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Sesamin Promotes Osteoblastic Differentiation and Protects Rats from Osteoporosis

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Background: Osteoporosis is a common osteopathy, resulting in fractures, especially in elder people. Sesamin has many pharmacological effects, including supplying calcium. However, how sesamin might prevent osteoporosis is still under study.

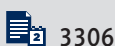
Material/Methods: Bone marrow stromal cells (BMSCs) extracted from rat femur were induced for osteoblastic differentiation. Cell proliferation, alkaline phosphatase (ALP), osterix (OSX), SRY-box 9 (SOX9), runt-related transcription factor 2 (RUNX2), osteocalcin (OCN), β -catenin, low density lipoprotein receptor-related protein 5 (LRP5), and glycogen synthase kinase-3 β (GSK-3 β) levels in BMSCs were detected in the presence or absence of sesamin (1 μ M or 10 μ M). In addition, FH535 (1 μ M) was used to silence Wnt/ β -catenin *in vitro*. Ovariectomized (OVX) rats were established and intragastrically administrated sesamin (80 mg/kg), and then the rat bones were analyzed by micro-computed tomography. Osteocalcin and collagen type I were measured in the rat femurs.

Results: Sesamin had no influence on BMSC proliferation. Higher sesamin concentration promoted Wnt/ β -catenin activity and enhanced more expressions of ALP, OSX, SOX9, RUNX2, and OCN, gradually and significantly ($P < 0.05$). Silencing Wnt/ β -catenin weakened the enhancement on RUNX2 and OCN expression. Sesamin (80 mg/kg) promoted bone structure in ovariectomized rats, and significantly enhanced osteocalcin and collagen type I expression ($P < 0.05$).

Conclusions: Sesamin promoted osteoblastic differentiation of rat BMSCs by regulating the Wnt/ β -catenin pathway, and improved rat bone structure. Sesamin could have therapeutic and preventive effects on osteoporosis.

MeSH Keywords: **Bone Marrow Cells • Cell Dedifferentiation • Osteoblasts • Osteoporosis • Sesame Oil • Wnt Signaling Pathway**

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Background

Osteoporosis is defined as a skeletal disorder with characteristics of compromising bone strength and predisposing bones to increased fracture risk, which are significant health risks to all people worldwide [1]. Osteoporosis has been reported to affect 15% of the white people in their 50s, and to affect 70% of those older than 80 years of age [2,3]. Osteoporosis incidence varies by gender and ethnicity, and has higher morbidity in females compared to males [4]. In the developed world, 2% to 8% of males and 9% to 38% of females are affected [5]. In the United States, 10 million individuals have osteoporosis, and more than 18 million people have low bone mass, which can lead to high risk for osteoporosis [6]. The International Osteoporosis Foundation predicted that by 2050, more than 50% of all osteoporotic fractures would occur in east and south-east Asia [7]. Especially in elder women, due to the lower level of estrogen after menopause, osteoporosis, usually with minor or no symptoms, is the common reason for broken bones [8]. Physiological, environmental, and modifiable lifestyle factors exert important influences on bone mass, although genetic factors are a primary risk factor. Many diseases or conditions are also risk factors for osteoporosis, including alcoholism, hyperthyroidism, anorexia, surgical removal of the ovaries, kidney disease [8]. Some medications can increase the incidence of bone loss such as chemotherapy, antiseizure medications, proton pump inhibitors, glucocorticosteroid medication, and selective serotonin reuptake inhibitors [8]. To prevent osteoporosis, a proper diet must be maintained. In addition, lifestyle changes, such as more exercise and stopping smoking and drinking alcohol, are also helpful, while medications that increase the rate of bone loss should also be avoided [8–11].

Bone marrow is the primary location of hematopoiesis; bone marrow is composed of hematopoietic cells, marrow adipose tissue, and supportive stromal cells [12]. Stroma of the bone marrow includes all the tissue not directly involved in the primary bone marrow function of hematopoiesis [13]. Stromal cells provide a microenvironment influencing the functions and differentiation of hematopoietic cells involved in hematopoiesis. Both osteoblasts and osteoclasts are types of bone marrow stromal cells (BMSCs) [14]. Osteoblasts have important roles in bone formation, involving the regulation on related proteins during differentiation such as osteocalcin (OCN), alkaline phosphatase (ALP), NS runt-related transcription factor 2 (RUNX2) [15].

In addition, many signal pathways are activated in osteoblastic differentiation. The Wnt/ β -catenin pathway is the signal transduction pathway, controlling myriad biological phenomena in all animals [16]. Osteoblastic differentiation has been confirmed as being regulated by the Wnt/ β -catenin pathway [17–19]. Here we primarily investigated the activity of Wnt/ β -catenin signal pathway in osteoblastic BMSCs.

Sesamin is a lignan separated from *Fagara* plants. Sesame oil is used as a dietary fat-reduction supplement [20,21]. According to Boulbaroud et al., sesame oil has preventive effects on bone loss, which was confirmed in an ovariectomized (OVX) rat model [22]. However, the mechanisms of sesame oil action on the bone formation process remain unclear. Orawan et al. used sesamin to treat human fetal osteoblast cells and human adipose-derived stem cells, and confirmed that sesamin could trigger osteoblast differentiation by activating the p38 and ERK/MAPK pathways [23]. Although sesamin is used widely as medicine, no evidence has shown the influence of sesamin on osteoblastic differentiation of rat BMSCs, not to mention shown protection or prevention from osteoporosis.

Our study is the first to use sesamin to directly treat BMSCs isolated from rat femur, and the first to measure the osteoblastic differentiation of BMSCs with or without silencing Wnt/ β -catenin. In addition, we established an OVX rat model, and carried out the intragastric administration of sesamin in rats. The therapeutic effect of sesamin on osteoporosis was analyzed.

Material and Methods

Sesamin preparation

Sesamin (S9314; Sigma-Aldrich LLC., USA) was dissolved in dimethylsulfoxide (DMSO; Sigma) and administrated as a 14.22 mM solution and stored at -20°C .

Cell culture

Sprague Dawley (SD) rats (4-weeks old) were bought from the Medical Laboratory Animal Center of the Second Affiliated Hospital of Inner Mongolia Medical University. All the animal experiments were performed in accordance with National Institutes of Health (NIH) Guidelines on Principles of Laboratory Animal Care (NIP Publication 85-23, revised 1996) and approved by the Ethic Committee of Laboratory Animal of Inner Mongolia Medical University (China), dated July 15, 2017; project serial number 2017032.

BMSCs were isolated from rat femur. The rat femur was separated and removed from surrounding soft tissue. The bone marrow was washed with α -Minimum Essential Medium (α -MEM; Thermo Fisher Scientific, Ltd., Shanghai, China). The bone marrow was cut into pieces and cultured with α -MEM containing 10% fetal bovine serum (FBS; Thermo), 100 U/mL penicillin, and 10 mg/mL streptomycin sulfate (Sigma). Non-adherent cells were removed, and adherent BMSCs were cultured and expanded for further experiments. Adherent BMSCs were passaged when cell culture confluence was above 95%. Then 1–3 generations of BMSCs were used for the study experiments.

Osteoblastic differentiation of BMSCs

There were 1×10^4 cells/well of BMSCs cultured in a 6-well plate (Corning Inc., Shanghai, China). BMSCs osteoblastic differentiation was induced with α -MEM containing 10% FBS, 50 μ M ascorbic acid (Sigma), 0.1 μ M DMSO, and 10 mM β -glycerol phosphate (Sigma) for 7 days.

Cell proliferation assay and ALP detection

Osteoblastic BMSCs were cultured in a 96-well plate with a density of 3×10^3 cells/well. After 24 hours, BMSCs were treated with sesamin (1 μ M or 10 μ M) for 3 days; 0.1% DMSO was used as negative control (NC). Cell proliferation assay was performed using the Caspase-8 Colorimetric Assay Kits (Abcam PLC., Shanghai, China) according to the product specifications. Optical density (OD) values were measured at 450 nm. Osteoblastic BMSCs were stained with NBT-BCIP[®] solution (Sigma) for detecting ALP in osteoblastic BMSCs according to the product specifications.

Immunohistochemistry for osterix (OSX) in osteoblastic BMSCs

After BMSCs osteoblastic differentiation, 1×10^5 cells/well were seeded in a 6-well plate with a cover slide. After 24 hours, the cells were treated with sesamin (1 μ M or 10 μ M) for 3 days. The cells were fixed with 4% paraformaldehyde (Beyotime Biotechnology, Shanghai, China) at 25°C for 30 minutes. The cells were washed with pre-cooled phosphate-buffered solution (PBS) (Beyotime), shaken at 25°C for 10 minutes, then incubated with 3% H₂O₂/methanol (Boster Biological Technology Co., Ltd., Wuhan, China) at 25°C for 20 minutes, blocked with goat serum (Solarbio Science and Technology Co., Ltd., Beijing, China) at 25°C for 30 minutes. Then incubated with rabbit anti-Sp7/osterix (OSX) antibody (1: 100) (ab22552, Abcam) at 4°C in a wet box, overnight. The cells were washed with pre-cooled PBS, and then incubated with goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (1: 100) (7071, Cell Signaling Technology, Inc., Shanghai, China) at 25°C for 30 minutes. Diaminobenzidine (DAB) (ZSGB-BIO, Beijing, China) and hematoxylin (Beyotime) staining followed and the slides were sealed with resin (Solarbio) and observed using a microscope (BX43; Olympus Optical Co., Ltd., Tokyo, Japan).

Immunofluorescence for SRY-box 9 (SOX9) in osteoblastic BMSCs

After BMSCs osteoblastic differentiation, 1×10^5 cells/well were seeded in a 6-well plate with a cover slide. After 24 hours, the cells were treated with sesamin (1 μ M or 10 μ M) for 3 days. The cells were fixed with 4% paraformaldehyde at 25°C for 30 minutes. Then cells were quenched with 30 mM

glycine (Sigma) in PBS at 25°C for 5 minutes, permeabilized with 0.5% Triton-X (Solarbio) in PBS at 25°C for 5 minutes, blocked with 5% non-fat milk and 2% bovine serum albumin (BSA) (Gen-View Scientific Inc., USA) in PBS at 25°C for 1 hour. Then the cells were incubated with Sox9 rabbit mAb (1: 100; #82630, Cell Signaling Technology, Inc.) at 25°C in a wet box, overnight. Then cells were washed with pre-cooling PBS, and incubated with Fluor[®] 594 goat anti-rabbit (1: 500; ZSGB-BIO) at 25°C for 1 hour in the dark. Cells were stained with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma) and observed with a confocal laser scanning microscopy (FV1000, Olympus). We analyzed the results with Image-Pro Plus software (Media Cybernetics, Rockville, MD, USA).

Blocking Wnt/ β -catenin in osteoblastic BMSCs

FH535 (Sigma) was dissolved in DMSO with the concentration as 10 mM and stored at -2°C. After BMSCs osteoblastic differentiation, 1×10^5 cells/well was seeded in a 6-well plate for 24 hours, and then treated with sesamin (1 μ M or 10 μ M) in the presence or absence of FH535 (1 μ M).

Western blot for RUNX2, OCN, Wnt/ β -catenin pathway in osteoblastic BMSCs

After BMSCs osteoblastic differentiation, 1×10^5 cells/well was seeded in a 6-well plate for 24 hours, and then treated with sesamin (1 μ M or 10 μ M) for 3 days. We then collected the cells and extracted proteins using Pierce[™] Universal Nuclease for Cell Lysis (Thermo) at 4°C for 20 minutes. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 10% separating gel and 5% stacking gel, and then transferred to polyvinylidene difluoride (PVDF) membrane (Merch Millipore Corporation, Shanghai, China), which was blocked with 5% non-fat milk at 25°C for 2 hours; then incubated with mouse anti-RUNX2 antibody (1: 5000; ab76956, Abcam), mouse anti-osteocalcin (OCN) antibody (1: 3000; ab13420, Abcam), mouse anti- β -actin antibody (1: 5000; ab8226, Abcam), rabbit anti-low density lipoprotein receptor-related protein 5 (LRP5) antibody (1: 1000; ab38311, Abcam), mouse anti-glycogen synthase kinase-3 β (GSK-3 β) antibody (1: 2000; ab93926, Abcam), rabbit anti- β -catenin antibody (1: 5000; ab32572, Abcam), respectively, at 4°C, overnight. Goat anti-rabbit or goat anti-mouse secondary antibody (1: 3000; Jackson Immuno Research Laboratories, Inc., USA) was added and incubated at 25°C for 1 hour. Electrochemiluminescence (ECL; Millipore) was used for analysis.

Ovariectomized rats

Eighteen female SD rats, aged 10 months, weighing 280.2 g to 91.5 g, were maintained on a standard rat diet and water ad libitum and kept in an animal chamber maintained at

22±2°C, 50±5% of humidity and 12 hours light-dark cycle. The 18 rats were randomly divided into 3 groups (n=6 per group): the OVX group, the OVX+S (sesamin) group and the Sham group. Rats in the OVX and OVX+S groups underwent bilateral ovariectomy to create OVX models with low estrogen levels. Rats in the Sham group had periovarian fatty tissue exteriorized. Rats in the OVX+S group were administered intragastrically with sesamin (80 mg/kg) once a day between 10:00 am and 12:00 pm. Rats in the OVX and Sham groups were administered intragastrically with 0.9% saline. Continuous feeding was performed for 12 weeks.

Micro-CT measurements of rat femurs

After 12 weeks, all study rats were euthanized with sodium pentobarbital (150 mg/kg) (Sigma). Rat femurs were separated, and the surrounding soft tissue was removed. Femur samples were fixed in 4% paraformaldehyde for 72 hours. The 3-dimensional microarchitecture of the femurs was analyzed with a micro-CT system (μ CT80; SCANCO Medical, AG, Switzerland) with energy settings of 55 kV and 145 μ A, including bone mineral density (BMD), trabecular number (Tb.N), and trabecular spacing (Tb.Sp).

Immunohistochemistry for OCN in rat femurs

Rat femur specimens were prepared for sections by 2 pathologists based on their consensus. Sectioned femur samples were dried at 68°C for 30 minutes. After regular de-waxing and gradual ethanol hydration, sections were incubated with 3% H₂O₂/methanol at 3°C for 10 minutes, and then washed with PBS. The sections were incubated in citrate buffer solution (0.01 M) (Boster) at 9°C for 20 minutes, and then the sections were cooled to 25°C, blocked with goat serum at 3°C for 10 minutes, and then incubated with mouse anti-osteocalcin (OCN) antibody (1: 100; ab13420, Abcam) at 4°C in a wet box, overnight. Then the sections were washed with PBS, and incubated with goat anti-rabbit IgG H&L (HRP) (1: 1000; ab6721, Abcam) at 37°C for 30 minutes. DAB staining was followed at 25°C for 10 minutes. Hematoxylin and eosin (H&E) (Beyotime) staining was carried on at 25°C for 2 minutes. After regular dehydration, the sections were sealed with resin and we analyzed the OD of positive OCN with Image-Pro Plus 6.0.

Immunofluorescence for collagen type I in rat femurs

Rat femur specimens were prepared for sections by 2 pathologists based on their consensus. Sectioned femur samples were incubated with testicular hyaluronidase for 30 minutes to expose collagen epitopes, and then incubated with immunofluorescent agents for 1 hour at 25°C using rabbit anti-collagen type I (1: 500; ab34710, Abcam) antibody. Then Fluor[®]488 goat anti-rabbit (1: 500; ZSGB) was added and sections incubated

at 25°C for 1 hour in the dark. Sections were stained with DAPI and observed with a confocal laser scanning microscopy. Results were analyzed with Image-Pro Plus software.

Statistical analyses

Quantitative data were showed as mean \pm standard deviations ($\chi \pm$ SDs). We analyzed the data using GraphPad "PRISM" software (version 3.0; GraphPad Software, Inc., La Jolla, CA, USA). One-way analysis of variance (ANOVA) or Student's *t*-tests was used to analyze statistically significant differences between groups. *P*<0.05 was significant.

Results

Sesamin without cytotoxicity on BMSCs

BMSCs were treated with sesamin (1 μ M or 10 μ M) after osteoblastic differentiation; 0.1% DMSO was used as the normal control (NC). Figure 1A shows the OD value among the NC group, the 1 μ M of sesamin group, and 10 μ M of sesamin group, with no significant differences (*F*=0.0683, *P*=0.9343). These results indicated that sesamin had no cytotoxicity effect on osteoblastic BMSCs.

Sesamin promoted BMSCs osteoblastic differentiation through Wnt/ β -catenin

Figure 1C shows that without FH535 (1 μ M) silencing, there was significant promotion of sesamin (1 μ M or 10 μ M) on Wnt/ β -catenin, as shown by the increase in β -catenin (*F*=128.60, *P*<0.0001) and LRP5 (*F*=191.3, *P*<0.0001) but a decrease in GSK-3 β (*F*=69.54, *P*<0.0001). Higher sesamin concentration promoted higher Wnt/ β -catenin activity, significantly (*P*<0.05). With FH535 (1 μ M) silencing, Wnt/ β -catenin activity was suppressed, which was demonstrated by no obvious changes in β -catenin, LRP5, or GSK-3 β .

Figure 1B shows the significant promotion of sesamin on RUNX2 (*F*=113.7, *P*<0.0001) and OCN (*F*=89.29, *P*<0.0001) without FH535 (1 μ M) silencing. Higher concentration of sesamin resulted in higher enhanced RUNX2 and OCN expressions, significantly (*P*<0.05). With FH535 silencing, the promotion effect of sesamin on RUNX2 and OCN were both weakened. Although shown as significantly increasing (*P*<0.05), it was not significant when compared to the NC group. Figure 2 shows the significant promotion effect of sesamin on ALP (Figure 2A) (*F*=23.81, *P*<0.0001), OSX (Figure 2B) (*F*=73.65, *P*<0.0001), and SOX9 (Figure 2C) (*F*=139.1, *P*<0.0001). With the higher concentration of sesamin, the promotion effect on BMSCs osteoblastic differentiation was significantly stronger (*P*<0.05).

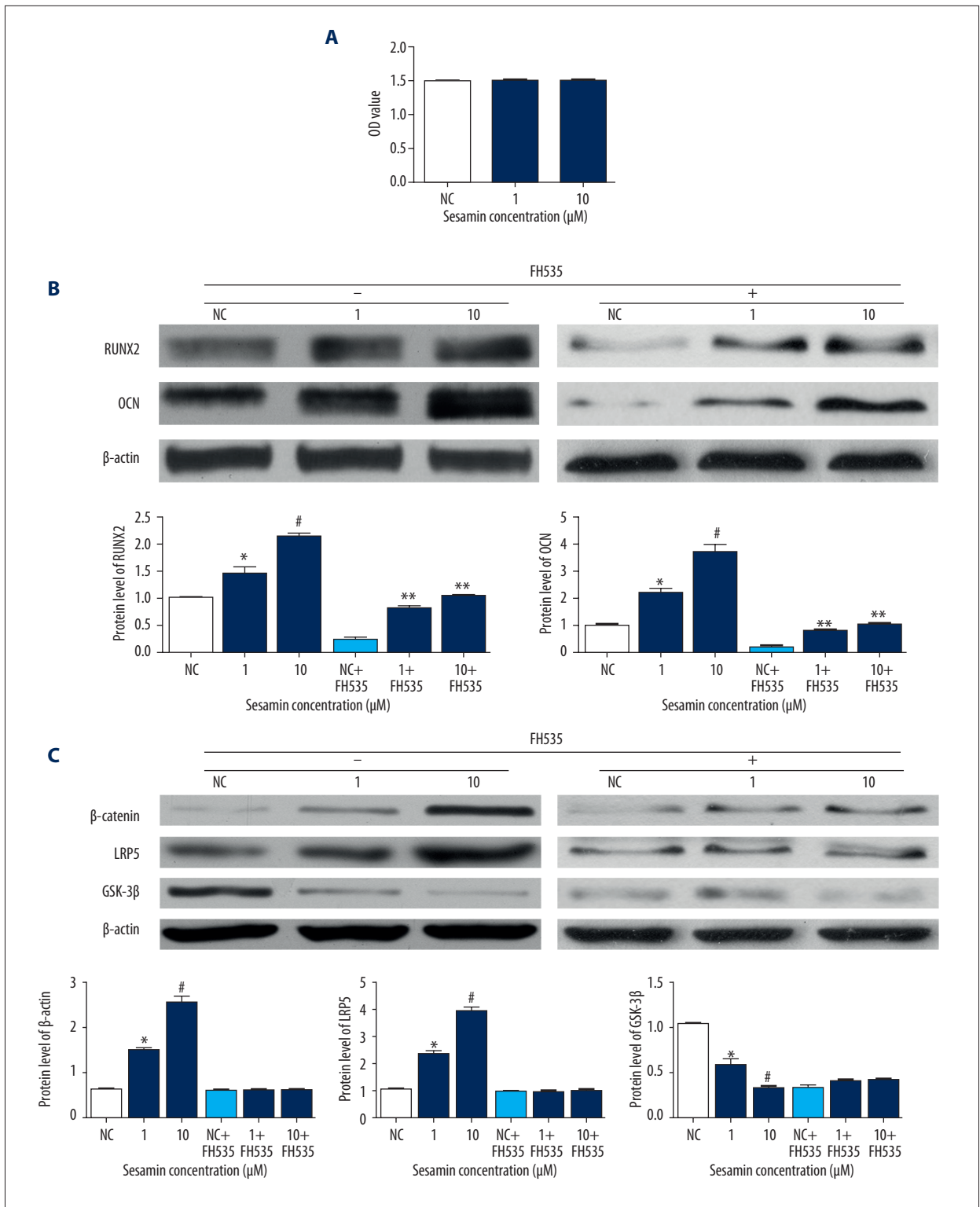


Figure 1. (A) BMSC proliferation; (B) RUNX2 and OCN detection in BMSCs; (C) Wnt/β-catenin pathway detection in BMSCs (NC group, negative control with 0.1% DMSO treatment; group 1 with 1 μM of sesamin treatment; group 10 with 10 μM of sesamin treatment; * comparing to NC group, $P < 0.05$; # comparing to NC group and group 1, $P < 0.05$; ** comparing to NC+FH535 group, $P < 0.05$). BMSCs – bone marrow stromal cells; RUNX2 – runt-related transcription factor 2; OCN – osteocalcin; LRP5 – low density lipoprotein receptor-related protein; GSK-3β – glycogen synthase kinase-3β.

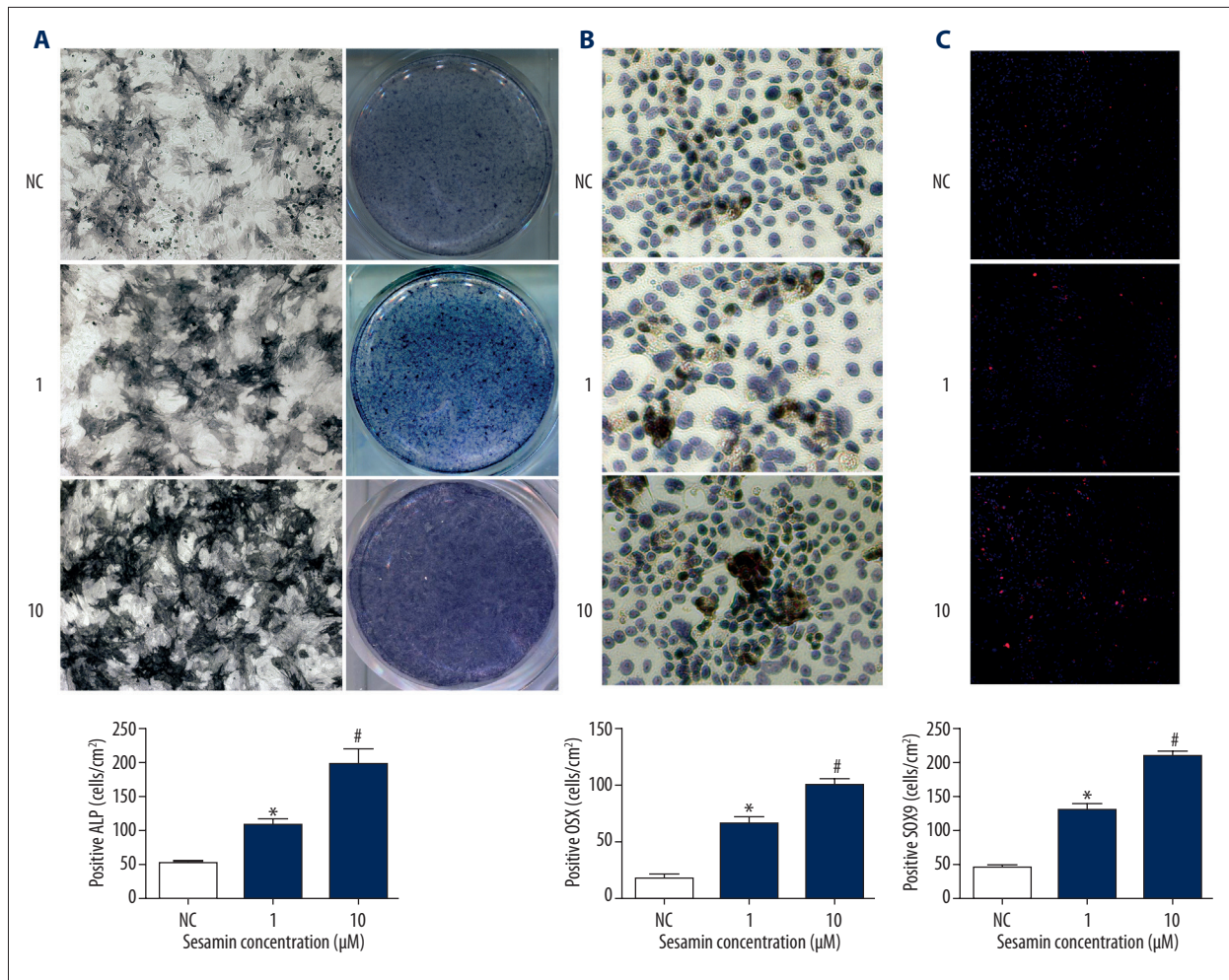


Figure 2. (A) ALP detection in osteoblastic BMSCs; (B) OSX detection in osteoblastic BMSCs; (C) SOX9 detection in osteoblastic BMSCs (NC group, negative control with 0.1% DMSO treatment; group 1 with 1 μM of sesamin treatment; group 10 with 10 μM of sesamin treatment; * comparing to NC group, $P < 0.05$; # comparing to NC group and group 1, $P < 0.05$). ALP – alkaline phosphatase; BMSCs – bone marrow stromal cells; OSX – osterix; SOX9 – SRY-box 9.

These results showed the promotion effect of sesamin on osteoblastic differentiation of BMSCs by activating Wnt/ β -catenin signaling pathway.

Sesamin might prevented osteoporosis

In Figure 3A, micro-CT showed lower BMD ($F=34.84$, $P=0.0003$) and Tb.N ($F=13.12$, $P=0.0037$) but higher Tb.Sp ($F=64.16$, $P < 0.0001$) in the OVX group and the OVX+S group compared to the Sham group, indicating the successful establishment of OVX rats. BMD ($t_2=6.737$, $P < 0.05$) of rats in the OVX+S group increased while Tb.Sp ($t_2=7.124$, $P < 0.05$) decreased compared to the OVX group, significantly, but with no significant difference in Tb.N. The OVX+S group and the Sham group showed similar results of BMD and Tb.N, but significant differences for Tb.Sp ($t_3=3.769$, $P < 0.05$). These results indicated a protection effect of sesamin (80 mg/kg) on bone structure.

Figure 3B shows significantly lower OCN in the OVX group ($t=5.083$, $P < 0.05$) and the OVX+S group ($t=3.995$, $P < 0.05$) compared to the Sham group. OCN in the OVX+S group was higher than the OVX group, but not significantly. Figure 3C shows lower collagen type I in the OVX group than the Sham group ($t=10.40$, $P < 0.05$). The OVX+S group and the Sham group showed similar levels of collagen type I. These results indicated the prevention effect of sesamin on OCN and collagen type I losses.

Discussion

In this study, we first used different concentration of sesamin (1 μM or 10 μM) to directly treat BMSCs isolated from SD rat femur *in vitro*, for a total of 3 days. The results showed that the expressions of ALP, OSX, SOX9, RUNX2, and OCN were both significantly enhanced after sesamin treatments.

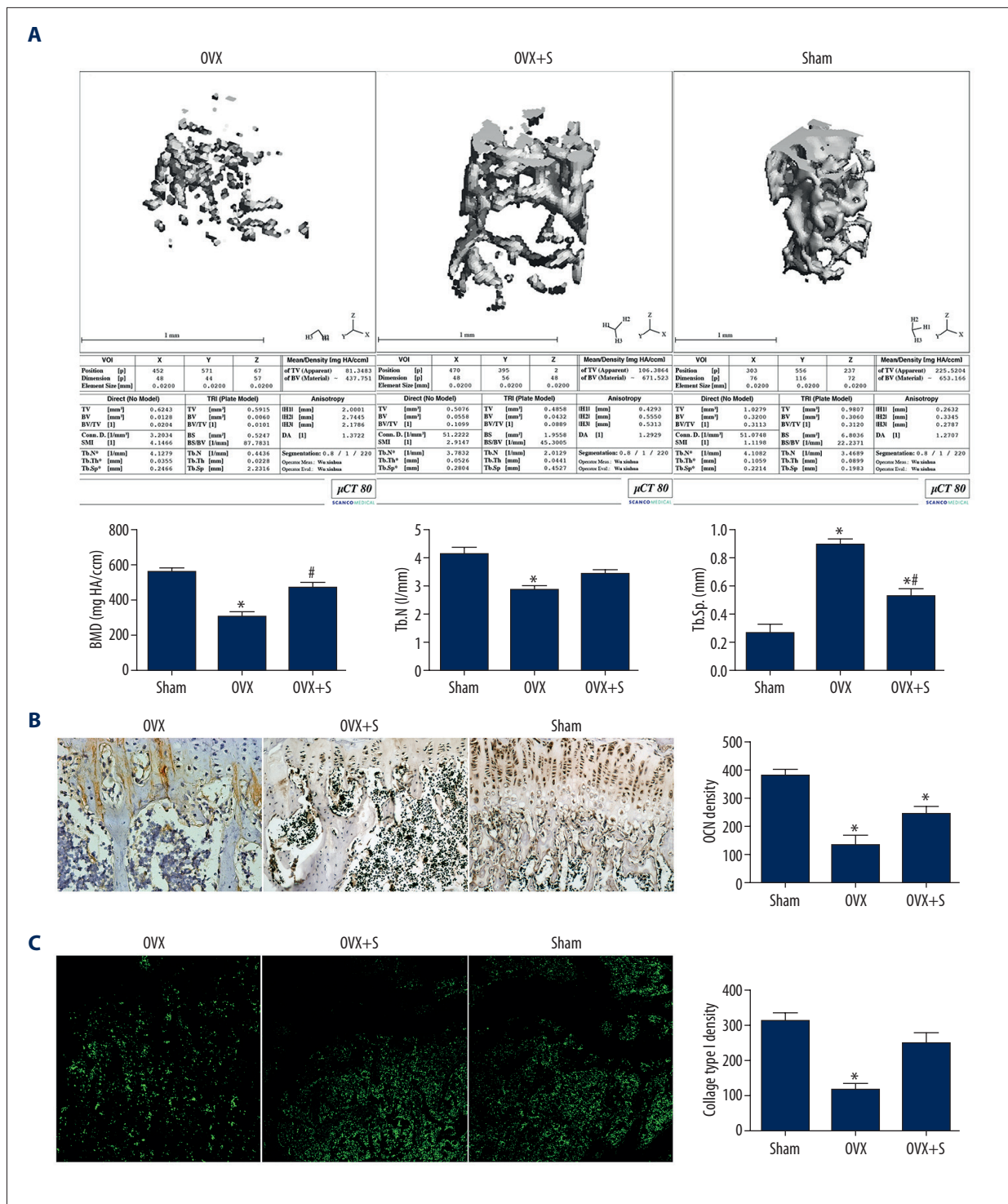


Figure 3. (A) Micro-CT results; (B) OCN detection in rat femurs; (C) Collage type I detection in rat femurs. OVX rats with intragastrical administration of 0.9% saline; OVX+S rats with intragastrical administration of 80 mg/kg of sesamin; Sham, rat with intragastrical administration of 0.9% saline; * comparing to Sham group, $P < 0.05$; # comparing to OVX group, $P < 0.05$. CT – computed tomography; OVX – ovariectomized; S – sesamin; OCN – osteocalcin.

In addition, 10 μM of sesamin promoted the aforementioned proteins expressions more than 1 μM of sesamin. By measuring the Wnt/ β -catenin pathway by western blotting, our results showed the upregulation of sesamin on β -catenin and LRP5 levels but downregulation on GSK-3 β levels, which was also concentration-dependent, which indicated activation of the Wnt/ β -catenin pathway. In order to confirm the critical role of Wnt/ β -catenin in sesamin promotion of BMSCs osteoblastic differentiation, we used FH535 (1 μM) to silence Wnt/ β -catenin and then measured related protein expressions. Western blotting results showed no significance enhancement of sesamin on RUNX2 and OCN expressions compared to those without FH535 treatment *in vitro*. These results indicated the promotion of sesamin on osteoblastic differentiation of BMSCs by regulating the Wnt/ β -catenin pathway. Second, in the animal experiments, micro-CT confirmed the successful establishment of an OVX rat model, which had lower BMD and Tb.N but higher Tb.Sp. Intra-gastric administration with 80 mg/kg of sesamin promoted bone structure by enhancing BMD and Tb.N but reducing Tb.Sp in the femurs of OVX rats. As well, sesamin promoted productions of OCN and collagen type I of the OVX rats. Both the cellular and animal experiments indicated the promotion effect of sesamin on osteoblastic differentiation and protection of rats from osteoporosis.

Some biomarkers have been confirmed in the development of osteoporosis. ALP [24], OSX [25], SOX9 [26], RUNX2 [27,28], and OCN [28] have been confirmed as having relationships to osteoporosis. Patients with osteoporosis often have lower ALP, OSX, SOX9, RUNX2, and OCN compared to healthy persons. In addition, RUNX2 is critical in regulating osteoblast and chondrocyte differentiation [29,30]. Therefore, ALP, OSX, SOX9, RUNX2, and OCN could be considered as biomarkers of osteoporosis or osteoblastic differentiation. Previous investigations have proven that the Wnt/ β -catenin pathway has a critical role in the pathogenesis and progression of osteoporosis, which has also been considered a target in treating osteoporosis [31–34]. In our study, we found that sesamin might be an effective reagent in therapy for osteoporosis. In our cellular experiments, sesamin treatments improved ALP, OSX, SOX9, RUNX2, and OCN levels, and activated the Wnt/ β -catenin signal pathway in BMSCs. Our animal experiments also provided evidence that sesamin treatments prevented the loss of OCN in the femur of rats with osteoporosis. Both these results not only align with previous research, but also indicate that sesamin

has the ability to improve osteoblastic differentiation, and has therapeutic and preventive effects on osteoporosis by regulating the Wnt/ β -catenin pathway.

Frequently, BMD is used as a proxy measure, and is considered to make up about 70% of bone strength [35]. All people experience a decreasing BMD at middle age [36]. Perimenopausal women experience more rapid bone loss in the early years, resulting in higher risk for fractures [37]. Hypogonadism is an important risk factor resulting in a decline of BMD in men [38]. Lower BMD is usually accompanied with lower Tb.N but higher Tb.Sp [39,40]. In our study, we established an OVX rat osteoporosis model, and measured the BMD, Tb.N, and Tb.Sp of rat femurs. Micro-CT results showed the osteoporosis rats had lower BMD and Tb.N but higher Tb.Sp. The treatment of sesamin prevented the decline of BMD and Tb.N, and the rising Tb.Sp. In addition, collagen type I in the femurs of osteoporosis rats was reduced obviously. The intra-gastric administration of sesamin promoted the secretion of collagen type I in the study rat femurs. These results confirmed the therapeutic and protective effects of sesamin on osteoporosis *in vivo*.

However, there were some limitations to our study. First, we only used BMSCs as the treatment targets of sesamin after osteoblastic differentiation *in vitro*. Osteoclastic and chondrogenic differentiation were lacking. Second, sesamin is the component isolated from the bark of *Fagara* plants and sesame oil. Whether the *Fagara* plants and sesame oil have the same therapeutic effect needs further studies. Last but not the least, whether sesamin has side-effects has not been determined. Further investigations should include larger number of samples and longer treating times.

Conclusions

Our study preliminarily confirmed that sesamin has the ability to promote osteoblastic differentiation of BMSCs by regulating the Wnt/ β -catenin pathway, and also indicated its potential therapeutic and preventive effects on osteoporosis.

Conflict of interests

None.

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