The Therapeutic Potential of the Wnt Signaling Pathway in Bone Disorders

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Abstract: The Wnt pathway plays a critical role in Development and differentiation of many tissues, such as the gut, hair follicles, and bone. Increasing evidence indicates that Wnts may function as key regulators in osteogenic differentiation of mesenchymal stem cells and bone formation. Conversely, aberrant Wnt signaling is associated with many osteogenic pathologies. For example, genetic alterations in the Wnt signaling pathway lead to osteoporosis and osteopenia, while inactivating mutations of Wnt inhibitors result in a hyperostotic skeleton with increased bone mineral density. Hyperparathyroidism causes osteopenia *via* induction of the Wnt signaling pathway. Lithium, often used to treat bipolar disorder, blocks a Wnt antagonist, decreasing the patient's risk of fractures. Thus, manipulating the Wnt pathway may offer plenty therapeutic opportunities in treating bone disorders. In fact, induction of the Wnt signaling pathway or inhibition of Wnt antagonists has shown promise in treating bone metabolic disorders, including osteoporosis. For example, antibodies targeting the Wnt inhibitor Sclerostin lead to increased bone mineral density in post-menopausal women. However, such therapies targeting the Wnt pathway are not without risk, as genetic alternations may lead to over-activation of Wnt/β-catenin and its association with many tumors. It is conceivable that targeting Wnt inhibitors may predispose the individuals to tumorigenic phenotypes, at least in bone. Here, we review the roles of Wnt signaling in bone metabolic and pathologic processes, as well as the therapeutic potential for targeting Wnt pathway and its associated risks in bone diseases.

Keywords: Wnt, osteoporosis, sclerostin, β-catenin, osteogenesis, fracture, osteogenic differentiation, LRP5/6.

INTRODUCTION

Discovered in the 1980s, the Wnt (Wingless and int-1) signaling cascade is involved in embryonic Development and homeostasis, through regulation of cell proliferation, differentiation, and apoptosis [1, 2]. The signaling pathway associated with tumorigenesis was first reported when the APC protein was found to interact with β-catenin in patients suffering from familial adenomatous polyposis (FAP) [1, 2]. The expression of Wnt proteins is regulated throughout Development [3]. It has been well established that Wnts play an integral role in many physiologic and pathologic processes.

Wnt genes encode a family of approximate 20 cysteine-rich secreted glycoproteins, which interact with cell surface receptors and trigger a cascade of intracellular events. There are three distinct pathways through which Wnt signaling can be transduced (Fig. **1**). The canonical pathway converges on the transcriptional regulator β-catenin (Table **1**). Wnt proteins, such as Wnt 1, Wnt 3a, or Wnt 8, bind to the Frizzled (Frz) receptors and co-receptor LRP5/6 (low-density lipoprotein-receptor-related protein 5/6), leading to β-catenin stabilization and translocation to the nucleus to initiate transcription [4-7]. Wnt-Frz interactions are often described as promiscuous, since a single Wnt protein may bind to multiple Frz proteins [8]. The Wnt-Frz/LRP5/6 interaction activates the associated kinases, which in turn phosporylates the intracellular protein disheveled (Dvl). Activation of Dvl inhibits glycogen synthase kinase 3 β (GSK3β), resulting in subsequent stabilization of β-catenin (Fig. **1**) [9]. Stabilized β-catenin then translocates to nucleus and forms a transcriptional complex with lymphoid-enhancer binding factor (Lef)/T-cell specific transcription factors (Tcfs) to regulate target gene expression [10]. Examples of the β-catenin/Tcf target genes include c-Myc, cyclin D1 and PPARδ [11-14]. In the absence of Wnt ligands, a complex consisting of GSK3β, Axin and adenomatous polyposis coli (APC), phosphorylates the N-terminal of β-catenin and initiates its proteosomal degradation [4, 5].

The non-canonical Wnt pathways, which occur independently of β-catenin, include the calcium dependent and cell polarity pathways (Fig. **1**). Well characterized non-canonical Wnts include Wnt 5a and Wnt 11 [7]. The calcium-dependent Wnt pathway plays important roles in embryonic Development, cell migration and motility, and possibly tumor suppression [5, 15]. In the calcium-dependent Wnt pathway, the Wnt ligands bind to Frz receptor and trigger intracellular calcium release by heterotrimeric G protein stimulation. The released intracellular calcium activates protein kinase C (PKC) or calcineurin (CaCN), which then turns on transcription factors Elk-1 and NF-AT [16]. The cell

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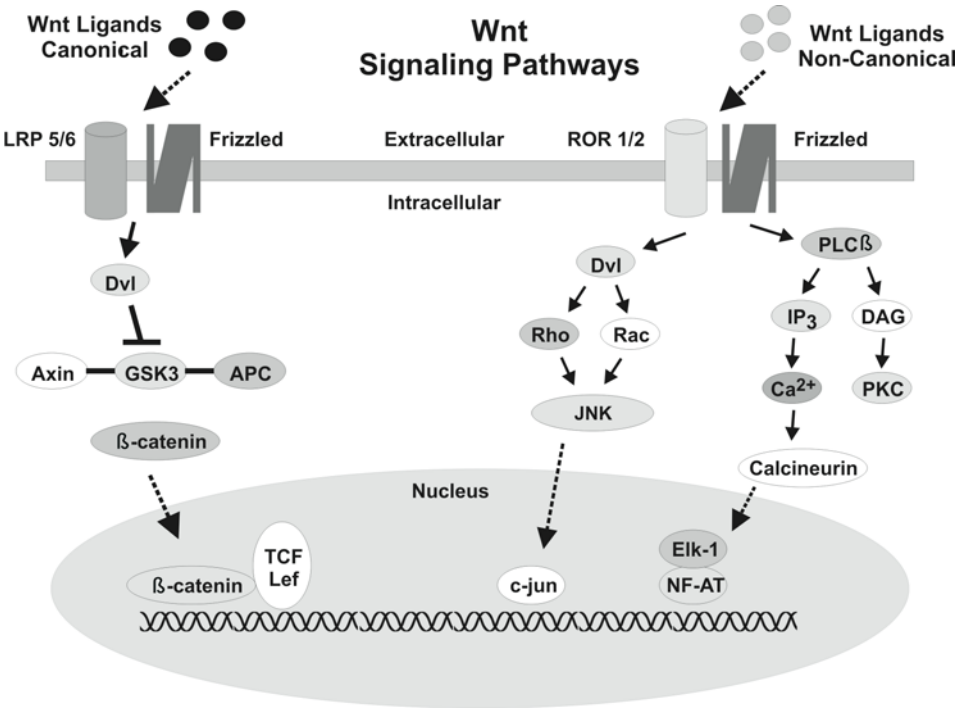

Fig. (1). Schematic representation of Wnt signaling pathways. Wnt ligands can activate either the canonical pathway (through β-catenin) or the non-canonical pathway (through Ca++ signaling or Rac/Rho pathway) to achieve distinct biological functions. In the canonical pathway, Wnt ligand binding of LRP 5/6 receptors induces them to interact with Frz proteins, leading to the phosphorylation of Disheveld (Dvl). Activating Dvl, then inhibits the Wnt antagonist complex, consisting of Axin, Glycogen Synthase Kinase 3 β (GSK3β), and Adenomatous Polyposis Coli (APC), which then stabilizes β-catenin and enables its translocation to the nucleus and interaction with the Tcf/Lef transcription factors. The non-canonical pathways include the calcium dependent and the cell polarity pathways. The calcium dependent pathway utilizes a heterotrimeric G protein to stimulate a cascade including phospholipase c, IP_3, and DAG which concludes in intracellular calcium release and calcineurin activation. In the cell polarity pathway, Frz activates Rho and Rac and proceeds through JNK pathway to activate the transcription factor c-Jun.

Table 1. Potential Therapeutic Strategies by Targeting Wnt Signaling at Different Levels

	Mechanism	Intervention	Therapeutic Effect	Reference
Agonize Wnt Signaling Molecules				
β-Catenin	Transmits Wnt signal to nucleus, binds TCF-Lef-1	DCA (activates β-catenin)	Enhanced Osteogenic Signaling and Differentiation	[96]
Antagonize Wnt Signaling Molecules				
Sclerostin	Inhibits Wnt coreceptors LRP 5/6	Anti-Sclerostin Abs	Decreased BMD in Spine and Hip	[4, 5]
Dkk-1	Bind, Internalize LRP Receptors	Anti-Dkk-1 Abs	Increased Bone Mass and Osteogenesis	[90]
SFRPs	Bind, competitively inhibit Frz Receptors	Small-molecule inhibitors of SFRPs	Increased Bone Formation and resultant Bone Density	[80, 92]
Tcf/Lef-1	Binds β-catenin, activates transcription	Quercetin (blocks the association of β-catenin to TCF/Lef-1)	Decreases Wnt Signaling and Wnt associated Osteogenesis	[97]
GSK-3	Phosphorylates β-catenin to lead to its degradation	Lithium and BIO (blocks GSK-3 to inhibit β-catenin degradation)	Increased BMD in ovariectomized rats, decreased fracture incidence	[87, 98, 99]
Targeting Wnt Cross Talk				
Gleevec	Tyrosine Kinase Inhibitor	Anti-Cancer Therapies	Downregulates β-catenin signaling pathway	[100]
PPARγ and Retinoic Acid	Stimulate Adipogenesis	Synthetic Agonists Ligands	Downregulates Wnt/β-catenin signaling pathway	[89, 101]

For therapeutic purpose, Wnt signaling can be targeted at the multiple levels of the signaling cascade, such as at the extracellular, cell membrane, and intracellular levels. Exemplary interventions targeting each signaling nodal point are given and discussed in the text.

polarity pathway functions through Frz receptors, activating Rho and Rac, which lead to c-jun NH2 terminal kinase (JNK) activation [17]. This pathway may modify the actin cytoskeleton, while also plays a role in cell proliferation, differentiation, and apoptosis [9, 18]. However, many aspects in the non-canonical Wnt signaling pathways are poorly understood.

In this review, we primarily focus on the canonical Wnt pathway. The Wnt pathway plays an important role in many physiologic and pathologic processes throughout the body. Abnormal Wnt signaling has been associated with many diseases, ranging from cancer to bone disorders. Investigations into the Wnt pathway have shed light on its role in osteogenesis, highlighting its role in associated pathologies. These lines of studies have also offered opportunities for therapeutic manipulation of the Wnt signaling components, from the receptors, signal transducers, and downstream targets to the antagonists.

WNT ANTAGONISTS

The negative regulation of the Wnt signaling pathway can occur both outside and within the cell (Fig. **2**). Extracellular inhibitors of the Wnt proteins include Wnt Inhibitory Factors (WIFs), Dickkopfs (Dkks), secreted frizzled-related proteins (SFRPs), Kremen1 and 2 (Krm 1/2), and Sclerostin (Sost) (Fig. **2** and Table **1**). These regulatory molecules act by either binding the Frz (SFRPs) or LRP 5/6 (Sclerostin, Dkks, Krm) receptors to prevent Wnt association, or by directly binding the Wnt proteins (WIFs) [5]. Dkk 1 and Dkk 2 are secreted proteins that bind to the LRPs, leading to a cross-linking and internalization of receptor [11]. The SFRPs inhibit Wnt signaling through sharing a similar ligand binding domain with Frz receptors [19]. The transmembrane proteins kremens are involved in promoting LRP inactivation [11]. Sclerostin is a secreted protein that binds and blocks the Wnt co-receptors LRP 5/6. Intracellular inhibitors include the GSK3β-Axin-APC complex and Chibby (Cby) (Fig. **2**). Cby competes with Tcf/Lef-1 to block β-catenin signaling [20]. This nuclear control involves modifying the Tcf and Lef transcription factors in the Wnt signaling cascade [21, 22]. The antagonist regulation of the Wnt cascade is critical in a variety of disease processes, and therefore, has tremendous potential in therapeutic regimens.

WNT SIGNALING IN NON-OSTEOGENIC DIFFERENTIATION

The Wnt signaling cascade plays a crucial role in many aspects of Development, and tissue renewal. Abnormalities in the Wnt signaling pathway may disrupt homeostasis by altering normal physiologic processes. Gut epithelium is the most dynamic example of self-renewal, as the new epithelial cells form in a matter of 3-5 days. In this process, stem cells

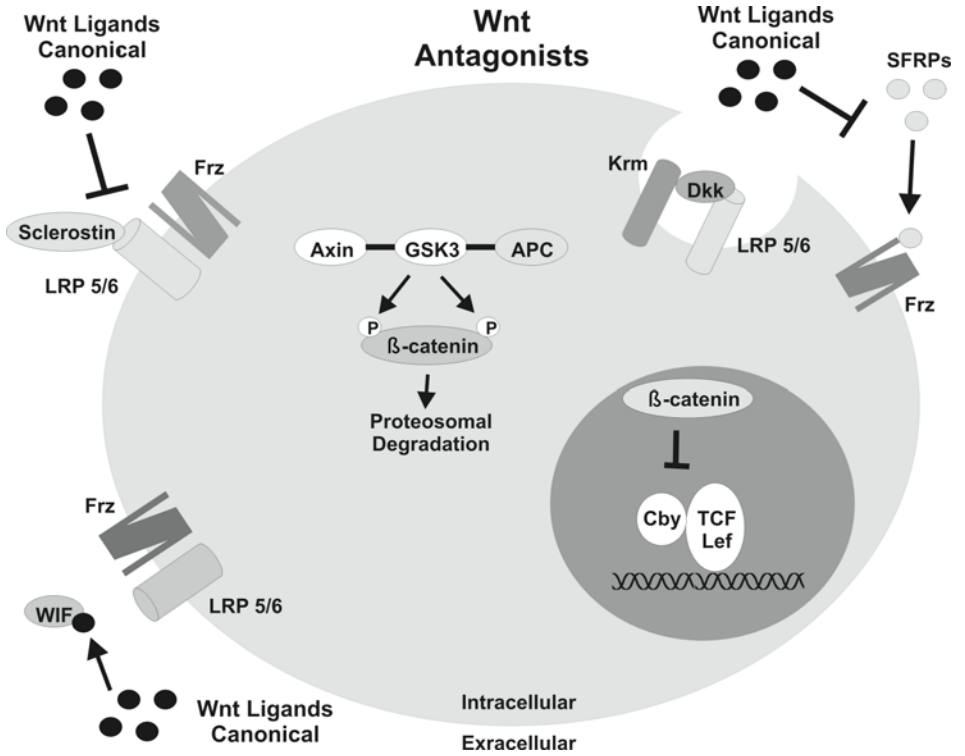

Fig. (2). Functions of some naturally occurring Wnt antagonists at cell surface. Wnt signaling is tightly regulated at cell surface level by several antagonists that can target receptors Frizzled (Frz), co-receptors (LRP5/6), or Wnt ligands themselves. Extracellular antagonists include Sclerostin, which binds and inhibits LRP 5/6, Wnt Inhibitory Factors (WIFs) that bind Wnt ligands, and secreted frizzled-related proteins (SFRPs) that bind Frz and competively inhibit the LRP interaction with Frz. Kremens (Krm) and Dickkopfs (Dkk) facilitate LRP inactivation through internalization of the receptor. The intracellular complex Glycogen Synthase Kinase 3 β (GSK3β), Axin, and Adenomatous Polyposis Coli (APC) inhibit β-catenin translocation to the nucleus. The recently discovered Chibby (Cby) blocks β-catenin association with its transcription factors in the nucleus.

form differentiated crypt precursors lining the base of the villi, enabling regeneration of the overlying epithelial layers [23]. Wnt signaling is an integral part in this process. Lack of Tcf4 or over-expression of the antagonist Dkk-1 leads to the absence of these progenitor cells, while Wnt stimulation results in cell proliferation and paneth cell terminal differentiation [23-25].

Another rapidly renewing cell regeneration cycle involving stem cells occurs within the hair follicles. Bulge stem cells migrate to the base of the follicle to undergo terminal differentiation and form the precortex [11]. Wnt signaling is critical in this differentiation process, activating bulge stem cells to initiate the cascade and hair follicle formation [11]. Mutants in the transcription factor Lef1 have few hair follicles, while the overexpression of β-catenin leads to excessive hair [26, 27]. Wnt signaling also functions as important regulators of the hematopoietic microenvironment. Wnt proteins and β-catenin overexpression promote proliferation and self-renewal of hematopoietic progenitor cells [28]. Furthermore, Tcf1 knockout leads to a decrease in thymocyte progenitors, impacting the production of T-lymphoid lineages [29].

WNT SIGNALING AND CROSS-TALK WITH OTHER PATHWAYS DURING OSTEOGENIC DIFFERENTIATION

The Wnt pathway plays an integral role in bone Development and osteogenic homeostasis [6, 9, 10]. Wnt ligands have been identified as critical mediators for limb bud initiation and Development, joint formation, and limb morphogenesis[9]. Experimental deficiencies of non-canonical Wnt signal transducers lead to abnormal bone formation throughout Development [30, 31]. Recent studies indicate that Wnts are important regulators of osteogenic differentiation of mesenchymal stem cells (MSCs). MSCs are pluripotent bone marrow stromal progenitor cells that retain the ability to differentiate into a variety of tissues, including bone, adipose, muscle, cartilage, and tendons (Fig. **3**). This differentiation process is tightly regulated by complex signaling events [32, 33]. Wnt signaling is crucial in regulating both osteogenic and adipogenic lineage-specific differentiation [6, 34], as Wnts can promote osteoblastic precursors into more differentiated osteoblasts and serve as negative regulators of adipogenesis (Fig. **3**) [35, 36]. Canonical Wnt/β-catenin, acts synergistically with the osteogenic regulator Runx2 and promotes the differentiation pathway toward osteogenic precursors [37, 38]. The non-canonical Wnt pathway has also been shown to suppress the adipogenic regulator PPARγ, and enhances Runx2, inducing osteogenesis [34, 39], as Wnt 5a is able to promote osteoblastogenesis, while acting as a co-repressor of PPARγ and subsequent adipogenesis [39]. These counter-regulatory functions are integral to terminal osteogenic differentiation (Fig. **3**).

Wnt proteins play an important role in regulating adipogenic, chondrogenic and myogenic Development [40, 41]. Canonical Wnt ligands are able to suppress both adipogenesis and chondrogenesis [37, 38]. Inhibition of Wnt signaling leads to transdifferentiation of myoblasts to adipocytes [42]. Both Wnt antagonists Dkk1 and Dkk2 are required for terminal differentiation of osteoblasts, suggesting a complex regulatory loop in the differentiation cascade [43, 44]. Nonetheless, Wnt proteins may play a key role in both promoting and regulating the complex cascade associated with MSC differentiation into mature osteocyte during regenerative processes.

Wnts cross-talk with bone morphogenetic proteins (BMPs) in regulating MSC differentiation. In particular, BMPs 2, 6, and 9 act as major osteogenic inducers [33]. Wnt3a acts synergistically to induce osteogenic differentiation with BMP9, while β-catenin knockdown or Frz antagonist overexpression blocks BMP9 induced osteogenic differentiation and bone formation, inducing chondrogenic matrix production [45]. Addition of BMPs to β-catenin has been proven to be critical for ectopic bone formation, although β-catenin alone is able to induce osteogenic differentiation, not bone formation [33, 46]. Both Wnt antagonist Dkk-1 and β-catenin null inhibit the BMP-2 induced bone formation, indicating that these pathways may cross-talk at the interaction of Smad-4 and β-catenin [47]. Thus, as two important mediators of osteogenesis, the cross-talk between Wnts and BMPs represents a critical step towards understanding and targeting this differentiation process.

During osteogenic regulation, Wnt signaling may cross-talk with the Hedgehog (Hh) and Notch signaling pathways. Hh is crucial for embryonic skeletal Development and endochondral bone formation [48, 49]. Wnt signaling regulates key mediators in the Hh signal transduction, Gli2 and Gli3 [50]. Notch signaling is also important for skeletal Development. A deficiency in the Notch signal mediator presenilin-1 leads to defects in the axial skeleton [51]. There appears to be a conserved domain between Notch signaling molecules and the transcription factors Tcf/Lef-1, enabling the Notch signaling mediators to inhibit the canonical Wnt pathway [52]. Other mechanisms associated with osteogenic induction through Wnt signaling involve activation of PKCδ, as well as, Src/ERK and PI3K/Akt signaling pathways [53]. Further investigations into the cross-talks between these signaling pathways with Wnt signaling are imperative to elicit the mechanisms underlying MSC differentiation and bone Development.

WNT SIGNALING IN OSTEOGENESIS AND OSTEOGENIC PATHOLOGIES

The canonical and non-canonical Wnt signaling pathways play an important role in bone metabolism and osteogenesis, and contribute to osteogenic pathologies, as shown in both animal studies and genetic disorders in humans. Many signaling components of the Wnt pathways thus may represent potential drug targets for treating pathologies of the skeletal system.

Wnt Co-Receptors as Regulators of Osteogenesis and Bone Mineral Density

Abnormalities in LRP5 have significant impacts on skeletal homeostasis. Osteoporosis pseudoglioma syndrome (OPPG) is caused by inactivating mutations of the *LRP5* gene [54]. The OPPG patients are characterized with phenotypes of osteoporosis and decreased bone mineral density [55]. Similar results have been seen in *LRP5* knockout mice [56, 57]. However, *LRP5* activating mutations lead to in-

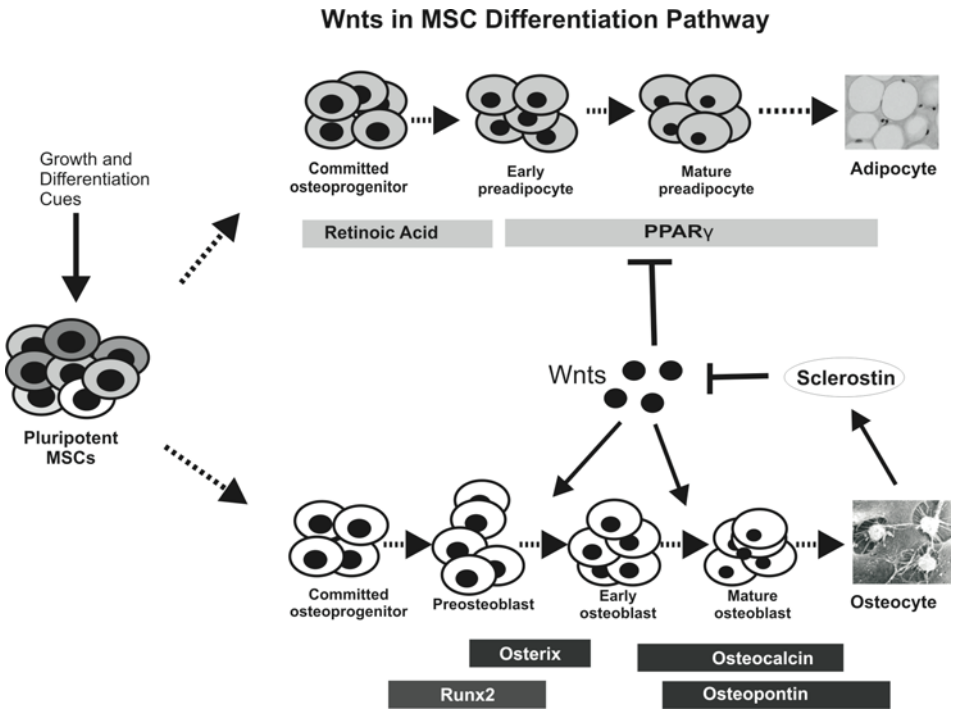

Fig. (3). The roles of Wnt signaling in regulating mesenchymal stem cell differentiation. Canonical Wnts can inhibit adipogenic differentiation, and promote osteogenic differentiation, which can be inhibited by sclerostin. Wnt signaling proteins are thought to regulate the late stages of ostegenic differentiation, while inhibiting adipogenesis in part through its interaction with PPARγ. Sclerostin is secreted by osteocytes in a negative feedback cycle to inhibit the Wny cascade. PPARγ and retinoic acid are critical regulators of adipogenesis, while Runx2, osterix, osteocalcin, and osteopontin are involved in the regulation of the osteogenic differentiation cascade.

creased bone mass, while knockout mutations reduce bone mineral density from a defect in osteoblast proliferation [55, 57, 58].

The role of LRP6 is analogous to that of LRP5 in initiating the Wnt signaling pathway. As a Wnt co-receptor, mutations in the *LRP6* gene results in a variety of phenotypes in adult bone homeostasis [59]. A mutation in the *LRP6* gene in humans leads to osteoporosis and other metabolic abnormalities [60]. In mice, a spontaneous missense mutation in the *LRP6* gene leads to defects in somitogenesis and reduced bone mass in adults [61]. Null mutations in the *LRP6* gene lead to prenatal lethality secondary to truncated distal limb and axial skeletal structures [59, 62]. The critical role of LRP6 in osteogenic Development is demonstrated when mutations of both LRP5 and LRP6 synergistically delay and prevent skeletal Development [63].

Although a few attentions have been paid to the Wnt ligands, there appears to be some associations of the extracellular Wnt agonists with metabolic abnormalities. *Wnt 10b* missense mutations in humans may be linked to obesity, while similar mutations in animals lead to a decrease in postnatal bone density [58, 64]. Furthermore, in mice overexpression of Wnt 10b results in increased bone mass [65]. Conditional *Wnt 7b* knockout causes defects in osteoblastogenesis and chondrogenesis [66]. Thus, therapeutic manipulations of extracellular Wnt receptor, either through mimicking Wnt ligands or using blocking antibodies against their receptors, hold tremendous potential for treating skeletal pathologies

Wnt Intracellular Signaling During Osteogenesis

As an important mediator of the canonical Wnt signaling pathway, β-catenin is crucial in bone formation and regeneration [36]. Conditional deletion of *β-catenin* in mice leads to impaired mineralization secondary to a deficiency of terminally differentiated osteblasts [67, 68]. This also impaired endochondral ossification and ectopic chondrocyte formation [4]. The osteopenia, or mildly decreased bone mineral density, resulting from *β-catenin* knockout is associated with increased osteoclasts [69]. These results point to the critical role for β-catenin in regulating the relationship between osteoblasts and osteoclasts.

The Tcf/Lef transcription factors are critical mediators in the Wnt pathway's regulation of osteogenesis through β-catenin. *Tcf1* null mice demonstrate low bone density with an increased number of osteoclasts [35]. This is similar to the impaired mineralization seen in β-catenin deficient mice, as would be expected from the interaction of these transcription factors with β-catenin. Further investigation into manipulating these intracellular mediators of Wnt signaling may enable us to target these nuclear mediators in the pathway using biological and/or chemical agents.

Wnt Extracellular Antagonists in Osteogenesis and Bone Pathologies

Sclerostin (Sost) is a protein secreted by osteocytes that binds to and blocks Wnt binding to co-receptors LRP5/6, leading to inhibiting osteogenic differentiation. It has been shown that sclerostin is able to indirectly inhibit BMP in-

duced osteogenic differentiation and bone formation through its association with LRP 5/6 [70]. During bone remodeling, osteocytes regulate bone formation by expressing sclerostin to complete a negative feedback cycle of Wnt signaling in mature osteoblasts [71]. Furthermore, mechanical stimulation of long bones reduces the expression of sclerostin and enhances bone formation, while mechanical unloading increases the sclerostin antagonistic effects and diminishes bone strength [72, 73].

Sclerostin represents one of the best documented examples that offer a link between Wnt antagonists and specific pathologies. The autosomal recessive diseases Sclerostosis and Van Buchen disease form secondary to homozygous mutations of *Sost* encoding the secreted protein sclerostin [74, 75]. These disorders are associated with a progressive increase in bone mass, hyperostotic skeleton and increased bone mineral density [76]. Sclerostin is directly involved in the regulation of osteoblastic differentiation through the antagonism of the Wnt signaling pathway [76]. Furthermore, specific polymorphisms of the *Sost* promoter have been implicated in osteoporosis [19]. Thus, sclerostin plays an important role in many facets of osteogenesis, as both a marker for osteogenic abnormalities and a therapeutic target.

Another group of extracellular regulators are the Dkk proteins, which play an important role in regulating bone matrix mineralization through the Wnt pathway. A mutation in the LRP5 receptor leads to Dkk1 resistance and increased bone density [55]. Furthermore, Dkk1 and Dkk2 expression levels correlate with matrix mineralization [4, 43, 77]. *Dkk1* knockout mice exhibit increased bone density, while *Dkk1* overexpression results in osteopenia [77, 78]. *Dkk2* null mice are also osteopenic with impaired mineralization [43], demonstrating the importance of both proteins in regulating bone Development and regeneration.

Although the antagonists to LRP 5/6 co-receptors are well established in their osteogenic regulatory roles, the Frizzled receptor antagonists play a key function in osteogenesis and osteogenic pathologies. Osteogenic abnormalities are associated with SFRPs, as increased incidence of hip osteoarthritis has been associated with alterations of the *Sfrp* gene [79]. *Sfrp1* knockout mice exhibit increased trabecular bone volume [80]. Furthermore, deficiencies in both sclerostin and Sfrp1 result in increased bone mass [58, 81]. Another antagonist to the Frz receptor, FrzB, can inhibit BMP9 induced osteogenic differentiation and bone formation [45]. Thus, these extracellular Wnt antagonists may be exploited as future therapeutic manipulations of osteogenesis.

Wnt Intracelullar Antagonists Regulate Bone Development and Regeneration

The Axin-GSK3β-APC complex has been shown to play a role in osteogenesis. Axin mutations lead to both osteogenic and chondrogenic abnormalities as familial tooth agenesis has been linked to Axin2 mutations [82]. Through the activation of the β-catenin signaling pathway, Axin 2 knockout mice present with increased osteoblast proliferation, craniosynostosis, increased chondrocyte differentiation, reduced limb length, and increased bone mass phenotypes [83-85]. GSK3β also regulates osteogenesis, as GSK3β inhibitors, such as lithium, are able to increase bone density and enhance osteoblast differentiation [86, 87]. Mice heterozygous for *Gsk3β* alleles exhibit increased bone mass [88]. It is thus conceivable that the Axin-GSK3β-APC complex may be potential drug targets.

OSTEOGENIC PATHOLOGIES AND THEIR THERAPEUTIC TARGETS IN THE WNT PATHWAY

There are an increased number of reports about the potential use of synthetic or natural compounds to enhance or inhibit the Wnt signaling pathway (Table **1**). There has been much investigation into the role of the Wnt pathway in many bone pathologies, such as osteoporosis, fracture healing, and hyperparathyroidism. Osteoporosis, defined as decreased bone mineral density 2.5 standard deviations below peak bone mass of young women, is the most prevalent bone pathology occurring in older women. Osteoporotic bones steadily lose bone mass, eventually leading to thin trabeculae, high marrow adipose content, and low tensile strength. Clinically, agents, such as bisphosphonates and SERMs, are used to block bone resorption. Furthermore, much attention has been paid to strategies enhancing bone regeneration in severe osteogenic abnormalities, such as critical sized defects, non-union fractures, and vertebral interventions. Thus, manipulation of both the intracellular and extracellular Wnt pathway has shown great potential in treating these and other osteogenic pathologies.

Extracellular Wnt Signaling Components as Therapeutic Targets

The extracellular Wnt receptors may be exploited as potential targets for pharmacologic therapies. For anticancer therapies, Wnt ligands have been targeted through antisense molecules, RNA interference, or neutralizing antibodies [89]. Recent investigation has focused on a similar strategy to induce the opposite effect in attempts to enhance the Wnt signaling pathway and osteogenesis, such as the possible use of synthetic receptor agonists or the inhibitors of the extracellular Wnt antagonists.

Investigations are undertaking to study the effects of blocking extracellular Wnt antagonists, such as DKK, SFRP, and sclerostin (Table **1**) [5]. One highly promising endeavor is to target sclerostin using humanized antibodies. As sclerostin is produced by mature osteoblasts, this intervention decreases bone resorption in animals [5, 90, 91]. Furthermore, since alterations in the *Sost* promoter are associated with osteoporosis, targeting sclerostin in osteoporosis has yielded promising results [19]. This strategy has been exemplified by the use of anti-sclerostin antibodies, which lead to increased bone mass [90]. Antibodies to sclerostin in estrogen deficient rats results in increased bone formation [90]. In a phase one clinical trial, a single dose of anti-sclerostin antibody given to post-menopausal women increased the bone mineral density of the lumbar spine and increased production of collagen type I [90].

The role of the extracellular Wnt pathway in osteogenic regeneration is demonstrated as both canonical and non-canonical Wnt ligands are upregulated throughout the early and late stages of fracture healing [9]. Exogenous treatment

with Dkk-1 inhibits both chondrogenesis and osteogenesis, while delaying the time in fracture healing [46, 90]. Exogenous inhibition of SFRP1 induces bone formation and increases bone density [92]. Thus, sclerostin, Dkks, and other extracellular Wnt antagonists may emerge as one of the most promising targeted therapies for osteogenic disorders.

Therapeutic Targets of the Intracellular Wnt Pathway in Bone Disorders

The intracellular molecules involved in the Wnt pathway also show promise in osteogenic therapeutic interventions. The Wnt intracellular targets have been investigated as possible anti-cancer therapeutic modalities. These strategies focus on the intracellular molecules, such as β-catenin and Tcf/Lef, or antagonists, such as Axin or GSK3β, to block the Wnt signaling cascade (Table **1**). These strategies involve overexpression or knockdown *via* transduction, antisense, or protein knockdown [93]. A particular study screened around 7000 compounds to identify a variety of molecules that inhibit the β-catenin and Tcf/Lef-1 complexes [94]. One example of a potential target is the β-catenin antagonist Cby, which inhibits complex formation between β-catenin and Tcf/Lef-1. This protein is required for MSC differentiation into adipocytes, and therefore, could enable drug-mediated shifts in the osteogenic cascade. Although synthetic β-catenin agonists or antagonists to Wnt inhibitors have potential, this study introduces an interesting strategy using naturally occurring proteins to target the Wnt pathway.

Targeting the interaction and signal propagation by β-catenin and Tcf/Lef-1 holds significant promise in therapies for osteogenic disorders (Table **1**). The canonical Wnt pathway's upregulation of β-catenin induced signaling plays a crucial role in chondrocyte maturation and mineralization, and resultant endochondral bone formation [46, 95]. As expected, β-catenin plays an integral role in fracture repair, as the fracture healing is inhibited in the conditional β-catenin knockout animals [46]. An example of exogenously enhancing the activity of β-catenin is the secondary bile acid deoxycholic acid (DCA). Exogenous administration of DCA is able to activate the β-catenin signaling pathway and its associated downstream molecules [96]. Conversely, Quercetin, an anti-tumor agent, antagonizes the Wnt signaling pathway *via* disrupting the association of β-catenin with Tcf/Lef-1 [97].

Wnt antagonists may also have potential therapeutic applications for bone metabolic disorders (Table **1**). One example involves the side effect from lithium, used to treat patients with Bipolar disorder. Lithium inhibits GSK3β, a Wnt protein antagonist. Lithium can enhance fracture healing in mice, while clinically it reduces the incidence of fractures [46, 98]. Its role in fracture healing is further demonstrated by increased β-catenin expression in fracture tissue in patients taking lithium [9]. Additionally, a similar GSK3β inhibitor was able to increase the bone mineral density in ovariectomized rats [87]. Exogenous administration of 6-bromoindirubin3'-oxime (BIO), another pharmacologic GSK3β inhibitor, activates the Wnt signaling pathway in MSCs [99]. These agents represent examples of how future Wnt-based therapies may be utilized to treat bone pathologies.

Pharmacologic Manipulations of Wnt Signaling Synergies with Other Pathways

During osteogenesis and bone regeneration, the Wnt/ β-catenin signaling pathway cross-talks with many other pathways associated with osteogenesis, such as the BMPs, Hh, and Notch [33] (Table **1**). Furthermore, it has been shown that tyrosine kinase inhibitors, including Gleevec, downregulate the β-catenin signaling pathway [100]. The adipogenic regulator PPARγ and retinoic acids are able to suppress the Wnt/ β-catenin signaling pathways [89, 101]. Utilizing these interactions of the Wnt pathway with other signaling pathways has significant potential in osteogenic and endocrine therapeutic strategies.

A well-documented example of cross-talk between the Wnt pathway and another important osteogenic pathway is seen with parathyroid hormone (PTH). PTH is an important endocrine calcium regulator that has a remarkable ability to induce bone formation through intermittent administration. The mechanism underlying its osteogenic induction is thought to prevent osteoblast apoptosis *via* inducing IGF and their TGF-β receptors [102-104]. It appears to induce TGFβ associated signaling protein Smad 3, leading to the induction of the Wnt signaling cascade [103]. Daily PTH treatment following fractures enhances the regeneration capabilities through, at least in part, upregulating the Wnt/ β-catenin signaling pathway [105]. It has been recently reported that PTH may exert its function by targeting the Wnt antagonist sclerostin [106, 107]. Interestingly, continuous administration, as is seen in primary hyperparathyroidism, leads to osteopenia, or decreased bone mineral density [108, 109]. Regardless, the osteogenic effects from PTH therapy seem to occur, at least in part, through the Wnt signaling cascades cross talk with the TGFβ signaling proteins.

POTENTIAL TUMORIGENIC EFFECT OF WNT-TARGETD THERAPIES

Although the Wnt signaling cascade has tremendous potential in the treatment of osteogenic abnormalities, caution is required, as Wnt signaling has been linked to tumorigenesis (Fig. **4**). Oncogenic mutations have been documented amongst the Wnt signaling mediators, including β-catenin, APC, Axin, Tcf/Lef1, Axins, and Wnt1 [110]. *APC* mutations are linked to patients with Familial Adenomatous Polyposis and sporadic colorectal cancers [111, 112]. Mutations in *β-catenin* and its associated transcription factors have been implicated in hair follicle and sebaceous gland tumors, as well as cancers of the hematopoietic system [113-115]. Exogenous activation of the Wnt/ β-catenin pathway with molecules, such as DCA, has been shown to enhance tumor growth [96]. The oncogenic characteristics of the Wnt signaling are secondary to their roles in promoting cell proliferation and cell cycle progression.

One potential risk with overstimulation of the Wnt pathway in bone would be the Development of osteosarcoma (OS). OS is the most common primary bone malignancy, characterized by a loss of differentiation and high propensity to metastasize [116, 117]. Although there are a variety of factors that have been linked to OS Development and prognosis, up-regulating the Wnt signaling pathway through silencing the Wnt inhibitory factor 1 (WIF1) may lead to a

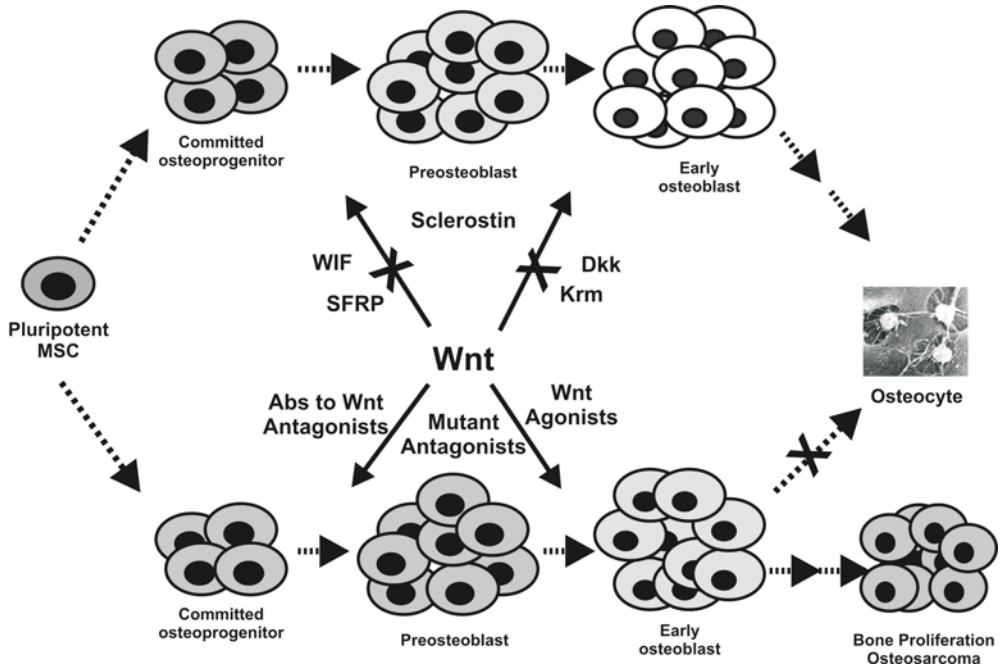

Fig. (4). The balance act of Wnt signaling in osteogenesis and bone tumorigenesis. The canonical Wnt pathway can promote osteogenic differentiation, which is inhibited by Wnt antagonists, such as Sclerostin, Dkk, SFRPs, Krms, and WIFs. However, an excessive activation of canonical Wnt signaling (e.g., by neutralizing antagonists by antibodies, loss of function mutations of antagonists, or over-expression of Wnt agonists) may lead to excessive cell proliferation and bone tumorigenesis. Blocks of Wnt signaling or osteogenic differentiation were indicated by "X".

lencing the Wnt inhibitory factor 1 (WIF1) may lead to a loss of differentiation and an increase in cell proliferation (Fig. **4**) [118]. Furthermore, OS often displays high levels of β-catenin, increasing their tumorigenic and/or metastatic potential [110, 119]. There have been reports of OS Development secondary to intermittent PTH, a known sclerostin antagonist [120, 121]. Thus, it remains to be thoroughly investigated about the tumorigenic potential of Wnt-targeted therapies, especially in long term use.

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ABBREVIATIONS

APC	=	adenomatous polyposis coli
BIO	=	6-bromoindirubin3'-oxime
BMP	=	bone morphogenetic protein
CaCN	=	calcineurin
Cby	=	Chibby
DAG	=	diacylglycerol
DCA	=	deoxycholic acid
Dkk	=	Dickkopf
Dvl	=	the Disheveled
Elk-1	=	Ets LiKe gene 1
ERK	=	extracellular-signal-regulated kinase
FAP	=	familial adenomatous polyposis
Frz	=	the Frizzled
GSK3β	=	glycogen synthase kinase 3 β
Hh	=	Hedgehog
IGF	=	Insulin-like growth factor
IP3	=	inositol 1,4,5-trisphosphate
JNK	=	c-jun NH2 terminal kinase
Krm 1/2	=	Kremen1 and 2
Lef	=	lymphoid-enhancer binding factor
LRP 5/6	=	low-density lipoprotein-receptor-related protein 5/6
MSCs	=	mesenchymal stem cells
NF-AT	=	Nuclear factor of activated T-cells
OPPG	=	Osteoporosis pseudoglioma syndrome
OS	=	osteosarcoma
PI3K	=	Phosphatidylinositol 3-kinase
PKC	=	protein kinase C
PPAR	=	peroxisome proliferator-activated receptor
PTH	=	parathyroid hormone

SERM = Selective Estrogen Receptor Modulator

SFRP = secreted frizzled-related protein

Sost = Sclerostin

Tcf = T-cell specific transcription factor

TGF β = transforming growth factor β

WIF = Wnt Inhibitory Factor

Wnt = Wingless and int-1

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