Role of Wnt Signaling in Bone Remodeling and Repair

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Abstract The Wnt genes encode a highly conserved class of signaling factors required for the development of several types of tissues including musculoskeletal and neural structures. There is increasing evidence that Wnt signaling is critical for bone mass accrual, bone remodeling, and fracture repair. Wnt proteins bind to cell-surface receptors and activate signaling pathways which control nuclear gene expression; this Wnt-regulated gene expression controls cell growth and differentiation. Many of the components of the Wnt pathway have recently been characterized, and specific loss-of-function or gain-of-function mutations in this pathway in mice and in humans have resulted in disorders of deficient or excess bone formation, respectively. Pharmacologically targeting components of the Wnt signaling pathway will allow for the manipulation of bone formation and remodeling and will have several orthopedic applications including enhancing bone formation in nonunion and osteoporosis and restricting pathologic bone formation in osteogenic tumors and heterotopic ossification.

The Wnt genes encode a highly conserved class of at least 19 secreted cysteine-rich signaling factors with multiple roles during embryonic development, adult tissue repair,

and tumorigenesis [1–3]. They provide a paradigm of the concept that cellular genes which are normally present during embryogenesis and which regulate tissue development are reactivated during tissue repair and can be aberrantly activated in cancers [1, 4]. Wnt genes and their signaling pathway play critical roles in cell fate determination, cell growth, and differentiation of several types of tissues. [2] Recent studies of human orthopedic diseases and specific mouse models suggest a clear role for Wnt signaling in the regulation of bone formation, repair, and remodeling [4–11]. Therefore, either enhancing or suppressing Wnt signaling by manipulating components of the signaling pathway may provide a potent therapeutic approach to treating disorders of excessive or diminished bone formation.

The Wnt signaling pathway

Wnt signaling proceeds through at least two distinct pathways, a traditional or canonical pathway and a non-canonical pathway. The canonical pathway signals through β -catenin, while the non-canonical pathway uses different mediators including G proteins [2, 12]. The vast majority of published studies, including those on the roles of Wnt signaling in osteogenesis, have examined the canonical pathway, and that will be the focus of this review.

The canonical pathway is initiated by Wnt ligands binding to a complex receptor composed of members of the frizzled gene family and low density lipoprotein receptor-related proteins (LRP5 and LRP6; Fig. 1). The binding of Wnt ligands to this receptor complex activates a signaling pathway transduced by the cytoplasmic proteins glycogen synthase kinase-3 β (GSK-3), adenomatous polyposis coli (APC), and β -catenin (Fig. 1). In the absence of Wnt signaling, cytoplasmic levels of β -catenin are low because it is phosphorylated by GSK-3. Phosphorylated β -catenin is

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Fig. 1. The canonical Wnt signaling pathway. Wnt proteins bind to a receptor complex composed of members of the frizzled gene family (*Fzd*) and low-density lipoprotein (*LDL*) receptor-related proteins (*LRP*). Receptor binding inhibits glycogen synthase kinase-3 β (*GSK-3*). In the absence of Wnt signaling, *GSK-3* phosphorylates β -catenin and targets it to a degradation pathway mediated by the APC protein (*APC*). In the presence of Wnt signaling, β -catenin escapes phosphorylation and degradation and accumulates in the cytoplasm. Excess β -catenin enters

the nucleus where it associates with the lymphoid enhancing factor (*Lef*)/T cell factor (*Tcf*) transcription factors to regulate gene expression. Secreted frizzled-related proteins (*Sfrps*) contain the ligand binding domain of frizzled but lack the transmembrane region; they can bind the Wnt ligands but cannot transduce the Wnt signal, and thus, effectively block Wnt signaling. Dickkopf (*DKK*) proteins are secreted factors which also block Wnt signaling by binding to the *LRP* receptors

targeted to a degradation pathway mediated by the APC protein. In the presence of Wnt signaling, *GSK-3* is inactivated, thus, preventing phosphorylation of β -catenin. Unphosphorylated β -catenin accumulates in the cytoplasm and enters the nucleus where it associates with the T cell factor (*Tcf*)/lymphoid enhancing factor (*Lef*) transcription factors to regulate gene expression (Fig. 1) [1, 2, 4, 5].

There are several antagonists to the Wnt signaling pathway (Fig. 1). Intracellular antagonists include *GSK-3* and APC-3. Extracellular antagonists include secreted frizzled-related proteins (*Sfrps*) which contain the ligand binding domain of frizzled but lack the transmembrane region [13, 14]. These proteins can bind the Wnt ligands but cannot transduce the Wnt signal, and thus, effectively block Wnt signaling. Dickkopf (*DKK*) proteins are secreted factors which interfere with Wnt signaling by binding to the *LRP-5* and *LRP-6* receptors [13, 15]. The latter two antagonists, *Sfrps* and *DKK*, are specific antagonists of the canonical Wnt signaling pathway and are proteins which may be commercially produced and used to block Wnt signaling. *GSK-3* and APC, in contrast, have more global roles in cellular function and are thus less likely to be targeted for specific blockage of Wnt signaling.

Wnt signaling in development and disease

Wnt genes were initially identified as genes potentially responsible for mouse mammary tumor virus-induced

carcinogenesis in mice [3]. They were subsequently noted to be present in several different species and important for the development of several different types of tissues including limbs and midbrain [1, 2]. Accumulating evidence suggests that Wnt signaling is highly regulated spatially and temporally. Specific Wnt genes are expressed at very precise times and locations; aberrant expression of Wnt signaling in the wrong tissue or at the wrong time in development can result in cancers [16].

Several human diseases have been linked to abnormal Wnt signaling. Mutations in APC, which impair its ability to degrade β -catenin, or mutations in β -catenin itself which stabilize β -catenin, both result in constitutively active Wnt signaling and have been linked to familial adenomatous polyposis and colon cancer [16, 17]. Increased Wnt signaling has been observed in several other cancers including osteosarcomas and Ewing's sarcoma [18, 19]. Mutations which impair Wnt signaling have been linked to the rare human disorder tetra-amelia marked by the absence of all four limbs and craniofacial abnormalities [20].

A specific role for Wnt signaling in bone formation was revealed by naturally occurring human mutations in the Wnt signaling pathway. Loss-of-function mutations in the Wnt co-receptor *LRP5*, which essentially blocks the canonical pathway, results in osteoporosis pseudoglioma syndrome [21] characterized by a low bone mass, an increased incidence of fragility fractures, and visual defects (Fig. 2a–e). In contrast, gain-of-function mutations in *LRP5*, which prevent inhibition

Fig. 2. Clinical characteristics of osteoporosis pseudoglioma syndrome (OPPG). **a** Photograph of the lower extremity of a 40-year-old woman with OPPG demonstrating angular deformity secondary to multiple childhood fragility fractures. **b** Lateral radiograph of the lumbar spine in a 10-year-old child with OPPG demonstrating abnormal vertebral body flattening and concavity. **c** Photograph of

the right eye of a 3-month-old child with OPPG demonstrating leukocoria (a retrolental membrane). **d, e** Iliac crest bone biopsies (both at 40 $\times$ magnification) from an unaffected 2.5-year-old child (control) (**d**) and from a 2.5-year-old child with OPPG (**e**). The control biopsy is substantially thicker than the OPPG biopsy. (Reproduced with permission from Elsevier [21])

of LRP5 by the inhibitor DKK, effectively increase Wnt signaling and are associated with a high bone mass phenotype characterized by increased bone mineral density, thickened femoral cortices, and increased vertebral body density (Fig. 3a–f) [22, 23]. The alternate phenotypes in these two disorders suggest that Wnt signaling increases bone formation and/or density, and understanding the mechanism by which this occurs may allow us to treat disorders of bone insufficiency and excess.

Roles in bone remodeling

The association of LRP5 mutations to bone mass disorders in humans led to several molecular studies to (1) confirm the role of Wnt signaling in bone regulation and (2) identify the mechanism by which Wnt regulates bone remodeling. Transgenic studies in which the LRP5 gene was disrupted in mice resulted in a phenotype identical to osteoporosis pseudoglioma syndrome in humans; mice demonstrated diminished bone formation and osteopaenia [24]. Mice engineered to express LRP mutations which are associated with high bone mass phenotypes in humans resulted in a similar phenotype with increases in both total and trabecular bone mineral density. A marked increase in vertebral compressive strength and femoral bending strength were

observed in these LRP5 mutants [25]. These findings suggest that Wnt signaling via LRP5 regulates bone accrual.

The above studies suggest that increased Wnt signaling results in increased bone formation, whereas diminished Wnt signaling results in decreased bone formation. Thus, manipulating the balance of Wnt agonists such as β -catenin or Wnt antagonists such as DKK and Sfrps to either increase or decrease Wnt signaling may effectively regulate the rate of bone formation [6–9, 26–29]. For example, stabilization of β -catenin in transgenic mice results in an osteopetrotic phenotype with increased bone mass and rib osteomata [7, 9]. In contrast, disrupting β -catenin expression in transgenic mice results in osteopaenia [6, 7, 9, 27]. Deletion of sFRP-1 in transgenic mice increases trabecular bone mineral density and volume with increased osteoblast proliferation and differentiation [26], whereas introducing sFRP-1 into osteoblasts blocks Wnt signaling and accelerates osteoblast cell death [26]. DKK overexpression in transgenic mice results in severe osteopaenia with a 49% reduction in the number of osteoblasts [28]; in contrast, mice engineered to lack DKK demonstrate significant increases in bone mass, bone formation, and osteoblast numbers. [29]

Several basic science studies have suggested that Wnt signaling enhances bone formation by regulating osteoblast and osteoclast proliferation and differentiation (Fig. 4). Removing β -catenin from osteoblast progenitor cells prevent

Fig. 3. Clinical characteristics of high bone density syndrome due to a mutation in LRP5. **a, b** Photographs of an affected patient at age 12 (**a**) and 45 years (**b**) show the development of a wide, deep mandible. **c** A large, lobulated torus palatinus in an affected member. **d–f**

Characteristic radiographic findings including an abnormally thick mandibular ramus (**d**), a thickened femoral cortex with narrowed medullary cavity (*arrow*; **e**), and dense vertebrae (**f**). Reproduced with permission from [22]

them from differentiating into mature osteocalcin-expressing osteoblasts [10, 11]. Interestingly, these progenitor cells differentiate into chondrocytes. This indicates that Wnt signaling acts as a master musculoskeletal switch preventing progenitor cells from differentiating down the chondrocyte lineage and targeting them instead to the osteoblast lineage [6, 9]. Furthermore, there is recent evidence that Wnt signaling, via β -catenin, can upregulate the expression of osteoprotegerin in osteoblasts [7, 27]. Because osteoprotegerin inhibits the differentiation of osteoclasts [30], Wnt signaling may increase bone mass partly by blocking osteoclast-mediated bone resorption (Fig. 4).

Fig. 4. Effects of Wnt signaling on osteoblast and osteoclast differentiation. Wnt-regulated bone formation and remodeling result from an enhancement of mesenchymal precursor cell differentiation into osteoblasts. Wnt signaling blocks the alternative chondrocyte differentiation pathway. Differentiating osteoblasts synthesize and secrete osteoprotegerin in response to Wnt signaling; osteoprotegerin blocks osteoclast differentiation. Thus, Wnt signals increase bone formation by enhancing osteoblast-mediated bone formation and inhibiting osteoclast-mediated bone resorption and chondrocyte differentiation

Conclusions

Studies on human bone mass disorders and genetic manipulation in transgenic mice have provided compelling evidence for a role for Wnt signaling in regulating bone mass, bone density, and remodeling. The increased understanding of the Wnt signaling pathway has identified specific regulators, such as DKK and Sfrps, which may be manipulated to either enhance or repress Wnt signaling. The established role of the Wnt pathway in osteoporosis, high bone mass disorders, and certain osteogenic tumors suggest that pharmacologic manipulation of this pathway with, for example, commercially produced sFRPs or DKK, may provide a powerful method to specifically intervene in the abnormal signaling in these diseases [5]. The orthopedic applications of such pharmacologic agents will likely be expanded to several other conditions of bone deficiency such as nonunion, or bone excess, such as heterotopic ossification.

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