Roles of oncogenes and tumor-suppressor genes in osteoclastogenesis (Review)

AKARI MINAMI[1*], MAKO OGINO[1*], NORIKO NAKANO[1], MAYUKO ICHIMURA[1], ATSUKO NAKANISHI[2], TOSHIYUKI MURAI[3,4], YASUKO KITAGISHI[1*] and SATORU MATSUDA[1*]

[1]Department of Food Science and Nutrition, Nara Women's University, Kita-Uoya Nishimachi, Nara 630-8506; [2]Department of Food and Nutrition, Faculty of Contemporary Human Life Science, Tezukayama University, Nara 631-8501; Departments of [3]Microbiology and Immunology, and [4]Genome Biology, Graduate School of Medicine, Osaka University, Suita 565-0871, Japan

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Abstract. Osteoporosis is a bone disease that poses a tremendous burden to health care. The receptor activator of nuclear factor-κB (RANK) and its ligand (RANKL) have been a major focus of this research field. RANKL signaling not only activates a variety of downstream signaling pathways required for osteoclast development, but crosstalk with other signaling pathways also adjusts bone homeostasis both in normal physiology and in bone disease. Consequently, novel drugs specifically targeting RANK-RANKL and their signaling pathways in osteoclasts are expected to revolutionize the treatment of various bone diseases such as osteoporosis. Osteoclasts are the exclusive cells involved in bone resorption. Abnormal activation of osteoclasts can lead to reduced bone density, resulting in osteopenia, osteoporosis and other bone disorders. To date, the mechanism of how osteoclast precursors differentiate into mature osteoclasts remains elusive. Cell proliferation and cell death may be key processes in the progression as well as other cell types. Oncogene products and tumor-suppressor molecules play a pivotal role in regulating the processes, which are important in regulating the configuration of bone disorders. Based on the understanding of these processes, promising alternatives to the use of medications against osteoporosis include specific diets with plant-derived supplements to modulate the expression and/or activity of these molecules. In this review, we summarize the progress of research with a focus on the modulatory roles of oncogene products and tumor-suppressor molecules and suggest the scope of further research concerning the prevention of osteoporosis in this field.

Contents

1. Introduction

Bone fracture is a health issue in patients with bone-related disorders such as osteoporosis as it is a common event (1). Healthy bone homeostasis depends on a balance between osteoblastic bone formation and osteoclastic bone resorption (2) (Fig. 1). Bone remodeling also requires a balance in growth, differentiation, and activity of osteoblasts and osteoclasts. Multinucleated osteoclasts resorb lamellar bone, and new bone is generated by osteoblasts. An imbalance can lead to impaired bone structure and/or small bone mass. Accordingly, osteoclasts are functionally indispensable for supporting bone health. Hyperactivation of osteoclasts and/or their increased number can lead to diseases characterized by bone loss, which is a key risk factor for bone fracture (3). Bone resorption leads to degradation of extracellular matrix which alters the environment of bone marrow stem cell binding and differentiation. On the other hand, bone morphogenetic proteins (BMPs), which

Correspondence to: Professor Satoru Matsuda, Department of Food Science and Nutrition, Nara Women's University, Kita-Uoya Nishimachi, Nara 630-8506, Japan
E-mail: smatsuda@cc.nara-wu.ac.jp

*Contributed equally

Abbreviations: BMP, bone morphogenetic proteins; CDK, cyclin-dependent kinase; CSF1, colony-stimulating factor 1; DHA, docosahexaenoic acid; GSK-3β, glycogen synthase kinase-3β; MAPK, mitogen-activated protein kinase; n-3 PUFA, ω-3 polyunsaturated fatty acid; NAD, nicotinamide adenine dinucleotide; NFATc1, nuclear factor of activated T cells 1; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog on chromosome 10; PUFAs, polyunsaturated fatty acids; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; RNAi, RNA interference; ROS, reactive oxygen species; TGF, transforming growth factor

Key words: osteoporosis, osteoclasts, Src, Myc, TP53, phosphatase and tensin homolog

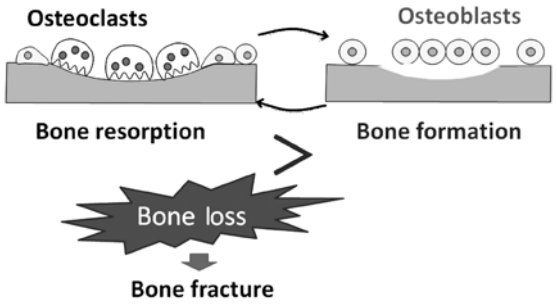

Figure 1. Schematic representation of bone homeostasis that depends on a balance between osteoblastic bone formation and osteoclastic bone resorption.

are members of the transforming growth factor (TGF)-β superfamily and in charge of the development and function of different cell types, induce bone formation (4). It has been shown that BMP signaling in osteoblasts regulates bone mass in mice, suggesting a role of BMP in osteoclastogenesis along with osteoblastic action (5). In addition, postmenopausal osteoporotic bone loss may largely result from the stimulation of bone resorption via increased osteoclast formation with insufficient osteoblastic bone formation (6). Clinically, medical orally available bisphosphonates targeting osteoclasts have been widely used to treat patients with osteoporosis and/or prevent osteoporotic fracture. Bisphosphonates principally inhibit the activation of osteoclasts by binding to hydroxyapatite (7); however, bisphosphonate-related side effects including hypocalcaemia, secondary hyperparathyroidism, renal toxicity, gastrointestinal tract problems and osteonecrosis have recently been reported (8). Colony-stimulating factor 1 (CSF1) is a growth factor required for the differentiation of monocyte-macrophage precursor cells into preosteoclasts (9). Osteoblasts produce CSF1 and receptor activator of nuclear factor-κB ligand (RANKL) which is essential for the early development of osteoclasts from precursor cells derived from monocyte/macrophage lineage originating from the liver and spleen (10). Membrane-bound RANKL invites osteoclasts and initiates and/or activates their differentiation to form multinucleated cells. The role of BMPs in osteoclast differentiation has also been demonstrated, which induce RANKL expression in osteoblasts. CSF1 is controlled by a Smad-signaling pathway (11). In addition, the involvement of the phosphatidylinositol 3-kinase (PI3K) and AKT signaling pathways has been shown to be critical both in osteoblast and in osteoclast differentiation in response to BMPs (11). Consequently, BMPs induce secretion of CSF1 dependent on PI3K/AKT signaling. Inhibition of PI3K/AKT signaling blocks the binding of Smads to the CSF1 BMP-responsive element present in the CSF1 promoter, resulting in attenuation of Smad-dependent CSF1 transcription (12).

Proto-oncogenes and tumor-suppressor genes, which are important in the normal development of cells, are involved in the regulation of the cell cycle and apoptosis (13). For example, the proto-oncogene *c-Src* has been implicated in the development and mature function of the nervous system (14). The protein product of the *c-Src* gene is a tyrosine protein kinase that is enriched in fetal neural tissues. Nuclear transcription of *c-Src* and other proto-oncogenes such as *N-ras*, *c-Myc* and *c-Fos* have been observed in proliferating and differentiating cells (15). In general, oncogenes are critically positioned in various growth factor receptor signaling pathways and are relevant in cancer development (16). *TP53* is a well-known tumor-related gene expressed ubiquitously in all cell types as an inactive transcription factor which undertakes activation in response to a variety of cellular stresses. *TP53* acts both as an oncogene and a tumor-suppressor gene. The effects of TP53 are mediated by different downstream effectors and target proteins. Among them, cyclin-dependent kinase (CDK) inhibitors such as p21 are key mediators of TP53 action, in which p21 may be involved in cell differentiation (17). In addition, p21 regulates cell cycle progression, cell differentiation and senescence (18). Phosphatase and tensin homolog (PTEN) is also a well-known tumor-suppressor gene product of the *pten* gene. PTEN is a dual-specificity phosphatase that has been shown to prevent cell proliferation and migration (19,20). The PTEN/AKT pathway appears to be important in the regulation of inflammatory responses (21).

2. Oncogenes are involved in osteoclastogenesis

Osteoclasts are multinucleated cells that are formed by the fusion of mononuclear osteoclasts, which is an essential process in bone resorption leading to bone remodeling (22). Mechanical response is known to regulate bone remodeling, yet the molecular events involved in the mechanical signal transduction are poorly understood. However, RANKL may be the most essential cytokine involved in the genesis of osteoclasts and/or osteoclast differentiation (23). The binding of RANKL to RANK provokes activation of signaling molecules including AKT and ERK that later induce the activation of transcription factors such as nuclear factor of activated T cells (NFATc1) and c-Fos to regulate the expression of genes required for osteoclast differentiation (24,25). The expression of c-Fos and NFATc1 is regulated by the ERK signaling pathway (26). Proto-oncogene product c-Fos is an essential factor for the induction of NFATc1, which is a master transcription factor that regulates the process of osteoclast differentiation by controlling osteoclast-specific genes (27,28). NFATc1 plays a role as a transcription factor required for regulating the expression of osteoclast-specific genes including TRAP and c-Src (29). c-Src tyrosine kinase (Fig. 2) is also required for the maintenance of osteoclasts and control of bone resorption through actin cytoskeleton turnover (30,31). Selective c-Src inhibitors induce osteoclast disruption and consequently reduce osteoclast numbers *in vivo*, which induce programmed cell death in mature osteoclasts (32,33). Caspase-3 and -9 are momentarily activated by treatment with c-Src inhibitors probably involving continual ERK1/2 phosphorylation (34). Both *c-Fos* and *c-Src* are proto-oncogenes. In addition, proto-oncogene *c-Myc* is strongly upregulated in RANKL-induced osteoclasts (Fig. 2), and is a transcription factor expressed at comparatively high levels in preosteoclasts (35). Consistent with this, a dominant-negative Myc could block RANKL-induced osteoclast formation (35). TRAP, a typical marker of osteoclast differentiation, is an enzyme that plays an active role in the process of bone resorption. The specific regulation of TRAP is performed at the transcriptional level by Myc, suggesting that Myc may play an active role in

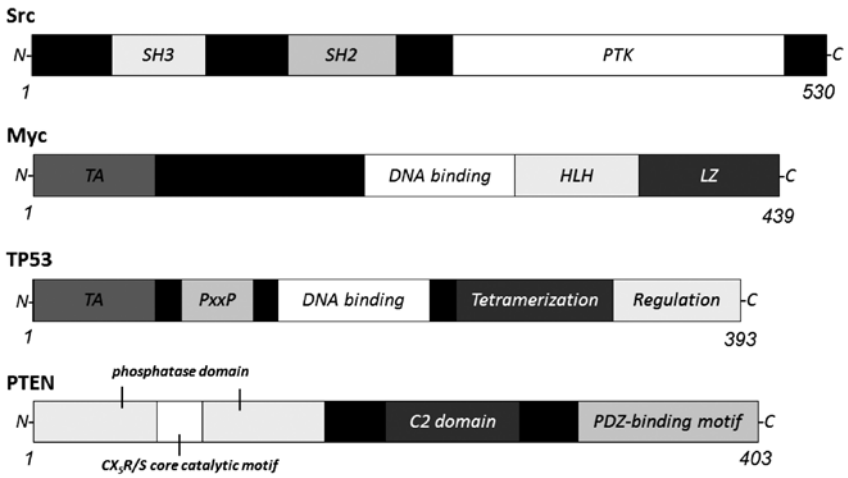

Figure 2. Schematic structures of Src, Myc, TP53 and PTEN protein. The predicted consensual domain structures for each protein are depicted. The functionally important sites are also shown. Note that the sizes of the protein are modified for clarity. SH3, Src homology 3 domain; SH2, Src homology 2 domain; PTK, protein tyrosine kinase domain; TA, transactivation domain; HLH, helix-loop-helix domain; LZ, leucine zipper domain; PxxP, proline-rich region; C2 domain, a protein structural domain involved in targeting proteins to cell membranes; PDZ, a common structural domain in signaling proteins (PSD95, Dlg and ZO-1).

suppressing the transcription of mature osteoclast genes (36). In addition, Myc has a function in the stimulation of FOXO1 which plays key roles in bone development and remodeling by stimulating osteoclast formation (37). In addition to PI3K/AKT signaling, small Ras GTPase also regulates osteoclast survival. Increased activity of Ras GTPase induces the binding with PI3K, whereas inhibition of Ras reduces PI3K-mediated osteoclast survival (38). Actually, the pharmacological inhibition of H-ras prevents the downstream mechanical repression of RANKL (39). Consistently, RNA interference (RNAi) of *H-ras* also retracts the mechanical repression of RANKL (39), suggesting that the mechanical repression of RANKL requires a specific form of Ras-GTP activity. Spatial arrangements in the lipid raft microdomain may be critical for downstream events in response to mechanical signals. In general, activation of Ras and the mitogen-activated protein kinase (MAPK) signaling pathway is known to underlie the proliferation and differentiation of different types of cell lineages including osteoclast progenitor cells (40).

3. Tumor-suppressor genes are involved in osteoclastogenesis

Treatment with RANKL was found to induce an accumulation of TP53 protein in a dose-dependent manner, consequently activating TP53 target genes (41). TP53 is a known tumor-suppressor molecule and also regulates osteoclast differentiation (Fig. 2). Mice with deficiency of the *TP53* gene display a high bone-mass phenotype (42). In addition, *TP53*-deficient mice have an improved ability to prefer osteoclast differentiation with increased expression of CSF1 (42,43). Therefore, TP53 acts as a regulator of osteoclastogenesis and subsequent bone remodeling (42,43). Bone loss induced by ovariectomy has been linked to enhanced bone turnover as a result of osteoclast activation (44), accompanied by increased expression of the senescence marker p16/p21 in bone associated with a decrease in Sirt1 (44). In general, bone cell senescence is associated with decreased Sirt1 expression and activation of TP53, p16 and p21 (45). Sirt1 is a candidate anti-aging gene which may suppress p16 expression through deacetylation (45). Although the TP53 and p16 pathways act separately to promote cellular senescence, their contribution can be cell type-dependent depending on the process (46). Cellular senescence is a process of aging involving a permanent growth arrest of mitotic cells and is different from apoptosis and/or programmed cell death.

PTEN (Fig. 2) is a frequently mutated tumor-suppressor gene in human cancers (47). PTEN has been found to regulate cell survival, growth, migration, adhesion, and invasiveness (48). PTEN adversely regulates PI3K/AKT signaling as a lipid phosphatase for the phosphatidylinositol 3,4,5-triphosphate second messenger. Inactivation of glycogen synthase kinase-3β (GSK-3β) via AKT plays an important role in RANKL-induced osteoclastogenesis (49). Downregulation of PTEN by RNAi increases AKT and GSK-3β phosphorylation by RANKL, thereby stimulating the development of osteoclasts (50). Notably, the phosphorylation defective mutant of PTEN at threonine 366 was found to result in increased osteoclastgenesis compared to that of wild-type PTEN. PTEN phosphorylation seems to reduce RANKL-induced osteoclastogenesis, whereas PTEN protein levels are unaffected (50). Treatment with GSK-3β inhibitor SB216763 was found to dose-dependently suppress PTEN phosphorylation consequently increasing AKT phosphorylation. RANKL stimulates activation of AKT, which in turn is consistent with the role of an increased level of PTEN in decreasing AKT activity. These data strongly suggest that inhibition of GSK-3β during RANKL-induced osteoclastogenesis decreases PTEN phosphorylation, contributing to osteoclast differentiation through subsequent AKT activation. PTEN overexpression also blocks RANKL-triggered AKT activation that is related to cell survival and cell migration. Thus, PTEN overexpression suppresses RANKL-mediated osteoclast differentiation. On the contrary, the dominant-negative form of PTEN induces osteoclast differentiation and migration. In this way, multiple roles for PTEN have been shown in RANKL-induced osteoclast precursor cells (50,51).

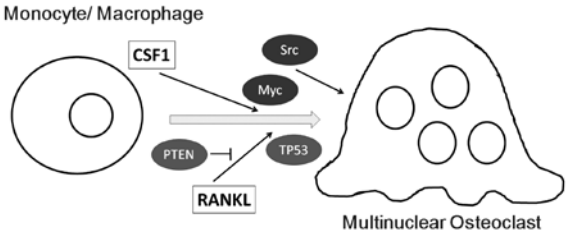

Figure 3. Schematic illustration of the tentative proposed model for osteoclastogenesis mediated by oncogenes and tumor-suppressor genes in addition to the stimulation of colony-stimulating factor 1 (CSF1) and receptor activator of nuclear factor-κB ligand (RANKL). Examples of molecules involved in osteoclastogenesis are shown. Note that some critical pathways have been omitted for clarity.

4. Strategy for the dietary treatment of osteoporosis

Potential therapeutic strategies exploit the observations made in the critical processes required for maintaining homeostasis of the metabolic condition characterized by osteoblast/osteoclast balance. Accordingly, dietary regulation of these cells is an important therapeutic strategy for preventing and/or treating bone disorders. As mentioned above, several oncogenes and tumor-suppressor genes are intensely involved in osteoclastogenesis and/or osteoporosis. Thus, it is a challenge to regulate the expression of these genes by dietary treatment. First of all, inhibition of oncogene expression such as c-*Myc* and *c-H-ras* by green tea and (-)-epigallocatechin gallate has been shown in mice (52). In addition, antioxidants such as retinoids (vitamin A), vitamin E (such as α-tocopheryl succinate), ascorbate (vitamin C) and carotenoids induce cell differentiation and growth inhibition in human cells by complex mechanisms including inhibition of the expression of c-*Myc* and *H-ras* and induction of *p21* genes (53). Moreover, dietary calorie restriction has been associated with reduced cancer risk, which is related to the abrogation of both Ras and PI3K signaling (54).

Praeruptorin A is the major bioactive component isolated from the dry root extract of *Peucedanum praeruptorum* Dunn, and has several biological activities such as anti-hypertensive activity by acting as a calcium channel blocker (55). Praeruptorin A attenuates the RANKL-induced phosphorylation of p38 without affecting JNK and ERK activity. The anti-osteoclastogenic action of praeruptorin A may be due to its potential to inhibit both the p38 and AKT signaling pathways that subsequently downregulate the expression of c-Fos and NFATc1 (56). Honokiol, a component of the Oriental herb *Magnolia officinalis*, inhibits RANKL-induced osteoclastogenesis with nuclear factor-κB (NF-κB) activation (57). Studies have shown that honokiol blocks TNF-induced phosphorylation, degradation and ubiquitination of IκBα through the inhibition of AKT (58). Expression of c-*Myc* is also downregulated by honokiol (59). Honokiol can diminish PI3K/AKT signaling by upregulation of PTEN expression (60). Magnolol, a honokiol isomer, has been shown to be equally active. Consumption of a blueberry-containing diet may contribute to the prevention of bone loss (61). Blueberries are an admirable source of dietary polyphenols such as phenolic acids and anthocyanins, which were found to significantly decrease the gene expression of *TP53* and *p21* in human HepG2 cells (62). Resveratrol was also found to activate Sirt1, a member of the sirtuin family of nicotinamide adenine dinucleotide (NAD)-dependent deacetylases (63). Stilbenes, which are related to resveratrol, have been shown to have an inhibitory effect on the expression of the c-*Myc* genes (64). In addition, resveratrol inhibits cell proliferation and induces cell apoptosis through regulation of *TP53* expression (65). An extract from thorns of the medicinal herb, *Gleditsia sinensis* caused an increase in cell cycle arrest during the G2/M phase, associated with increased TP53 levels (66). Treatment with an ethanol extract of the thorns of *Gleditsia sinensis* was also found to be associated with upregulation of p21 levels (67). Both *TP53* and *p21* mRNA levels were increased following treatment with the Chinese herb Kanglaite, an extract from Coix seed (68). Kanglaite appears to extend the half-life of TP53 protein (68). A ginsenoside, one of the components of American ginseng herb, was found to activate TP53 (69). In addition, apoptosis induction by thymoquinone, the most abundant component in black seed, was found to be associated with an increase in *TP53* mRNA and downstream TP53 target genes (70,71). Treatment with an extract of *Magnolia officinalis* upregulated the expression of p21 and p27 (72). Baicalin, a herb-derived flavonoid compound, enhanced the expression of p27 (73,74). Treatment with an extract of *Saussurea involucrate* was also found to induce p21 and p27 expression, independent of the TP53 pathway (75). Treatment with triptolide, a purified extract from the herb *Tripterygium wilfordii* Hook F resulted in increased p21 expression (76,77). Curcumin, an active ingredient derived from the root of the plant *Curcuma longa*, restored PTEN expression (78). In contrast, various components of the herb rosemary inhibited the expression of PTEN in K562 myeloid cells (79).

Dietary intake of indole-3-carbinol was found to upregulate PTEN in an animal model (80). Indole-3-carbinol is a promising cancer-preventive phytochemical found in various vegetables such as broccoli (81). In addition, PTEN expression at the mRNA and protein levels was found to be elevated in experimental animals fed whey protein which has been shown to possess multiple health benefits (82). It has also been reported that DHA and EPA raise the level of PTEN in breast cancer cells, providing a mechanism for the beneficial effects of fish oils on breast cancer cells (83,84). Fish oil rich in polyunsaturated fatty acids may induce PTEN expression by activation of peroxisome proliferator-activated receptor (PPAR) (85,86), which attenuates cellular damage playing an important role in the activation of anti-apoptotic signaling (87). The information discussed here may also be useful for supporting the design of further research concerning the prevention of osteoporosis.

5. Future perspectives

It will be a challenge to elucidate how to utilize natural compounds for the correction of critical processes required for maintaining cellular homeostasis and metabolic conditions to prevent osteoporosis (Fig. 3). Identification of effective target molecules relevant for osteoporosis allows the screening for natural products capable of modulating targets. In addition, combination therapy using two or more food ingredients is a promising therapeutic strategy over traditional approaches. For example, resveratrol and curcumin synergistically induce

apoptosis by increasing the level of p21 and decreasing the level of c-Myc (88). The information here may provide further insight into the molecular mechanisms of special diets underlying the daily use of certain foods as a therapeutic strategy for osteoporosis. This may also provide the basis for the development of rational dietary treatments against other diseases. Future studies are required to demonstrate whether oncogenes, tumor-suppressor genes and/or their downstream targets could be used to modulate the cellular composition of tissue including bone, thereby enhancing metabolic stability. Knowledge of the local determinants of the phenotype of osteoclasts in critical lesions and how they interact with the risk factors of bone disorders may lead to significantly improved therapies with reduced side effects.

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