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REVIEW

The regulation of osteoclast differentiation by Wnt signals

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Wnt ligands activate β-catenin-dependent canonical and –independent noncanonical signaling pathways. Wnt regulates many physiological events such as the development of organs and bone metabolism. In contrast, failed signaling leads to pathological conditions including cancer and osteoporosis. Analyses of loss-of-function mutations in the low-density lipoprotein receptor-related protein (*Lrp*) 5 gene revealed that Lrp5 acted as a co-receptor of Wnt/β-catenin signals and positively regulated bone mass in humans and mice. Many players in Wnt signals including sclerostin, an osteocyte-derived Wnt antagonist, also have since been found to influence bone mass. Bone mass is regulated by the activities of bone-forming osteoblasts, -resorbing osteoclasts and matrix-embedded osteocytes. The roles of Wnt/β-catenin signals in osteoblastogenesis and osteoclastogenesis have been established by the findings of a large number of *in vitro and in vivo* studies. In contrast, the roles of noncanonical Wnt signals in bone metabolism are only now being examined. In this review, we introduced and discussed recent information on the roles of Wnt signals in bone resorption.

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Introduction

Osteoclasts, multinucleated giant cells, are differentiated from monocyte-macrophage lineage cells.[1] This differentiation is regulated by osteoblast-lineage cells including osteocytes. Osteoblasts express two cytokines that are essential for osteoclastogenesis, colony-stimulating factor 1 (Csf1) and receptor activator of nuclear factor-κB (NF-κB) ligand (Rankl, encoded by the *Tnfsf11* gene).[2–4] Rankl is inducibly expressed by osteoblasts[5,6] and osteocytes[7,8] in response to bone resorption-stimulating factors such as $1\alpha,25(OH)_2D_3$ and parathyroid hormone. Csf1 is constitutively expressed in osteoblasts. In contrast, osteoblasts and osteocytes also express osteoprotegerin (Opg, encoded by the *Tnfrsf11b* gene),[9] which has been shown to inhibit the binding of Rankl to its receptor Rank (encoded by the *Tnfrsf11a* gene).[10] Opg negatively regulates osteoclast differentiation.

The binding of Rankl to Rank in osteoclast precursors is known to activate several signaling pathways such as mitogen-activated protein kinases (Mapks), NF-κB, c-Fos and nuclear factor-activated T-cell cytoplasmic 1 (Nfatc1).[11] Consequently, osteoclasts develop and express osteoclast-related genes such as tartrate-resistant acid phosphatase, cathepsin K and calcitonin receptors.

Glass *et al.*[12] previously reported the importance of Wnt/β-catenin signals in osteoclast differentiation *in vivo*. They demonstrated that mice expressing a dominant active form of β-catenin in osteoblasts (DA-β-catenin mutant mice) exhibited a high bone mass phenotype with impaired osteoclast differentiation. The expression of Opg was also higher in DA-β-catenin mutant mice. These findings indicated that Wnt/β-catenin signals in osteoblasts induced the expression of Opg, which, in turn, inhibited osteoclast differentiation.

Wnt/β-catenin signals also regulate osteoblast differentiation to increase bone mass.[13] Song *et al.*[14] generated pre-osteoblast-specific β-catenin conditional knockout mice (β-catenin cKO), in which the β-catenin gene was postnatally deleted, and analyzed their bone phenotypes. β-catenin cKO exhibited a low bone mass phenotype with increased bone marrow adiposity. *Ex vivo* studies showed that the osteoblast differentiation was suppressed in bone marrow stromal cells isolated from β-catenin cKO. In contrast, the adipocyte differentiation was enhanced in those cells. These findings suggest that Wnt/β-catenin signals determine the cell fate of preosteoblasts from osteoblasts to adipocytes.

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Subsequent studies found that β-catenin-independent noncanonical signals were also involved in osteoclastogenesis. In this review, we introduced recent information on the roles of β-catenin-dependent and β-catenin-independent Wnt signals in osteoclast differentiation.

Wnt Signaling Pathways

Wnt ligands activate two signaling pathways, the β-catenin-dependent canonical and β-catenin-independent noncanonical pathways, in various types of cells.[15,16]

The canonical Wnt pathway: in the absence of canonical Wnt ligands including Wnt3a, β-catenin is effectively degraded by a β-catenin destruction complex consisting of axin, adenomatous polyposis coli, glycogen synthase kinase 3 and casein kinase 1. Phosphorylated β-catenin is degraded by the destruction complex through the ubiquitin-proteasome pathway (**Figure 1a**). The binding of Wnt ligands to the receptor complex of frizzled and Lrp5/6 inactivates the β-catenin destruction complex, which, in turn, induces the cytosolic accumulation and nuclear translocation of β-catenin in target cells. The nuclear β-catenin with members of the T-cell factor (Tcf)/lymphoid enhancer factor family initiates the transcription of target genes (**Figure 1b**).

The noncanonical Wnt pathway: several Wnt ligands including Wnt5a activate the Wnt/Ca^{2+} and Wnt/planar cell polarity pathways. An increase in cytosolic Ca^{2+} has been shown to activate Ca^{2+}-sensitive enzymes such as Ca^{2+}-calmodulin-dependent protein kinase II and protein kinase C.[17–19] Small G proteins including Rho and Rac and c-Jun N-terminal kinase (Jnk) were previously reported to have roles in the planar cell polarity pathway.[20–22] Receptor tyrosine kinase-like orphan receptor (Ror) 1 and Ror2 act as alternative receptors or co-receptors for Wnt5a.[23–25]

Wnt/β-Catenin Signals in Osteoblasts

Wnt/β-catenin signaling in osteoblasts is known to influence osteoclast differentiation *in vivo*.[12] DA-β-catenin mutant mice had a high bone mass phenotype. A histomorphometric analysis revealed that osteoclast differentiation was impaired in DA-β-catenin mutant mice. In contrast, the number of osteoblasts was normal in these mutant mice. Using a microarray analysis, *Opg* was found to be downregulated in *Lrp5*$^{-/-}$ calvariae and upregulated in DA-β-catenin mutant mouse calvariae. This study also demonstrated that β-catenin with TCF1 was recruited to the *Opg* promoter to induce the transcription of *Opg* (**Figure 1b**). However, the identity of the Wnt ligands that induced the expression of *Opg* remained unclear until recently.

Genome-wide association studies (GWAS) demonstrated that the *Wnt16* locus reproducibly associated with bone mineral density, cortical bone thickness and non-vertebral fractures.[26–31] These findings suggested that Wnt16 regulated bone formation and resorption to maintain bone mass in humans. Movérare-Skrtic *et al.*[32] recently discussed the roles of Wnt16 in bone metabolism. Wnt16 was shown to act on osteoblasts and induce the expression of *Opg* expression (**Figure 2**). The expression level of *Wnt16* mRNA in cortical bone was the highest among various tissues including trabecular bone. In cell cultures, primary osteoblasts strongly expressed *Wnt16*, whereas bone marrow-derived macrophages and osteoclasts did not. The treatment of MC3T3E1 cells, an osteoblastic cell line, with recombinant Wnt16 induced the expression of *Opg* mRNA due to the activation of Wnt/β-catenin signaling. The bone mass of cortical bone in *Wnt16*$^{-/-}$ mice was reduced, whereas that of trabecular bone was not. This finding supported previous data from the GWAS analysis and indicated that Wnt16 is a candidate ligand that induces the expression of *Opg* in bone tissues. Although Wnt16 was shown to activate Wnt/β-catenin signaling in

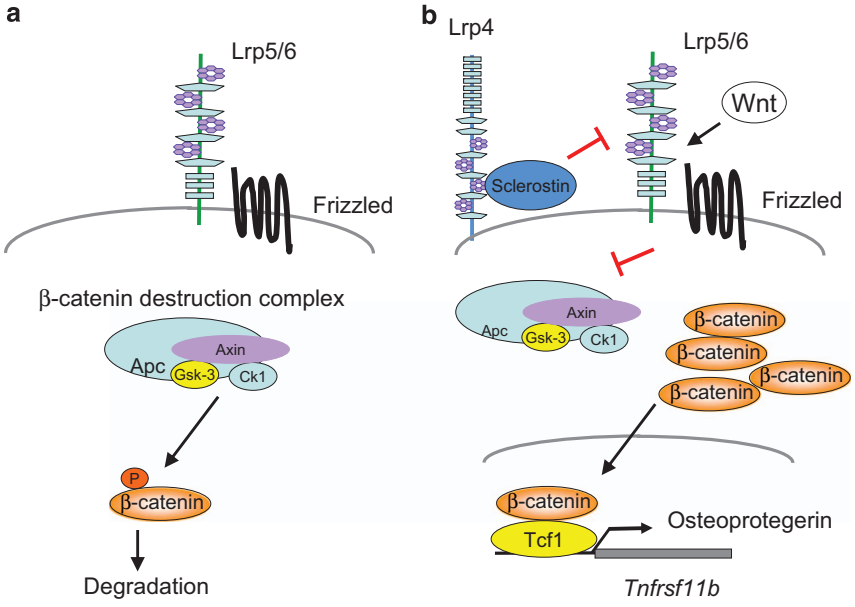

Figure 1 Roles of Wnt/β-catenin signals in osteoclast differentiation. (**a**) In the absence of Wnt ligands, the β-catenin destruction complex phosphorylated β-catenin. Phosphorylated β-catenin is degraded by the ubiquitin-proteasome pathway. (**b**) Binding of Wnt ligands with the receptor complex of frizzled and Lrp5/6 inactivates the β-catenin destruction complex, which, in turn, induces the cytosolic accumulation and nuclear translocation of β-catenin. Nuclear β-catenin with Tcf1 induces the transcription of the *Tnfrsf11b* gene. Sclerostin, an osteocyte-derived inhibitor, inhibits Wnt/β-catenin signals. Lrp4 facilitates the inhibitory action of sclerostin.

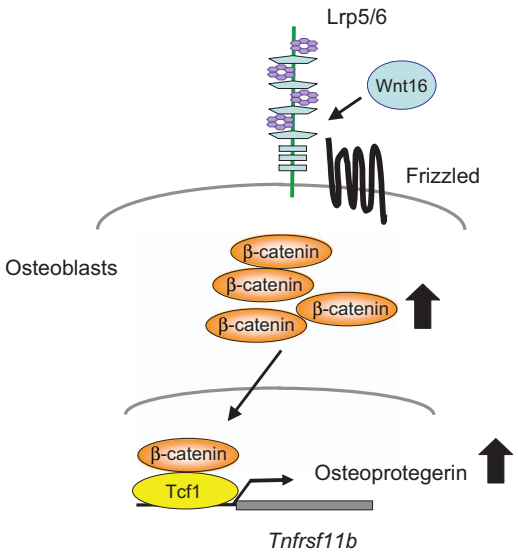

Figure 2 Effects of Wnt16 through osteoblasts on osteoclast differentiation. Wnt16 activates Wnt/β-catenin signals in osteoblasts, which, in turn, induces the expression of osteoprotegerin. Osteoprotegerin interferes the binding of Rankl with Rank and inhibits osteoclast differentiation.

osteoblasts in cultures, the mineralization of *Wnt16*$^{-/-}$ osteoblasts was similar to that of wild-type osteoblasts.[32] In addition, the expression of osteoblast marker genes, such as *Runx2* and *Alpl* (encoding alkaline phosphatase), and histomorphometric parameters for bone formation remained unchanged in the cortical bone of *Wnt16*$^{-/-}$ mice. In contrast, the expression of *Opg* mRNA was significantly lower in the cortical bone of *Wnt16*$^{-/-}$ mice. These findings indicated that Wnt16, secreted from osteoblasts, induced the expression of *Opg* in cortical bone, thereby predominantly regulating bone resorption *in vivo*. This study also showed the direct effects of Wnt16 on RANKL-induced osteoclast formation. This topic is discussed in a later part of this review.

Noncanonical Signals by Wnt4 in Osteoblast-Lineage Cells

An analysis of single nucleotide polymorphisms identified the *WNT4* gene as one of the five genes associated with the cross-sectional area of vertebrae in caucasian men aged more than 65 years in the osteoporotic fractures in men study.[33] GWAS subsequently confirmed that the *WNT4* gene was associated with bone mineral density.[26] Prior to these human studies being conducted, the role of Wnt4 in the differentiation of mesenchymal stem cells (MSCs) into osteoblasts was examined.[34] The overexpression of Wnt4 in MSCs promoted their osteoblastic differentiation in cultures. Wnt4 was shown to enhance bone morphogenetic protein signals via p38 Mapk and promoted osteoblastogenesis. The transplantation of MSCs expressing Wnt4 enhanced the restoration of alveolar bone and calvarial bone defects. Recently, the overexpression of Wnt4 in osteoblasts is reported to impact osteoclast differentiation.[35] This topic is discussed in a later part of this review.

Lrp4 and Sclerostin

Lrp4 has been identified as a receptor of agrin, a motor neuron-derived ligand.[36] Agrin binds to Lrp4 and forms a complex with muscle-specific kinase (Musk) to develop neuromuscular synapses. Lrp4 has also been shown to bind Wise (Wnt modulator in surface ectoderm, also called sost domain-containing protein 1, *Sostdc1*).[37] Choi *et al.*[38] previously reported that Lrp4-bound dickkopf 1 (Dkk1) and sclerostin *in vitro.* They generated Lrp4-deficient mice (Lrp4-ECD), in which the transmembrane domain and intracellular domain of Lrp4 were deleted, and analyzed their bone phenotypes. Lrp4-ECD mice grew and exhibited a low bone mass at 10 weeks of age. The phenotype of Lrp4-ECD mice was largely different from that of Lrp4-null mice (described below). This discrepancy was attributed to the interaction between the mutant form of Lrp4 and its ligands in Lrp4-ECD mice. Leupin *et al.*[39] searched for binding partners of sclerostin using tandem affinity purification proteomic approaches and identified Lrp4 as a new binding partner. The expression of Lrp4 was shown to enhance the inhibitory effects of sclerostin on Wnt/β-catenin signaling in HEK293 cells.[39] An immunohistochemical study demonstrated that Lrp4 was expressed in the osteoblasts and osteocytes of human bone tissues. Notably, two mutations in the *LRP4* gene (R1170W and W1186S) were identified in sclerosteosis patients. Both mutations were detected in the third propeller domain of LRP4. These mutant forms of LRP4 reduced the binding capacity and inhibitory effects of sclerostin. These findings indicated that Lrp4 bound sclerostin through the third propeller domain (**Figure 1b**).

Two recent studies demonstrated the important roles of Lrp4 in bone metabolism using cKO mice.[40,41] Chang *et al.*[40] generated mice lacking *Lrp4* in their osteoblasts and osteocytes (*Lrp4*$^{flox/flox}$; osteocalcin-cre mice) and osteocytes only (*Lrp4*$^{flox/flox}$; *Dmp1*-cre mice). These cKO mice showed a high bone mass phenotype with a larger number of osteoblasts and a smaller number of osteoclasts. Serum levels of sclerostin in these mutant mice were higher compared with those in control mice, suggesting that Lrp4 retained sclerostin in bone tissues. Chang *et al.*[40] also developed anti-Lrp4 antibodies that bound to the third propeller domain of Lrp4. These antibodies inhibited the facilitative effects of Lrp4 on sclerostin. As discussed above, Lrp4 is involved in Musk signaling to form neuromuscular synapses. The anti-Lrp4 antibodies did not inhibit the binding of Lrp4 to Agrin in C2C12 cell cultures. Furthermore, treatments with anti-Lrp4 antibodies increased bone mass in 7-month-old rats. These findings suggested the applicability of anti-Lrp4 antibodies to the treatment of osteoporosis, similar to anti-sclerostin antibodies.

Xiong *et al.*[41] analyzed bone phenotypes in two Lrp4 mutant mice, osteoblast-specific *Lrp4* knockout mice (*Lrp4*$^{flox/flox}$; osteocalcin-cre mice) and muscle-rescued *Lrp4*-null mice (mr-*Lrp4*mitt). As *Lrp4*-null mice die at birth because of defects in neuromuscular junctions, *Lrp4*-null mice were rescued by crossing with transgenic mice expressing human skeletal α-*actin* promoter-driven *Lrp4*. The bone mass of trabecular and cortical bones was high in these mutant mice. Histomorphometric analysis revealed that parameters for bone formation were increased. Serum levels of sclerostin were higher in these mutant mice. The inhibitory effects of sclerostin and Wise, but not Dkk1, on alkaline phosphatase expression disappeared in bone marrow stromal cells isolated from mr-*Lrp4*mitt mice. This finding confirmed the roles of Lrp4 in the inhibitory actions of sclerostin and Wise. Furthermore, the number of osteoclasts and serum levels of deoxypyridinoline, a

marker for bone resorption, were lower in both mutant mice. The treatment of bone marrow stromal cells from control mice, but not from mr-Lrp4mitt mice, with sclerostin increased the expression of *Rankl* and decreased that of *Opg* mRNA. This finding suggested that the expression of *Rankl* as well as *Opg* is regulated by the Wnt/β-catenin signaling pathway; however, the underlying mechanism has not yet been elucidated in detail. Thus, Lrp4 represents a new therapeutic target for increasing bone mass. We anticipate that anti-Lrp4 antibodies will be used in the future treatment of osteoporosis.

Wnt/β-Catenin Signals in Osteoclast Precursors

β-catenin signals in osteoclast precursors have been reported to have roles in osteoclast differentiation.[42] DA-β-catenin mice and β-catenin cKO mice were generated using mice expressing Cre recombinases under the *Ppar-γ* promoter. Bone mass was higher in osteoclast precursor-specific DA-β-catenin and β-catenin cKO mice due to impaired osteoclast formation. Furthermore, the Rankl and Csf1-induced formation of osteoclasts was diminished in bone marrow-derived macrophage cultures isolated from these mice. These findings indicated that β-catenin signals in osteoclast precursors were required for their proliferation, whereas the sustained activation of β-catenin signals inhibited osteoclast formation. In contrast, we and others showed that the treatment of osteoclast precursors with Wnt3a had no effect on the Rankl-induced formation of osteoclasts; however, Wnt3a induced the cytosolic accumulation of β-catenin in these cells.[43,44] Our finding was different from those in a former study using DA-β-catenin. This difference was attributed to the different methods used to activate Wnt/β-catenin signals. The expression of DA-β-catenin activated Wnt/β-catenin signals in cells more strongly than treatment with recombinant Wnt3a. Thus, Wei *et al.*[42] reported that finely balanced β-catenin signals in osteoclast precursors may be required for proper osteoclastogenesis.

Noncanonical Wnt Signals by Wnt5a in Osteoclast Differentiation

Wnt5a, a typical noncanonical Wnt ligand, activates β-catenin-independent signals in various types of cells. Ror1 and Ror2 have been shown to function as receptors or co-receptors of Wnt5a.[23] In *in vitro* experiments, we found that osteoblasts strongly expressed Wnt5a, whereas bone marrow macrophages expressed receptor components of Wnt5a such as *Frizzleds 1, 2* and *5*, and *Ror2*.[43] Immunofluorescence studies confirmed that osteoblasts expressing Runx2 and Osterix had positive signals for anti-Wnt5a antibodies on the bone surfaces of bone tissues.

Femurs isolated from 8-week-old *Wnt5a*$^{+/-}$ and *Ror2*$^{+/-}$ mice were analyzed to clarify the roles of Ror2-mediated signals in bone formation and resorption, because *Wnt5a*-deficient mice and *Ror2*-deficient mice die during the perinatal period. The findings of micro-computed tomography and a histomorphometric analysis revealed that *Wnt5a*$^{+/-}$ mice had a lower bone mass with impaired bone formation and resorption, whereas *Ror2*$^{+/-}$ mice had a higher bone mass with decreased bone resorption. The number of osteoclasts in both heterozygous mice was significantly lower than that in wild-type mice. To further clarify the roles of Ror2 signals in osteoclast formation, osteoclast precursor-specific *Ror2* cKO mice were generated by crossing *Rank*$^{Cre/+}$ mice with *Ror2*$^{flox/flox}$ mice. *Ror2* cKO showed a high bone mass with impaired osteoclast differentiation. The number of osteoclasts was also lower in osteoblast-specific *Wnt5a* cKO mice, which were generated by crossing *Wnt5a*$^{flox/flox}$ with osterix-Cre mice. Taken together, these findings indicated that Wnt5a-Ror2 signals between osteoblasts and osteoclast precursors were critical for osteoclast differentiation.

We then attempted to elucidate the mechanism by which Ror2 signals positively regulate osteoclast differentiation. Osteoclasts were formed in bone marrow macrophage cultures treated with Rankl and Csf1. The addition of recombinant Wnt5a into the cultures enhanced the formation of osteoclasts. In contrast, the addition of Wnt3a, a typical canonical Wnt ligand, had no effect. The treatment with Wnt5a did not enhance Rankl-induced osteoclast formation in cultures of osteoclast precursors isolated from *Ror2*$^{-/-}$ mice.

During efforts to clarify how Ror2 signals enhance Rankl-induced osteoclast formation, we found that the expression of *Rank* mRNA as well as *Ctsk* (encoding cathepsin K), *Calcr* (encoding the calcitonin receptor) and *Acp5* (encoding tartrate-resistant acid phosphatase) mRNAs was lower in tibiae from *Ror2* cKO mice. The expression of *Csf1r* (encoding c-fms) mRNA remained unchanged. Furthermore, recombinant Wnt5a increased the expression of *Rank* mRNA in cultures of osteoclast precursors in the presence of Csf1. We also generated *Rank*-enhanced green fluorescence protein (EGFP) mice, in which EGFP was expressed by the *Rank* promoter-driven Cre recombinase. Using bone marrow cells isolated from these mice, the number of Rank-expressing cells was higher in bone marrow cell cultures treated with Csf1 plus Wnt5a than in those treated with Csf1 only.

A reporter assay using the *Rank* promoter revealed that the cluster of Sp1 sites on the proximal *Rank* promoter was important for Wnt5a-induced *Rank* expression.[43] Chromatin immunoprecipitation assays further showed that the recruitment of c-Jun to the cluster region of Sp1 sites in the *Rank* promoter was critical for the Wnt5a-induced expression of Rank. Therefore, Wnt5a secreted from osteoblasts enhanced the expression of *Rank* in osteoclast precursors through the c-Jun–Sp1 interaction, thereby enhancing Rankl-induced osteoclast formation (**Figure 3**). We previously reported that osteoclast precursors highly expressing Rank were present in the vicinity of alkaline phosphatase-positive osteoblasts, even in bone tissues of *Rankl*-deficient mice.[45] Wnt5a together with Csf1 secreted from osteoblasts may have roles in the localization of osteoclast precursors in the vicinity of osteoblasts.

Noncanonical Signals by Wnt16 in Osteoclast Differentiation

As discussed above, Wnt16 activated Wnt/β-catenin signals, which, in turn, induced the expression of *Opg* in osteoblasts. In contrast, the treatment of osteoclast precursors with Wnt16 failed to induce the cytosolic accumulation of β-catenin or the expression of *Axin2*, a target gene of Wnt/β-catenin signals.[32] Alternatively, Wnt16 markedly phosphorylated Jnk and c-Jun proteins, both of which were activated by noncanonical Wnt signals in osteoclast precursors.[45] Wnt16 inhibited

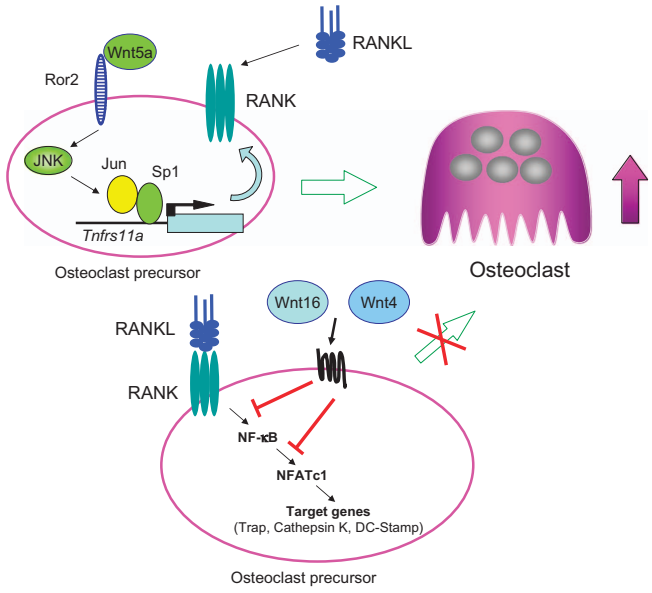

Figure 3 Roles of noncanonical Wnt signals in osteoclast differentiation. Wnt5a secreted from osteoblasts binds to Ror2 in osteoclast precursors. Ror2-mediated signals promote the expression of Rank in the precursors, thereby enhancing Rankl-induced osteoclast differentiation. Wnt4 and Wnt16 are also secreted from osteoblasts and inhibit the Rankl-induced activation of NF-κB and NFATc1 signals, which, in turn, inhibit osteoclast differentiation.

Rankl-induced osteoclast formation in human and mouse osteoclast precursors in culture. Wnt16 also inhibited the Rankl-induced activation of NF-κB signals and suppressed the Rankl-induced expression of *Nfatc1*. These findings suggested that Wnt16 also directly acted on osteoclast precursors, thereby inhibiting osteoclastogenesis (**Figure 3**). Although Wnt16 activated c-Jun in osteoclast precursors, the molecular relationship between c-Jun and the inhibitory effects of Wnt16 has not yet been clarified. We previously reported that Wnt16 failed to bind GST-soluble Ror2 containing the extracellular domain of Ror2.[45] Movérare-Skrtic *et al.*[32] showed that Wnt16 failed to activate Ror2 signals using reporter assays; however, Wnt16 induced the phosphorylation of Jnk in osteoclast precursors. A previous study demonstrated that the activation of c-Jun was important for the Rankl-induced formation of osteoclasts.[46] Taken together, these findings indicated that the inhibitory effects of Wnt16 on osteoclast formation were not mediated by the Ror2-Jnk signaling axis in osteoclast precursors. Further studies are needed to elucidate the mechanism by which Wnt16 inhibits Rankl-induced signals.

Noncanonical Signaling by Wnt4 in Osteoclast Differentiation

Yu *et al.*[35] generated *Wnt4* transgenic (*Wnt4*-Tg) mice, in which *Wnt4* was expressed in osteoblasts, and analyzed their bone phenotype. Bone mass was higher in *Wnt4*-Tg mice than in control mice. A histomorphometric analysis showed that the number of osteoblasts in bone tissues was moderately higher in 3-month-old *Wnt4*-Tg mice. *Wnt4*-Tg mice were protected from ovariectomy-induced and tumor necrosis factor (TNF)-α-induced bone loss. The number of osteoclasts was significantly increased in ovariectomized and *Tnf*-α transgenic mice. The overexpression of Wnt4 in the osteoblasts of these mouse models decreased the number of osteoclasts. These findings suggested that Wnt4 secreted from osteoblasts inhibited osteoclast formation.

The inhibitory action of Wnt4 was then examined. The treatment of osteoclast precursors with Wnt4 inhibited the Rankl-induced phosphorylation of transforming growth factor-activated kinase 1, thereby suppressing the activation of NF-κB signals. Wnt4 also suppressed Rankl-induced *Nfatc1* expression in osteoclast precursors. Furthermore, Wnt4 failed to activate Wnt/β-catenin signals in these cells. These findings indicated that Wnt4 activated noncanonical signals, which, in turn, inhibited NF-κB and Nfatc1 signals in osteoclast precursors (**Figure 3**). The administration of Wnt4 proteins to mice also prevented ovariectomy-induced bone loss.

Conclusion

Wnt ligands and antagonists constitute a signal network in bone tissues and regulate osteoclast differentiation. Wnt5a activates the Ror2-Jnk-c-Jun signaling axis and enhances Rankl-induced osteoclast formation. In contrast, Wnt16 and Wnt4 inhibit osteoclast formation by inhibiting Rankl–Rank signals. In addition to the expression of Opg by Wnt/β-catenin signals in osteoblasts, Wnt ligands secreted from osteoblasts tightly regulate osteoclast differentiation through noncanonical Wnt signals. The relationship between Wnt ligands and their receptors and the signaling pathways in osteoclasts need to be clarified in more detail in future studies. Thus, an analysis of noncanonical Wnt signals as well as the canonical signals may lead to the development of new therapeutic drugs for osteoporosis.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocr Rev* 1999; **20**: 345–357.
2. Wagner EF, Karsenty G. Genetic control of skeletal development. *Curr Opin Genet Dev* 2001; **11**: 527–532.
3. Takahashi N, Akatsu T, Udagawa N, Sasaki T, Yamaguchi A, Moseley JM *et al.* Osteoblastic cells are involved in osteoclast formation. *Endocrinology* 1998; **123**: 2600–2602.
4. Teitelbaum SL, Ross FP. Genetic regulation of osteoclast development and function. *Nat Rev Genet* 2003; **4**: 638–649.
5. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S *et al.* Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci USA* 1998; **95**: 3597–3602.
6. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T *et al.* Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998; **93**: 165–176.
7. Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ *et al.* Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat Med* 2001; **17**: 1231–1234.
8. Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA. Matrix-embedded cells control osteoclast formation. *Nat Med* 2011; **17**: 1235–1241.
9. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R *et al.* Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309–319.

10. Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T *et al.* RANK is essential for osteoclast and lymph node development. *Genes Dev* 1999; **13**: 2412–2424.
11. Takayanagi H. The role of NFAT in osteoclast formation. *Ann NY Acad Sci* 2007; **1116**: 227–237.
12. Glass 2nd DA , Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H *et al.* Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell* 2005; **8**: 751–764.
13. Krishnan V, Bryant HU, MacDougald OA. Regulation of bone mass by Wnt signaling. *J Clin Invest* 2006; **116**: 1202–1209.
14. Song L, Liu M, Ono N, Bringhurst FR, Kronenberg HM, Guo J. Loss of wnt/β-catenin signaling causes cell fate shift of preosteoblasts from osteoblasts to adipocytes. *J Bone Miner Res* 2012; **27**: 2344–2358.
15. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 2004; **20**: 781–810.
16. Gordon MD, Nusse R. Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. *J Biol Chem* 2006; **281**: 22429–22433.
17. Weeraratna AT, Jiang Y, Hostetter G, Rosenblatt K, Duray P, Bittner M *et al.* Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell* 2002; **1**: 279–288.
18. Dejmek J, Safholm A, Kamp Nielsen C, Andersson T, Leandersson K. Wnt-5a/Ca2 + -induced NFAT activity is counteracted by Wnt-5a/Yes-Cdc42-casein kinase 1alpha signaling in human mammary epithelial cells. *Mol Cell Biol* 2006; **26**: 6024–6036.
19. Ishitani T, Ninomiya-Tsuji J, Matsumoto K. Regulation of lymphoid enhancer factor 1/T-cell factor by mitogen-activated protein kinase-related Nemo-like kinase-dependent phosphorylation in Wnt/beta-catenin signaling. *Mol Cell Biol* 2003; **23**: 1379–1389.
20. Schambony A, Wedlich D. Wnt-5 A/Ror2 regulate expression of XPAPC through an alternative noncanonical signaling pathway. *Dev Cell* 2007; **12**: 779–792.
21. Qian D, Jones C, Rzadzinska A, Mark S, Zhang X, Steel KP *et al.* Wnt5a functions in planar cell polarity regulation in mice. *Dev Biol* 2007; **306**: 121–133.
22. Yamamoto S, Nishimura O, Misaki K, Nishita M, Minami Y, Yonemura S *et al.* Cthrc1 selectively activates the planar cell polarity pathway of Wnt signaling by stabilizing the Wnt-receptor complex. *Dev Cell* 2008; **15**: 23–36.
23. Mikels A, Minami Y, Nusse R. Ror2 receptor requires tyrosine kinase activity to mediate Wnt5A signaling. *J Biol Chem* 2009; **284**: 30167–30176.
24. Oishi I, Suzuki H, Onishi N, Takada R, Kani S, Ohkawara B *et al.* The receptor tyrosine kinase Ror2 is involved in non-canonical Wnt5a/JNK signalling pathway. *Genes Cells* 2006; **8**: 645–654.
25. Nomachi A, Nishita M, Inaba D, Enomoto M, Hamasaki M, Minami Y. Receptor tyrosine kinase Ror2 mediates Wnt5a-induced polarized cell migration by activating c-Jun N-terminal kinase via actin-binding protein filamin A. *J Biol Chem* 2008; **283**: 27973–27981.
26. Estrada K, Styrkarsdottir U, Evangelou E, Hsu Y-H, Duncan E, Ntzani EE *et al.* Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nat Genet* 2012; **44**: 491–501.
27. Medina-Gomez C, Kemp JP, Estrada K, Eriksson J, Liu J, Reppe S *et al.* Meta-analysis of genome-wide scans for total body BMD in children and adults reveals allelic heterogeneity and age-specific effects at the WNT16 locus. *PLoS Genet* 2012; **8**: e1002718.
28. Zheng HF, Tobias JH, Duncan E, Evans DM, Eriksson J, Paternoster L *et al.* WNT16 influences bone mineral density, cortical bone thickness, bone strength, and osteoporotic fracture risk. *PLoS Genet* 2012; **8**: e1002745.
29. Koller DL, Zheng HF, Karasik D, Yerges-Armstrong L, Liu CT, McGuigan F *et al.* Meta-analysis of genome-wide studies identifies WNT16 and ESR1 SNPs associated with bone mineral density in premenopausal women. *J Bone Miner Res* 2013; **28**: 547–558.
30. García-Ibarbia C, Pérez-Núñez MI, Olmos JM, Valero C, Pérez-Aguilar MD, Hernández JL *et al.* Missense polymorphisms of the WNT16 gene are associated with bone mass, hip geometry and fractures. *Osteoporos Int* 2013; **24**: 2449–2454.
31. Hendrickx G, Boudin E, Fijałkowski I, Nielsen TL, Andersen M, Brixen K *et al.* Variation in the Kozak sequence of WNT16 results in an increased translation and is associated with osteoporosis related parameters. *Bone* 2014; **59**: 57–65.
32. Movérare-Skrtic S, Henning P, Liu X, Nagano K, Saito H, Börjesson AE *et al.* Osteoblast-derived WNT16 represses osteoclastogenesis and prevents cortical bone fragility fractures. *Nat Med* 2014; **20**: 1279–1288.
33. Zmuda JM, Yerges-Armstrong LM, Moffett SP, Klei L, Kammerer CM, Roeder K *et al.* Genetic analysis of vertebral trabecular bone density and cross-sectional area in older men. *Osteoporos Int* 2011; **22**: 1079–1090.
34. Chang J, Sonoyama W, Wang Z, Jin Q, Zhang C, Krebsbach PH *et al.* Noncanonical Wnt-4 signaling enhances bone regeneration of mesenchymal stem cells in craniofacial defects through activation of p38 MAPK. *J Biol Chem* 2007; **282**: 30938–30948.
35. Yu B, Chang J, Liu Y, Li J, Kevork K, Al-Hezaimi K *et al.* Wnt4 signaling prevents skeletal aging and inflammation by inhibiting nuclear factor-κB. *Nat Med* 2014; **20**: 1009–1017.
36. Kim N, Stiegler AL, Cameron TO, Hallock PT, Gomez AM, Huang JH *et al.* Lrp4 is a receptor for Agrin and forms a complex with MuSK. *Cell* 2008; **135**: 334–342.
37. Ohazama A, Johnson EB, Ota MS, Choi HY, Porntaveetus T, Oommen S *et al.* Lrp4 modulates extracellular integration of cell signaling pathways in development. *PLoS One* 2008; **3**: e4092.
38. Choi HY, Dieckmann M, Herz J, Niemeier A. Lrp4, a novel receptor for Dickkopf 1 and sclerostin, is expressed by osteoblasts and regulates bone growth and turnover *in vivo*. *PLoS One* 2009; **4**: e7930.
39. Leupin O, Piters E, Halleux C, Hu S, Kramer I, Morvan F *et al.* Bone overgrowth-associated mutations in the LRP4 gene impair sclerostin facilitator function. *J Biol Chem* 2011; **286**: 19489–19500.
40. Chang MK, Kramer I, Huber T, Kinzel B, Guth-Gundel S, Leupin O *et al.* Disruption of Lrp4 function by genetic deletion or pharmacological blockade increases bone mass and serum sclerostin levels. *Proc Natl Acad Sci USA* 2014; **111**: E5187–E5195.
41. Xiong L, Junga J-U, Wua H, Xiaa W-F, Pana J-X, Shena C *et al.* Lrp4 in osteoblasts suppresses bone formation and promotes osteoclastogenesis and bone resorption. *Proc Natl Acad Sci USA* 2015; **112**: 3487–3492.
42. Wei W, Zeve D, Suh JM, Wang X, Du Y, Zerwekh JE *et al.* Biphasic and dosage-dependent regulation of osteoclastogenesis by beta-catenin. *Mol Cell Biol* 2011; **31**: 4706–4719.
43. Maeda K, Kobayashi Y, Udagawa N, Uehara S, Ishihara A, Mizoguchi T *et al.* Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. *Nat Med* 2012; **18**: 405–412.
44. Yamane T, Kunisada T, Tsukamoto H, Yamazaki H, Niwa H, Takada S *et al.* Wnt signaling regulates hemopoiesis through stromal cells. *J Immunol* 2001; **167**: 765–772.
45. Mizoguchi T, Muto A, Udagawa N, Arai A, Yamashita T, Hosoya A *et al.* Identification of cell cycle-arrested quiescent osteoclast precursors *in vivo*. *J Cell Biol* 2009; **184**: 541–554.
46. Ikeda F, Nishimura R, Matsubara T, Tanaka S, Inoue J, Reddy SV *et al.* Critical roles of c-Jun signaling in regulation of NFAT family and RANKL-regulated osteoclast differentiation. *J Clin Invest* 2004; **114**: 475–484.