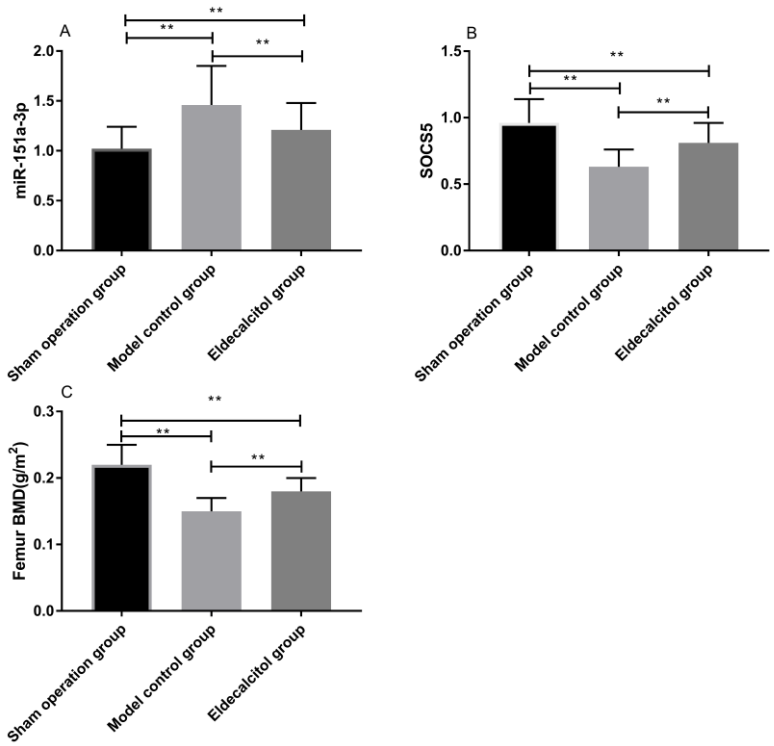
The collected data were statistically analyzed via SPSS20.0 (Chicago SPSS Co., Ltd.) medical statistical analysis software, and the pictures were drawn via GraphPad Prism 7 (San Diego GraphPad Software Co., Ltd.). The measurement data were represented by mean±standard deviation (Meas±SD). Comparison between the two groups was under independent-samples t test, comparison between multiple groups was checked by one-way analysis of variance and expressed as f, and post hoc pairwise comparison was under LSD-t test. There was statistical difference when $P < 0.05$.

## RESULTS

### Effect of Eldecalcitol on Rats

By comparing miR-151a-3p, SOCS5 and BMD of rats in each group, we found that miR-151a-3p in the MCG and the EG was dramatically higher than that in the SOG, SOCS5 and BMD were markedly lower than those in the SOG, miR-151a-3p in the EG was obviously lower than that in the MCG, and SOCS5 and BMD were obviously higher than those in the MCG. (Figure 1)

**Figure 1**
**Effect of Eldecalcitol on rats**



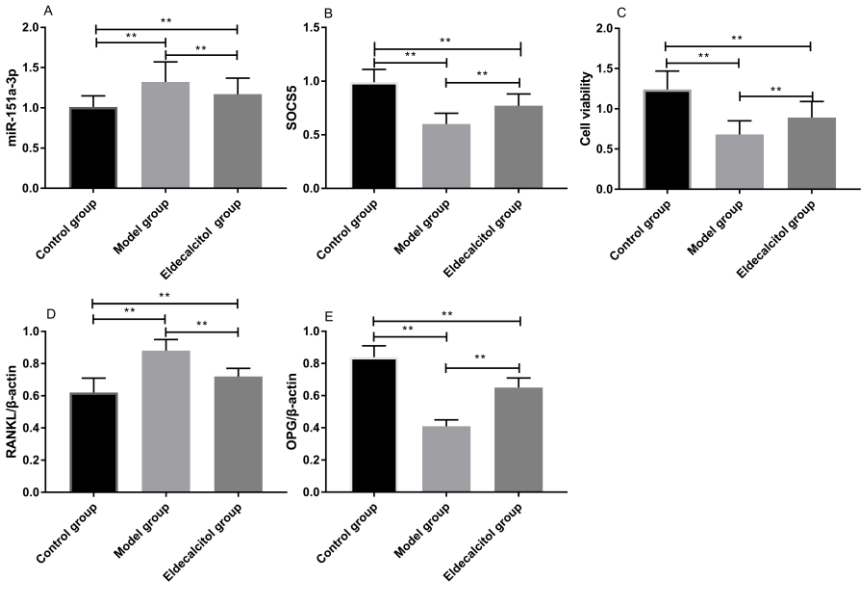

A. miR-151a-3p in model rats is remarkably higher than that in normal rats, and its expression reduces after eldecalcitol treatment. B. SOCS5 expression in model rats is remarkably lower than that in normal rats, and it increases after Eldecalcitol treatment. C. BMD of femoral head in model rats isremarkably lower

than that in normal rats, and it increases after Eldecalcitol treatment.

## Effect of Eldecalcitol on Model Cells

Comparing the miR-151a-3p expression, SOCS5, cell viability, RANKL and OPG of cells in each group, we found that miR-151a-3p and RANKL in the MG and the EG were dramatically higher than those in the CG, SOCS5, cell viability and OPG were remarkably lower than those in the CG, and miR-151a-3p and RANKL in the EG were markedly lower than those in the MG, and SOCS5, cell viability and OPG were significantly higher than those in the CG. (Figure 2)

**Figure 2**
**Effect of Eldecalcitol on model cells**

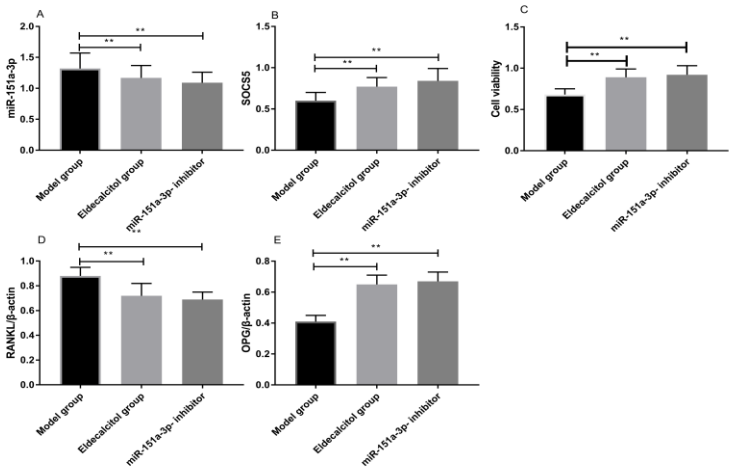



A. miR-151a-3p in model cells is dramatically higher than that in normal cells, and its expression decreases after eldecalcitol treatment. B. SOCS5 expression in model cells is dramatically lower than that in normal cells, and increases after Eldecalcitol treatment. C. Cell activity of model cells is dramatically lower than that of normal cells, and its expression increases after Eldecalcitol treatment. D. RANKL expression in model cells is dramatically higher than that in normal cells, and it decreases after Eldecalcitol treatment. E.OPG expression of model cells is dramatically lower than that of normal cells, and it increases after Eldecalcitol treatment.

## Effect of Inhibition of miR-151A-3P Cells

After comparing the expression levels of miR-151a-3p, SOCS5, cell viability, RANKL and OPG of cells in each group, we found that miR-151a-3p and RANKL in the MG were dramatically higher than those in the EG and miR-151a-3p-inhibitor group, while SOCS5, cell viability and OPG were dramatically lower than those in the EG and the miR-151a-3p-inhibitor group. (Figure 3)

**Figure 3**
**Effect of inhibition of miR-151a-3p cells**

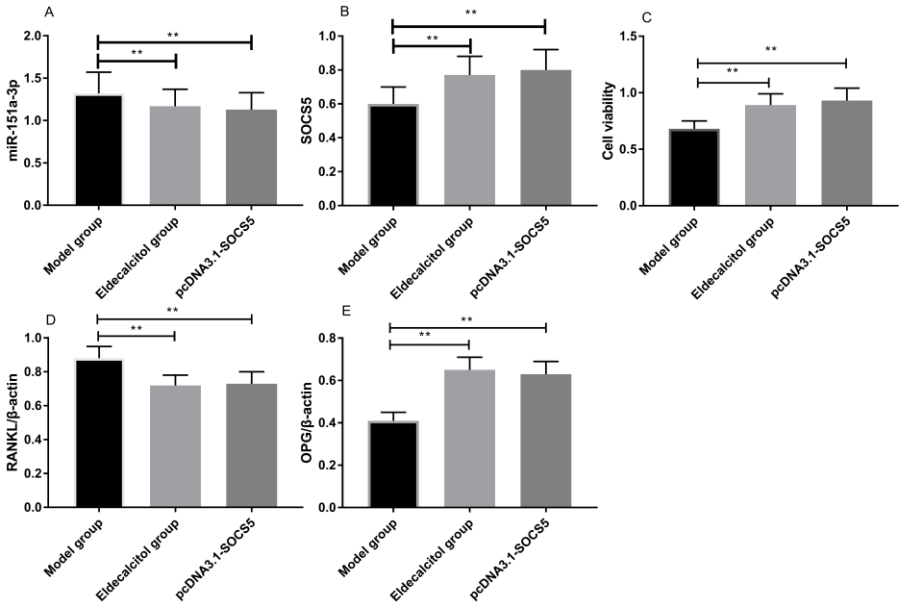



A. miR-151a-3p reduces significantly after miR-151a-3p inhibitor is transfected into cells B. SOCS5 expression increases significantly after cells are transfected with miR-151a-3p inhibitor. C. The cell activity increases significantly after transfecting miR-151a-3p inhibitor. D. RANKL decreases significantly after cells are transfected with miR-151a-3p inhibitor. E. OPG expression increases significantly after cells are transfected with miR-151a-3p inhibitor.

## Effect of Promotion of SOCS5 on Cells

After comparing the expression levels of miR-151a-3p, SOCS5, cell viability, RANKL and OPG of cells in each group, we found that miR-151a-3p and RANKL in the MG were dramatically higher than those in the EG and the pcDNA3.1-SOCS5 group, while SOCS5, cell viability and OPG were dramatically lower than those in the EG and the pcDNA3.1-SOCS5 group. (Figure 4)

**Figure 4**
**Effect of promotion of SOCS5 on cells**

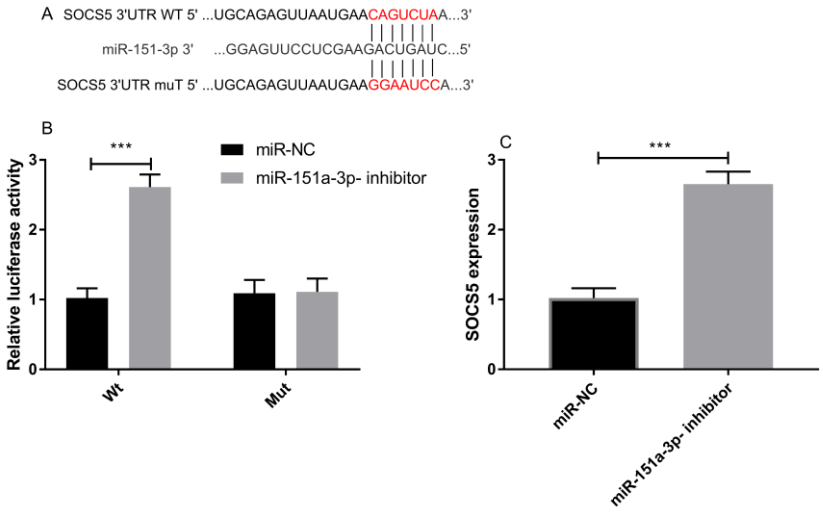



A. miR-151a-3p reduces significantly after cells are transfected with SOCS5 over-expression plasmid. B. SOCS5 expression increases significantly after transfecting SOCS5 over-expression plasmid. C. Cell activity increases significantly after transfecting SOCS5 over-expression plasmid. D. RANKL decreases significantly after transfecting SOCS5 over-expression plasmid. E. OPG expression increases significantly after transfecting SOCS5 over-expression plasmid.

### Dual-luciferase Report

To further verify the relationship between miR-151a-3p and SOCS5, we first predicted the target gene downstream of miR-151a-3p by Targetscan7.2 and found that there was a targeted binding site between SOCS5 and miR-151a-3P. Hence, we conducted a dual-luciferase activity test and found that the SOCS5 Wt luciferase activity reduced dramatically after transfection of miR-151a-3p, but had no remarkable effect on SOCS5 Mut luciferase activity. The detection found that the SOCS5 expression increased obviously after transfecting miR-151a-3p-inhibitor. (Figure 5)

**Figure 5**
**Dual-luciferase report**




A. There is a targeted binding site between miR-151a-3p and SOCS5. B. Results of dual-luciferase report C. Protein expression of SOCS5 increases significantly after transfection of miR-151a-3p-inhibitor.

## DISCUSSION

microRNA (miR) is a kind of microRNA that has attracted much attention in recent years. It has been found that miR can participate in the development and progression of many diseases, so it is expected to become a new diagnostic prediction indicator and a potential therapeutic target for some diseases [18, 19]. However, miR can often affect the progress and development of diseases by regulating the expression of target genes or target proteins downstream of miR. Osteoporosis is also regulated by some miR and target genes, such as miR-320a high expression in PMOP, which can inhibit osteoblast apoptosis by reducing the level of MAP9 downstream of miR-320a [20, 21]. Hence, these miR and target genes are also expected to become potential therapeutic targets and participate in the treatment process. Eldecalcitol is an active vitamin D3 analogue, which has anti-absorption properties, improves osteoblast activity and inhibits osteoblast differentiation [22].

In our study, we first investigated the effect of Eldecalcitol on PMOP rats, and found that the femoral head BMD of rats decreased significantly after ovariectomy;after 4 weeks of Eldecalcitol intervention, it improved significantly, which was lower than that of ovariectomized rats, but significantly higher than that of those without intervention; this also suggested that Eldecalcitol could improve the symptoms of PMOP. At the same time, we also found that compared with normal rats, the miR-151a-3p and SOCS5 expression levels in osteoporosis rats increased significantly, while the miR-151a-3p expression decreased significantly and SOCS5 increased significantly through Eldecalcito's intervention. Hence, we suspect that miR-151a-3p and SOCS5 may participate in Eldecalcito's restoration of osteoporosis. Next, we detected RANKL and OPG proteins of Eldecalcito-treated MC3T3-E1 cells. RANKL and OPG are proteins related to osteoblasts and osteoclasts. OPG can inhibit the formation of osteoclasts and induce apoptosis of osteoclasts, thus inhibiting bone resorption, enhancing BMD and bone strength. RANKL can promote bone resorption by prolonging the life of osteoclasts and inhibiting their apoptosis [23, 24]. Our study found that after MC3T3-E1 cells induced osteogenic differentiation, RANKL expression increased, OPG expression decreased,

and cell activity decreased markedly. The reduced RANKL expression, the increased OPG expression and the significantly increased cell activity of Eldecalcito treated model cells indicated Eldecalcito's repair effect on bone cells, and we also discovered that the miR-151a-3p expression reduced dramatically and SOCS5 expression increased dramatically.

In order to verify whether miR-151a-3p and SOCS5 participate in the repair process of PMOP, we transfected miR-151a-3p inhibitor into model cells, and found that miR-151a-3p expression decreased, SOCS5 expression significantly increased, RANKL expression decreased, OPG expression increased, and cell activity increased. Simultaneously, we also transfected the model cells with SOCS5 over-expression plasmid, and discovered that the SOCS5 expression in the transfected cells increased significantly, while the miR-151a-3p expression decreased, RANKL expression decreased and OPG expression increased;this also showed that inhibiting the miR-151a-3p expression or promoting SOCS5 could promote the cell activity of model cells and inhibit the bone absorption process. Finally, we found that miR-151a-3p had a certain relationship with SOCS5 in the repair process of Eldecalcito, so we verified the relationship by dual-luciferase report. We found that the activity of SOCS5 Wt luciferase decreased obviously after miR-151a-3p was inhibited, but it had no significant effect on SOCS5 Mut luciferase activity, which also showed that miR-151a-3p had a targeting relationship with SOCS5.

However, there are still some deficiencies in this study. First of all, the doses used in our study are all based on previous studies. Therefore, the influence of different doses on the study has not been discussed. Secondly, some studies mentioned that Eldecalcitol might cause some adverse reactions such as hypercalcemia in the treatment process [25, 26], but adverse reactions were found in our study and in rats, so we hoped that clinical experiments could be carried out in the follow-up study to observe the effect of Eldecalcitol and improve our view. In the end, we have not predicted and verified the specific signal paths affected, and also hopes to carry out corresponding research in the future.

In conclusion, Eldecalcitol plays a part in PMOP by inhibiting miR-151a-3p and promoting the SOCS5 level.

## REFERENCES

1. Kanis J A, Cooper C, Rizzoli R, et al. Executive summary of the European guidance for the diagnosis and management of osteoporosis in postmenopausal women[J]. Calcified tissue international, 2019, 104(3): 235-238.
2. Peck W A. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis[J]. Am J Med, 1993, 94(6): 646-650.
3. Faienza M F, Chiarito M, D'amato G, et al. Monoclonal antibodies for treating osteoporosis[J]. Expert opinion on biological therapy, 2018, 18(2): 149-157.
4. Wang Y, Tao Y, Hyman M E, et al. Osteoporosis in china[J]. Osteoporosis international, 2009, 20(10): 1651-1662.
5. Chen P, Li Z, Hu Y. Prevalence of osteoporosis in China: a meta-analysis and systematic review[J]. BMC public health, 2016, 16(1): 1039.
6. Yan G, Huang Y, Cao H, et al. Association of breastfeeding and postmenopausal osteoporosis in Chinese women: a community-based retrospective study[J]. BMC women's health, 2019, 19(1): 1-7.
7. Yang Y, Li C, Wang J, et al. Ubiquitylomes Analysis of the Whole blood in Postmenopausal Osteoporosis Patients and healthy Postmenopausal Women[J]. Orthopaedic surgery, 2019, 11(6): 1187-1200.
8. Hasegawa T, Yamamoto T, Sakai S, et al. Histological effects of the combined administration of eldecalcitol and a parathyroid hormone in the metaphyseal trabeculae of ovariectomized rats[J]. Journal of Histochemistry & Cytochemistry, 2019, 67(3): 169-184.
9. Saito S, Sugo Y, Tsuburai T, et al. Activated vitamin D3 formulations can be safely used as concomitant medication for prevention of denosumab‐induced hypocalcemia in women with postmenopausal osteoporosis[J]. Journal of Obstetrics and Gynaecology Research, 2019, 45(4): 908-914.
10. Iba K, Sonoda T, Takada J, et al. Further significant effects of eldecalcitol on bone resorption markers and bone mineral density in postmenopausal osteoporosis patients having undergone long-term bisphosphonate treatment[J]. Journal of bone and mineral metabolism, 2017, 35(2): 171-176.
11. Saito K, Miyakoshi N, Matsunaga T, et al. Eldecalcitol improves muscle strength and dynamic balance in postmenopausal women with osteoporosis: an open-label randomized controlled study[J]. Journal of bone and mineral metabolism, 2016, 34(5): 547-554.
12. Li C, Xu L, Jian L, et al. Stattic inhibits RANKL-mediated osteoclastogenesis by suppressing activation of STAT3 and NF-κB pathways[J]. International immunopharmacology, 2018, 58: 136-144.
13. Kim M H, Jung K, Nam K H, et al. Salvia plebeia R. Br. inhibits signal transduction of IL-6 and prevents ovariectomy-induced bone loss by suppressing osteoclastogenesis[J]. Archives of pharmacal research, 2016, 39(12): 1671-1681.
14. Zhu J, Tang Y, Wu Q, et al. HIF‐1α facilitates osteocyte‐mediated osteoclastogenesis by activating JAK2/STAT3 pathway in vitro[J]. Journal of cellular physiology, 2019, 234(11): 21182-21192.
15. Fu Y, Xu Y, Chen S, et al. MiR-151a-3p Promotes Postmenopausal Osteoporosis by Targeting SOCS5 and Activating JAK2/STAT3 Signaling[J].

Rejuvenation research, 2019.
16. Xu L, Shen L, Yu X, et al. Effects of irisin on osteoblast apoptosis and osteoporosis in postmenopausal osteoporosis rats through upregulating Nrf2 and inhibiting NLRP3 inflammasome[J]. Experimental and Therapeutic Medicine, 2020, 19(2): 1084-1090.
17. Bu J, Du J, Shi L, et al. Eldecalcitol effects on osteoblastic differentiation and function in the presence or absence of osteoclastic bone resorption[J]. Experimental and therapeutic medicine, 2019, 18(3): 2111-2121.
18. He Y, Chen C, Shi Z. The biological roles and clinical implications of microRNAs in clear cell renal cell carcinoma[J]. Journal of cellular physiology, 2018, 233(6): 4458-4465.
19. Moridikia A, Mirzaei H, Sahebkar A, et al. MicroRNAs: Potential candidates for diagnosis and treatment of colorectal cancer[J]. Journal of Cellular Physiology, 2018, 233(2): 901-913.
20. Kong Y, Nie Z K, Li F, et al. MiR-320a was highly expressed in postmenopausal osteoporosis and acts as a negative regulator in MC3T3E1 cells by reducing MAP9 and inhibiting PI3K/AKT signaling pathway[J]. Experimental and molecular pathology, 2019, 110: 104282.
21. Wang W W, Yang L, Wu J, et al. The function of miR-218 and miR-618 in postmenopausal osteoporosis[J]. European review for medical and pharmacological sciences, 2017, 21(24): 5534-5541.
22. Bu J, Du J, Shi L, et al. Eldecalcitol effects on osteoblastic differentiation and function in the presence or absence of osteoclastic bone resorption[J]. Experimental and therapeutic medicine, 2019, 18(3): 2111-2121.
23. Xu X J, Shen L, Yang Y P, et al. Serum βcatenin levels associated with the ratio of RANKL/OPG in patients with postmenopausal osteoporosis[J]. International journal of endocrinology, 2013, 2013.
24. Zhao Y, Wang H L, Li T T, et al. Baicalin Ameliorates Dexamethasone-Induced Osteoporosis by Regulation of the RANK/RANKL/OPG Signaling Pathway[J]. Drug Design, Development and Therapy, 2020, 14: 195.
25. Aihara S, Yamada S, Oka H, et al. Hypercalcemia and acute kidney injury induced by eldecalcitol in patients with osteoporosis: a case series of 32 patients at a single facility[J]. Renal failure, 2019, 41(1): 88-97.
26. Saito H, Kakihata H, Nishida Y, et al. The safety and effectiveness profile of eldecalcitol in a prospective, post-marketing observational study in Japanese patients with osteoporosis: interim report[J]. Journal of bone and mineral metabolism, 2017, 35(4): 456-463.