Role and Application of Wnt/β-catenin Signaling Pathway in Osteogenic Differentiation of Bone Marrow Mesenchymal Stem Cells

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Abstract. The Wnt/β-catenin signaling pathway is a crucial signaling mechanism in multicellular organisms, regulating a variety of cellular behaviors. It acts a significant role in the differentiation of bone marrow mesenchymal stem cells (MSCs) into osteoblasts and in maintaining bone homeostasis. This involves promoting gene expression that is relevant and interacting with other pathways to boost osteogenic differentiation when inhibiting the differentiation of cartilage and adipocytes. This paper reviews the role of the MSCs' osteogenic differentiation and the Wnt/β-catenin signaling pathway, explores the relationship between abnormalities in this pathway and various skeletal diseases, and discusses The Wnt signaling pathway can potentially be targeted in order to create medicinal strategies for these conditions, offering new insights for the treatment of skeletal disorders.

Keywords: Wnt/β-catenin, osteogenic differentiation, bone marrow mesenchymal stem cells.

1. Introduction

Bone is a dynamic tissue which provides structural assistance and defense to surrounding organs, the storage of essential minerals like calcium and phosphate requires continuous remodeling to maintain mechanical strength and calcium homeostasis. The differentiation of bone marrow mesenchymal (BM-MSCs) stem cells results in the formation of bone, BM-MSCs can divide into osteoblasts, chondrocytes, and adipocytes [1]. Controlling the differentiation of BM-MSCs is crucial for bone formation, with several signaling channels, notably the route of Wnt signaling.

The Wnt signaling pathway is vital for regulating various cellular behaviors, mediated by Wnt proteins that activate this pathway. The Int-1 gene was first identified in mouse mammary tumors in 1982, and in 1987, Int-1 was found to be homologous to the Drosophila Wingless gene, leading to the merged nomenclature of Wnt [2]. A group of glycoproteins secreted that contain cysteine is encoded by Wnt genes, approximately 40 kDa in size, intercellular signaling molecules that are linked to diverse signaling pathways and produce a wide range of biological effects related to cell proliferation, differentiation, survival, migration, and other cellular behaviors [3]. Linking abnormalities in the Wnt signaling to various diseases; for instance, mutations of the APC gene, a Wnt signaling inhibitor, are commonly linked to colorectal cancer [4]. Additionally, the pathway is also crucial in development of the embryo [4, 5].

2. Wnt/β-catenin Signaling Pathway

2.1. Wnt Proteins

Wnt proteins are widely distributed in multicellular organisms. The number of known mammalian Wnt proteins is 19, which play a role in the Wnt signaling pathways, with some Wnt proteins capable of regulating both pathways simultaneously [5]. The secretion and transport of Wnt proteins are tightly regulated processes, involving post-translational lipid modifications. Within the endoplasmic reticulum, palmitoyltransferase modifies specific cysteine and serine residues in the Wnt receptor-

binding domains, resulting in a hydrophobic Wnt protein that can effectively interact with cellular lipid membranes—an essential step for Wnt secretion and signaling [6].

2.2. Wnt Protein Binding Receptors

Wnt receptors typically form a complex consisting of the tissue-specific frizzled receptor (Fzd) and the LRP5/6 co-receptor [7]. Fzd, the primary receptor for Wnt, is a heptameric transmembrane protein having an extracellular component, cysteine-rich N-terminal domain (CRD) that has a hydrophobic structure conducive to binding lipids on Wnt proteins [8]. LRP5/6, a single-transmembrane co-receptor, is vital for Wnt secretion and signaling during embryonic development. Multiple Wnt-binding sites are present in the extracellular domain of LRP6, which facilitate the binding of various Wnt proteins, which in turn causes Wnt binding to Fzd [7].

2.3. Wnt/β-catenin Signaling

The involvement of β-catenin in the Wnt signaling pathway enables it to be classified into pathways that are either classical or non-classical. The classical Wnt signaling is characterized by the central role of β-catenin (Fig. 1). In the absence of Wnt protein binding, a complex comprising APC, CK1, Axin, and GSK3β exists in the cytoplasm. APC and Axin are involved in the binding of β-catenin in this complex, phosphorylated by CK1 and GSK3β, and subsequently degraded via the ubiquitin-proteasome pathway. After the Wnt interacts with LRP5/6 and the Fzd, Axin is broken down and β-catenin is released from the complex through the translocation of CK1 and GSK3β to the cell membrane. Once within the nucleus, β-catenin engages in a complex interaction with the transcription factor TCF/LEF after building up in the cytoplasm, displacing histone deacetylase and activating target genes' expression [9]. Research shows that a variety of human illnesses are connected to the Wnt signaling pathway, metabolic conditions, and cancers, highlighting its multifunctionality and essential functions that are involved in a variety of both normal and abnormal activities [10].

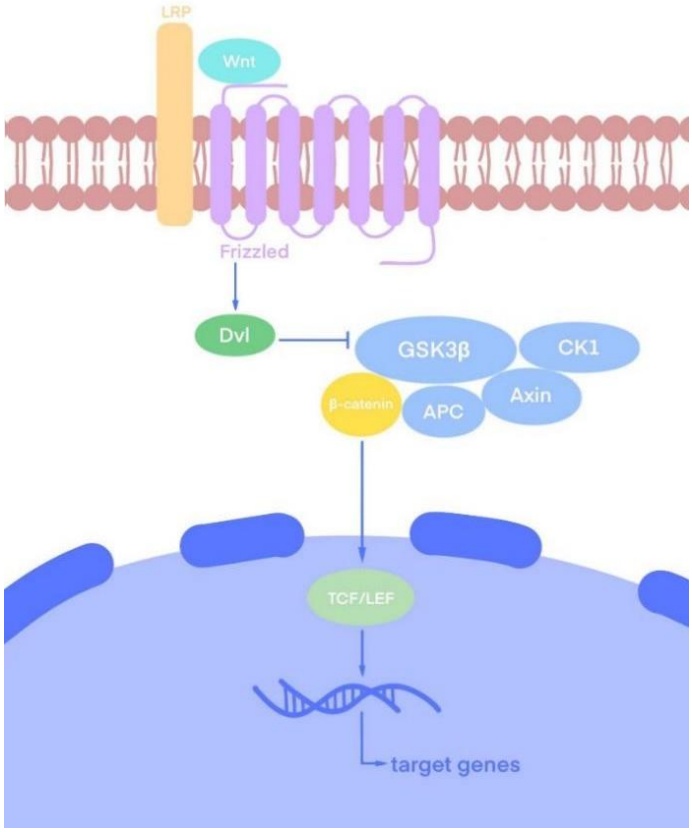

Figure 1. Wnt/β-catenin signaling pathway

3. Regulatory Mechanisms of the Wnt/β-catenin Signaling Associated with BM-MSC Osteogenic Differentiation

3.1. Effects of Changes in the Structure of the Wnt/β-catenin Signaling Pathway on BM-MSC Differentiation

Experimental evidence indicates that alterations in the composition of Skeletal development is greatly impacted by the Wnt/β-catenin signaling system. The Wnt/β-catenin pathway is one of the signaling pathways that controls the fate of BM-MSCs; it prevents the cells from differentiating into adipocytes and cartilage while encouraging the cells to differentiate into osteoblasts. Table 1 summarizes the effects of changes in the components of the Wnt/β-catenin signaling pathway on bone.

Table 1. Effects of changes in Wnt/β-catenin signaling components on bone

Compositional changes	Skeletal changes	References
Loss of beta-catenin function	Increased cartilage, decreased bone mass, increased bone resorption	[11, 12]
Wnt10b features gained	Increased bone mass and bone strength to resist aging-related bone loss	[13]
Fzd8 loss of function	Increased osteoclast formation	[14]
Fzd9 loss of function	Decreased bone formation	[15]
LRP5 loss of function	Bone loss, osteoporosis pseudoglioma syndrome	[16]
Acquired Functional Mutations in LRP5 Result in Dkk1 Inhibition	High bone mass	[17]
Loss of GSK3β function	Damaged cartilage and increased bone mass	[18, 19]

3.2. Role of the Wnt/β-catenin Signaling Pathway on the Expression of Genes Related to Osteogenic Differentiation

It has been proven by studies that Wnt signaling contributes to osteogenic differentiation while inhibiting the differentiation of BM-MSCs into cartilage and adipocytes through various mechanisms. The Wnt/β-catenin pathway can directly activate bone-related transcription-related molecules like Runx2, osterix, and Dlx5, which are crucial for osteoblastic differentiation [13, 20]. The transcriptional coactivator TAZ is a crucial factor in this differentiation process, promoting the conversion of BM-MSCs into osteoblasts while inhibiting adipocyte formation [21]. Additionally, ZBP1, a DNA-binding protein, which facilitates the translocation of β-catenin to the nucleus, where it interacts with the SP7 and Runx2 promoters, promoting osteoblast differentiation and inhibiting the adipogenesis of BM-MSCs [22]. Conversely, serpinB2, a serine protease inhibitor, hinders osteogenic differentiation, but the Wnt/β-catenin signaling suppresses serpinB2, thereby promoting osteoblastic differentiation [23]. Furthermore, β-catenin inhibits adipocyte differentiation by downregulating CEBPα and PPARγ [13, 24]. The Wnt signaling also regulates cellular metabolism in osteoblasts [25]. Notably, the classical Wnt pathway inhibits osteoclast genesis by increasing the levels of osteoprotegerin, which pPlays a critical role in maintaining bone homeostasis [26].

3.3. Interaction of the Wnt/β-catenin Signaling Pathway with Other Signaling Pathways

The classical Wnt signaling pathway, mediated by Wnt3a, stabilizes TAZ and stimulates the osteogenic differentiation through prevention of TAZ degradation through the Hippo signaling pathway [27]. Additionally, the Piezo1/2 pathway contributes to osteoblast differentiation by

regulating osteoblast-related gene expression via signaling pathways like ERK and P38, while further engaging the Hippo pathway. Piezo1/2 can activate β-catenin, further improving Runx2 activation to regulate osteoblast genes' expression [28]. Within the JAK/STAT signaling pathway, STAT3 promotes the binding of SOCS3 to β-catenin, leading to the inhibition of β-catenin activity [29]. Sclerostin (SOST), a Wnt/β-catenin signaling antagonist, is regulated by PHD2, which is involved in cytosolic oxygen sensing. HIF-α, modulated by PHD2, reduces SOST expression, consequently boosting the Wnt/β-catenin pathway's activity [30]. The BMP signaling exhibits a synergistic effect with the Wnt/β-catenin pathway, encouraging osteogenic differentiation through the RANKL-OPG pathway [31]. Furthermore, Wnt3a activates mTORC2 and AKT via LRP5 and RAC1, leading to increased activity of glycolysis-related enzymes and promoting osteoblast formation through enhanced glucose metabolism [32].

4. Relationship Between the Wnt/β-catenin Signaling and Skeletal Diseases

4.1. Skeletal Diseases Associated with the Wnt/β-catenin Pathway

Abnormal functioning of the Wnt/β-catenin pathway has been linked to various skeletal diseases. This section examines the relationship between the Wnt/β-catenin signaling pathway and two specific conditions: osteoporosis and osteoarthritis.

4.2. Relationship Between Wnt/β-catenin Signaling Pathway and Osteoporosis

Degenerative diseases like osteoporosis cause abnormal microarchitecture in bone tissue and low bone mass, which increases the risk of fractures and increases the fragility of bones. This condition occurs when osteoclasts overtake osteoblasts in bone resorption. Osteoporosis is the result of abnormalities in the Wnt/beta-catenin signaling pathway, as demonstrated by research; for example, mutations in the Wnt1 gene result in decreased levels of β-catenin, reducing Wnt/β-catenin signaling. This impairment affects the differentiation of BM-MSCs into osteoblasts, which ultimately leads to decreased bone mass and osteoporosis [33].

4.3. Relationship Between Wnt/β-catenin Signaling Pathway and Osteoarthritis

Osteoarthritis is a prevalent age-related degenerative joint disease marked by cartilage damage, synovial inflammation, and chondrosclerosis. Given that the Wnt/β-catenin pathway promotes the differentiation of BM-MSCs into osteoblasts, β-Catenin is essential for developing osteoarthritis. If β-catenin is activated or overexpressed, it may lead to abnormal bone mass increases and reduced cartilage formation, which can help to progress osteoarthritis [34]. In contrast, Wnt16 inhibition of the Wnt/β-catenin pathway can mitigate cartilage damage in osteoarthritis through interactions with the PCP/JNK-mTORC1-PTHrP pathway. [35]. Additionally, Runx1, a key transcriptional regulator of cartilage formation, inhibits the Wnt/β-catenin pathway by decreasing the levels of active β-catenin. Overexpression of WIF-1 can further promote chondrocyte proliferation and inhibit apoptosis by blocking the Wnt/β-catenin signaling, Minimizing the generation of reactive oxygen species (ROS) and matrix metalloproteinase (MMP) secretion. This approach offers a novel therapeutic strategy for osteoarthritis treatment [36].

5. Application of the Wnt/β-catenin Signaling in the Treatment of Bone Diseases

The role of the Wnt/β-catenin pathway in bone formation has been extensively studied, and targeting this pathway holds potential for treating skeletal diseases.

5.1. Targeting the Wnt/β-catenin Signaling Pathway

Current treatments for osteoporosis primarily focus on inhibiting bone resorption or using hormones to promote bone formation. Considering the pivotal function of the Wnt/β-catenin pathway in osteogenic differentiation, activating or inhibiting its antagonists presents a promising therapeutic

strategy for bone metabolic diseases. For instance, inhibiting Wnt antagonists such as Dkk1 can enhance the activity of the Wnt/β-catenin signaling pathway, leading to increased bone mass. However, safety concerns arise due to the association of this pathway with tumorigenesis [37]. In the context of osteoarthritis, which is linked to overactive β-catenin, therapeutic benefits can arise from targeting the Wnt/β-catenin pathway. Reducing nuclear β-catenin levels or decreasing the β-catenin/TCF complex's transcriptional activity can help mitigate cartilage degradation in osteoarthritis [38].

5.2. Modulation of the Wnt/β-catenin Signaling Pathway for the Treatment of Skeletal Diseases

Therapeutic effects were achieved using monoclonal antibodies against sclerostin in osteoporosis, while Wnt pathway inhibitors have also shown potential benefits in osteoarthritis. Encouraging the Wnt signaling pathway to be activated in the bone microenvironment by inhibiting sclerostin is an effective treatment for osteoporosis, even though its duration may be limited [37, 39]. Additionally, upregulation of Dkk1 has been found to enhance chondrocyte differentiation and alleviate symptoms of osteoarthritis [40].

6. Conclusion

The Wnt/β-catenin signaling is a vital component of regulating cellular behavior, The differentiation of BM-MSCs into osteoblasts is a process that is regulated by the pathway, which ultimately determines the fate of the cell. Throughout osteogenic differentiation, genes that play a role in the formation of bones being transcribed is facilitated by the Wnt/β-catenin signaling and it has interactions with other signaling pathways., such as Hippo and TGF-β/BMP, to facilitate osteoblast formation while inhibiting the differentiation of chondrocytes and adipocytes, thereby maintaining bone homeostasis.

The Wnt/β-catenin signaling pathway is implicated in various skeletal diseases. In osteoporosis, inhibition of this pathway contributes to disease progression, while overactivation can trigger osteoarthritis. Furthermore, rheumatoid arthritis and related skeletal disorders have been connected to the Wnt/β-catenin pathway. Understanding how Wnt/β-catenin contributes to bone diseases can lead to the creation of targeted drugs and new therapeutic approaches.

Current treatments for osteoporosis, such as sclerostin inhibition, have shown some success, but they face challenges, including limited duration of effectiveness and potential reductions in bone resorption. Continued research might offer perceptions into More efficacious and suitable therapeutic modalities.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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