

*Forum Minireview***Pharmacological Topics of Bone Metabolism:  
Antiresorptive Microbial Compounds That Inhibit Osteoclast  
Differentiation, Function, and Survival**Je-Tae Woo<sup>1,\*</sup>, Takayuki Yonezawa<sup>1</sup>, Byung-Yoon Cha<sup>1</sup>, Toshiaki Teruya<sup>1</sup>, and Kazuo Nagai<sup>1</sup><sup>1</sup>Department of Biological Chemistry, Chubu University, Matsumoto 1200, Kasugai 487-8501, Japan

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**Abstract.** The mass and function of bones depends on the maintenance of a complicated balance between osteoclast-mediated bone resorption and osteoblast-mediated bone formation. Osteoporosis typically reflects an imbalance in skeletal turnover, such that bone resorption exceeds bone formation. Osteoclasts are target cells for anti-osteoporosis therapies. To discover new types of antiresorptive agents, we screened for natural compounds that regulate osteoclast differentiation, function, and survival. As a result, we identified reveromycin A, destruxins, mevastatin, FK506, cyclosporin A, prodigiosins, concanamycins, and symbioimine among microbial natural compounds. In this review, we discuss the mechanisms of action of these compounds on osteoclasts.

**Keywords:** microbial metabolite, antiresorptive agent, bone resorption, osteoclast, osteoporosis, bone metabolism

**Introduction**

Bone is a specialized tissue that confers multiple mechanical and metabolic functions to the skeleton in all higher vertebrates. It is composed of an organic and mineralized matrix. The crystalline salts deposited in the organic matrix of bone under cellular control are primarily calcium and phosphate in the form of hydroxyapatite. Bone contains two distinct cell types, namely osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells). Bone is constantly destroyed or resorbed by osteoclasts and then replaced by osteoblasts in a physiologic process referred to as bone remodeling. Imbalance between bone resorption and bone formation results in several bone diseases, including osteoporosis, hyperparathyroidism, metastatic bone disease, and hypercalcemia of malignancies. Reduction of the bone mass is a characteristic of osteoporosis, and it increases both the fragility of bone and its susceptibility to fractures. Osteoporosis occurs in older men as well as in women as a result of estrogen deficiency after meno-

pause. Most bone disorders, including osteoporosis, are due to increased bone resorption. Inhibition of bone resorption can be accomplished by reducing either osteoclast generation or osteoclast activity.

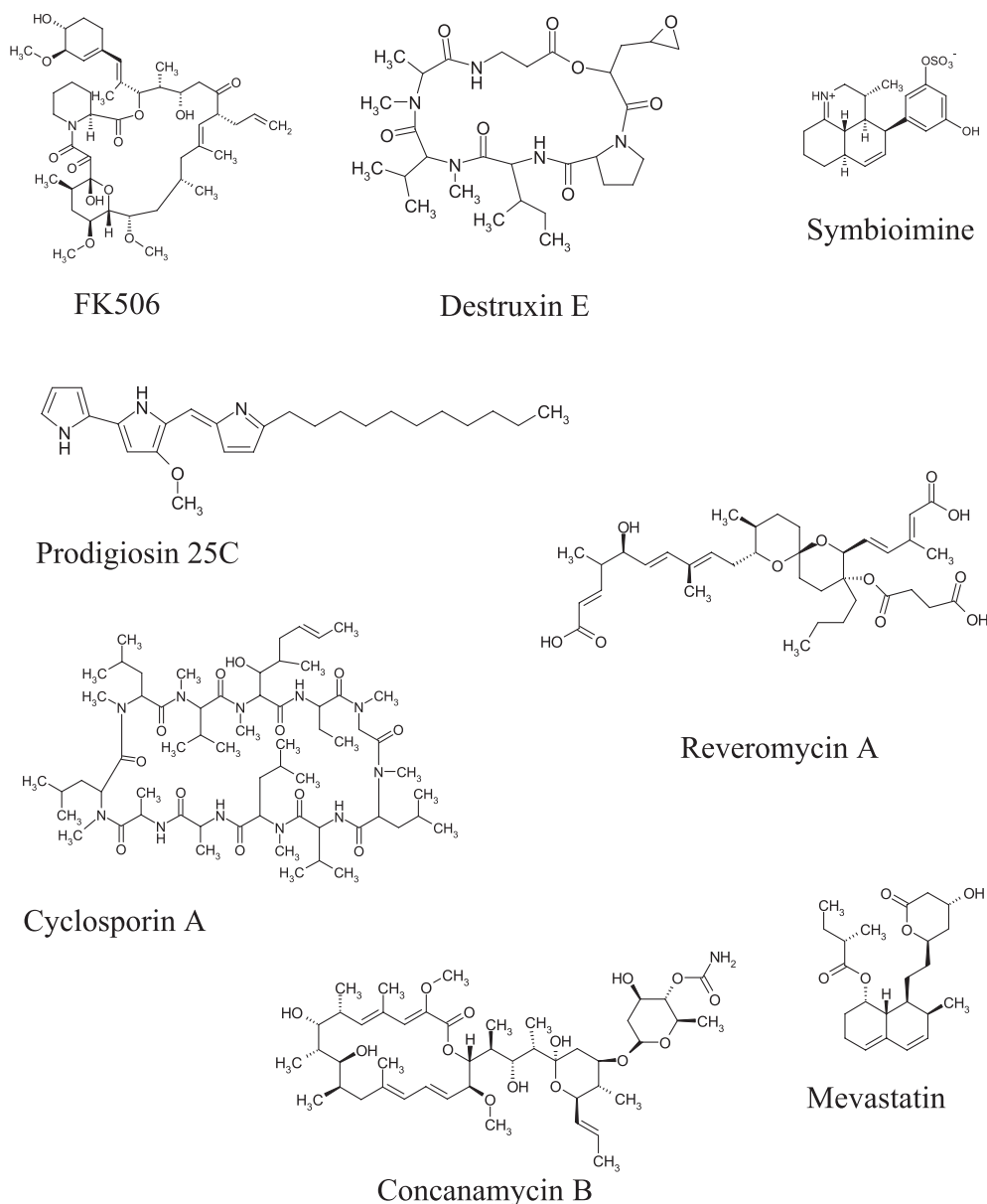
Osteoclast-mediated bone resorption occurs by a multistep process as follows (1 – 5): a) commitment of progenitor cells to precursor cells; b) differentiation of mononuclear preosteoclasts from hemopoietic cells of the monocyte-macrophage lineage; c) fusion of mononuclear preosteoclasts into mature multinucleated osteoclasts; d) attachment of mature osteoclasts to RGD-sequence-containing proteins on the mineralized bone surface through vitronectin receptors; e) polarization of cytoplasmic structures, such as ruffled borders and clear zones; and f) secretion of acids and lysosomal enzymes into the resorbing site beneath the ruffled border, which results in dissolution of apatite crystals and degradation of the organic matrix.

Natural compounds that specifically inhibit these steps could be developed as antiresorptive drugs for the treatment of metabolic bone disorders characterized by excessive osteoclastic bone resorption. Several microbial natural compounds are known to regulate osteoclast differentiation and/or function, such as c-Src tyrosine kinase inhibitors (herbimycin A, genistein), a phos-

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**Fig. 1.** Structures of microbial natural compounds.

phatidyl inositol 3-kinase inhibitor (wortmannin), vitamin K<sub>2</sub>, and protease inhibitors (E-64, leupeptin, and others). Among these metabolites, vitamin K<sub>2</sub> is already used clinically as a drug for the prevention and treatment of osteoporosis. To discover other natural compounds that may act as antiresorptive agents, we screened for microbial natural compounds that can inhibit the differentiation and function of osteoclasts. As a result, we identified reveromycin A, destruxins, metastatin, FK506, cyclosporin A, prodigiosins, concanamycins, and symbioimine among microbial natural compounds (Figs. 1 and 2). In this review, we discuss the cellular and molecular mechanisms of the actions of these

compounds on osteoclasts.

### Proapoptotic activity of reveromycin A on osteoclasts

In a screening for new types of antiresorptive agents, we found that reveromycin A specifically induced apoptosis in osteoclasts (6). Reveromycin A is a natural compound with three carboxylic groups in its structure that was isolated from the genus *Streptomyces*. It has been reported to inhibit EGF-dependent responses of mouse epidermal cells, proliferation of human tumor cells, and protein synthesis in mammalian cells (7). Recent studies have revealed that a major target of

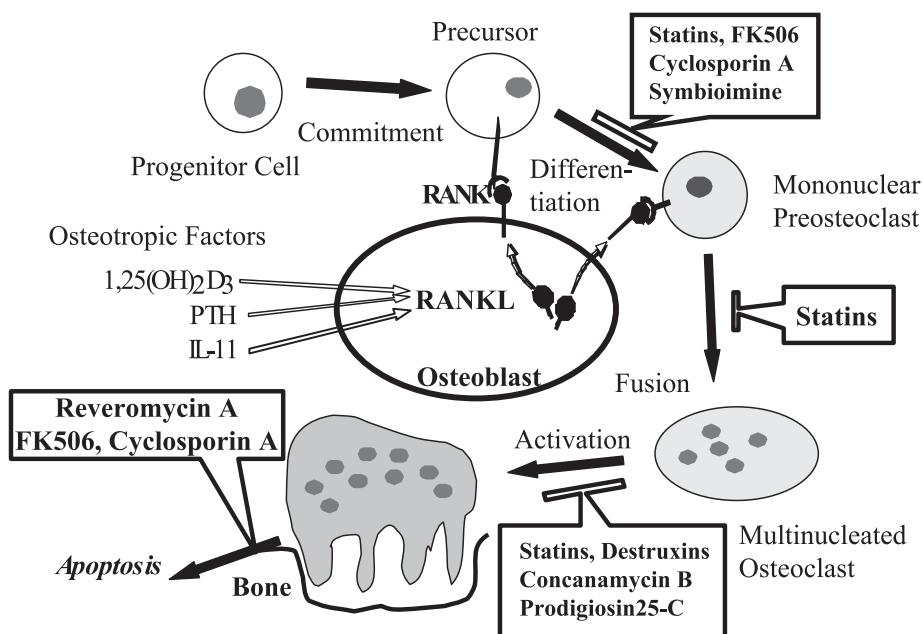


Fig. 2. Microbial natural compounds regulate osteoclast differentiation and function.

reveromycin A action in yeasts is isoleucyl-tRNA synthetase (8). We showed that reveromycin A induced apoptosis in functional (polarized) osteoclasts, but not in non-functional osteoclasts, osteoclast progenitor cells, or osteoblasts (6). We further demonstrated that isoleucyl-tRNA synthetase was also one of the major targets of reveromycin A in osteoclasts (6). Reveromycin A was incorporated into osteoclasts with both actin rings and acidified intracellular organelles. The pro-apoptotic activity of reveromycin A was prevented by disruption of the actin rings by calcitonin or disruption of an acidic microcompartment by a vacuolar-type proton ATPase inhibitor (concanamycin A). These observations suggest that the specificity of reveromycin A for activated osteoclasts may result from acidic conditions that suppress the dissociation of protons from its carboxylic acid moieties and subsequently increase the amounts of non-polar forms of reveromycin A, which are more cell-permeable (Fig. 3).

Bisphosphonates, which are widely used for the treatment of osteoporosis (9), are also incorporated into functional osteoclasts. Following their incorporation, these bisphosphonates disrupt the actin rings and induce apoptosis in osteoclasts (10–12). Bisphosphonate incorporation into functional osteoclasts is regulated by acidification of the extracellular microenvironment. This aspect of the incorporation mechanism of bisphosphonates is very similar to that of reveromycin A. However, recent studies have shown that alendronate, a bisphosphonate, impairs the ability of parathyroid

hormone (PTH) to increase bone mineral density in both women and men with osteoporosis (13, 14). Since alendronate decreases overall bone turnover by continuous inhibition of bone resorption, bisphosphonates will probably not be used in combination with anabolic drugs, such as PTH. Antiresorptive drugs with mechanisms that differ from that of bisphosphonates need to be developed for use with anabolic drugs. Reveromycin A, which does not accumulate in bone minerals, may be a candidate for combination treatments with anabolic drugs.

We further showed that reveromycin A suppressed resorption pit formation by osteoclasts on dentine slices as well as PTH-stimulated <sup>45</sup>Ca release in organ cultures. Furthermore, reveromycin A dramatically prevented bone loss in the distal metaphysis in ovariectomized mice. Muguroma et al. showed that reveromycin A inhibits osteolytic bone metastasis of lung cancer cells (SBC-5 cells) through an antiosteoclastic activity (15). The inhibitory effect of reveromycin A on bone metastasis is caused by directly inducing apoptosis of osteoclasts and indirectly inhibiting tumor cell-derived PTHrP production without affecting the number of proliferating tumor cells (15). Furthermore, in a rat osteoporosis model with a low calcium intake, reveromycin A reduced the number of osteoclasts in the bone and inhibited bone resorption. The decrease of the number of osteoclasts by reveromycin A may result from inducing apoptosis of osteoclasts. Although further studies are required to determine whether co-treatment

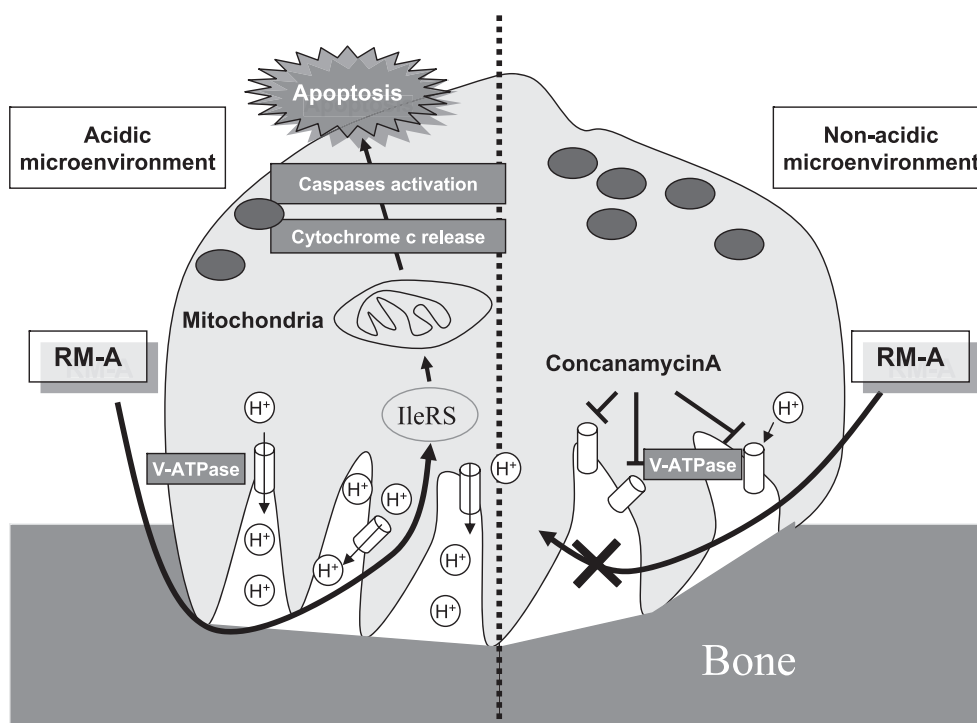


Fig. 3. Possible mechanism of action of reveromycin A on osteoclasts. IleRS: Isoleucyl-tRNA synthetase.

of reveromycin A with PTH can enhance the effect of PTH on bone mineral density, reveromycin A may be a unique antiresorptive agent for the treatment of bone disorders including osteolytic bone metastasis.

#### Disruptive activity of destruxins on actin rings in osteoclasts

Calcitonin inhibits bone resorption by disrupting the morphological structures of polarized osteoclasts via the cAMP-protein kinase A (PKA) pathway, without affecting osteoclast survival (16). Continuous treatment with calcitonin eventually leads to decreased inhibitory effects on osteoclastic bone resorption (escape phenomenon). This escape phenomenon is known to involve downregulation of the calcitonin receptor, which is derived from activation of the PKA pathway (17, 18). Compounds that exert calcitonin-like activity without affecting the PKA pathway would be viewed as new types of antiresorptive agents. Therefore, to discover compounds with calcitonin-like activity, we screened for natural compounds among microbial metabolites that inhibit the bone-resorbing activity of osteoclasts without affecting their survival. As a result, we identified destruxins B and E, which were isolated from an entomopathogenic or phytopathogenic fungus, respectively (19). Destruxins, like calcitonin, reversibly inhibit bone resorption by disrupting morphological structures with

actin rings and ruffled borders in polarized osteoclasts. However, they did not affect non-polarized osteoclasts, osteoclast progenitor cells, preosteoclasts, and other adherent cell lines (A549, B16, C33A, L929, MCF7, and ST2 cells). These results suggest that destruxins specifically act on polarized osteoclasts with cytoskeletal structures, rather than on the general cytoskeleton of the cells. Since they reversibly induce morphological changes in osteoclasts without affecting cell survival, the effects of destruxins on polarized osteoclasts are very similar to those of calcitonin. However, calcitonin inhibits the fusion of preosteoclasts into multinuclear osteoclasts, whereas destruxins do not. These findings suggest that the targets of destruxins are as yet unknown signaling pathways involved in the maintenance of polarization to exert bone-resorbing activity. Destruxins are also known to increase intracellular calcium levels. The possibility of calcium ion involvement in the activity of destruxins toward inducing structural disorders has not been excluded. It will be important to identify the target molecules and mechanisms of action of destruxins and to elucidate how destruxins affect the morphological structures and bone-resorbing activity of functional osteoclasts.

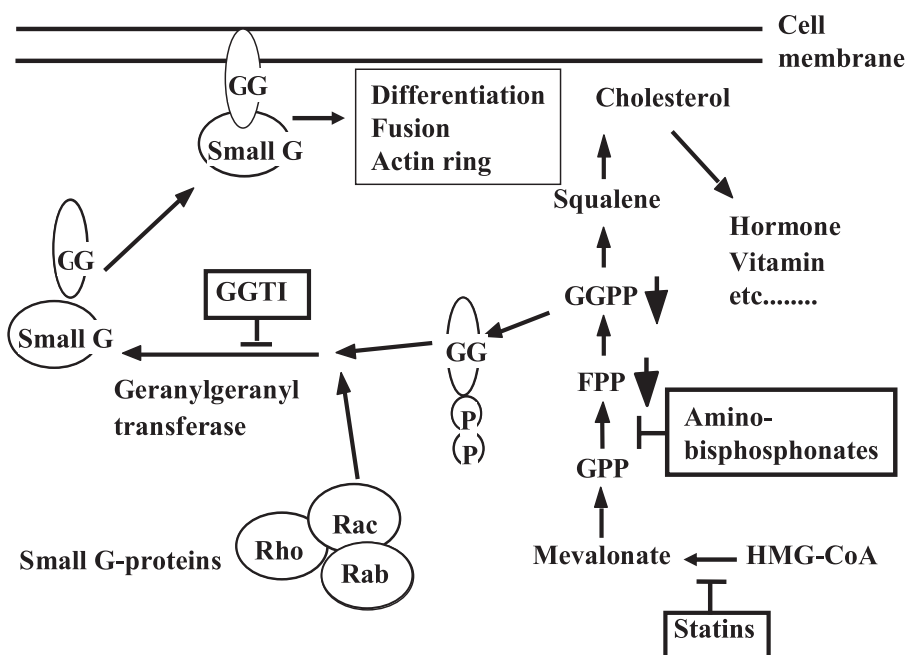
### Inhibitory effects of statins on osteoclast differentiation, function, and survival

Mevastatin (compactin), a kind of statin, was isolated from *Penicillium citrinum*. It is known to inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), which catalyzes the synthesis of mevalonic acid lactone (MVA) from HMG-CoA. Its derivatives, such as lovastatin and pravastatin, are clinically used as antihyperlipidemic drugs for the treatment of arteriosclerosis.

Recent studies have revealed that nitrogen-containing bisphosphonates, which represent pyrophosphate analogs, inhibit multinucleated osteoclast formation, suppress osteoclastic bone resorption, and induce apoptosis in mature osteoclasts (9–12). Although the exact mechanisms of action of nitrogen-containing bisphosphonates and pyrophosphates on osteoclast differentiation and survival are not known, recently it has been proposed that nitrogen-containing bisphosphonates inhibit the enzyme activity of farnesyl and geranylgeranyl pyrophosphate synthase and affect the farnesylation and geranylgeranylation of small G-proteins. In fact, the effect of nitrogen-containing bisphosphonates is rescued by geranylgeraniol and mimicked by specific geranylgeranyl transferase inhibitors. These results suggest that the geranylgeranylation of small G-proteins plays important roles in osteoclast differentiation, survival, and function.

Statin that inhibit the synthesis of mevalonate, have similar effects on osteoclasts to that of nitrogen-containing bisphosphonates. We found that mevastatin

reversibly inhibits the fusion of preosteoclasts into multinucleated osteoclasts, disrupts the actin rings in mature osteoclasts, and suppresses bone resorption activity without promoting apoptosis in osteoclasts (20). Recently, we also showed that mevastatin inhibits mononuclear preosteoclast formation induced by receptor activator of nuclear factor  $\kappa$ B (NF- $\kappa$ B) ligand (RANKL) or tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in cultures of mouse marrow-derived macrophage colony stimulating factor-dependent monocytes or mouse monocyte cell line RAW264.7 cells (21). This inhibition of mevalonate synthesis by statins also prevents the synthesis of its downstream intermediates, namely, farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Similar to nitrogen-containing bisphosphonates, statins can also exert these effects on osteoclasts by preventing the geranylgeranylation of small GTP-binding proteins. We further showed that geranylgeranyl pyrophosphate rescues the inhibitory effects of statins on osteoclast differentiation, fusion, survival, and function and that a specific geranylgeranyl transferase I inhibitor (GGTI), but not a farnesyl transferase inhibitor (FTI), was found to inhibit the formation of multinucleated osteoclasts in bone marrow cultures (21). In our studies, GGTI-2166 significantly also inhibited the incorporation of [ $^3$ H]geranylgeraniol into 21–26-kDa proteins, which represent geranylgeranylated small G-proteins. These findings suggest that the inhibitory effects of statins on osteoclasts result from decreased geranylgeranylation of prenylated G-proteins (Fig. 4). Recent studies have revealed that G-proteins, such as Rab, Rho, and Rac, are involved in the organization of morpho-



**Fig. 4.** Possible mechanism of action of statins on osteoclast differentiation and function. GG: geranylgeranyl, P: mono-phosphate, GGTI: geranylgeranyl transferase inhibitor, Small G: small G-protein, HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A, GPP: geranyl pyrophosphate, GGPP: geranylgeranyl pyrophosphate, FPP: farnesyl pyrophosphate.

logical structures and in bone resorption (22, 23). Rab is known to colocalize with c-src and V-ATPase on ruffled borders. We further demonstrated that toxin B, which inactivates Rho, Rac, and Cdc42 family proteins, inhibited the preosteoclast formation induced by RANKL or TNF- $\alpha$ . These findings suggest that preosteoclast formation and osteoclast function are regulated by these G-proteins (Fig. 4). Further studies are necessary to identify the specific small G-proteins involved in osteoclast formation.

In a study examining the downstream events during signal transduction of geranylgeranylated proteins, Fisher et al. found that lovastatin activates a 34-kDa kinase in murine osteoclasts and that this activation is blocked by geranylgeraniol or MVA (24). Activation of this 34-kDa kinase may play an important role in the effect of lovastatin on bone resorption. Further studies are required to determine which geranylgeranylated proteins and signaling pathways downstream of small G-proteins are involved in actin ring stability in osteoclasts.

Mevastatin also reversibly inhibited osteoclastic bone resorption on bone slices as well as  $^{45}\text{Ca}$  release in bone organ cultures stimulated by osteotropic factors such as interleukin-1 $\beta$ , 1 $\alpha$ ,25-dihydroxyvitamin D $_3$  (calcitriol), and PTH. These factors are known to induce the expression of RANKL in osteoblasts and support osteoclast differentiation and function. Therefore, the inhibitory effects of mevastatin are likely to be a consequence of the inhibition of osteoclast differentiation, fusion, and polarization by blocking the geranylgeranylation of small G-proteins.

Antiresorptive agents have not been proven to significantly increase bone formation. Therefore, the ideal agent would not only improve bone strength by preventing bone loss, but also stimulate bone formation for prevention of fractures. Most of the current therapies available for the treatment of osteoporosis prevent bone loss rather than stimulate its formation. Statins were reported to have bone anabolic effects (25). In vitro studies as well as some animal studies have revealed that statins increase bone mass by enhancing bone morphogenetic protein-2 expression in osteoblasts (26). Although several case-control studies have suggested that statins reduce the risk of fractures by increasing bone formation, other studies have failed to show a benefit in fracture reduction. One possible reason for these conflicting preclinical results, as well as those of clinical studies, is the liver-specific nature of statins. To use statins for the treatment of osteoporosis, we need to develop bone-specific statins or use bone-targeted delivery systems. Furthermore, double-blind randomized controlled trials are required.

## Effects of FK506 and cyclosporin A on osteoclast differentiation and survival

The calcineurin/nuclear factor of activated T cells (NFAT) signaling pathway is known to be involved in a wide range of biological responses in a variety of different cell types. Several studies have shown that the immunosuppressant drugs FK506 (tacrolimus) and cyclosporin A inhibit osteoclast differentiation in bone marrow cell cultures and osteoclastic bone resorption in bone organ cultures induced by PTH, calcitriol, prostaglandins, and cytokines. Takayanagi et al. recently showed that NFATc1 expression was induced in osteoclast precursors via both TNF receptor-associated factor 6 and c-fos (a member of the heterodimeric transcription factor complex AP-1) signaling pathway by RANKL stimulation (27). We further found that FK506 and cyclosporin A inhibited osteoclast differentiation and induced apoptosis in osteoclasts (28). Furthermore, we reported that the calcineurin/nuclear factor of activated T cells signaling pathway regulates osteoclastogenesis in RAW264.7 cells (29). These observations suggest that calcineurin is an essential downstream mediator of the RANKL-induced signal transduction pathway for osteoclast differentiation, and indicate that activation of the NFATc1 transcription factor is sufficient to induce osteoclast differentiation and to express bone resorptory function.

## Effects of prodigiosins, concanamycins, and symbioimine on osteoclast differentiation and/or function

Prodigiosins, which are secondary metabolites of bacterial origin, are red pigments produced by many strains of the bacterium *Serratia marcescens*. They have been reported to have antifungal, immunosuppressive, antiproliferative, and pro-apoptotic activities. We previously reported that prodigiosin 25-C uncouples vacuolar type H(+)-ATPase, inhibits vacuolar acidification, and affects glycoprotein processing and that concanamycin B inhibits the acidification of endosomes and lysosomes induced by V-ATPase in macrophage J774 (30, 31). We further found that prodigiosin 25-C and concanamycin B inhibit osteoclastic bone resorption on bone slices (32, 33). Prodigiosin 25-C and concanamycin B are an uncoupler and a specific inhibitor, respectively, of vacuolar H $^{+}$ -ATPase. The inhibitory effect of these two compounds on osteoclast function may be caused by disrupting the acidified microenvironment of the bone resorption site. We found that symbioimine, an amphoteric iminium metabolite from the dinoflagellate *Symbiodinium* sp., inhibits osteoclast differentiation in cultures of murine monocytic cell line

RAW264 cells in the presence of RANKL (34). Further studies are necessary to identify its target molecule and elucidate its mechanism of action for osteoclast differentiation.

## Conclusions

Microbial metabolites that contain structurally diverse chemical compounds usually are known to have some form of biological activity. Thus, microbial natural compounds have been invaluable for discovering drugs and lead compounds. In our screening program, we have identified microbial natural compounds, reveromycin A, destruxins, statins, FK506, cyclosporin A, simbioimine, and prodigiosins, that inhibit osteoclast differentiation, function, and/or survival. The mechanism of action of reveromycin A for inducing apoptosis in functional osteoclasts is different from that of bisphosphonates, which impair the anabolic effect of PTH. Reveromycin A may be a unique candidate for combination treatment with anabolic agents. Statins have dual activities, an antiresorptive effect on osteoclasts and a stimulative effect on osteoblasts. However, in order to be recommended as new therapeutic agents with both antiresorptive and anabolic effects, the development of bone-specific statins and clinical trials are required. Although the mechanisms of action of destruxins and simbioimine have not been elucidated, further studies on these metabolites may provide new insights into the mechanisms of osteoclast differentiation and functions.

## References

- Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature*. 2003;423:337–342.
- Takahashi N, Ejiri S, Yanagisawa S, Ozawa H. Regulation of osteoclast polarization. *Odontology*. 2007;95:1–9.
- Rodan GA, Martin TJ. Therapeutic approaches to bone diseases. *Science*. 2000;289:1508–1514.
- Teitelbaum, SL. Bone resorption by osteoclasts. *Science*. 2000;289:1504–1508.
- Chambers, TJ. Regulation of the differentiation and function of osteoclasts. *J Pathol*. 2000;192:4–13.
- Woo JT, Kawatani M, Kato M, Shinki T, Yonezawa T, Kanoh N, et al. Reveromycin A, an agent for osteoporosis, inhibits bone resorption by inducing apoptosis specifically in osteoclasts. *Proc Natl Acad Sci U S A*. 2006;103:4729–4734.
- Takahashi H, Yamashita Y, Takaoka H, Nakamura J, Yoshihama M, Osada, H. Inhibitory action of reveromycin A on TGF- $\alpha$ -dependent growth of ovarian carcinoma BG-1 in vitro and in vivo. *Oncol Res*. 1997;9:7–11.
- Miyamoto Y, Machida K, Mizunuma M, Emoto Y, Sato N, Miyahara K, et al. Identification of *saccharomyces cerevisiae* Isoleucyl-tRNA synthetase as a target of the G<sub>i</sub>-specific Inhibitor reveromycin A. *J Biol Chem*. 2002;277:28810–28814.
- Russell RG. Bisphosphonates: from bench to bedside. *Ann N Y Acad Sci*. 2006;1068:367–401.
- Rogers MJ. New insights into the molecular mechanisms of action of bisphosphonates. *Curr Pharm Des*. 2003;9:2643–2658.
- Reszka AA, Rodan GA. Bisphosphonate mechanism of action. *Curr Rheumatol Rep*. 2003;5:65–74.
- Milner RJ, Farese J, Henry CJ, Selting K, Fan TM, de Lorimier LP. Bisphosphonates and cancer. *J Vet Intern Med*. 2004;18:597–604.
- Black DM, Greenspan SL, Ensrud KE, Palermo L, McGowan JA, Lang TF, et al. The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. *N Engl J Med*. 2003;349:1207–1215.
- Finkelstein JS, Hayes A, Hunzelman JL, Wyland JJ, Lee H, Neer RM. The effects of parathyroid hormone, alendronate, or both in men with osteoporosis. *N Engl J Med*. 2003;349:1216–1226.
- Muguruma H, Yano S, Kakiuchi S, Uehara H, Kawatani M, Osada H, et al. Reveromycin A inhibits osteolytic bone metastasis of small-cell lung cancer cells, SBC-5, through an antiosteoclastic activity. *Clin Cancer Res*. 2005;11:8822–8828.
- Huang CL, Sun L, Moonga BS, Zaidi M. Molecular physiology and pharmacology of calcitonin. *Cell Mol Biol (Noisy-le-grand)*. 2006;52:33–43.
- Suzuki H, Nakamura I, Takahashi N, Ikuhara T, Matsuzaki K, Isogai Y, et al. Calcitonin-induced changes in the cytoskeleton are mediated by a signal pathway associated with protein kinase A in osteoclasts. *Endocrinology*. 199;137:4685–4690.
- Takahashi S, Goldring S, Katz M, Hilsenbeck S, Williams R, Roodman GD. Downregulation of calcitonin receptor mRNA expression by calcitonin during human osteoclast-like cell differentiation. *J Clin Invest*. 1995;95:167–171.
- Nakagawa H, Takami M, Udagawa N, Sawae Y, Suda K, Sasaki T, et al. Destruxins, cyclodepsipeptides, block the formation of actin rings and prominent clear zones and ruffled borders in osteoclasts. *Bone*. 2003;33:443–455.
- Woo JT, Kasai M, Stern PH, Nagai K. Compactin suppresses bone resorption by inhibiting the fusion of pre-fusion osteoclasts and disrupting the actin ring in osteoclasts. *J Bone Miner Res*. 2000;15:650–662.
- Woo JT, Nakagawa H, Krecic AM, Nagai K, Hamilton AD, Sehti SM, et al. Inhibitory effects of mevastatin and a geranylgeranyl transferase I inhibitor (GGTI-2166) on mononuclear osteoclast formation induced by receptor activator of NF kappa B ligand (RANKL) or tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). *Biochem Pharmacol*. 2005;69:87–95.
- Reszka AA, Rodan GA. Nitrogen-containing bisphosphonate mechanism of action. *Mini Rev Med Chem*. 2004;4:711–719.
- Russell RG. Bisphosphonates: mode of action and pharmacology. *Pediatrics*. 2007;119 2:S150–S162.
- Fisher JE, Rogers MJ, Halasy JM, Luckman SP, Hughes DE, Masarachia PJ, et al. Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. *Proc Natl Acad Sci U S A*. 1999;96:133–138.
- Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, et al. Stimulation of bone formation in vitro and in rodents by statins. *Science*. 1999;286:1946–1949.
- Jadhav SB, Jain GKS. Statins and osteoporosis: new role for old drugs. *J Pharm Pharmacol*. 2006;58:3–18.
- Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida

- H, et al. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev Cell*. 2002;3:889–901.
- 28 Igarashi K, Hirotani H, Woo JT, Stern PH. Cyclosporine A and FK506 induce osteoclast apoptosis in mouse bone marrow cell cultures. *Bone*. 2004;35:47–56.
- 29 Hirotani H, Tuohy NA, Woo JT, Stern PH, Clipstone NA. The calcineurin/NFAT signaling pathway regulates osteoclastogenesis in RAW264.7 cells. *J Biol Chem*. 2004;279:13984–13992.
- 30 Kataoka T, Muroi M, Ohkuma S, Waritani T, Magae J, Takatsuki A, et al. Prodigiosin 25-C uncouples vacuolar type H(+)-ATPase, inhibits vacuolar acidification and affects glycoprotein processing. *FEBS Lett*. 1995;359:53–59.
- 31 Woo JT, Shinohara C, Sakai K, Hasumi K, Endo A. Inhibition of the acidification of endosomes and lysosomes by the antibiotic concanamycin B in macrophage J774. *Eur J Biochem*. 1992; 207:383–389.
- 32 Woo JT, Ohba Y, Tagami K, Sumitani K, Yamaguchi K, Tsuji T. Concanamycin B, a vacuolar H(+)-ATPase specific inhibitor suppresses bone resorption in vitro. *Biol Pharm Bull*. 1996; 19:297–299.
- 33 Woo JT, Ooba Y, Tagami K, Sumitani, K, Kataoka T, Nagai K. Prodigiosin 25-C and metacycloprodigiosin suppresses bone resorption by osteoclasts. *Biosci Biotech Biochem*. 1997;59: 350–352.
- 34 Kita M, Kondo M, Koyama T, Yamada K, Matsumoto T, Lee KH, et al. Symbioimine exhibiting inhibitory effect of osteoclast differentiation, from the symbiotic marine Dinoflagellate symbiodinium sp. *J Am Chem Soc*. 2004;126:4794–4795.