Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Ginsenoside Rc alleviates osteoporosis by the TGF-β/Smad signaling pathway

Shanfu Wang, Bing Xu, Heng Yin, Zhen Hua, Yang Shao, Jianwei Wang*

Jiangsu CM Clinical Innovation Center of Degenerative Bone & Joint Disease, Wuxi TCM Hospital Affiliated to Nanjing University of Chinese Medicine, Wuxi 214000, Jiangsu, China

Article Info

Article history:

Received: January 02, 2023
Accepted: February 17, 2024
Published: March 31, 2024

Use your device to scan and read the article online

Abstract

Osteoporosis is a common chronic bone disorder in postmenopausal women. Ginsenosides are primary active components in ginseng and the effects of various ginsenoside variants in osteoporosis treatment have been widely revealed. We planned to explore the impact of ginsenoside Rc on bone resorption in an osteoporosis rat model. We used ovariectomized rats to assess the potential impact of ginsenoside Rc on osteoporosis. μ-CT was implemented for analyzing the microstructure of the distal left femur in rats. H&E staining together with Masson staining were applied for bone histomorphometry evaluation. ELISA kits were implemented to detect serum concentrations of TRACP-5b, OCN, CTX, as well as PINP. Ginsenoside Rc treatment lessened the serum levels of TRACP-5b as well as CTX, while increasing serum levels of OCN, and PINP of OVX rats. Moreover, we found that ginsenoside Rc contributed to the synthesis of type I collagen via increasing Col1a1 and Col1a2 levels in femur tissues of ovariectomized rats. Our findings also revealed that ginsenoside Rc activated the TGF-β/Smad pathway by increasing TGF-β as well as phosphorylated Smad2/3 protein levels. Ginsenoside Rc alleviates osteoporosis in rats through promoting the TGF-β/Smad pathway.

Keywords: Ginsenoside Rc, Type I collagen, Osteoblast, The TGF-β/Smad pathway, Osteoporosis.

1. Introduction

Osteoporosis is a prevalent chronic disease that results in an elevated risk of bone fractures in postmenopausal women [1]. Osteoporosis has features of decreased bone mass, injured bone structure and calcium malabsorption [2, 3]. The risk factors for osteoporosis include malnutrition, hormone fluctuation, inflammation, and mechanical stress [4]. Currently, most therapeutic agents for osteoporosis are based on inhibition of bone resorption [5, 6].

A previous study has revealed that the coupled dynamic balance between bone formation and bone resorption belongs to the basis for keeping the stability of bone morphology [5]. Type I collagen is composed of 2 α1 chains as well as 1 α2 chain, accounting for approximately 80-90% of the bone organic matter, which promotes bone homeostasis [7]. In addition, type I collagen belongs to the central part of bone extracellular matrix [8]. Increasing studies have revealed that the decreased synthesis of type I collagen along with the change in its morphology and stability will aggravate osteoporosis [9, 10].

Recently, the vital functions of Chinese herbs in osteoporosis treatment have been revealed. Guilu erxian glue comprises *Testudinis Plastrum*, *Ginseng root*, *Cornu Cervi* and *Lycii Fructus*, and has been indicated to exert favorable effects on degenerative joint diseases and perimenopausal syndrome [11]. Ginseng is one of the most valuable medicinal herbs. The primary active components in ginseng are ginsenosides containing two parts: the scaffold (sapogenin) and the sugar side chain(s). More than 600 ginsenosides have been identified, and the most common sapogenins are protopanaxadiols as well as protopanaxatriols [12]. Many variants of ginsenosides have been identified to exert protective roles in osteoporosis, for example, ginsenoside Rb2 reduces oxidative damage as well as bone-resorbing cytokines during osteogenesis to exert anti-osteoporotic effects [13]. Ginsenoside Rg3 promotes osteogenic differentiation by facilitating autophagy to attenuate ovariectomy-induced osteoporosis [14]. Ginsenoside Rh2 suppresses osteogenic differentiation from bone marrow macrophages by downregulation of NF-κB [15].

Ginsenoside Rc (molecular formula: $C_{53}H_{90}O_{22}$; CAS: 11021-14-0) exerts an anti-inflammatory effect on synovial cells in rheumatoid arthritis patients [16], a systemic autoimmune disease that can lead to cartilage destruction and systemic osteoporosis, which indicates the putative association of ginsenoside Rc with osteoporosis. We hypothesized that ginsenoside Rc can alleviate osteoporosis and investigated the impacts of ginsenoside Rc on bone microstructure as well as serum bone turnover markers of ovariectomized (OVX) rats. Moreover, influence of ginsenoside Rc on expression of osteoblast markers in primary

* Corresponding author.
E-mail address: wangjianwei1963@hotmail.com (J. Wang).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.3.14

human osteoblasts as well as the downstream pathway of ginsenoside Rc were investigated.

2. Materials and methods

2.1. OVX rat model

Fifty Sprague-Dawley rats (about 220 g; 3-month-old) could be acquired from Vital River (Beijing, China) and fed at 20-24°C, 45-60% humidity, as well as 12 h light/dark cycle. Rats were separated into 5 groups: sham group, OVX group, OVX+estrogen group, OVX+ginsenoside Rc group, as well as OVX+ginsenoside Rc+SB431542 group, with 10 rats in each group. The rats received fast for 12 h before surgery. 10% chloral hydrate solution (0.3 mL/100 g) was intraperitoneally injected into the rats for anesthesia. In addition to sham operation group (n=10), all the other experimental rats underwent lower abdominal incisions to remove their ovaries (n=40). Some rats in the positive control group were daily administrated with 17β-estradiol (2 μg/kg) [17]. Ten weeks after operation, rats in the OVX group received intraperitoneal treatment with ginsenoside Rc (20 mg/kg, purity ≥ 98%, Aladdin, n=20) or 10% DMSO (n=10) every 2 days for 4 weeks. Rats in OVX+ginsenoside Rc+SB431542 group (n=10) received an intraperitoneal administration of ginsenoside Rc (20 mg/kg) and SB431542 (2.5 mg/kg), the inhibitor of TGF-β [18], every 2 days for 4 weeks. By the end of the trial, rats received sacrifice using thiopental (100 mg/kg). Abdominal aorta puncture was used to collect blood, and serum was separated for the evaluation of bone turnover markers. Femur tissues were collected for detection of synthesis of Collagen. The animal study was approved by the ethics committee of Wuxi Hospital Affiliated to Nanjing University of Chinese Medicine (Jiangsu, China), and implemented in line with the ARRIVE guidelines.

2.2. Cell culture

Primary human calvarial osteoblasts (Cat. #4600) could be acquired from ScienCell Research Laboratories (Carlsbad, CA, USA). A total of 5×10^5 cells were plated onto 30-mm-diameter culture dishes including DMEM (Invitrogen) which contained 10% fetal bovine serum, antibiotic-antimycotic reagent (100 units of penicillin G, 100 μg mL^{-1} of streptomycin sulfate, along with 0.25 μg mL^{-1} of amphotericin B), as well as 2 mM L-glutamine at 37°C with 5% CO_2 and 95% air humidity. For some experiments, the culture medium was added with 10 μmol/L of ginsenoside Rc and 10 μmol/L of SB431542. Cell viability was detected by MTT assay [19].

2.3. RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

This method could be performed as a previous study described [9]. In brief, total RNA received extraction from femur tissues and human osteoblasts by TRIzol reagent (Takara, Japan). A spectrophotometer was employed to measure the concentrations of total RNA. For each sample, 1 μg of RNA received reverse transcription to cDNA by M-MLV reverse transcriptase. RT-qPCR was conducted in an iCycler iQ5 system. GAPDH acted to be the internal reference. The standard curve method was utilized to analyze the gene expression. Primer sequences are listed as follows:

Alp (rat): F: 5'-TGGTTACTGCTGATCACCC-3'; R: 5'-TTGTTGTGAGCATAATCCACC-3'.
Col1a1 (rat): F: 5'-CAAGGACTATGAAGTTGATGC-3'; R: 5'-ACCAGTAGAGAAATCGCAGT-3'.
Col1a2 (rat): F: 5'-GCAACATGCCAATCTTTCCT-3'; R: 5'-TCAACACCATCTCTGCCTC-3'.
Gapdh (rat): F: 5'-AACTCCCATTCTTCCACCT-3'; R: 5'-TTGTCATACCAGGAAATGAGC-3'.
ALP (human): F: 5'-CAGAAGAAGGACAAACTGGG-3'; R: 5'-ATTGTATGTCTTGGACAGAGC-3'.
OCN (human): F: 5'-CTTTGTGTCCAAGCAGGAG-3'; R: 5'-CTCCCAGCCATTGATACAG-3'.
COL1A1 (human): F: 5'-AAAGATGGAGAGGCTGGAG-3'; R: 5'-ATCACCCTTAGCACCATCG-3'.
COL1A2 (human): F: 5'-GAAGGCTCTAGAAAGAACCCA-3'; R: 5'-CCAGTAGTAACCACTGCTCC-3'.
GAPDH (human): F: 5'-TCAAGATCATCAGCAATGCC-3'; R: 5'-CGATACCAAAGTTGTCATGGA-3'.

2.4. Western blot analysis

RNA immunoprecipitation assay lysis buffer (Beyotime, Shanghai, China) was used for extracting proteins from femur tissues and human osteoblasts. The protein concentrations were assessed by a bicinchoninic acid protein assay kit (Beyotime, China), and then received subjection to 10% sodium dodecyl sulfate, polyacrylamide gel electrophoresis gel and shifted onto polyvinylidene difluoride membranes (Millipore, USA). Membranes were then sealed with 5% nonfat milk, followed by treatment with the primary antibodies at 4°C overnight. The primary antibodies were: COL1A1 (sc-293182, Santa Cruz Biotechnology), COL1A2 (sc-393573, Santa Cruz Biotechnology), ALP (ab224335, Abcam), OCN (ab133612, Abcam), TGF-β (ab215715, Abcam), Smad3 (ab40854, Abcam), p-Smad3 (ab51177, Abcam), Smad2 (ab40855, Abcam), p-Smad2 (ab188334, Abcam), BMP2 (ab214821, Abcam) and β-actin (ab6276, Abcam). Next, the membranes were cultured with secondary antibodies at room temperature for 1 h. β-actin acted to be the internal control. An enhanced chemiluminescence detection kit (Thermo Fisher Scientific) was adopted for visualizing protein bands and the ImageJ software was used for densitometry analysis of the band intensity.

2.5. Bone histomorphometry evaluation

The proximal femur was immobilized in 10% methanal for 48 hours and received decalcification for four weeks, followed by being dehydrated and embedded in paraffin. Next, paraffin sections received deparaffinization and were subjected to hematoxylin and eosin (H&E) staining as well as Masson staining.

2.6. Microstructural analysis

μ-CT (CT 40, Switzerland) at 55 kVp was conducted for microstructural analysis on the left distal femur of rats. The region of interest (ROI) could be considered to be 2.1 cm under the growth plate of the distal femur. The structural parameters of the ROI could be obtained from the 3-dimensional reconstructed image through the image analysis program based on μ-CT workstation. Parameters contained bone mineral density (BMD), connectivity density (COD), bone surface-to-volume ratio (BS/BV), bone volume over total volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) as well as structural model index (SMI) was measured.

2.7. ALP activity assay
Rat femur tissues were harvested followed by culture for 72 h. After washing, an alkaline phosphatase assay kit (Nanjing Jiancheng Bioengineering Institute, China) could be adopted for measuring relative ALP activity using chemical colorimetry. The absorbance at 405 nm from each group was detected by virtue of a spectrophotometer.

2.8. Enzyme-linked immunosorbent assay (ELISA)
The blood was gathered, followed by receiving centrifugation to obtain the serum. The serum levels of tartrate-resistant acid phosphatase-5b (TRACP-5b), osteocalcin (OCN), C-terminal telopeptide of type 1 collagen (CTX), as well as procollagen I N-terminal propeptide (PINP) could be quantified using corresponding ELISA kits.

2.9. Statistical analysis
Statistical analysis was implemented by SPSS 19.0 software (IBM, USA). Data were exhibited as mean ± standard deviation. Differences were analyzed by t-test or one-way analysis of variance followed by Tukey's post hoc test. $P < 0.05$ meant statistically significant.

3. Results
3.1. Ginsenoside Rc increases serum levels of OCN, and PINP levels, and reduces TRACP-5b together with CTX levels in OVX rats
Relative to sham group, the distal femurs of OVX rats exhibited less osteoid staining, indicating the successful establishment of OVX rodents. Estrogen or ginsenoside Rc treatment alleviates OP symptoms (Fig 1A). To determine whether ginsenoside Rc exerts effects on osteoporosis development, we measured the serum levels of bone turnover markers. OCN and PINP are bone formation markers, and TRACP-5b and CTX are bone absorption markers. As presented in Fig 1B-E, the decreased serum contents of OCN, PINP, and increased serum contents of TRACP-5b and CTX in OVX group were rescued by estrogen or ginsenoside Rc treatment. Furthermore, H&E staining illustrated that relative to the sham group, the trabecular region presented smaller, thinner, and sparser in the OVX group. Estrogen or ginsenoside Rc treatment rescued the effects of OVX surgery on histomorphology of femurs (Fig 1F). The BMD, BV/TV, COD, Tb.N, together with Tb.Th in the ROI in OVX rats presented apparently reduced relative to sham rats, while the BS/BV, SMI, and Tb.Sp presented significantly higher (Table 1). Treatment of estrogen rescued the effects of ovariectomy on these parameters in rats. Interestingly, ginsenoside Rc had rescue effects on these parameters except Tb.Th.

3.2.Ginsenoside Rc promotes the synthesis of collagen I
Fig 2A indicated that ginsenoside Rc induced upregulation of Alp mRNA in femurs of OVX rats. As presented in Fig 2B, the ALP activity was decreased in femur tissues of OVX rats, while ginsenoside Rc treatment reversed such effect. Col1a1 and Col1a2 mRNA as well as protein levels presented reduced in OVX rats but were increased after treatment of ginsenoside Rc (Fig 2C-E).

3.3. Effects of ginsenoside Rc on the TGF-β/Smad pathway
The TGF-β/Smad pathway is activated during osteogenic differentiation [20] and is inhibited during osteoporotic progression [21]. As indicated in Fig 3A-B, TGF-β, BMP2, p-Smad2, along with p-Smad3 protein levels were

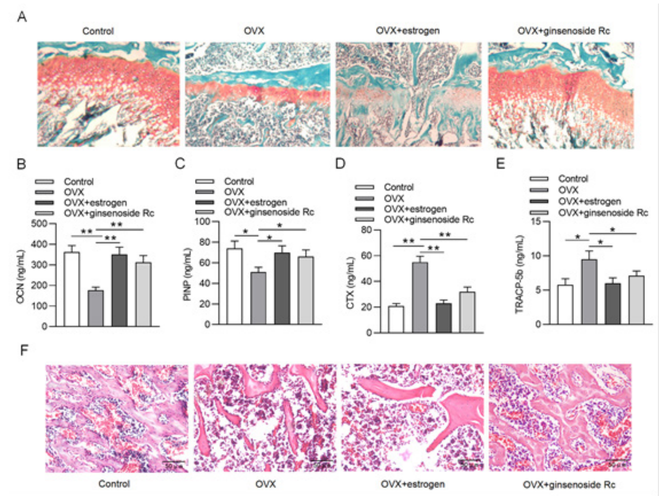

Fig. 1. Effects of ginsenoside Rc on serum TRACP-5b, OCN, CTX, PINP levels in OVX rats. (A) The representative images of the distal femurs in rats of the control, OVX, OVX+estrogen, OVX+ginsenoside Rc groups were revealed by Masson staining. (B-E) Analysis of biochemical parameters (TRACP-5b, OCN, CTX, PINP) in serum of rats. (F) H&E staining revealed histomorphology changes in femurs of OVX rats. There are 10 rats in each group.$^{*}p < 0.05$, $^{**}p < 0.01$.

Table1. Microarchitectural quantitative analysis results of an ROI in the femur trabecular bone.

	Control	OVX	OVX+estrogen	OVX+ginsenoside Rc
BMD (mgHA/ccm)	655.17±19.4	489.79±17.5*	612.87±15.1$^{\#}$	598.67±14.9$^{\#\#}$
BV/TV (%)	0.29±0.03	0.04±0.01***	0.13±0.02$^{\#\#\#}$	0.08±0.01$^{\#\#}$
SMI	1.15±0.18	2.81±0.26**	1.78±0.19$^{\#}$	2.04±0.23$^{\#\#}$
COD (mm^{-3})	168.42±14.47	14.11±1.45***	82.33±5.99$^{\#\#\#}$	47.17±3.72$^{\#\#\#}$
BS/BV (mm^{-1})	34.53±1.18	58.21±3.75*	42.51±2.17$^{\#\#}$	45.81±2.81$^{\#\#}$
Tb.N (mm^{-1})	4.95±0.51	0.99±0.08***	3.58±0.28$^{\#\#\#}$	2.04±0.16$^{\#\#}$
Tb.Sp (mm)	0.20±0.014	1.03±0.072***	0.27±0.019$^{\#\#\#}$	0.49±0.032$^{\#\#}$
Tb.Th (mm)	0.071±0.006	0.055±0.004*	0.065±0.005$^{\#\#}$	0.059±0.004

Student's t-test was conducted for comparisons between 2 groups. ROI: region of interest, OVX: ovariectomy, BMD: bone mineral density, BV/TV: bone volume over total volume, SMI: structural model index, COD: connectivity density, BS/BV: bone surface to volume ratio, Tb.N: trabecular number, Tb.Sp: trabecular separation, Tb.Th: trabecular thickness. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. the control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. the OVX group.

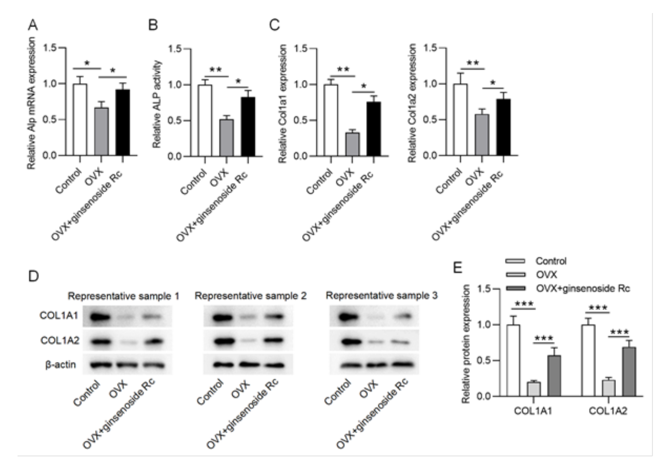

Fig. 2. Ginsenoside Rc promotes the synthesis of COL1A1 and COL1A2 in femur tissues of OVX rats. (A) RT-qPCR was conducted to reveal Alp mRNA expression in femur tissues of rats of the control, OVX, and OVX+ginsenoside Rc groups. (B) ALP activity in femur tissues was evaluated by an ALP Test Kit. (C) RT-qPCR analysis was conducted to measure Col1a1 and Col1a2 mRNA expression in rat femur tissues. (D-E) The protein levels of COL1A1 and COL1A2 were evaluated by western blot. There are 10 rats in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

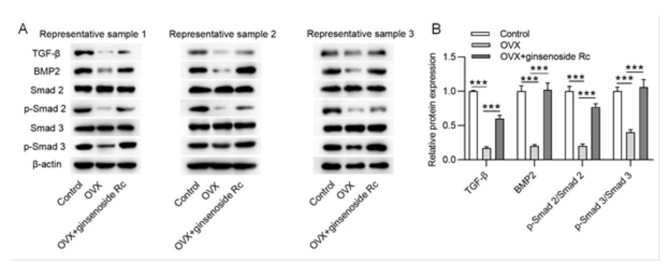

Fig. 3. Effects of ginsenoside Rc on the TGF-β/Smad pathway. (A-B) The protein levels of TGF-β, BMP2, Smad2, p-Smad2, Smad3 and p-Smad3 in femur tissues were measured by western blot analysis. There are 10 rats in each group. $^{***}p < 0.001$.

inhibited in femur tissues of OVX rats, whereas ginsenoside Rc treatment significantly increased their protein levels in OVX rats.

3.4. Repression of TGF-β/Smad pathway rescues the impacts of ginsenoside Rc

The elevated mRNA expression and activity of ALP caused by ginsenoside Rc were rescued by SB431542 (Fig 4A-B). Increased Col1a1 and Col1a2 mRNA levels stimulated by ginsenoside Rc were rescued by SB431542 (Fig 4C-D). Similarly, COL1A1 and COL1A2 protein levels were elevated in OVX+ginsenoside Rc group. However, up-regulation of COL1A1 and COL1A2 was rescued by SB431542 (Fig 4E). Figure 4F revealed that SB431542 successfully rescued the ginsenoside Rc-induced activation of the TGF-β/Smad pathway.

3.5. Ginsenoside Rc promoted osteoblast markers expression by the TGF-β pathway

Reactive activity of ALP was increased by ginsenoside Rc treatment and was decreased by SB431542 treatment in human osteoblasts (Fig 5A). Fig 5B-C displayed that the mRNA together with protein levels of osteoblast markers including ALP, OCN, COL1A1 as well as COL1A2 were increased by ginsenoside Rc and were decreased by

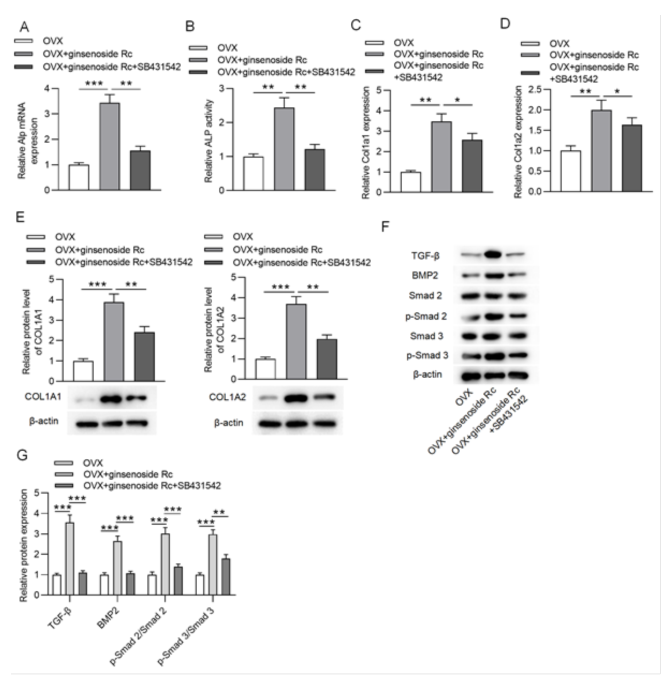

Fig. 4. Inactivation of the TGF-β/Smad pathway rescues the effects of ginsenoside Rc. (A-B) Alp mRNA expression and ALP activity in femur tissues were detected. (C-D) RT-qPCR revealed Col1a1 and Col1a2 mRNA expression in femur tissues of rats in 3 groups: OVX, OVX+ginsenoside Rc, OVX+ginsenoside Rc+SB431542 groups. (E) The protein levels of COL1A1 and COL1A2 in femur tissues of rats in 3 groups: OVX, OVX+ginsenoside Rc, OVX+ginsenoside Rc+SB431542 groups were measured by western blot. (F) The protein levels of TGF-β, BMP2, Smad2, p-Smad2, Smad3 and p-Smad3 in femur tissues of rast in the OVX, OVX+ginsenoside Rc, OVX+ginsenoside Rc+SB431542 groups were measured by western blot analysis. There are 10 rats in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

SB431542 in human osteoblasts. Figure 5D revealed that ginsenoside Rc or SB431542 possessed no influence on the viability of human osteoblasts.

4. Discussion

Bone remodeling includes bone resorption modulated by osteoclasts together with bone formation modulated by osteoblasts [22]. Excessive bone resorption is the main cause of osteoporosis. Osteoclasts originate from hematopoietic precursor cells, which can differentiate into multinucleated cells [23]. When osteoclasts adsorb on the bone surface, hydrolytic enzymes, synthesized as well as secreted by osteoclasts, degrade bone minerals along with collagen matrices. In addition, the serum levels of bone resorption markers reflect the degradation degree of bone mineral as well as collagen matrices [24]. In the current research, serum OCN and PINP levels presented decreased in OVX group, whereas ginsenoside Rc treatment restored the decrease of OCN and PINP. Serum levels of bone absorption markers, TRACP-5b and CTX, were increased in OVX group, and ginsenoside Rc treatment rescued the increase of TRACP-5b and CTX.

Increasingly studies have revealed that type I collagen has a vital function in bone homeostasis and belongs to the major part of bone extracellular matrix [7, 8]. The alterations in synthesis, morphology, and stability of type I collagen are closely associated with the progression of osteoporosis [9, 10]. TGF-β affects the synthesis

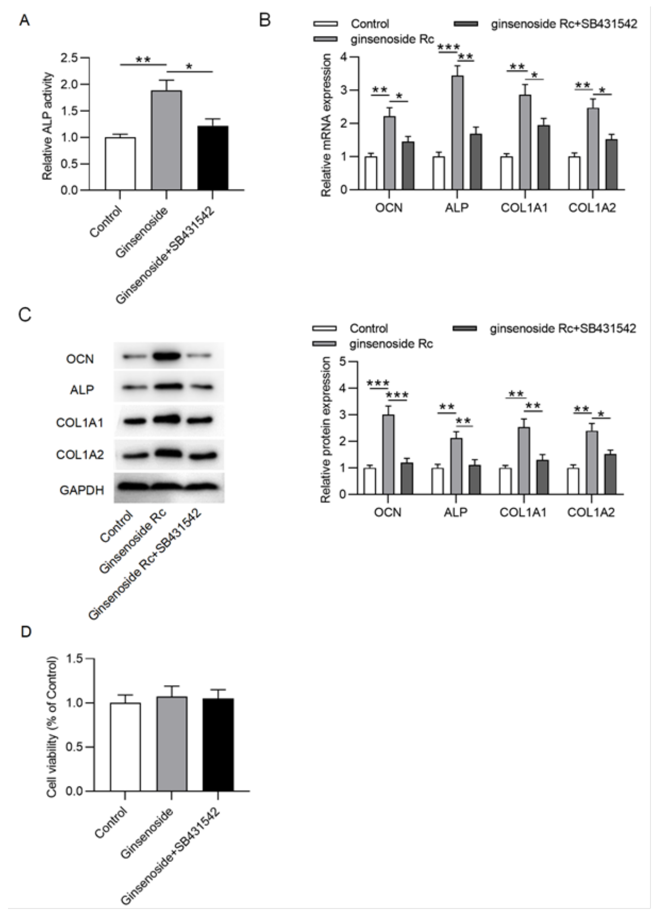

Fig. 5. Ginsenoside Rc promoted expression of osteoblast markers by the TGF-β pathway. (A) ALP activity in human osteoblasts was evaluated by an ALP Test Kit. (B-C) RT-qPCR and western blotting analyses were conducted to measure the mRNA and protein expression of ALP, OCN, COL1A1 and COL1A2 in human osteoblasts. (D) Viability of human osteoblasts under treatment of ginsenoside Rc or SB431542 was detected by MTT assay. Each assay was performed using 3 biological samples 3 times. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

and metabolism of type I collagen [25]. TGF-β cytokine belongs to a multi-functional protein released by non-parenchymal cells and is closely linked to several cellular processes containing cell proliferation, differentiation as well as angiogenesis [26]. TGF-β combines with corresponding receptors on cell surface, which in turn induces the SMAD pathway. Accumulating studies have revealed the close relationship between TGF-β/SMAD pathway and progression of osteoporosis. For instance, rhubarb ameliorates tubulointerstitial fibrosis in chronic kidney disease via repressing the TGF-β/Smad pathway [27]. MiR-224 promotes vascular remodeling by inducing the TGF-β/Smad pathway in acute coronary syndrome]28]. MOTS-c facilitates osteogenic differentiation of bone marrow mesenchymal stem cells in osteoporosis through the TGF-β/Smad pathway [29]. Moreover, a recent study indicated that MOTS-c alleviates osteoporosis development by increasing the levels of COL1A1 and COL1A2 in a TGF-β/Smad7 signaling pathway-dependent manner]10]. Our findings innovatively revealed that ginsenoside Rc activates the TGF-β/Smad3 pathway. Suppressed TGF-β/Smad3 pathway rescues the impacts of ginsenoside Rc on Alp, Col1a1 as well as Col1a2 levels in osteoporotic rats. In addition, ginsenoside Rc promoted expression of osteoblast markers, but did not affect viability in primary human osteoblasts.

Moreover, ginsenoside Rc has been revealed to upregulate ACE2 [28], and upregulation of ACE2 had a therapeutic value for ovariectomy-induced osteoporosis in rats [30]. Ginsenoside Rc can suppress the activation of TBK1 [16], and inhibitor of TBK1 can be used to suppress osteoclastogenesis and to prevent OVX-induced bone loss [31]. Oxidative stress is implicated in postmenopausal osteoporosis and catalase, an oxidative stress marker, is decreased in postmenopausal osteoporosis [32]. Ginsenoside Rc can induce the upregulation of catalase [33]. ACE2 and catalase were associated with the TGF-β pathway [34, 35]. It can be inferred that ACE2, TBK1 and catalase are involved in the therapeutic effect of ginsenoside Rc on osteoporosis. Nevertheless, this study had several limitations. Firstly, clinical data was lacking. Secondly, more pathways underlying the protective effect of ginsenoside Rc on osteoporosis need investigation.

5. Conclusions

This study revealed the protective role of ginsenoside Rc on osteoporosis via increasing the synthesis of type I collagen in a TGF-β/Smad3 pathway-dependent way. These findings may provide theoretical basis for finding novel strategies for osteoporosis.

Abbreviations

TRACP-5b: tartrate-resistant acid phosphatase-5b; OCN: osteocalcin; CTX: C-terminal telopeptide of type 1 collagen; PINP: procollagen I N-terminal propeptide; ROI: region of interest; BMD: bone mineral density; SMI: structural model index; COD: connectivity density; BS/BV: bone surface to volume ratio; BV/TV: bone volume over total volume; Tb.N: trabecular number; Tb.Th: trabecular thickness; Tb.Sp: trabecular separation.

Declarations

Ethics declarations

Ethics approval and consent to participate

The animal study was approved by the ethics committee of Wuxi Hospital Affiliated to Nanjing University of Chinese Medicine (Jiangsu, China). The study was carried out in compliance with the ARRIVE guidelines. All the study was conducted in accordance with all the national/institutional/international guidelines.

Consent for publication

Not applicable.

Availability of data and materials

All data from this study are available in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by Key Item of Wuxi Wei Planning Commission Project (No. QNRC068, QNRC042, Q201609, Q201708, Q201945), the National Natural Science Foundation (No. 81973878, 81873320), and Jiangsu Natural Science Foundation (No. BK20180167) and the Foundation of Jiangsu CM Clinical Innovation Center of Degenerative Bone & Joint Disease (Jiangsu Science and Education of Traditional Chinese Medicine

[2021] No. 4).

Authors' contributions

WS and WJ conceived of the presented idea. WF, XB, YH and WJ developed the theory and performed the computations. HZ and SY verified the analytical methods. WF and WJ supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

AcknowledgementS

Not applicable.

References

1. Lane NE (2006) Epidemiology, etiology, and diagnosis of osteoporosis. American journal of obstetrics and gynecology 194: S3-11. doi: 10.1016/j.ajog.2005.08.047
2. Eastell R, Szulc P (2017) Use of bone turnover markers in postmenopausal osteoporosis. The lancet Diabetes & endocrinology 5 (11): 908-923. doi: 10.1016/s2213-8587(17)30184-5
3. Licata AA (2013) Bone density, bone quality, and FRAX: changing concepts in osteoporosis management. American journal of obstetrics and gynecology 208 (2): 92-96. doi: 10.1016/j.ajog.2012.10.874
4. Nagaraja MP, Risin D (2013) The current state of bone loss research: data from spaceflight and microgravity simulators. Journal of cellular biochemistry 114 (5): 1001-1008. doi: 10.1002/jcb.24454
5. Marie PJ, Kassem M (2011) Osteoblasts in osteoporosis: past, emerging, and future anabolic targets. European journal of endocrinology 165 (1): 1-10. doi: 10.1530/eje-11-0132
6. Lorentzon M (2019) Treating osteoporosis to prevent fractures: current concepts and future developments. Journal of internal medicine 285 (4): 381-394. doi: 10.1111/joim.12873
7. Reuter MS, Schwabe GC, Ehlers C, Marschall C, Reis A, Thiel C, Graul-Neumann L (2013) Two novel distinct COL1A2 mutations highlight the complexity of genotype-phenotype correlations in osteogenesis imperfecta and related connective tissue disorders. European journal of medical genetics 56 (12): 669-673. doi: 10.1016/j.ejmg.2013.10.002
8. Jones CA, Liang L, Lin D, Jiao Y, Sun B (2014) The spatial-temporal characteristics of type I collagen-based extracellular matrix. Soft matter 10 (44): 8855-8863. doi: 10.1039/c4sm01772b
9. Xia G, Zhao Y, Yu Z, Tian Y, Wang Y, Wang S, Wang J, Xue C (2015) Phosphorylated Peptides from Antarctic Krill (Euphausia superba) Prevent Estrogen Deficiency Induced Osteoporosis by Inhibiting Bone Resorption in Ovariectomized Rats. Journal of agricultural and food chemistry 63 (43): 9550-9557. doi: 10.1021/acs.jafc.5b04263
10. Che N, Qiu W, Wang JK, Sun XX, Xu LX, Liu R, Gu L (2019) MOTS-c improves osteoporosis by promoting the synthesis of type I collagen in osteoblasts via TGF-β/SMAD signaling pathway. European review for medical and pharmacological sciences 23 (8): 3183-3189. doi: 10.26355/eurrev_201904_17676
11. Tsai CC, Chou YY, Chen YM, Tang YJ, Ho HC, Chen DY (2014) Effect of the herbal drug guilu erxian jiao on muscle strength, articular pain, and disability in elderly men with knee osteoarthritis. Evidence-based complementary and alternative medicine : eCAM 2014: 297458. doi: 10.1155/2014/297458
12. Yang WZ, Ye M, Qiao X, Liu CF, Miao WJ, Bo T, Tao HY, Guo DA (2012) A strategy for efficient discovery of new natural compounds by integrating orthogonal column chromatography and liquid chromatography/mass spectrometry analysis: Its application in Panax ginseng, Panax quinquefolium and Panax notoginseng to characterize 437 potential new ginsenosides. Analytica chimica acta 739: 56-66. doi: 10.1016/j.aca.2012.06.017
13. Huang Q, Gao B, Jie Q, Wei BY, Fan J, Zhang HY, Zhang JK, Li XJ, Shi J, Luo ZJ, Yang L, Liu J (2014) Ginsenoside-Rb2 displays anti-osteoporosis effects through reducing oxidative damage and bone-resorbing cytokines during osteogenesis. Bone 66: 306-314. doi: 10.1016/j.bone.2014.06.010
14. Zhang X, Huang F, Chen X, Wu X, Zhu J (2020) Ginsenoside Rg3 attenuates ovariectomy-induced osteoporosis via AMPK/mTOR signaling pathway. Drug development research 81 (7): 875-884. doi: 10.1002/ddr.21705
15. He L, Lee J, Jang JH, Lee SH, Nan MH, Oh BC, Lee SG, Kim HH, Soung NK, Ahn JS, Kim BY (2012) Ginsenoside Rh2 inhibits osteoclastogenesis through down-regulation of NF-κB, NFATc1 and c-Fos. Bone 50 (6): 1207-1213. doi: 10.1016/j.bone.2012.03.022
16. Yu T, Yang Y, Kwak YS, Song GG, Kim MY, Rhee MH, Cho JY (2017) Panax ginsengGinsenoside Rc from exerts anti-inflammatory activity by targeting TANK-binding kinase 1/interferon regulatory factor-3 and p38/ATF-2. Journal of ginseng research 41 (2): 127-133. doi: 10.1016/j.jgr.2016.02.001
17. Wang N, Sun LY, Zhang SC, Wei R, Xie F, Liu J, Yan Y, Duan MJ, Sun LL, Sun YH, Niu HF, Zhang R, Ai J (2015) MicroRNA-23a participates in estrogen deficiency induced gap junction remodeling of rats by targeting GJA1. International journal of biological sciences 11 (4): 390-403. doi: 10.7150/ijbs.10930
18. Inman GJ, Nicolás FJ, Callahan JF, Harling JD, Gaster LM, Reith AD, Laping NJ, Hill CS (2002) SB-431542 is a potent and specific inhibitor of transforming growth factor-beta superfamily type I activin receptor-like kinase (ALK) receptors ALK4, ALK5, and ALK7. Molecular pharmacology 62 (1): 65-74. doi: 10.1124/mol.62.1.65
19. Oliveira FA, Matos AA, Santesso MR, Tokuhara CK, Leite AL, Bagnato VS, Machado MA, Peres-Buzalaf C, Oliveira RC (2016) Low intensity lasers differently induce primary human osteoblast proliferation and differentiation. Journal of photochemistry and photobiology B, Biology 163: 14-21. doi: 10.1016/j.jphotobiol.2016.08.006
20. Yu J, Xu L, Li K, Xie N, Xi Y, Wang Y, Zheng X, Chen X, Wang M, Ye X (2017) Zinc-modified Calcium Silicate Coatings Promote Osteogenic Differentiation through TGF-β/Smad Pathway and Osseointegration in Osteopenic Rabbits. Scientific reports 7 (1): 3440. doi: 10.1038/s41598-017-03661-5
21. Li H, Fan J, Fan L, Li T, Yang Y, Xu H, Deng L, Li J, Li T, Weng X, Wang S, Chunhua Zhao R (2018) MiRNA-10b Reciprocally Stimulates Osteogenesis and Inhibits Adipogenesis Partly through the TGF-β/SMAD2 Signaling Pathway. Aging and disease 9 (6): 1058-1073. doi: 10.14336/ad.2018.0214
22. Henriksen K, Neutzsky-Wulff AV, Bonewald LF, Karsdal MA (2009) Local communication on and within bone controls bone remodeling. Bone 44 (6): 1026-1033. doi: 10.1016/j.bone.2009.03.671
23. Lee JW, Asai M, Jeon SK, Iimura T, Yonezawa T, Cha BY, Woo JT, Yamaguchi A (2015) Rosmarinic acid exerts an antiosteoporotic effect in the RANKL-induced mouse model of bone loss by promotion of osteoblastic differentiation and inhibition of osteoclastic differentiation. Molecular nutrition & food research 59 (3): 386-400. doi: 10.1002/mnfr.201400164
24. Boyle WJ, Simonet WS, Lacey DL (2003) Osteoclast differentiation and activation. Nature 423 (6937): 337-342. doi: 10.1038/nature01658
25. Rahimi RA, Leof EB (2007) TGF-beta signaling: a tale of two responses. Journal of cellular biochemistry 102 (3): 593-608. doi: 10.1002/jcb.21501
26. Yan J, Bao H, Fan YJ, Jiang ZL, Qi YX, Han Y (2020) Platelet-

derived microvesicles promote endothelial progenitor cell proliferation in intimal injury by delivering TGF-β1. The FEBS journal 287 (23): 5196-5217. doi: 10.1111/febs.15293
27. Zhang ZH, Li MH, Liu D, Chen H, Chen DQ, Tan NH, Ma SC, Zhao YY (2018) Rhubarb Protect Against Tubulointerstitial Fibrosis by Inhibiting TGF-β/Smad Pathway and Improving Abnormal Metabolome in Chronic Kidney Disease. Frontiers in pharmacology 9: 1029. doi: 10.3389/fphar.2018.01029
28. Wang Y, Fu W, Xue Y, Lu Z, Li Y, Yu P, Yu X, Xu H, Sui D (2021) Ginsenoside Rc Ameliorates Endothelial Insulin Resistance via Upregulation of Angiotensin-Converting Enzyme 2. Frontiers in pharmacology 12: 620524. doi: 10.3389/fphar.2021.620524
29. Hu BT, Chen WZ (2018) MOTS-c improves osteoporosis by promoting osteogenic differentiation of bone marrow mesenchymal stem cells via TGF-β/Smad pathway. European review for medical and pharmacological sciences 22 (21): 7156-7163. doi: 10.26355/eurrev_201811_16247
30. Abuohashish HM, Ahmed MM, Sabry D, Khattab MM, Al-Rejaie SS (2017) Angiotensin (1-7) ameliorates the structural and biochemical alterations of ovariectomy-induced osteoporosis in rats via activation of ACE-2/Mas receptor axis. Scientific reports 7 (1): 2293. doi: 10.1038/s41598-017-02570-x
31. Zhang Y, Guan H, Li J, Fang Z, Chen W, Li F (2015) Amlexanox Suppresses Osteoclastogenesis and Prevents Ovariectomy-Induced Bone Loss. Scientific reports 5: 13575. doi: 10.1038/srep13575
32. Zhao F, Guo L, Wang X, Zhang Y (2021) Correlation of oxidative stress-related biomarkers with postmenopausal osteoporosis: a systematic review and meta-analysis. Archives of osteoporosis 16 (1): 4. doi: 10.1007/s11657-020-00854-w
33. Kim DH, Park CH, Park D, Choi YJ, Park MH, Chung KW, Kim SR, Lee JS, Chung HY (2014) Ginsenoside Rc modulates Akt/FoxO1 pathways and suppresses oxidative stress. Archives of pharmacal research 37 (6): 813-820. doi: 10.1007/s12272-013-0223-2
34. Chou CH, Chuang LY, Lu CY, Guh JY (2013) Interaction between TGF-β and ACE2-Ang-(1-7)-Mas pathway in high glucose-cultured NRK-52E cells. Molecular and cellular endocrinology 366 (1): 21-30. doi: 10.1016/j.mce.2012.11.004
35. Odajima N, Betsuyaku T, Nagai K, Moriyama C, Wang DH, Takigawa T, Ogino K, Nishimura M (2010) The role of catalase in pulmonary fibrosis. Respiratory research 11 (1): 183. doi: 10.1186/1465-9921-11-183