Special Issue: Osteoimmunology and vascular biology

Mini Review

Regulatory mechanism of osteoclastogenesis by Wnt signaling

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Bone-resorbing osteoclasts develop from monocyte-macrophage lineage cells under the regulation of bone-forming 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. Wnt proteins (Wnts) play a central role in the development of organs and tissues. There are two pathways of Wnt signaling. β-catenin-dependent canonical and β-catenin-independent noncanonical pathways. The discovery that loss-of-function mutations in low density lipoprotein receptor-related protein 5 (LRP5), a Wnt co-receptor, led to low bone mass in humans revealed the possible role of Wnt signaling in bone formation: Wnts act on osteoblast precursor cells and promote their differentiation into osteoblasts through the β-catenin-dependent canonical pathway. In addition, Wnts suppress bone resorption by the up-regulation of OPG expression and the down-regulation of RANKL expression in osteoblasts through the same pathway. In contrast, the activation of the β-catenin-independent noncanonical pathway enhances the RANKL-induced osteoclastogenesis. Recent studies have shown that the β-catenin independent noncanonical pathway is also involved in bone resorption induced by arthritis. This review summarizes the regulatory mechanism of bone resorption by Wnt signals.

Rec./Acc.10/17/2011, pp413-419

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Key words Osteoclast, osteoblast, Wnt, Ror, LRP5/6

Introduction

Bone remodeling is a dynamic process orchestrated by osteoblasts and osteoclasts. Osteoblasts, mononuclear cells responsible for bone formation, are differentiated from mesenchymal progenitor cells. Osteoclasts, multinuclear cells responsible for bone resorption, are derived from monocyte-macrophage lineage cells[1,2]. Besides their roles in bone formation, osteoblasts regulate osteoclastic bone resorption. Osteoblasts express two cytokines

required for osteoclast differentiation and function, macrophage-colony stimulating factor (M-CSF, also called CSF-1) and receptor activator of NF-κB ligand (RANKL)[1,3]. M-CSF is constitutively expressed in osteoblasts, whereas RANKL is inducibly expressed in osteoblasts in response to bone resorption-stimulating factors such as parathyroid hormone (PTH) and 1α,25 dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$]. Osteoblasts also express a negative regulator of bone resorption, osteoprotegerin (OPG), which inhibits the interaction between RANK and RANKL by acting as a decoy receptor of RANKL[1,2].

Wnt proteins (Wnts) are a family of 19 glycoproteins, which play a central role in the early development of organs and tissues[4,5]. 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: the β-catenin-dependent canonical pathway and the 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[6]. Loss-of-function mutations in LRP5 reduce the number of osteoblasts and cause osteoporosis.

It has been shown that the β-catenin-dependent canonical pathway in osteoblasts/stromal cells suppresses osteoclastogenesis through the up-regulation of OPG expression and the down-regulation of RANKL expression[7,8]. In addition, the activation of the noncanonical pathway in osteoclast precursors enhances RANKL-induced osteoclastic differentiation[9]. The noncanonical Wnt pathway has been shown to be involved in osteoclastogenesis induced by arthritis. 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 Wnt signals. The word "osteoblasts" is used as a generic name for osteoblast-lineage cells in this article.

Regulation of osteoclast differentiation and function

Experiments with an osteopetrotic op/op mouse model have established that an osteoblast product, M-CSF, is crucial for osteoclast formation. 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[10]. Administration of recombinant human M-CSF restored impaired bone resorption in op/op mice[11]. Calvarial osteoblasts obtained from op/op mice failed to support osteoclast development in cocultures

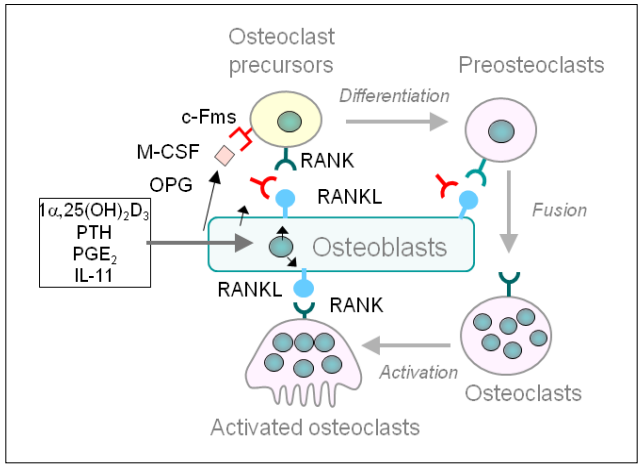

Fig.1 Regulation of osteoclast differentiation and function by osteoblasts

Bone resorption-stimulating factors such as 1α,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 fuse with each other to form multinucleated osteoclasts in response to RANKL stimulation. Mature osteoclasts also express RANK, and RANKL induces bone-resorbing activity of mature osteoclasts via binding to RANK.

with normal spleen cells, but the addition of M-CSF to cocultures induced osteoclast formation in response to 1α,25$(OH)_2D_3$[12]. These findings indicate that M-CSF produced by osteoblasts plays an essential role in osteoclast development (Fig.1). 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[13].

RANKL is a member of the tumor necrosis factor (TNF) family (TNF superfamily member 11, TNFSF11), which is also identified as "osteoclast differentiation factor"[14,15]. In contrast to M-CSF, the expression of RANKL by osteoblasts is inducible (Fig.1). Osteoblasts express RANKL as a membrane-associated form in response to stimuli of bone resorption-stimulating hormones and factors such as 1α,25$(OH)_2D_3$, PTH, prostaglandin E_2 (PGE_2), and interleukin 11 (IL-11). 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 osteo-

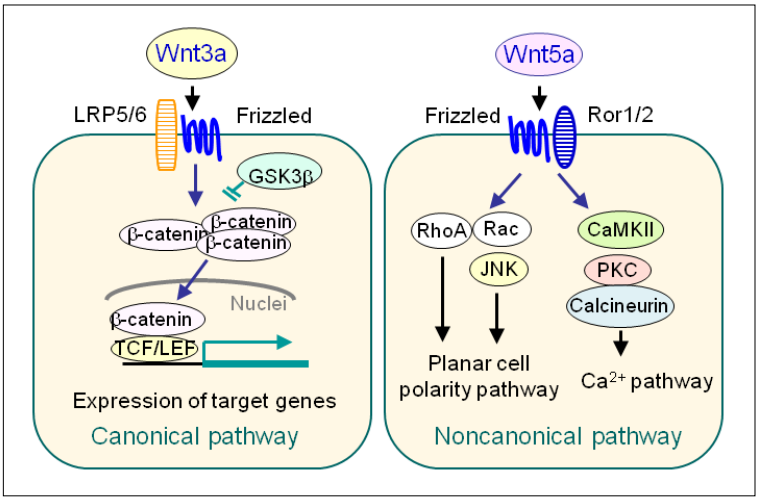

Fig.2 Wnt signaling pathways
Canonical Wnt pathway: In the absence of Wnts, GSK-3β phosphorylates β-catenin in target cells. Canonical Wnts (Wnt3a) bind to the receptor complex of Frizzled and LRP5 or LRP6, inhibit GSK-3β and promote the accumulation of β-catenin. The accumulated β-catenin translocates 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.

blasts and differentiate into osteoclasts in the presence of M-CSF (Fig.1). 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[16,17]. 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. Both RANKL-deficient (RANKL$^{-/-}$) mice[18] and RANK$^{-/-}$ mice[19] 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[20,21]. Administration of OPG[22] or blocking antibodies against RANKL[23] to normal mice strongly suppressed bone-resorbing activity of osteoclasts, as well as osteoclast differentiation.

Recently the interesting finding regarding the source of RANKL has been reported by two independent research groups[24,25]. They generated mice with osteocyte specific deletion of RANKL gene (osteocyte-specific RANKL cKO) and analyzed bone phenotype of those mice. Bone volume of osteocyte-specific RANKL cKO was dramatically increased with aging due to the suppression of osteoclastogenesis. Using a series of Cre-deleter strains, they also showed that RANKL expression in the osteoblast-lineage cells plays an essential role in osteoclast formation[24,25]. Taken together, these findings confirm the notion that RANKL expressed by osteoblast-lineage cells plays an essential role in osteoclastogenesis.

Wnt signalling pathways

Wnts are a family of 19 molecules in mammals[4,5]. In general, Wnts can be divided into two classes: the canonical Wnt class (e.g., Wnt1, Wnt3a, Wnt7a, and Wnt8) and the noncanonical Wnt class (e.g., Wnt5a, and Wnt11). The canonical Wnt class ligands such as Wnt1, Wnt3a, and Wnt7a activate the canonical pathway through the formation of a complex of Wnt, Frizzled, and LRP5 or LRP6[26] (Fig.2). 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. Nuclear β-catenin interacts with T-cell 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[4,5,26]. 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. Loss-of-function mutations in LRP5 reduce the number of osteoblasts and cause osteoporosis[6]. Thus, the canonical Wnt ligands act on osteoblast precursor cells and promote their differentiation into osteoblasts through the β-catenin-dependent canonical pathway. A recent report has also suggested that LRP5 signals control bone formation by inhibiting serotonin synthesis in the duodenum[27]. More recently, Cui et al.[28] have generated mice with osteocyte-specific expression of inducible Lrp5 mutations, and showed that the LRP5 signal in bone but not in intestine is important for the regulation of bone mass. There is a controversy over the issue where LRP5 functions to regulate bone mass.

Noncanonical Wnt signalling is classified into two subpathways:

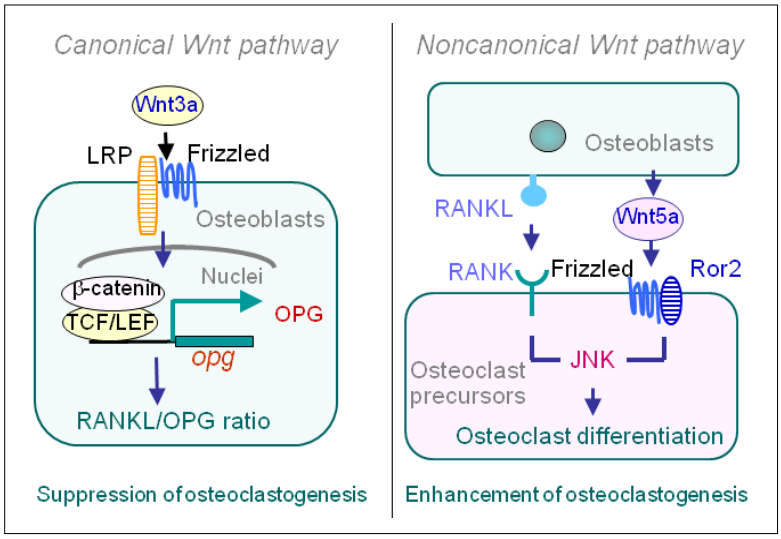

Fig.3 Regulation of osteoclastogenesis by Wnt signals

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. Thus, the noncanonical Wnt pathway enhances osteoclastogenesis.

the Wnt/Ca^{2+} and Wnt/planer cell polarity pathways[26] (Fig.2). Both pathways are activated by the noncanonical Wnt 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)[29-32]. The planer cell polarity pathway is mediated through RhoA-dependent and c-Jun N-terminal kinase (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[33-35]. The mammalian Ror family consists of two structurally related proteins, Ror1 and Ror2. Ror1 and Ror2 act as alternative receptors or coreceptors for Wnt5a[36]. Wnt5a binds to the cysteine-rich domain of Ror2, which then associates with Frizzled2 and activates JNK in cultured cells[37]. Recently, it was shown that Frizzled2 was internalized through a clathrin-mediated route in response to Wnt5a, and Ror1 or Ror2 were required for this action[38]. These results suggest that both Ror2 and Ror1 mediate Wnt5a signaling by activating the noncanonical Wnt pathway (Fig.2).

Role of canonical Wnt signals in osteoclastogenesis

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[39] (Fig.3). 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.[7] developed mice expressing a stabilized form of β-catenin in their osteoblasts (β-catenin mutant mice). The β-catenin mutant mice developed osteopetrosis with tooth eruption defects and a decreased number of osteoclasts. The number of osteoblasts and other parameters of osteoblast function remained unchanged in those mice. Micro-array analysis showed that OPG was up-regulated in osteoblasts in the β-catenin mutant mice[7] (Fig.3). When β-catenin was inactivated selectively in mature osteoblasts using α1(I) collagen Cre mice, the osteopetrotic disorder was decreased due to enhancement of bone resorption. Thus, activation of the canonical Wnt pathway stimulated OPG expression in osteoblasts. In addition, the canonical Wnt pathway suppressed the expression of RANKL in MC3T3E1 cells and MG-63 cells[8]. 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.

LRP5 and LRP6 are expressed in bone marrow macrophages, suggesting that Wnt3a can stimulate canonical Wnt pathways in osteoclast precursors[9]. Wnt3a stimulated cytosolic β-catenin accumulation in bone marrow macrophages, but showed no ef-

fect on osteoclast formation in bone marrow macrophage cultures treated with RANKL and M-CSF[9]. 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.

Role of noncanonical Wnt signals in osteoclastogenesis

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[9] (Fig.3). 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 (Fig.3). 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[40]. 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.

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. 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. 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. These results suggest that Wnt pathway plays an important role in osteoclastogenesis under not only physiological but also pathological conditions such as rheumatoid arthritis, and, thereby, that the Wnt5a-Ror2 pathway could be a therapeutic target for bone diseases with abnormal bone resorption.

Financial disclosure

The authors declare that they have no financial or commercial conflict of interests.

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