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Regulatory Mechanism of Osteoclastogenesis by RANKL and Wnt Signals

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1. ABSTRACT

Osteoclasts develop from monocyte-macrophage lineage cells under the regulation of osteoblasts. Osteoblasts express two cytokines essential for osteoclastogenesis, macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-κB ligand (RANKL). Osteoblasts also produce osteoprotegerin (OPG), a decoy receptor for RANKL, which inhibits the interaction between RANKL and RANK, a receptor of RANKL. Bone resorption-stimulating factors act on osteoblasts to regulate RANKL and OPG expression. Nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) is a master transcription factor for osteoclast differentiation. The immunoreceptor tyrosine-based activation motif (ITAM)-mediated signal was discovered as a co-stimulatory signal in RANKL-induced osteoclastogenesis. Wnt proteins activate two pathways: β-catenin-dependent canonical and β-catenin-independent noncanonical pathways. Wnt proteins promote differentiation of osteoblasts through the canonical pathway. The canonical pathway in osteoblasts also suppresses osteoclastogenesis through up-regulation of OPG expression and down-regulation of RANKL expression. In contrast, activation of the noncanonical pathway in osteoclast precursors enhances RANKL-induced osteoclastic differentiation. Thus, Wnt signals in osteoblasts and osteoclast precursors play important roles in osteoclastogenesis. This review summarizes the regulatory mechanism of osteoclastogenesis by RANKL and Wnt signals.

2. INTRODUCTION

Osteoblasts and osteoclasts are specialized cells responsible for bone formation and resorption, respectively. Osteoblasts produce bone matrix proteins and lead mineralization of the tissue. Osteoclasts, the multinucleated giant cells that resorb bone, develop from hematopoietic cells of the monocyte-macrophage lineage (1-3). We developed a mouse coculture system of osteoblasts/stromal cells and hematopoietic cells in which osteoclasts are formed in response to bone resorption-stimulating factors such as 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], parathyroid hormone (PTH), prostaglandin E_2 (PGE_2), and interleukin 11 (IL-11) (1,4). A series of experiments using this coculture system established the concept that osteoblasts/stromal cells are closely involved in osteoclast development (5).

Advances in bone cell biology have elucidated the precise mechanism by which osteoblasts regulate osteoclast differentiation and function. Osteoblasts express two cytokines, macrophage colony-stimulating factor (M-CSF, also called CSF-1) and receptor activator of nuclear factor κB ligand (RANKL), essential for osteoclast differentiation (1,6). Bone resorption-stimulating factors all stimulate RANKL expression in osteoblasts/stromal cells. The identification of nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), a master transcription factor for osteoclastogenesis, provided major insights into the molecular mechanism of osteoclast differentiation (7). The discovery of immunoreceptor tyrosine-based activation

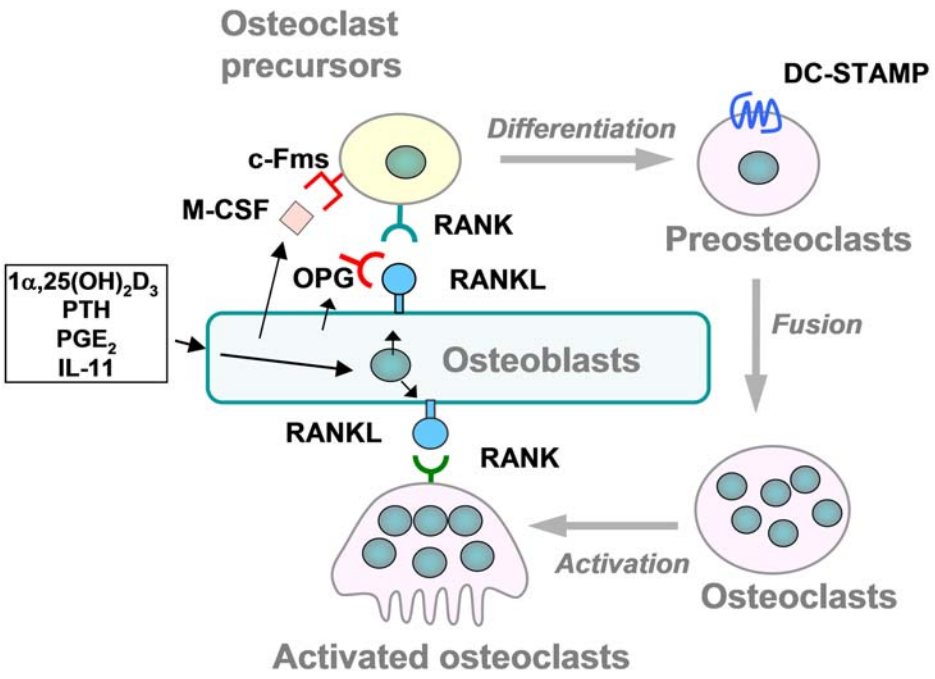

Figure 1. Regulation of osteoclast differentiation and function by osteoblasts. Bone resorption-stimulating factors such as $1\alpha,25(OH)_2D_3$, PTH, PGE_2 and IL-11 act on osteoblasts to induce expression of RANKL. Osteoblasts constitutively produce M-CSF. Osteoclast precursors of the monocyte-macrophage lineage express RANK and c-Fms. Osteoclast precursors recognize RANKL expressed by osteoblasts through cell-to-cell interaction, and differentiate into mononuclear preosteoclasts in the presence of M-CSF. Mononuclear preosteoclasts express DC-STAMP, which is essential for the fusion between preosteoclasts to form multinucleated osteoclasts. Mature osteoclasts also express RANK, and RANKL induces bone-resorbing activity of mature osteoclasts via binding to RANK.

motif (ITAM)-mediated signals as a co-stimulatory signal in RANKL-induced osteoclastogenesis has confirmed that osteoblasts play another important role in osteoclastogenesis (7).

Wnt proteins (Wnts) are a family of 19 glycoproteins (8, 9). Wnt binds to two distinct receptor complexes: a complex of Frizzled and low density lipoprotein receptor-related protein 5/6 (LRP5/6) and another complex of Frizzled and receptor tyrosine kinase orphan receptors (Rors). The binding of Wnts to the receptors activates two classes of signalling pathways: a □-catenin-dependent canonical pathway and a β- catenin-independent noncanonical pathway. The importance of the canonical pathway in bone biology has been emphasized by the identification of a link between bone mass and mutations in the LRP5 gene (10). Loss-of-function mutations in LRP5 reduce the number of osteoblasts and cause osteoporosis.

Recent findings have shown that the β-catenin-dependent canonical pathway in osteoblasts/stromal cells suppresses osteoclastogenesis through the up-regulation of osteoprotegerin (OPG) expression and the down-regulation of RANKL expression (11,12). In addition, the activation of the noncanonical pathway in osteoclast precursors enhances RANKL-induced osteoclastic differentiation, in a cell-autonomous manner (13). These results suggest that Wnts play important roles not only in bone formation, but also in bone resorption. In this review, we summarize the regulatory mechanism of osteoclastogenesis by RANKL and Wnt signals. The word "osteoblasts" is used as a generic name for osteoblast lineage cells, including bone marrow stromal cells, in this article.

3. REGULATION OF OSTEOCLAST DIFFERENTIATION AND FUNCTION

3.1. M-CSF

Experiments with an osteopetrotic *op/op* mouse model have established that an osteoblast product, M-CSF, is crucial for osteoclast formation (14) (Figure 1). The M-CSF gene of *op/op* mice cannot functionally code active M-CSF protein due to an extra thymidine insertion in the coding region of the M-CSF gene. Administration of recombinant human M-CSF restored impaired bone resorption in *op/op* mice (15). Calvarial osteoblasts obtained from *op/op* mice failed to support osteoclast development in cocultures with normal spleen cells, but the addition of M-CSF to cocultures induced osteoclast formation in response to $1\alpha,25(OH)_2D_3$ (16). These findings indicate that M-CSF produced by osteoblasts plays an essential role in osteoclast development. M-CSF is involved in not only proliferation of osteoclast progenitors of the monocyte/macrophage lineage, but also their differentiation into osteoclasts. Osteoblasts constitutively express M-CSF (17) (Figure 1).

3.2. RANKL

RANKL is a member of the tumor necrosis factor (TNF) family (TNF superfamily member 11, TNFSF11), which is also identified as "osteoclast differentiation factor" (18-21) (Figure 1). In contrast to M-CSF, the expression of RANKL by osteoblasts is inducible. Osteoblasts express RANKL as a membrane-associated form in response to stimuli of bone resorption-stimulating hormones and factors such as $1\alpha,25(OH)_2D_3$, PTH, PGE_2, and IL-11 (1,6,18). Osteoclast precursors express c-Fms (M-CSF receptors) and RANK (TNF receptor superfamily member 11A, TNFRSF11A). Osteoclast precursors recognize RANKL through cell-to-cell interactions with osteoblasts and differentiate into osteoclasts in the presence of M-CSF. RANKL expressed by osteoblasts also stimulates the survival and bone-resorbing activity of osteoclasts. Thus, RANKL is a cytokine responsible for the whole life cycle of osteoclasts. Osteoblasts also produce OPG (TNFRSF11B), a soluble decoy receptor for RANKL, which inhibits osteoclastogenesis by blocking RANKL-RANK interaction (1, 6, 22, 23). OPG production by osteoblasts is also regulated by osteotropic factors. Most bone resorption-stimulating hormones and cytokines down-regulate the expression of OPG in osteoblasts (1, 6). Both RANKL-deficient ($RANKL^{-/-}$) mice (24) and $RANK^{-/-}$ mice (25) developed severe osteopetrosis with no osteoclasts in bone tissues. In contrast, $OPG^{-/-}$ mice exhibited severe trabecular and cortical bone porosity with enhanced osteoclastic bone resorption (26, 27). Administration of OPG or blocking antibodies against RANKL to normal mice strongly suppressed bone-resorbing activity of osteoclasts, as well as osteoclast differentiation (22, 28) (Figure 1). Taken together, these findings confirm the notion that RANKL expressed by osteoblasts also plays an essential role in osteoclast differentiation and function in humans.

3.3. DC-STAMP

Multinucleated osteoclasts are formed by cell-cell fusion of mononuclear preosteoclasts. Recent studies have shown that the dendritic cell-specific transmembrane protein (DC-STAMP), a seven transmembrane protein, is responsible for the cell-cell fusion of osteoclasts (29, 30). DC-STAMP expression in osteoclast precursors was up-regulated during differentiation into osteoclasts. No multinucleated osteoclasts were observed in DC-STAMP knockout ($DC\text{-}STAMP^{-/-}$) mice, but many mononuclear preosteoclasts expressing osteoclast-specific markers were detected in those bone tissues. Bone-resorbing activity was considerably lower in $DC\text{-}STAMP^{-/-}$ osteoclasts than in wild-type osteoclasts (30). $DC\text{-}STAMP^{-/-}$ mice developed mild osteopetrosis. These results suggest that DC-STAMP is an essential molecule for osteoclast fusion, and multinucleated osteoclasts have higher bone-resorbing activity than mononuclear preosteoclasts. Thus, osteoclasts are specifically differentiated multinucleated cells specialized for bone resorption.

4. SIGNAL TRANSDUCTION IN OSTEOCLASTOGENESIS

4.1. NFATc1

The cytoplasmic tail of RANK interacts with TNF receptor-associated factor 1 (TRAF1), TRAF2, TRAF3, TRAF5 and TRAF6 (31, 32). Of these, TRAF6-mediated signals appear to be important for osteoclast differentiation and function because $TRAF6^{-/-}$ mice develop osteopetrosis (33, 34). TRAF6-mediated signals in osteoclast precursors enhance activation of nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK), and induction of c-Fos. Mice deficient in both the p50 and p52 subunits of NF-κB (35, 36) and those lacking c-Fos (37) develop osteopetrosis due to an early block of differentiation in the osteoclast lineage. Using mice lacking JNK1 or JNK2, David *et al.* (38) showed that JNK1 activation essentially modulate osteoclastogenesis. Ikeda *et al.* (39) also reported that RANK-mediated signaling on osteoclasts was inhibited by overexpression of dominant-negative JNK1. These results suggest that JNK1 plays a role in osteoclastogenesis.

RAW264.7, a transformed mouse myeloid cell line, differentiates into osteoclasts in response to RANKL. Ishida *et al.* (40) reported that another transcription factor, NFATc1 (also called NFAT2), was specifically induced in RAW264.7 cells by treatment with RANKL. The translocation of NFATc1 into the nucleus is an essential step in autoamplification, followed by exerting its function. Calcineurin is a Ca^{2+}-dependent phosphatase which is activated by calcium-calmodulin signalling. The calcineurin inhibitor cyclosporine A inhibited NFATc1 activity and suppressed osteoclast differentiation. Antisense oligonucleotides to NFATc1 also suppressed osteoclast differentiation. Using a cDNA microarray, Takayanagi *et al.* (41) independently identified NFATc1 as a master transcription factor for osteoclast differentiation. RANKL evoked Ca^{2+} oscillations that led them to calcineurin-mediated activation of NFATc1 (7, 41).

4.2. Co-stimulatory ITAM signals

ITAM is an important signalling component for a number of receptors in the immune system, including T-cell, B-cell, NK-cell, and Fc receptors (42, 43). Kim *et al.* (44) succeeded in the molecular cloning of a new member of the leukocyte receptor complex (LRC) family specifically expressed by osteoclasts, and named it "the osteoclast-associated receptor (OSCAR)" (Figure 2). Genes in the LRC family produce immunoglobulin-like surface receptors and play critical roles in the regulation of both innate and adaptive immune responses (45). OSCAR is detected specifically in murine mononuclear preosteoclasts or multinucleated osteoclasts (44). The putative ligand of OSCAR was shown to be expressed by osteoblasts. Addition of a soluble form of OSCAR in cocultures with osteoblasts inhibited the formation of osteoclasts from bone marrow precursor cells in the presence of bone-resorbing factors (44). These results suggest that OSCAR may be an important bone-specific regulator of osteoclast differentiation. However, the physiological role of OSCAR in osteoclastogenesis remains to be elucidated.

Intracellular calcium oscillation is an important stimulus for NFATc1 activation. Koga *et al.* (46) showed that molecules containing ITAM, such as DNAX-activating protein 12 (DAP12) and Fc receptor common γ chain

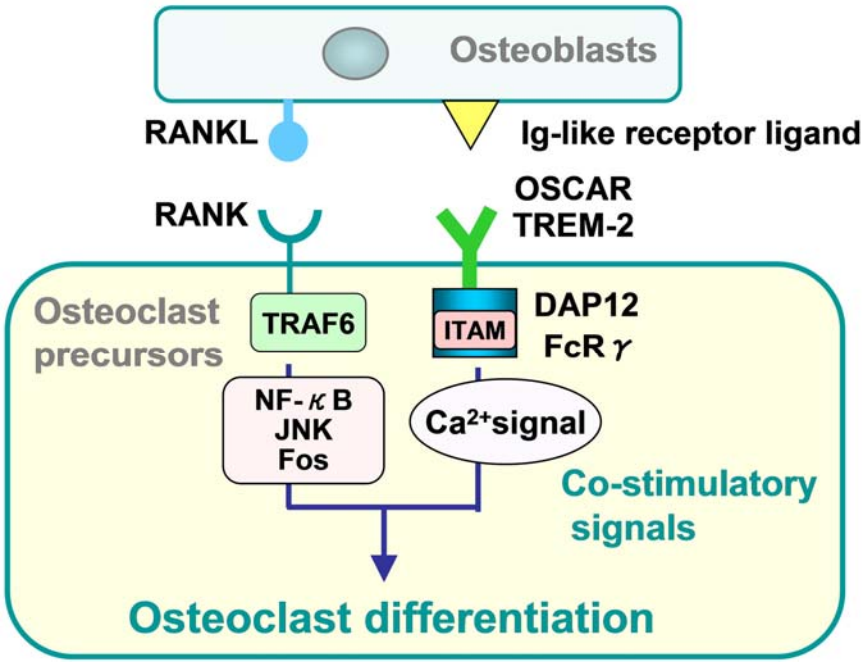

Figure 2. Intracellular signals in osteoclast precursors induced by osteoblasts. Osteoclast differentiation is induced by signals mediated by c-Fms, RANK and its co-stimulatory immunoglobulin-like receptors such as OSCAR and TREM-2. c-Fms has a tyrosine kinase domain in the cytoplasmic region, and tyrosine kinase-mediated signals regulate proliferation of osteoclast progenitors and their differentiation into osteoclasts. The binding of RANKL to RANK results in the recruitment of TRAF family members including TRAF2 and TRAF6. RANK-mediated signals induce c-Fos expression and NF-κB activation. In collaboration with c-Fms-mediated signals, RANK-mediated signals induce autoamplification of NFATc1, a key transcription factor for osteoclastogenesis. Activation of AP-1 is a prerequisite for the robust induction of NFATc1. RANK activation also induces the phosphorylation of ITAM in DAP12 and FcRγ, adaptor proteins associated with immunoglobulin-like receptors. Osteoblasts express unidentified ligands of immunoglobulin-like receptors, which stimulate phosphorylation of ITAM in DAP12 and FcRγ. Phosphorylation of ITAM results in the activation of calcium signalling. ITAM-induced signals also activate calcineurin, a phosphatase, which dephosphorylates NFATc1 (activation of NFATc1). Induction of NFATc1 and its activation in osteoclast precursors induce their differentiation into osteoclasts.

(FcRγ), facilitate the calcium-mobilizing mechanism during osteoclastogenesis (Figure 2). FcRγ and DAP12 are adaptor molecules that associate with immunoglobulin-like receptors such as OSCAR, triggering receptors expressed on myeloid cells 2 (TREM2), signal-regulatory protein β1 (SIRPβ1) and paired immunoglobulin-like receptor A (PIR-A). OSCAR and PIR-A use FcRγ, while TREM2 and SIRPβ1 associate with DAP12. Deficiency in both FcRγ and DAP12 caused osteopetrotic phenotypes in mice (46). ITAM-mediated signals cooperate with RANK to stimulate calcium oscillation through ITAM phosphorylation and the resulting activation of spleen tyrosine kinase (SYK) and phospholipase Cγ (PLCγ). Recently, it was shown that RANK and ITAM signalling induced the formation of Tec family non-receptor tyrosine kinases [Tec/Bruton's tyrosine kinase (Btk)] and B cell linker protein (BLNK), and resulted in PLCγ-mediated activation, an essential calcium signal for osteoclastogenesis (47). Because this pathway is crucial for the robust induction of NFATc1 that leads to osteoclastogenesis, these signals are known as co-stimulatory signals for RANK in osteoclastogenesis. Although the ligands for of these receptors will have to be identified in the future, osteoblasts have been proposed to express molecules which stimulate ITAM signals. Recent studies have shown that the system of semaphoplin 6D and its receptor plexin A1 has important roles in osteoclast differentiation through activation of DAP12-mediated ITAM signals (48). This series of experiments has established a new research area, "Osteoimmunology" (7).

5. WNT SIGNALLING PATHWAYS

5.1. Canonical and noncanonical Wnt signals

In the absence of Wnt signalling, glycogen synthase kinase-3β (GSK-3β) phosphorylates β-catenin in the target cells (8,9) (Figure 3). Phosphorylated β-catenin is degraded through the ubiquitin-proteasome pathway. Wnt1 class ligands such as Wnt1 and Wnt3a activate the canonical pathway through the formation of a complex of Wnt, Frizzled, and LRP5 or LRP6 (49). This complex in turn promotes the phosphorylation of GSK-3β, which inhibits the kinase activity of GSK-3β. Inactivation of GSK-3β induces the accumulation of β-catenin in the target cells, followed by translocation of accumulated β-catenin into the nucleus. Accumulation of β-catenin leads to its translocation into the nucleus, where it interacts with T-cell

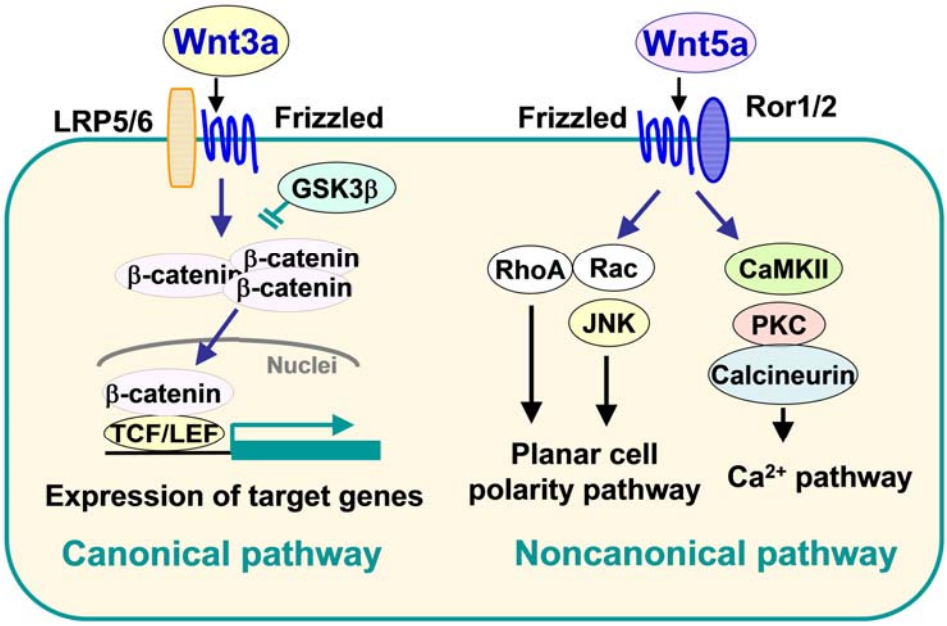

Figure 3. Wnt signalling pathways. Canonical Wnt pathway: In the absence of Wnts, GSK-3β phosphorylates β-catenin in target cells. Canonical Wnts (Wnt3a) bind to the receptor complex of Frizzled and LRP5 or LRP6, inhibit GSK-3β and promote the accumulation of β-catenin. The accumulated β-catenin translocates into the nucleus and together with TCF/LEF induces the expression of Wnt target genes. Noncanonical Wnt pathway: Noncanonical Wnts (Wnt5a) bind to the receptor complex of Frizzled and Ror1 or Ror2. This binding activates the planar cell polarity pathway through RhoA, Rac and JNK-dependent signals. Noncanonical Wnts also activate PKC- and calcineurin-dependent signals.

factor/lymphoid enhancer factor (TCF/LEF) family members to initiate transcription of the target genes. LRP5 and LRP6 act as Wnt coreceptors for the canonical pathway (8,9,49). The importance of the canonical pathway in bone biology has been emphasized by the identification of a link between bone mass and mutations in the LRP5 gene (10). Loss-of-function mutations in LRP5 reduce the number of osteoblasts and cause osteoporosis (10, 50). A recent report has suggested that LRP5 signals control bone formation by inhibiting serotonin synthesis in the duodenum (51).

Noncanonical Wnt signalling is classified into two subpathways: the Wnt/Ca^{2+} and Wnt/planer cell polarity pathways (49). Both pathways are activated by Wnt5a class ligands such as Wnt5a and Wnt11. The Ca^{2+} pathway activates Ca^{2+}-sensitive enzymes, such as Ca^{2+}-calmodulin-dependent protein kinase II (CAMKII) and protein kinase C (PKC) (52-55). The planer cell polarity pathway is mediated through JNK-dependent signals, and this pathway plays a critical role in morphogenetic processes, including convergent-extension movements during gastrulation and the alignment of the sensory hair cells of the inner ears (56-58). The mammalian Ror family consists of two structurally related proteins, Ror1 and Ror2. Ror1 and Ror2 act as alternative receptors or coreceptors for Wnt5a (59). Wnt5a binds to the cysteine-rich domain of Ror2, which then associates with Frizzled2 and activates JNK in cultured cells (60). Recently, Sato *et al.* (61) showed that Frizzled2 was internalized through a clathrin-mediated route in response to Wnt5a, and Ror1 or Ror2 were required for this action. These results suggest that both Ror2 and Ror1 mediate Wnt5a signaling by activating the non-canonical Wnt pathway.

5.2. Role of canonical Wnt signals in bone resorption

Osteoclasts are formed in cocultures of osteoblasts and bone marrow cells in the presence of bone resorption-stimulating factors such as $1\alpha,25(OH)_2D_3$ and PTH. Wnt3a strongly inhibits $1\alpha,25(OH)_2D_3$-induced osteoclast formation in cocultures of stromal ST2 cells and bone marrow cells (62). However, Wnt3a fails to inhibit RANKL-induced osteoclast formation in bone marrow macrophage cultures. These results suggest that the inhibitory effect of Wnt3a on osteoclast formation is mediated by osteoblasts. Glass *et al.* (11) developed mice expressing a stabilized form of β-catenin in their osteoblasts (β-catenin mutant mice), and reported that bone mass was increased, but the number of osteoblasts and other parameters of osteoblast function remained unchanged in those mice (Figure 4). Interestingly, the β-catenin mutant mice developed osteopetrosis with tooth eruption defects and a decreased number of osteoclasts. Urinary deoxypyridinoline, a marker of osteoclastic bone resorption, was also decreased in the β-catenin mutant mice. Micro-array analysis comparing gene expression changes in $LRP5^{-/-}$ mice and β-catenin mutant mice showed that OPG was up-regulated in osteoblasts in those mice (11). When β-catenin was inactivated selectively in mature osteoblasts using α1(I) collagen *Cre* mice, the bone mass was decreased due to enhancement of bone resorption. Activation of the canonical Wnt pathway stimulated OPG

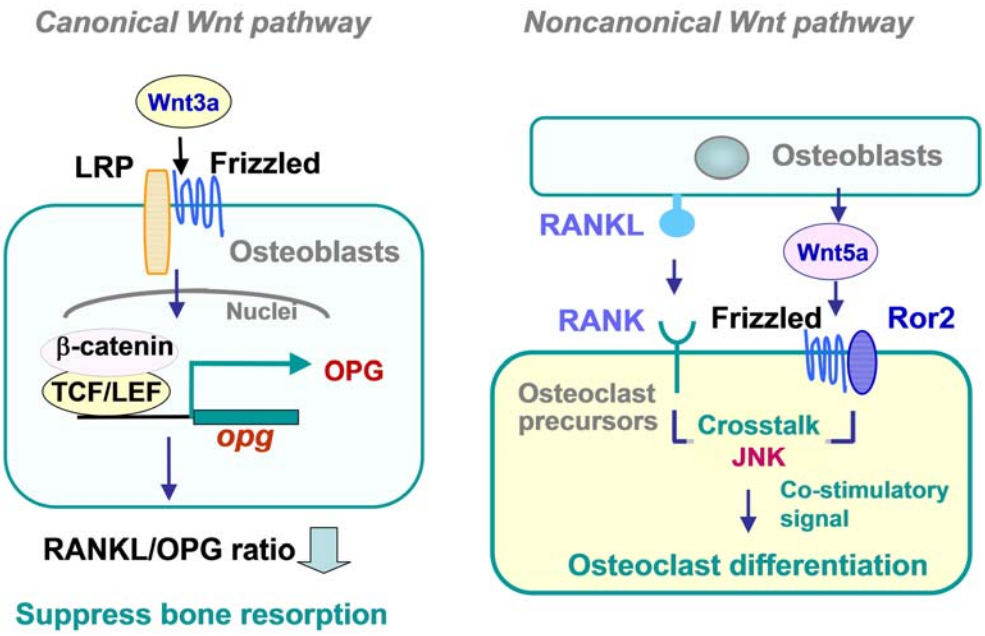

Figure 4. Regulation of osteoclast differentiation by Wnt signals. Canonical Wnt pathway**:** Canonical Wnts (Wnt3a) bind to the receptor complex of Frizzled and LRP5 or LRP6, inhibit GSK-3β and promote the accumulation of β-catenin in osteoblasts. The accumulated β-catenin translocates into the nucleus and together with TCF/LEF induces the expression of OPG. Thus, the canonical pathway in osteoblasts suppresses osteoclastogenesis through down-regulation of the RANKL/OPG ratio. Noncanonical Wnt pathway: Osteoblasts express both RANKL and noncanonical Wnt5a. Osteoclast precursors express RANK and Ror2 but not Ror1. Wnt5a enhances RANKL-induced osteoclastic differentiation of precursor cells. Wnt5a binds to the receptor complex of Frizzled and Ror2, and stimulates the noncanonical Wnt signals such as PKC in osteoclast precursors. Wnt5a enhances RANKL-induced phosphorylation of JNK in osteoclast precursors, suggesting that JNK is involved in the crosstalk between RANK- and Ror2-mediated signals. The noncanonical Wnt pathway is a newly proposed co-stimulatory signal in osteoclastogenesis.

expression in osteoblasts. In addition, the canonical Wnt pathway suppressed the expression of RANKL in MC3T3E1 cells and MG-63 cells (12). These results suggest that the activation of the canonical Wnt pathway in osteoblasts suppresses bone resorption through up-regulation of OPG expression and down-regulation of RANKL expression (Figure 4).

LRP5 and LRP6 are expressed in bone marrow macrophages, suggesting that Wnt3a can stimulate canonical Wnt pathways in osteoclast precursors (13). Wnt3a stimulated cytosolic β-catenin accumulation in bone marrow macrophages, but showed no effect on osteoclast formation in bone marrow macrophage cultures treated with RANKL and M-CSF (13). When β-catenin in bone marrow macrophages was depleted by short hairpin RNAs, those macrophages normally differentiated into osteoclasts in response to RANKL and M-CSF. These results suggest that the canonical Wnt pathway plays important roles in osteoblasts, but not in osteoclast precursors, to regulate bone resorption.

5.3. Role of noncanonical Wnt signals in bone resorption

Wnt5a stimulates the noncanonical Wnt pathway in target cells. A recent study showed that Wnt5a enhanced RANKL-induced osteoclast formation in mouse bone marrow macrophage cultures (13) (Figure 4). Wnt3a showed no effect on osteoclast formation in the same culture system. Mouse bone marrow macrophages expressed Frizzleds 2 and 5 and Ror2, the receptor components of Wnt5a. Knock-down of Ror2 by short hairpin RNA abolished the synergistic effect of Wnt5a on osteoclast formation, suggesting that the synergistic effect of Wnt5a on osteoclast formation is mediated by the Wnt5a-Ror2 axis. Wnt5a stimulated phosphorylation of PKC and enhanced RANKL-induced phosphorylation of JNK in bone marrow macrophages. Thus, JNK appeared to be involved in the crosstalk between RANK- and Ror2-medated signals. Accumulation of β-catenin was not induced by Wnt5a in osteoclast precursors. RT-PCR analysis also revealed that osteoblasts expressed higher amounts of Wnt5a than bone marrow macrophages. These results suggest that Wnt5a produced by osteoblasts enhances osteoclast differentiation through the noncanonical Wnt pathway in osteoclast precursors. It was also reported that synovial tissues from rheumatoid arthritis patients produce large amounts of Wnt5a (63). This suggests that Wnt5a secreted from the synovial tissue is involved in bone destruction in rheumatoid arthritis. These results suggest that Wnt5a promotes RANKL-induced osteoclast formation through Ror2 expressed by osteoclast precursors in physiological and pathological situations (Figure 4).

Ror1 and Ror2 exhibit different expression patterns in the developing limbs, where the expression of Ror2 is broad compared with that of Ror1 (64). $Ror2^{-/-}$ mice exhibit a perinatal lethality and profound skeletal abnormalities with shortened limbs (65). Ror2 is expressed in chondrocytes of developing cartilage anlagen, but not in osteogenic cells during intramembranous ossification. $Wnt5a^{-/-}$ mice also die during the perinatal period and this is associated with severe reductions in length of the individual skeletal elements (66). Reduced proliferation of progenitor cells was observed in the progress zone of forelimbs in $Wnt5a^{-/-}$ mice, and Wnt5a stimulated proliferation and differentiation of chondrocytes. These results suggest that Wnt5a-Ror2 signaling regulates chondrogenesis. However, it is still not known how this signaling pathway regulates bone remodeling. Our findings suggest that defects of Wnt5a-Ror2 signals in osteoclast precursors may be involved in the abnormality of developing limbs in these deficient mice.

6. CONCLUSIONS

Osteoblasts closely regulate osteoclast differentiation and function through the expression of two cytokines, M-CSF and RANKL. Osteoblasts also express OPG, a soluble decoy receptor of RANKL, as an inhibitor of osteoclastogenesis. Most bone resorbing factors enhance RANKL expression and suppress OPG expression in osteoblasts. NFATc1 has been identified as a master transcription factor for osteoclast differentiation. Recent studies have also shown that immunoglobulin-like receptor-mediated ITAM signals are involved in the robust induction of NFATc1 in osteoclast precursors. Osteoblasts have been proposed to express molecules which stimulate ITAM signals. ITAM signals are known as co-stimulatory signals for RANK in osteoclastogenesis.

Wnt signalling regulates physiological development of organs, whereas deregulated signals induce diseases including cancer, arthritis and osteoporosis. Wnts mediate biological processes via two signalling pathways: the β-catenin-dependent canonical and β-catenin-independent noncanonical pathways. Wnts act on osteoblast precursors and promote their differentiation into mature osteoblasts through the canonical pathway. The canonical pathway in osteoblasts suppresses osteoclastogenesis through down-regulation of the RANKL/OPG ratio. In contrast, the activation of the noncanonical pathway in osteoclast precursors enhances RANKL-induced osteoclastic differentiation in a cell-autonomous manner. Osteoclast precursors express Ror2, a coreceptor for noncanonical signalling, while osteoblasts express Wnt5a, a Ror2 ligand. Synovial tissues from rheumatoid arthritis patients produce large amounts of Wnt5a. Based on these findings, we propose that the noncanonical Wnt pathway is a newly discovered co-stimulatory signal in osteoclastogenesis. This co-stimulatory signal plays an important role under not only physiological, but also pathological, conditions such as rheumatoid arthritis. These results also suggest that the Wnt5a-Ror2 pathway could be a therapeutic target for bone diseases with abnormal bone resorption.

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Abbreviations: M-CSF, macrophage colony-stimulating factor; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; NFATc1, nuclear factor of activated T-cells, cytoplasmic 1; ITAM, immunoreceptor tyrosine-based activation motif; $1\alpha,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; PTH, parathyroid hormone; PGE_2, prostaglandin E_2; IL-11, interleukin 11; LRP, low density lipoprotein receptor-related protein; Ror, receptor tyrosine kinase orphan receptor; TNF, tumor necrosis factor; DC-STAMP, dendritic cell-specific transmembrane protein; TRAF, TNF receptor-associated factor; NF-κB, nuclear factor-κB; JNK, c-Jun N-terminal kinase; LRC, leukocyte receptor complex; OSCAR, osteoclast-associated receptor; DAP12, DNAX-activating protein 12; FcRγ, Fc receptor common γ chain; TREM2, triggering receptors expressed on myeloid cells 2; SIRPβ1, signal-regulatory protein β1; PIR-A, paired immunoglobulin-like receptor A; SYK, spleen tyrosine kinase; PLCγ, phospholipase Cγ; Btk, Bruton's tyrosine kinase; BLNK, B cell linker protein; GSK-3β, glycogen synthase kinase-3β; TCF/LEF, T-cell factor/lymphoid enhancer factor, CAMKII, Ca^{2+}-calmodulin-dependent protein kinase II; PKC, protein kinase C

Key words: Osteoclasts, RANKL, RANK, Wnt, Ror, Frizzled, LRP

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