MINI REVIEW

Roles of Wnt signaling in bone formation and resorption

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1. Introduction

Bone remodeling is a dynamic process orchestrated by bone-forming osteoblasts and bone-resorbing osteoclasts. Osteoblasts are mononuclear cells responsible for bone formation. Osteoblasts are differentiated from mesenchymal progenitor cells at sites of membranous bone formation and endochon-
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 [5]. Wnt proteins (Wnts) play a central role in the early development of organs and tissues [6]. Recent studies have established that Wnt-mediated signals have important roles in bone remodeling in both physiological and pathological conditions. In this review, we will introduce the molecules involved in the Wnt signaling pathway in detail, and focus on the role of Wnt signaling in bone formation and bone resorption.

2. Molecules involved in Wnt signaling

2.1. Ligands

Wnts are a family of 19 molecules in mammals, each consisting of 350–400 amino acids [7]. Wnts possess 22–24 conserved cysteine residues, and show 20–80% amino acid identity among the family members. Xenopus Wnts (XWnts) are divided into two classes: the canonical Wnt class (e.g., XWnt1, XWnt3a and XWnt8) and the non-canonical Wnt class (e.g., XWnt4, XWnt5a, and XWnt11) [7]. However, this classification of XWnts cannot be applied in the other species. Non-canonical Wnt5a activated the canonical pathway in HEK293 cells co-expressing frizzled 4 and LRP5 [8]. This finding suggests that combination of frizzleds and their co-receptors determines the Wnt signaling pathway to be activated.

2.2. Receptors

Frizzled is a seven transmembrane receptor of Wnts. The Frizzled family consists of 10 members in mammals with wide-ranged in the size from 500 to 700 amino acids [9]. There is a cysteine-rich domain (CRD) in the amino terminal extracellular region in Frizzled. The CRD is necessary for binding of Wnts. The KTXXXW motif located after the seventh hydrophobic domain is highly conserved in Frizzled members and is essential for activation of the canonical Wnt pathway (Fig. 1).

2.3. Co-receptors

LRP5 and LRP6 are transmembrane proteins with 1615 and 1613 amino acids, respectively. Both proteins belong to the LDLR family. Unlike other LDLR family members, LRPs 5 and 6 act as co-receptors of Frizzleds to induce the canonical Wnt pathway. The amino-terminal extracellular domain of LRP is composed of four YWTD repeat domains, four epidermal

![Figure 1](image_url)
growth factor (EGF)-like domains and an LDLR-like domain (LDLRLD) [10]. A single YWTD repeat domain consists of six tandem YWTD sequences. The first YWPD repeat domain binds to Wnt, and the third one binds to Dickkop (DKK). The binding of DKK to LRP interferes with the binding of Wnt and LRP and inhibits the canonical Wnt signals.

Receptor tyrosine kinase orphan receptor (ROR)1 and ROR2 belong to the tyrosine kinase (Trk) receptor family [11]. These proteins were first isolated as homologues of Trk neurotrophin receptors. Ror1 and Ror2 genes encode 882 and 909 amino acids, respectively. ORs are expressed in a variety of organs, including skeletal tissues. The extracellular region of ROR consists of an immunoglobulin-like domain (IgLD), a CRD and a kringle domain (KD). Wnt proteins bind to the CRD of ROR. The intracellular region of ROR harbors a tyrosine kinase domain (TKD) and a proline-rich domain (PRD).

2.4. Antagonists and agonists

Secreted frizzled-related proteins (SFRPs) are a family of Wnt antagonists. This family consists of five members with ranging in size from 286 to 329 amino acids. SFRP contains a CRD in the amino-terminal region and a netrin-like domain (NTR) in the carboxy-terminal region [12]. SFRP directly binds to Wnt and inhibits the interaction between Wnt and Frizzled. The CRD of SFRP is the binding site of Wnt. The DKK family is composed of four members with 206—366 amino acids. Among DKK family members, DKK1 and DKK4 inhibit Wnt signaling pathways. DKK contains two characteristic CRDs (CRD1 and CRD2). CRD2 is highly conserved among all members of this family. Unlike SFRP, DKK1 binds to LRP 6 through CRD1. The binding of DKK1 to LRP6 inhibits the activation of the canonical Wnt pathway (Fig. 2) [13].

Figure 2  Wnt signaling pathways. In the absence of Wnts, GSK-3β phosphorylates β-catenin in the target cells. APC and axin act as scaffolds for GSK-3β and β-catenin. Phosphorylated β-catenin is degraded by ubiquitin/proteosomes. There are two pathways for Wnt signaling: (A) β-catenin-dependent canonical pathway and (B) β-catenin-independent non-canonical pathway. (A) Canonical Wnts 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. (B) Non-canonical Wnts bind to the receptor complex of Frizzled and ROR1/2. This binding activates the planar cell polarity pathway through RhoA, Rac and JNK-dependent signals. Non-canonical Wnts also activate protein kinase C (PKC)- and calcineurin-dependent signals. Canonical Wnts also can activate the non-canonical pathway.

2.5. Others

Kremen proteins are single-pass transmembrane receptors for DKK1. The interaction of DKK1 with Kremen 1 is required for the inhibitory effect of DKK1 on the canonical Wnt pathway. DKK1, LRP6 and Kremen form a ternary complex. The formation of the ternary complex promotes endocytosis of the complex and the removal of LRP5s from the plasma membrane, resulting in the shutdown of the canonical Wnt pathway [13]. There are three other proteins named Wnt inhibitory factor-1, Wnt modulator in surface ectoderm and Cerberus, which inhibit the Wnt signaling pathways. The roles of those proteins in Wnt signaling are described in more detail in a review article [11].

3. Wnt signaling pathways

Wnt binds to two distinct receptor complexes: a complex of Frizzled and LRP5/6 and another complex of Frizzled and RORs. The binding of Wnt to the receptors activate two classes of signaling pathways: a β-catenin-mediated canonical pathway and a β-catenin-independent non-canonical pathway [6]. In the absence of Wnt signaling, glycogen synthase kinase-3β (GSK-3β) phosphorylates β-catenin in the target cells (Fig. 2). Adenomatous polyposis coli (APC) and axin act as scaffold proteins allowing the association of GSK-3β with β-catenin. Phosphorylated β-catenin is degraded through the ubiquitin-proteosome 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. This complex in turn promotes the phosphorylation of GSK-3β, which inhibits the kinase activity of GSK-3β. Inactivation of GSK-3β induces the accu-
mulation of β-catenin in the target cells, followed by translocation of accumulated β-catenin into the nucleus. The nuclear β-catenin, together with transcription factors, T-cell factor/lymphoid enhancer factor (TCF/LEF) family members, induces the expression of the Wnt target genes (Fig. 2). In the other pathway, Wnt5a binds to a receptor complex of Frizzled and ROR1/2 (Fig. 2). The binding of Wnt5a to a receptor complex activates heterotrimeric G proteins, which increase intracellular calcium via protein kinase C (PKC)—and calcium-dependent mechanisms [14–17]. Wnt5a also activates the planar cell polarity pathway through Rho- and Rac/c-Jun amino-terminal kinase (JNK)-dependent signals [18–21].

4. Role of Wnt signaling in bone formation

Loss-of-function mutations in human LRPS are associated with osteoporosis-pseudoglioma syndrome, which is characterized by severe juvenile-onset osteopenia, and congenital or infant-onset blindness [22]. In contrast, mutations in the amino-terminal region of LRPS (e.g., G171V) that reduce the binding affinity of LRPS to DKK1 lead to high bone mass [23,24]. Recently, a loss-of-function mutation in human LRPS was discovered in a family suffering from the early-onset of coronary artery disease [25]. This mutation also leads to low bone mass in the patients. The bone phenotypes found in humans with LRPS and LRPS6 mutations are largely in accord with the data from animal models. Lrp5+/− mice show a low bone mass phenotype [26]. The low bone mass in Lrp5+/− mice is further exacerbated by loss of a single allele of Lrp6 [27]. The ringelschwartz (rs) mutant mouse has a naturally occurring arginine-to-tryptophan mutation at amino acid 886 in Lrp6 that attenuates efficient signaling via the canonical Wnt pathway [28]. In addition to defects of the axial skeleton, digits and neural tube formation, rs/rs mice show a delay in bone mass (Fig. 3).

Effects of Wnt antagonists on bone mass have also been examined in vivo. Sfrp1−/− mice have an increase in trabecular bone, and this increase is more remarkable in the female mice [30]. The number of osteoblasts and the rate of bone formation are increased in Sfrp1−/− mice due to the suppression of osteoblast apoptosis. These results suggest that the canonical Wnt pathway is involved in the proliferation and differentiation of osteoblasts. Dkk1−/− mice are embryonic lethal due to defects of head induction and limb formation defects. Dkk1−/− mice have an increased bone mass due to an increase in bone formation with an increased number of osteoblasts and increased rate of bone formation [31]. In contrast, Dkk2−/− mice develop osteopenia with an increase in osteoid matrix [32]. Osteoblasts obtained from Dkk2−/− mice showed mineralization defects in culture. These results suggest that DKK1 acts as an antagonist, while DKK2 acts as an agonist in the canonical Wnt pathway in osteoblast lineage cells. Dkk2−/− mice also have an increased number of osteoclasts in vivo (Fig. 3) [33]. The precise role of DKK2 action in bone resorption is described in the next section.

Figure 3 Roles of Wnt signaling in bone remodeling. (A) Canonical Wnt signaling promotes differentiation of osteoblast precursors into mature osteoblasts, which in turn increases bone formation. The canonical Wnt signaling also induces the up-regulation of OPG expression and down-regulation of RANKL expression in osteoblasts, resulting in the inhibition of bone resorption. Thus, activation of the canonical Wnt pathway leads to a net increase in bone mass. On the other hand, Wnt5a-induced activation of the non-canonical pathway in osteoclast precursors enhances RANKL-induced osteoclast formation. Non-canonical Wnts also induced the production of inflammatory cytokines in synovial cells from RA patients. The non-canonical Wnt signaling promotes bone destruction in inflammatory diseases. (B) Phenotypes of bone in mice lacking Wnt signaling molecules.
via a PKC-dependent mechanism [33]. Wnt3a activates both the canonical and non-canonical Wnt pathways, while Wnt7b activates only non-canonical Wnt signaling in ST2 cells. This suggests that the non-canonical Wnt pathway is also involved in osteoblast differentiation. This notion is supported by in vivo and ex vivo analyses of Pckα−/− mice and conditional knockout mice with osteoblast-specific deletion of Wnt7b (Wnt7b mutant mice). Both Pckα−/− mice and Wnt7b mutant mice exhibited a deficit in bone formation during embryonic development [33]. Bone nodule formation is decreased in osteoblasts in culture obtained from Pckα−/− and Wnt7b mutant mice. These findings indicate that non-canonical Wnt signaling is also involved in osteoblastogenesis. The regulation of bone formation by Wnts seems to be more complicated than previously believed.

5. Role of Wnt signaling in bone resorption

Osteoclasts are formed in cocultures of osteoblastic stromal cells and bone marrow cells in the presence of bone resorption stimulating factors such as 1α,25(OH)2D3 and PTH [3]. Wnt3a strongly inhibits 1α,25(OH)2D3-induced osteoclast formation in cocultures of stromal ST2 cells and bone marrow cells [34]. However, Wnt3a fails to inhibit RANKL-induced osteoclast formation in bone marrow macrophage cultures [34]. These results suggest that the inhibitory effect of Wnt3a on osteoclast formation is mediated by stromal cells. Glass et al. [35] developed mice expressing a stabilized form of β-catenin in 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. Interestingly, the β-catenin mutant mice developed osteopetrosis with tooth eruption defects and a decreased number of osteoclasts. Urinary deoxypyridinolone, 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 mRNA expression of OPG, a decoy receptor of RANKL, is up-regulated in osteoblasts in those mice [39]. When β-catenin was inactivated selectively in mature osteoblasts using α1(I) collagen Cre mice, the bone mass was decreased due to the enhancement of bone resorption [35]. Activation of the canonical Wnt pathway was shown to stimulate OPG expression in osteoblasts. In addition, the canonical Wnt pathway suppresses the expression of RANKL in MC3T3E1 cells and MG-63 cells [36]. 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 (Fig. 3). DKK2, an agonist of Wnt signaling, affects bone resorption as well. Osteoblasts from Dkk2−/− mice expressed higher levels of M-CSF and RANKL than wild-type osteoblasts [32]. The expression of OPG remained unchanged in the mutant osteoblasts. Osteoclast precursors from Dkk2−/− mice in culture differentiated normally into osteoclasts in the presence of RANKL and M-CSF. These results suggest that DKK2 acts on osteoblasts and suppresses the expression of RANKL and M-CSF. It was reported that SFRP1 inhibited the differentiation of macrophages into osteoclasts in the presence of RANKL and M-CSF [37]. In addition, anti-SFRP1 antibodies promoted 1α,25(OH)2D3-induced osteoclast formation in the cocultures of osteoblasts and spleen cells. SFRP1 has been shown to binds to RANKL. RANKL-induced osteoclast formation was increased in cultures of bone marrow cells derived from Sfrp1−/− mice [30]. These results suggest that SFRP1 inhibits RANKL–RANK interaction. Overall, the canonical Wnt pathway inhibits osteoclast formation.

Wnt5a has been shown to stimulate the non-canonical Wnt pathway in the target cells. Recent study has shown that Wnt5a enhances RANKL-induced osteoclast formation in mouse bone marrow macrophage cultures [38]. Wnt3a showed no effect on osteoclast formation in the same culture system. Mouse bone marrow macrophages expressed Frizzled 2 and 5 and ROR2, the receptor components of Wnt5a. Knock-down of ROR2 by ROR2-specific 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. Wnt3a induced the accumulation of β-catenin in bone marrow macrophages, but Wnt5a did not. Wnt5a stimulated phosphorylation of PKC and enhanced RANKL-induced phosphorylation of JNK in bone marrow macrophages [38]. These results suggest that Wnt5a stimulates the non-canonical Wnt pathway but not the canonical pathway in osteoclast precursors. It was reported that synovial tissues from rheumatoid arthritis (RA) patients produce a large amounts of Wnt5a [39]. RT-PCR analysis of Wnt expression revealed that osteoclasts express higher amounts of Wnt5a than bone marrow macrophages [38]. These results suggest that Wnt5a secreted from osteoblasts and RA synovial tissues promotes RANKL-induced osteoclast formation through ROR2 expressed by osteoclast precursors in physiological and pathological situations (Fig. 3).

6. Roles of Wnt signaling in inflammatory diseases

Expression of several Wnts and Frizzleds has been identified in RA synovial tissues. The expression levels of Wnt1, Wnt5a, and frizzled5 are higher in the synovial tissue of RA patients than in those of osteoarthritis patients [39]. Wnt7b, Wnt4 and Wnt5a proteins were also detected in synovial tissues of RA patients [40] and RA model mice [41]. Wnt5a stimulated the production of pro-inflammatory cytokines such as IL-6 and IL-8 in synovial cells [42]. Treatment of RA patient-derived synovial cells with antibodies against frizzled 5, one of the receptors for Wnt5a, diminished the production of IL-15 and RANKL [42]. These findings suggest that Wnt5a promotes pro-inflammatory cytokine production and enhances bone resorption through the production of RANKL in the pathogenesis of RA. In addition, as described above, Wnt5a enhanced osteoclast formation in mouse bone marrow macrophage cultures. These results suggest that Wnt5a is involved in bone destruction in chronic inflammatory diseases such as RA and periodontitis. Using human TNFα transgenic mice as an RA model, DKK1 was shown to play important roles in bone formation. Treatment of the transgenic mice with anti-DKK1 antibodies generated osteopetrosis without any change in the clinical signs of inflammation [41]. DKK1 has been shown to be induced by TNFα in inflammatory tissues. These findings suggest that DKK1 induced by TNFα hampers bone formation and enhances bone resorption in inflammatory diseases.
through blockade of the canonical Wnt pathway. Thus, the canonical and non-canonical Wnt pathways play important roles in bone destruction in inflammatory diseases. Molecules involved in Wnt signaling may be therapeutic targets for the treatment of patients suffering from RA and periodontitis.

7. Conclusions

Wnts play central roles in the early development of organs and tissues. In addition, recent studies have established that Wnts play potential roles in bone remodeling in both physiological and pathological conditions. The canonical Wnt pathway enhances differentiation of precursors into osteoblasts. This signaling pathway also suppresses bone resorption through up-regulation of OPG expression and down-regulation of RANKL expression in osteoblasts. Together, these endpoints of the canonical Wnt pathway lead to an increase in bone mass. In contrast, the non-canonical Wnt pathway induced by Wnt5a enhances the formation of osteoclast from the precursor cells. The activation of this signaling pathway appears to enhance bone destruction in inflammatory diseases. Thus, Wnt signaling clearly plays multiple important roles in the development of bone and in the onset and progression of certain bone diseases. The molecular mechanism of Wnt action in human bone diseases is not fully understood. Further studies on Wnt signaling will be needed to establish new therapeutic strategies for the treatment of bone disease.

References

gene leads to an increase in bone formation and bone mass. J Bone Miner Res 2006;21:934–45.


