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Ginsenoside Rg2 inhibits osteoclastogenesis by downregulating the NFATc1, c-Fos, and MAPK pathways

Sung-Hoon Lee[1,#], Shin-Young Park[2,#], Jung Ha Kim[3], Nacksung Kim[3,] & Junwon Lee[1,*]*

[1]Department of Life Science and Genetic Engineering, Graduate School of PaiChai University, Daejeon 35345, [2]Division of Software Engineering, PaiChai University, Daejeon 35345, [3]Department of Pharmacology, Chonnam National University Medical School, Gwangju 61469, Korea

Ginsenosides, among the most active components of ginseng, exhibit several therapeutic effects against cancer, diabetes, and other metabolic diseases. However, the molecular mechanism underlying the anti-osteoporotic activity of ginsenoside Rg2, a major ginsenoside, has not been clearly elucidated. This study aimed to determine the effects of ginsenoside Rg2 on receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclast formation. Results indicate that ginsenoside Rg2 inhibits RANKL-induced osteoclast differentiation of bone marrow macrophages (BMMs) without cytotoxicity. Pretreatment with ginsenoside Rg2 significantly reduced the RANKL-induced gene expression of *c-fos* and nuclear factor of activated T-cells (*Nfatc1*), as well as osteoclast-specific markers tartrate-resistant acid phosphatase (TRAP, *Acp5*) and osteoclast-associated receptor (*Oscar*). Moreover, RANKL-induced phosphorylation of mitogen-activated protein kinases (MAPKs) was decreased by ginsenoside Rg2 in BMM. Therefore, we suggest that ginsenoside Rg2 suppresses RANKL-induced osteoclast differentiation through the regulation of MAPK signaling-mediated osteoclast markers and could be developed as a therapeutic drug for the prevention and treatment of osteoporosis. [BMB Reports 2023; 56(10): 551-556]

INTRODUCTION

Coordination between osteoblasts, responsible for bone formation, and osteoclasts, tasked with bone resorption, is important in maintaining bone homeostasis and the structural integrity of the skeleton. The essential components, macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κB ligand (RANKL), play a defining role in steering hematopoietic stem cells towards the osteoclast lineage for bone tissue production (1, 2). The progression of commonly observed bone disorders like osteoporosis, rheumatoid arthritis, and lytic bone metastasis is driven by the gradual and excessive bone resorption carried out by osteoclasts (3, 4). Osteoporosis is a systemic skeletal condition marked by the reduction of bone mass and diminished bone mineral density, and compromised bone strength. Osteoporosis is usually considered an age-related manifestation, notably among postmenopausal women. Several studies have indicated that osteoclast populations and activity increase further after menopause during the pathogenesis of osteoporosis (5, 6). Its management is one of the greatest challenges faced by modern medicine. Bone resorption inhibitors, such as estrogen, calcitonin, and bisphosphonates, are traditional therapeutic agents for osteoporosis. Although these drugs seem to be the most effective methods to lower the bone loss rate after menopause, they can be accompanied by serious side effects such as venous thromboembolism, rhinitis, breast cancer, or esophageal ulcers (7, 8).

Belonging to the TNF-family, RANKL holds significance as a key cytokine promoting osteoclast formation (9). Interaction between RANKL and the receptor for RANK (known as RANK) on osteoclast precursors promotes the enlistment of TNF receptor-associated factor 6 (TRAF6), subsequently initiating cascading downstream signaling pathways, including NF-κB, Akt, c-jun N-terminal protein kinase (JNK), p38, and extracellular signal-related kinase (ERK), to promote the upregulation of genes associated with osteoclastogenesis, including *c-fos*, osteoclast-associated receptor (*Oscar*), tartrate-resistant acid phosphatase (TRAP, *Acp5*), and nuclear factor of activated T cells c1 (*Nfatc1*) (10-12). Among the genes that are stimulated, NFATc1 plays a particularly significant role as a crucial regulator of osteoclastogenesis. (13).

Natural products are valuable resources for developing new herbal drugs, such as the widely used ginseng. The roots of *Panax ginseng* Meyer (Araliaceae) are rich in highly bioactive phytomedicine widely used in traditional Korean, Chinese, and Japanese medicine. Studies so far have identified approximately 30 different forms of ginsenosides as the dominant active component exhibiting a variety of pharmacological effects on

*Corresponding authors. Nacksung Kim, Tel: +82-61-379-2835; Fax: +82-61-373-6974; E-mail: naksungkim@jnu.ac.kr; Junwon Lee, Tel: +82-42-520-5914; Fax: +82-70-4850-8479; E-mail: junwon@pcu.ac.kr
#These authors contributed equally to this work.

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cancer and on the cardiovascular, endocrine, nervous, and immune systems, among others (14-17). Although the anti-osteoporotic effects of ginseng have been examined (18, 19), the precise underlying molecular mechanism remains unknown. Ginsenoside Rg2 is a protopanaxatrial-type compound and one of the major active components found in the roots, stem, and leaves of ginseng. Several recent studies have suggested that ginsenoside Rg2 exerts various biological effects, including anti-apoptotic and anti-diabetic effects (20, 21). Furthermore, Rg2 plays a role in cardiovascular protection, neuroprotection, and inflammatory factor regulation (22, 23). Osteoclastogenesis is closely related to inflammatory regulation (24), but the role of Rg2 on the process of osteoclastogenesis remains unexplored.

Therefore, this study assessed the impacts of ginsenoside Rg2 on osteoclast differentiation induced by RANKL and elucidated the underlying mechanisms by which Rg2 inhibits osteoclastogenesis. The results demonstrated that Rg2 is a ginsenoside component with anti-osteoporotic activity.

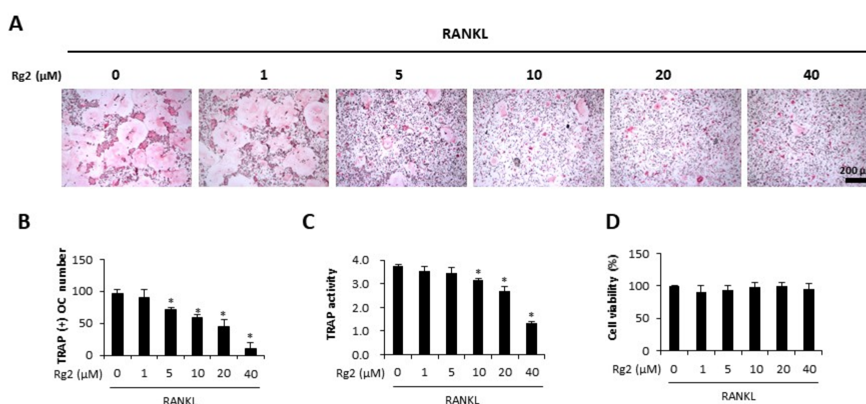

Fig. 1. Ginsenoside Rg2 inhibits osteoclast differentiation from BMMs. (A-D) Mouse bone marrow macrophages (BMMs) were cultured for 3 d with RANKL (100 ng/ml) at the indicated concentration of ginsenoside Rg2; (A) cultured cells were fixed and subjected to TRAP staining; (B) multinucleated osteoclasts were counted; (C) BMMs were stimulated with RANKL (100 ng/ml) for 3 d in the presence of ginsenoside Rg2 at the indicated concentrations, followed by TRAP solution assay; and (D) BMMs were treated with RANKL (100 ng/ml) and ginsenoside Rg2 for 3 d at the indicated concentration. Data are represented as mean ± standard error of the mean (SEM). *$P < 0.05$ compared to control. Bar: 200 μm.

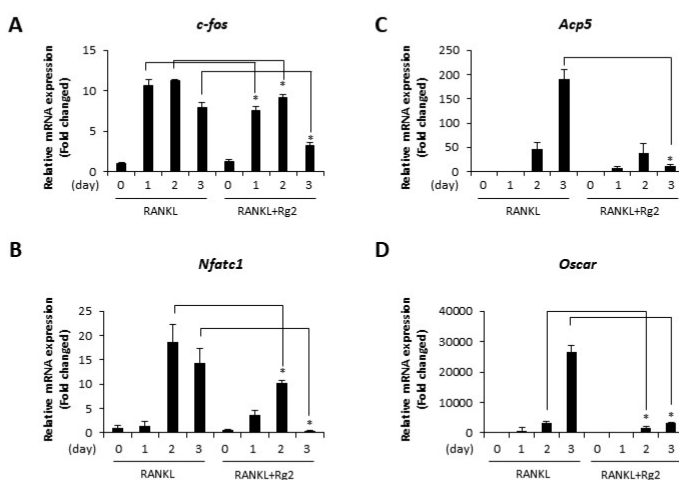

Fig. 2. Ginsenoside Rg2 suppresses RANKL-induced mRNA expression of c-fos, Nfatc1, Acp5, and Oscar in BMMs. (A-D) BMMs were pretreated with ginsenoside Rg2 (40 μM) or vehicle (DMSO) for 6 h and were then stimulated with RANKL (100 ng/ml) for the indicated time period. *c-fos*, *Nfatc1*, *Acp5*, and *Oscar* expression levels were analyzed by real-time PCR. Data are represented as mean ± standard error of the mean (SEM). *$P < 0.05$ compared to control.

RESULTS

Ginsenoside Rg2 inhibits osteoclast differentiation in bone marrow macrophages (BMMs) induced by RANKL

First, the impact of ginsenoside Rg2 on the osteoclast differentiation of BMMs was examined under RANKL influence using tartrate-resistant acid phosphatase (TRAP) staining. RANKL-induced osteoclast differentiation was determined based on the emergence of multinucleated cells positive for TRAP staining was observed. Ginsenoside Rg2 demonstrated a dose-dependent decrease in osteoclast differentiation, as reported (Fig. 1A, B). The TRAP assay further supported the suppressive impacts of Rg2 on osteoclast differentiation induced by RANKL (Fig. 1C). These findings suggest that Rg2 strongly inhibited osteoclast differentiation of BMMs. Following this, the cytotoxicity of Rg2-treated BMMs was evaluated using the MTT assay. The results indicated that Rg2 did not display any cytotoxicity, further substantiating the inhibitory property of ginsenoside Rg2 (Fig. 1D).

Ginsenoside Rg2 suppresses osteoclast-specific transcription factors induced by RANKL

Given that the pivotal osteoclast-specific transcription factors include c-Fos and NFATc1 (25, 26), their expression was analyzed to comprehend the inhibitory mechanism by which ginsenoside Rg2 counteracts osteoclastogenesis. qPCR analyses showed that administration of ginsenoside Rg2 before RANKL stimulation notably inhibited the expression of both *c-fos* and *Nfatc1* at the transcriptional level (Fig. 2A, B), as well as osteoclast-specific gene expression of *Acp5* and *Oscar* (Fig. 2C, D). Furthermore, Rg2 strongly attenuated c-Fos and NFATc1 protein expression (Fig. 3). These results indicate that ginsenoside Rg2 suppresses the mRNA expression of osteoclast-specific genes induced by RANKL, achieved by downregulating the expression of c-Fos and NFATc1.

Ginsenoside Rg2 suppresses phosphorylation of the MAPK pathway induced by RANKL in BMMs

Phosphorylation of the MAPK pathway, including p38, ERK, and JNK, was observed within 5 min of RANKL treatment to BMMs (27). Earlier studies have shown that inhibition of MAPK kinases suppresses osteoclastogenesis-related factors, such as TRAP and OSCAR, indicating that MAPK signaling is positively involved in RANKL-induced osteoclastogenesis (28, 29). Therefore, we demonstrated the role of ginsenoside Rg2 on the phosphorylation of the MAPK pathway induced by RANKL. As expected, the phosphorylation of JNK, ERK, and p38 stimulated by RANKL was found to be significantly suppressed by Rg2 in BMM (Fig. 4A, B). These results suggest that Rg2 negatively contributed to osteoclastogenesis induced by RANKL through the inhibition of p38, JNK, and ERK phosphorylation.

DISCUSSION

Enhanced RANKL signaling leads to heightened osteoclast formation and bone resorption. Consequently, the reduction of RANKL expression or modulation of its downstream signaling presents a promising avenue for addressing pathological bone loss therapeutically. Multiple transcription factors, such as c-Fos, NF-κB, Spi-1 (PU.1), and NFATc1, are involved in osteoclast development, with each exerting its influence at distinct phases of osteoclast differentiation (30). In particular, the absence of

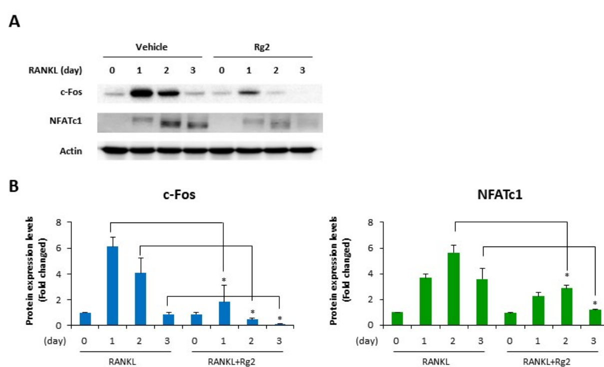

Fig. 3. Effect of ginsenoside Rg2 on RANKL-induced protein levels of c-FOS and NFATc1 in BMMs. (A) BMMs were pretreated with ginsenoside Rg2 (40 μM) or vehicle (DMSO) for 6 h and then stimulated with RANKL (100 ng/ml) for 3 d. Cells were lysed at the indicated times, and immune blot analyses were performed using anti-c-Fos, anti-NFATc1, or anti-actin antibodies to detect the respective protein bands. (B) Band intensity was quantified with Quantity Ones software. Data are represented as mean ± standard error of the mean (SEM). *P < 0.05 compared to control.

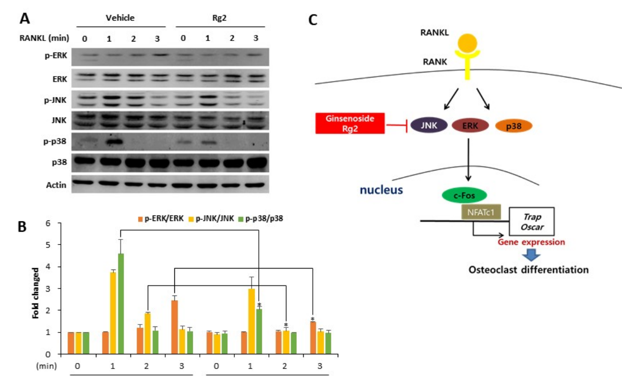

Fig. 4. Effect of ginsenoside Rg2 on RANKL-induced phosphorylation of MAPK pathways in BMMs. (A) BMMs were pretreated with ginsenoside Rg2 (40 μM) or vehicle (DMSO) for 6 h and then stimulated with RANKL (100 ng/ml) for the indicated times. The whole-cell lysates were extracted and subjected to western blotting analyses with anti-p-ERK, anti-ERK, anti-p-JNK, anti-JNK, anti-p-p38, and anti-p38 antibodies. (B) Band intensity was quantified with Quantity Ones software. Data are represented as mean ± standard error of the mean (SEM). *P < 0.05 compared to control. (C) A working model for the action of ginsenoside Rg2 on BMMs.

c-Fos and NFATc1 significantly disrupts the process of osteoclastogenesis (31). Ginsenoside Rg2 constitutes a component found in red ginseng and belongs to the propanaxatriol saponin group, having various biological effects (32). The present study found that ginsenoside Rg2 inhibit osteoclast differentiation induced by RANKL in BMM. The findings demonstrated that Rg2 suppressed the formation of osteoclasts from their precursors induced by RANKL, without causing cytotoxic effects.

Transcription factors c-Fos and NFATc1 have demonstrated pivotal roles in osteoclast formation; their absence halts the process of osteoclastogenesis (6, 27, 33). These factors become active in response to RANKL/RANK signaling, triggering the transformation of osteoclast precursors into fully developed osteoclasts. Notably, c-Fos is a key constituent of the AP-1 transcription factor, contributes to osteoclast differentiation by inducing NFATc1 expression (26, 33, 34). NFATc1 functions as a central transcription factor in osteoclast differentiation, driving the activation of multiple osteoclast-specific genes like *Acp5* and *Oscar*. Moreover, it has the capacity to trigger osteoclast differentiation independently of RANKL presence (35). In this study, at both mRNA and protein levels, ginsenoside Rg2 prominently suppressed the expression of c-Fos and NFATc1. These findings suggest that Rg2's inhibitory impact on osteoclast differentiation is associated with reduced expression of c-Fos and NFATc1. While the RANKL receptor RANK lacks inherent enzymatic activity within its intracellular domain, it communicates signals by enlisting adapters like TRAF6, subsequently triggering multiple signaling cascades (36, 37). RANKL is recognized for its ability to initiate MAPK pathways, encompassing ERK, JNK, and p38 (38). Demonstrably, inhibiting ERK has been established to reduce the formation of osteoclasts (39, 40) but induce c-Fos for osteoclastogenesis (41). In contrast, dominant-negative JNK inhibits osteoclastogenesis stimulated by RANKL (42), with significant inhibition of p38 occurs during the initial phases of osteoclast formation (43). The inhibition of ERK, JNK, and p38, resulting in the suppression of osteoclastogenesis and c-Fos expression, highlights the significant involvement of MAPK signaling pathways in osteoclast formation. The downregulation of c-Fos expression by ginsenoside Rg2 (Fig. 2A) implies that ginsenoside Rg2 efficiently inhibits the MAPK pathway to regulate osteoclast differentiation.

In conclusion, ginsenoside Rg2 effectively averted osteoclastogenesis induced by RANKL in primary bone marrow cells. Furthermore, this study clarified the molecular mechanisms that underlie the inhibitory impacts of ginsenoside Rg2. The findings imply that the therapeutic influence of ginsenoside Rg2 is linked to the decrease in c-Fos and NFATc1 expression, along with ERK, JNK, and p38 phosphorylation, resulting in reduced expression of *Acp5* and *Oscar* (Fig. 4C). Therefore, we suggest that ginsenoside Rg2 has the potential to serve as an herbal remedy for preventing and treating osteoporosis.

MATERIALS AND METHODS

Reagents and antibodies

Alpha-modified Eagle's minimum essential medium (α-MEM) and fetal bovine serum (FBS) were purchased from HyClone Laboratories (Waltham, MA, USA). Recombinant human M-CSF and soluble recombinant mouse RANKL were purchased from PeproTech (London, United Kingdom). Purified ginsenoside Rg2 (purity $\geq$ 98.0%, HPLC) was purchased from Hongjiu Biotechnology (Jilin, China). Penicillin-streptomycin solution and trypsin-EDTA were purchased from WelGENE Inc. (Daegu, Korea). The antibodies used were as follows: anti-phospho-p38 (#4631), anti-p38 (#9212), anti-phospho-JNK (#9255), anti-JNK (#9252), anti-phospho-ERK (#9101), and anti-ERK (#4695) were purchased from Cell Signaling Technology (Beverly, MA, USA); anti-NFATc1 (#sc-7294) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-c-Fos (Ab-2) (4-17) was purchased from Calbiochem (San Diego, CA, USA); and anti-actin (#A2228) was obtained from MilliporeSigma (Burlington, MA, USA). All the other chemicals were of analytical grade.

Osteoclast differentiation

Mouse bone marrow cells were obtained from the femurs and tibiae of 6- to 8-week-old ICR mice and incubated in α-MEM complete medium supplemented with 10% FBS and 100 U/ml penicillin in a 100 mm dish in the presence of M-CSF (30 ng/ml) for 3 d. Adherent cells were used as BMM osteoclast precursors after the removal of non-adherent cells. To generate osteoclasts, BMMs (1×10^5 cells/well) were cultured in complete medium containing M-CSF (30 ng/ml) and RANKL (100 ng/ml) in a 96-well plate, with or without ginsenoside Rg2. Following this, the cells were fixed in 10% formalin for 15 min, permeabilized with 0.1% Triton X-100, and stained with tartrate-resistant acid phosphatase (TRAP) solution. $TRAP^+$ multinuclear cells (MNCs) with more than three nuclei were counted as osteoclasts.

Cell viability assay

Cytotoxicity was measured using a MTT assay. BMMs (1×10^4 cells/well) were cultured with or without ginsenoside Rg2 at various concentrations (0-40 μM) for 48 h in the presence or absence of M-CSF (30 ng/ml) and RANKL (100 ng/ml) in 96-well plates (200 μl/well). MTT (100 μl/ml, 0.5 mg/ml) (MilliporeSigma) solution was added; after 4 h, 100 μl/ml of extraction buffer was added in 96-well plates (200 μl/well) and preserved for 2 h. The optical density (OD) was recorded at 570 nm with a 96-well plate reader.

Real-time PCR

Total RNA was extracted from the cultured cells using TRIZOL (Qiagen, Hilden, Germany). cDNA was synthesized from 1 μg of total RNA using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and random primers (Promega Corporation, Madison, WI, USA). qPCR was performed using SYBR

Green PCR Master Mix (Qiagen) according to the manufacturer's protocols. Relative differences in PCR results were evaluated using the comparative cycle threshold method. The primers used were as follows: *c-fos*, 5′-ATG GGC TCT CCT GTC AAC ACA-3′ and 5′-TGG CAA TCT CAG TCT GCA ACG CAG-3′; *Nfatc1*, 5′-CTC GAA AGA CAG CAC TGG AGC AT-3′ and 5′-CGG CTG CCT TCC GTC TCA TAG-3′; *Oscar*, 5′-CTG CTG GTA ACG GAT CAG CTC CCC AGA-3′ and 5′-CCA AGG AGC CAG AAC CTT CGA AAC T-3′; *Acp5*, 5′-CAG TTG GCA GCA GCC AAG GAG GAC-3′ and 5′-GTC CCT CAG GAG TCT AGG TAT CAC-3′; and *Gapdh*, 5′-TGA CCA CAG TCC ATG CCA TCA CTG-3′ and 5′-CAG GAG ACA ACC TGG TCC TCA GTG-3′. All reactions were performed in triplicate and normalized to the housekeeping gene *Gapdh*.

Western blot analysis

Cells were harvested after 1X PBS washing and lysed in extraction buffer (50 mM Tris-HCl [pH 8.0], 0.5% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, and protease inhibitors). Protein samples were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (MilliporeSigma). The membranes were then incubated with primary antibodies against anti-NFATc1 (1:1,000), anti-c-Fos (1:1,000), anti-actin (1:3,000), anti-phospho-ERK (1:2,000), anti-ERK (1:1,000), anti-phospho-JNK (1:500), anti-JNK (1:1,000), anti-phospho-p38 (1:500) and anti-p38 (1:1,000). Horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences, Piscataway, NJ, USA) were probed and developed with ECL solution (MilliporeSigma). Signals were detected and analyzed using an LAS3000 luminescent image analyzer (Fuji, Tokyo, Japan).

Statistical analysis

Statistical analyses were performed using SPSS software (version 17.0). Comparisons between experimental groups were performed using one-way ANOVA with Tukey's multiple post-hoc test or Student's *t*-test. All measurements were performed at least in triplicate; all quantitative data have been expressed as mean ± standard error of the mean (SEM). $P < 0.05$ were considered statistically significant.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Novack DV and Teitelbaum SL (2008) The osteoclast: friend or foe? Annu Rev Pathol 3, 457-484
2. Nakashima T, Hayashi M, Fukunaga T et al (2011) Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat Med 17, 1231-1234
3. Rodan GA and Martin TJ (2000) Therapeutic approaches to bone diseases. Science 289, 1508-1514
4. Rivollier A, Mazzorana M, Tebib J et al (2004) Immature dendritic cell transdifferentiation into osteoclasts: a novel pathway sustained by the rheumatoid arthritis microenvironment. Blood 104, 4029-4037
5. Roodman GD (1999) Cell biology of the osteoclast. Exp Hematol 27, 1229-1241
6. Takeshita S, Namba N, Zhao JJ et al (2002) SHIP-deficient mice are severely osteoporotic due to increased numbers of hyper-resorptive osteoclasts. Nat Med 8, 943-949
7. Genant HK, Baylink DJ and Gallagher JC (1989) Estrogens in the prevention of osteoporosis in postmenopausal women. Am J Obstet Gynecol 161, 1842-1846
8. Reid IR (2002) Pharmacotherapy of osteoporosis in postmenopausal women: focus on safety. Expert Opin Drug Saf 1, 93-107
9. Kong YY, Yoshida H, Sarosi I et al (1999) OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 397, 315-323
10. Lee ZH and Kim HH (2003) Signal transduction by receptor activator of nuclear factor kappa B in osteoclasts. Biochem Biophys Res Commun 305, 211-214
11. Lee SE, Woo KM, Kim SY et al (2002) The phosphatidylinositol 3-kinase, p38, and extracellular signal-regulated kinase pathways are involved in osteoclast differentiation. Bone 30, 71-77
12. Li J, Sarosi I, Yan XQ et al (2000) RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. Proc Natl Acad Sci U S A 97, 1566-1571
13. Walsh MC, Kim N, Kadono Y et al (2006) Osteoimmunology: interplay between the immune system and bone metabolism. Annu Rev Immunol 24, 33-63
14. Chang Y, Huang WJ, Tien LT and Wang SJ (2008) Ginsenosides Rg1 and Rb1 enhance glutamate release through activation of protein kinase A in rat cerebrocortical nerve terminals (synaptosomes). Eur J Pharmacol 578, 28-36
15. Chen WF, Lau WS, Cheung PY, Guo DA and Wong MS (2006) Activation of insulin-like growth factor I receptor-mediated pathway by ginsenoside Rg1. Br J Pharmacol 147, 542-551
16. Lau WS, Chan RY, Guo DA and Wong MS (2008) Ginsenoside Rg1 exerts estrogen-like activities via ligand-independent activation of ERalpha pathway. J Steroid Biochem Mol Biol 108, 64-71
17. Pan XY, Guo H, Han J et al (2012) Ginsenoside Rg3 attenuates cell migration via inhibition of aquaporin 1 expression in PC-3M prostate cancer cells. Eur J Pharmacol 683, 27-34
18. Kim HR, Cui Y, Hong SJ et al (2008) Effect of ginseng mixture on osteoporosis in ovariectomized rats. Immuno-

pharmacol Immunotoxicol 30, 333-345
19. Liu J, Shiono J, Shimizu K et al (2009) 20(R)-ginsenoside Rh2, not 20(S), is a selective osteoclastgenesis inhibitor without any cytotoxicity. Bioorg Med Chem Lett 19, 3320-3323
20. Yuan HD, Kim DY, Quan HY, Kim SJ, Jung MS and Chung SH (2012) Ginsenoside Rg2 induces orphan nuclear receptor SHP gene expression and inactivates GSK3beta via AMP-activated protein kinase to inhibit hepatic glucose production in HepG2 cells. Chem Biol Interact 195, 35-42
21. Zhang G, Liu A, Zhou Y, San X, Jin T and Jin Y (2008) Panax ginseng ginsenoside-Rg2 protects memory impairment via anti-apoptosis in a rat model with vascular dementia. J Ethnopharmacol 115, 441-448
22. Xue Q, Yu T, Wang Z et al (2023) Protective effect and mechanism of ginsenoside Rg2 on atherosclerosis. J Ginseng Res 47, 237-245
23. Zhou L, Wang F, Sun R et al (2016) SIRT5 promotes IDH2 desuccinylation and G6PD deglutarylation to enhance cellular antioxidant defense. EMBO Rep 17, 811-822
24. Yao F, Xue Q, Li K, Cao X, Sun L and Liu Y (2019) Phenolic compounds and ginsenosides in ginseng shoots and their antioxidant and anti-inflammatory capacities in LPS-induced RAW264.7 mouse macrophages. Int J Mol Sci 20, 2951
25. Wang ZQ, Ovitt C, Grigoriadis AE, Mohle-Steinlein U, Ruther U and Wagner EF (1992) Bone and haematopoietic defects in mice lacking c-fos. Nature 360, 741-745
26. Takayanagi H, Kim S, Koga T et al (2002) Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev Cell 3, 889-901
27. Lee JH, Jin H, Shim HE, Kim HN, Ha H and Lee ZH (2010) Epigallocatechin-3-gallate inhibits osteoclastogenesis by down-regulating c-Fos expression and suppressing the nuclear factor-kappaB signal. Mol Pharmacol 77, 17-25
28. Nedeva IR, Vitale M, Elson A, Hoyland JA and Bella J (2021) Role of OSCAR signaling in osteoclastogenesis and bone disease. Front Cell Dev Biol 9, 641162
29. Kim HN, Baek JK, Park SB et al (2019) Anti-inflammatory effect of Vaccinium oldhamii stems through inhibition of NF-kappaB and MAPK/ATF2 signaling activation in LPS-stimulated RAW264.7 cells. BMC Complement Altern Med 19, 291
30. Teitelbaum SL and Ross FP (2003) Genetic regulation of osteoclast development and function. Nat Rev Genet 4, 638-649
31. Teitelbaum SL (2004) RANKing c-Jun in osteoclast development. J Clin Invest 114, 463-465
32. Kiefer D and Pantuso T (2003) Panax ginseng. Am Fam Physician 68, 1539-1542
33. Grigoriadis AE, Wang ZQ, Cecchini MG et al (1994) c-Fos: a key regulator of osteoclast-macrophage lineage determination and bone remodeling. Science 266, 443-448
34. Yamashita T, Yao Z, Li F et al (2007) NF-kappaB p50 and p52 regulate receptor activator of NF-kappaB ligand (RANKL) and tumor necrosis factor-induced osteoclast precursor differentiation by activating c-Fos and NFATc1. J Biol Chem 282, 18245-18253
35. Wagner EF and Eferl R (2005) Fos/AP-1 proteins in bone and the immune system. Immunol Rev 208, 126-140
36. Gohda J, Akiyama T, Koga T, Takayanagi H, Tanaka S and Inoue J (2005) RANK-mediated amplification of TRAF6 signaling leads to NFATc1 induction during osteoclastogenesis. EMBO J 24, 790-799
37. Takayanagi H (2007) Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. Nat Rev Immunol 7, 292-304
38. Boyle WJ, Simonet WS and Lacey DL (2003) Osteoclast differentiation and activation. Nature 423, 337-342
39. Ang E, Liu Q, Qi M et al (2011) Mangiferin attenuates osteoclastogenesis, bone resorption, and RANKL-induced activation of NF-kappaB and ERK. J Cell Biochem 112, 89-97
40. Kim HJ, Lee Y, Chang EJ et al (2007) Suppression of osteoclastogenesis by N,N-dimethyl-D-erythro-sphingosine: a sphingosine kinase inhibition-independent action. Mol Pharmacol 72, 418-428
41. Monje P, Hernandez-Losa J, Lyons RJ, Castellone MD and Gutkind JS (2005) Regulation of the transcriptional activity of c-Fos by ERK. A novel role for the prolyl isomerase PIN1. J Biol Chem 280, 35081-35084
42. Ikeda F, Nishimura R, Matsubara T et al (2004) Critical roles of c-Jun signaling in regulation of NFAT family and RANKL-regulated osteoclast differentiation. J Clin Invest 114, 475-484
43. Matsumoto M, Sudo T, Saito T, Osada H and Tsujimoto M (2000) Involvement of p38 mitogen-activated protein kinase signaling pathway in osteoclastogenesis mediated by receptor activator of NF-kappa B ligand (RANKL). J Biol Chem 275, 31155-31161