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Review Article

Wnt Signaling in Bone

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Abstract. Wnt signaling is involved not only in embryonic development but also in maintenance of homeostasis in postnatal tissues. Multiple lines of evidence have increased understanding of the roles of Wnt signaling in bone since mutations in the *LRP5* gene were identified in human bone diseases. Canonical Wnt signaling promotes mesenchymal progenitor cells to differentiate into osteoblasts. The canonical Wnt/β-catenin pathway possibly through Lrp6, a co-receptor for Wnts as well as Lrp5, in osteoblasts regulates bone resorption by increasing the OPG/RANKL ratio. However, endogenous inhibitors of Wnt signaling including sclerostin block bone formation. Regulation of sclerostin appears to be one of the mechanisms of PTH anabolic actions on bone. Since sclerostin is almost exclusively expressed in osteocytes, inhibition of sclerostin is the most promising design. Surprisingly, Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum, but not by directly promoting bone formation. Pharmacological intervention may be considered in many components of the canonical Wnt signaling pathway, although adverse effects and tumorigenicity to other tissues are important. More studies will be needed to fully understand how the Wnt signaling pathway actually influences bone metabolism and to assure the safety of new interventions.

Key words: Wnt signaling, LRP5, LRP6, sclerostin, bone metabolism

Introduction

Bone mass increases in childhood and adolescence until it reaches the peak. Increasing the peak bone mass is important to prevent osteoporosis and fractures in later life, when bone mass gradually declines. Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture, and it is a common health issue with the increasing size of our aging population (1). Genetic factors contribute to the variance in bone strength (2). Loss-of-function and gain-of-function mutations in the human *low-density lipoprotein receptor-related protein 5 (LRP5)* gene have been shown to be associated with osteoporosis-pseudoglioma syndrome (OPPG) and high bone mass (HBM) phenotypes, respectively (3–5). A mutation in the *LRP6* gene was recently identified in a family with risk factors of metabolic syndrome as well as osteoporosis (6). The above findings emphasize the importance of canonical Wnt signaling in bone metabolism because both LRP5

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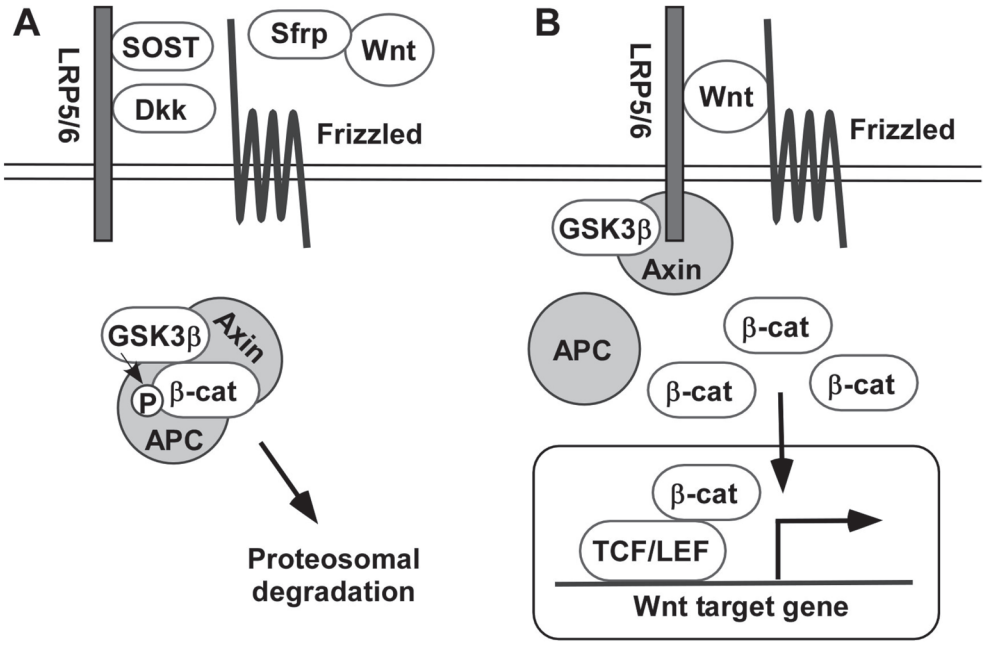

Fig. 1 Simplified view of the canonical Wnt signaling pathway (Modified from ref. 9). (A) In the absence of Wnts, β-catenin (β-cat) forms a complex with GSK-3β, Axin and APC and is phosphorylated by mainly GSK-3β. Phosphorylated β-catenin is conjugated with ubiquitin and then degraded by proteosome. Dkk, sclerostin (SOST) and Sfrp are secreted Wnt inhibitors; the two former molecules bind to LRP5/6, and the latter associates with Wnts. (B) When Wnts bind to Frizzled and LRP5 or LRP6 in a ternary complex at the cell surface, Axin is recruited away from the β-catenin destruction machine to LRP5 or LRP6, leading to the accumulation of β-catenin. Accumulated β-catenin translocates into the nucleus and activates LEF/ TCF-mediated gene transcription.

and LRP6 are thought to be co-receptors of Wnts (7–10). In this review, we describe roles of canonical Wnt signaling components in bone.

Wnt Signaling

Wnt molecules are a family of secreted cysteine-rich glycoproteins that activate at least three distinct pathways: the canonical (β-catenin-dependent), Ca^{2+} and planar polarity pathways. Of the three, the canonical pathway has been well elucidated (11; http://www.stanford.edu/~rnusse/wntwindow.html). Briefly, in the absence of Wnts, β-catenin forms a complex with Axin, adenomatous polyposis coli (APC) and glycogen synthase kinase 3β (GSK-3β) and is phosphorylated by mainly GSK-3β, resulting in proteosomal degradation (Fig. 1A). Dickkopfs (Dkks), secreted frizzled-related proteins (Sfrps) and sclerostin are secreted Wnt inhibitors. When Wnts bind to Frizzled and LRP5 or LRP6 in a ternary complex at the cell surface, Axin is recruited away from the β-catenin destruction complex to LRP5 or LRP6, allowing β-catenin to accumulate and translocate into the nucleus where it activates lymphoid enhancer factor (LEF)/T-cell factor (TCF)-mediated gene transcription (Fig. 1B).

Table 1 Roles of Wnt in mouse tissue development (Modified from ref. 12)

Gene	Knockout phenotypes
Wnt1, Wnt3a	Defects in expansion of neural crest and CNS progenitors
Wnt1, Wnt4	Decrease in thymocyte number
Wnt3a	Paraxial mesoderm defects, tail bud defects
Wnt4	Absence of Mullerian duct, defects in adrenal gland development
Wnt5a	Truncated limbs, defects in lung morphogenesis, chondrocytes differentiation defects
Wnt7a	Abnormal development in females and regression failure of the Mullerian duct in males
Wnt7b	Placental development defects, lung hypoplasia
Wnt11	Ureteric branching defects, kidney hypoplasia

Table 2 Wnt signaling components associated with human diseases (Modified from ref. 11)

Gene	Human disease
WNT3	LOF, tetra-amelia
WNT4	LOF, Mullerian duct regression, virilization
SOST	LOF, high bone mass, sclerosteosis, van Buchem disease
LRP5	GOF, high bone mass; LOF, osteoporosis-pseudoglioma syndrome, FEVR
LRP6	LOF, osteoporosis, coronary disease, hypertension, diabetes, hyperlipidemia
FZD4	LOF, FEVR
Axin2	LOF, tooth agenesis, colorectal cancer
APC	LOF, familial adenomatous polyposis, colorectal cancer
β-catenin	GOF, colon cancer

LOF, loss-of-function; GOF, gain-of-function; FEVR, familial exudative vitreoretinopathy; FZD, frizzled.

Wnt Signaling Components in Development and Disease

Wnt signaling is important in embryo development. Loss of a single Wnt gene can produce various phenotypes that range from embryonic lethality and central nerve system (CNS) abnormalities to kidney and limb defects (12) (Table 1). Some Wnts have a specific role in the developmental process, while others show redundancy in embryogenesis. That signaling is also involved in developing cancers and diseases, including colon cancer, coronary disease, tetra-amelia, Mullerian duct regression, eye vascular defects and abnormal bone mass (11) (Table 2).

LRP5

Loss-of-function mutations in the *LRP5* gene cause OPPG, a rare autosomal recessive disorder characterized by early onset osteoporosis and blindness (3). The patients display reduced bone mass and skeletal fragility. On the other hand, gain-of-function mutations in the *LRP5* gene are associated with the autosomal dominant HBM phenotype (4, 5). Several association studies suggest that *LRP5* polymorphisms are linked to bone mineral density (BMD) and fracture rate in the general population (13, 14). Recently, a genome-wide association study and a large-scale analysis have also demonstrated that *LRP5* variants are associated with BMD and fracture risk (15, 16). Human bone phenotypes caused by *LRP5* loss-of-function mutations are

reproduced in mice lacking *Lrp5* (17). *Lrp5*$^{-/-}$ mice exhibit a low bone mass and decreased proliferation of osteoblasts (bone forming cell). However, surprisingly, osteoblast-specific *Lrp5* deficiency does not produce a low bone mass (18). Lrp5 has recently been shown to control bone formation by inhibiting serotonin synthesis in the duodenum (18). Lrp5 inhibits expression of tryptophan hydroxylase 1, the rate-limiting biosynthetic enzyme for serotonin in enterochromaffin cells of the duodenum. Serotonin acts in an endocrine fashion on osteoblasts through the serotonin receptor 1b and cAMP response element binding (CREB) protein, a transcription factor, to inhibit their proliferation (18). The above study in mice demonstrates that LRP5 in the gut but not bone regulates osteoblast proliferation.

LRP6

Mutant mice lacking *Lrp6* display compound defects caused by mutations in various Wnt genes, including Wnt1, Wnt3a and Wnt7a (19), and die during the perinatal period. Heterozygosity for the *Lrp6*-null allele further decreases BMD in mice lacking *Lrp5* (20). We previously identified a point mutation, *ringelschwanz* (*rs*), in the *Lrp6* gene in spontaneous missense mutant mice with delay in the appearance of ossification centers and reduced bone mass in adults (21). *Lrp6*$^{rs/rs}$ mice exhibit reduced trabecular bone mass associated with an expanded eroded (resorbed) surface (22). Urinary excretion of deoxypyridinoline, a bone resorption marker, is higher in *Lrp6*$^{rs/rs}$ mice, while the levels of serum osteocalcin, a bone formation marker, are unchanged between *Lrp6*$^{rs/rs}$ mice and wild-type littermates. Expression of the receptor activator of nuclear factor-κ B ligand (*Rankl*), an essential molecule for the differentiation and activity of osteoclast (bone resorbing cell), is enhanced in *Lrp6*$^{rs/rs}$ osteoblasts both *in vivo* and *in vitro*, and osteoclastogenesis and bone-resorbing activity *in vitro* are facilitated in *Lrp6*$^{rs/rs}$ cells (22). Taken together, Lrp6-mediated signaling regulates bone mass, at least partly through the regulation of bone resorption. A study in humans demonstrated that a mutation in the *LRP6* gene results in osteoporosis as well as early coronary disease, hyperlipidemia, hypertension and diabetes (7).

Wnt

Wnt consists of 19 members (11); which of them are involved in bone metabolism has not been fully elucidated. Osteoblasts, chondrocytes, myocytes and adipocytes originate from mesenchymal stem cells. Adipogenesis is thought to be the default pathway for mesenchymal stem cells that do not receive an appropriate stimulation to differentiate into other cells. Wnt1 and Wnt10b inhibit adipogenesis in preadipocyte cells (23). Some Wnts, including Wnt7b and Wnt10b, have been shown to function in bone homeostasis in mice. Wnt7b is expressed in osteoblasts (24), and removal of *Wnt7b* in skeletal progenitor cells in mice leads to defects in chondrogenesis and osteoblastogenesis (25). *Wnt10b*$^{-/-}$ mice have decreased trabecular bone with a reduced bone formation rate (26). The above results suggest that Wnt7b and Wnt10b are endogenous regulators of bone formation.

β-Catenin

β-Catenin plays different roles at various stages of osteoblast development. Deletion of β-catenin in mesenchymal precursors of chondrocytes (cartilage forming cells) and osteoblasts blocks osteoblast differentiation (27, 28). Thus, β-catenin is required for osteoblast differentiation in early stages. In osteoblasts, β-catenin plays another vital role. Inactivation of *β-catenin* in osteoblasts using *α1 (I) collage-Cre* mice leads to low bone mass caused by increased bone resorption through enhanced expression of *osteoprotegerin (Opg)*, an antagonist for RANKL

(29). Deficiency of *β-catenin* in mature osteoblasts using *osteocalcin-Cre* mice produces severe osteopenia with increased osteoclasts (30). *In vitro*, osteoblasts lacking *β-catenin* exhibit elevated expression of *Rankl* and diminished expression of *Opg* (30). The above findings suggest that β-catenin in osteoblasts regulates osteoclastogenesis and osteoclast function.

Wnt Inhibitors: Sclerostin, Dkk, Sfrp

Sclerostin encoded by the *SOST* gene is a secreted Wnt antagonist. Several studies have shown that sclerostin binds to LRP5 and LRP6 to inhibit Wnt/β-catenin signaling (31, 32). Loss-of-function mutations and decreased expression of the *SOST* gene in humans are associated with sclerosteosis and van Buchem disease, respectively (33–35). Targeting and overexpressing of the *Sost* gene in mice lead to an increase and a reduction in bone mass with altered bone formation, respectively (36). Of note, sclerostin is almost exclusively expressed in osteocytes (37), which lie within the bone matrix and are derived from osteoblasts. Thus, sclerostin might be a promising therapeutic target molecule. Sclerostin monoclonal antibody treatment increases bone formation markers in postmenopausal women (38) and bone formation, bone mass and bone strength in a rat model of postmenopausal osteoporosis (39). Intermittent administration of PTH stimulates bone formation, but the molecular and cellular mechanisms underlying this effect are not completely understood (40). PTH treatment reduces the expression of sclerostin (41, 42), possibly alleviating endogenous Wnt inhibition and enhancing bone formation. Transgenic mice expressing a constitutively active PTH receptor exclusively in osteocytes display increased bone mass (43). The above studies suggest that PTH receptor signaling in osteocytes affects bone metabolism, at least in part, by controlling sclerostin expression.

Dkks are also secreted glycoproteins. Dkk1, Dkk2 and Dkk4 inhibit Wnt/β-catenin signaling by binding to LRP5 and LRP6. *Dkk1*$^{+/-}$ mice exhibit an HBM caused by an increase in bone formation (44). Mice overexpressing Dkk1 in osteoblasts develop osteopenia because of reduced osteoblast number and bone formation (45), indicating that Dkk1 negatively regulates bone formation. Unexpectedly, *Dkk2*$^{-/-}$ mice are osteopenic with impaired mineralization (46). They exhibit enhanced osteoclastogenesis with the up-regulation of *Rankl* expression, indicating that Dkk2 affects both bone formation and bone resorption. Sfrps are Wnt antagonists that block the interaction between Wnts and frizzled receptors, and adult *Sfrp1*$^{-/-}$ mice exhibit an increase in trabecular bone accrual (47).

Conclusion

A wide variety of findings have revealed that canonical Wnt signaling plays crucial roles in bone. It favors the commitment of mesenchymal progenitor cells to osteoblasts and also regulates bone resorption by increasing the OPG/RANKL ratio in osteoblasts. Wnt inhibitors including sclerostin mainly block bone formation. Inhibition of sclerostin might be the most promising therapeutic design to augment bone mass. Surprisingly, Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum, but not by directly promoting bone formation. More studies will be needed to fully understand how the Wnt signaling pathway actually controls bone metabolism.

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