Wnt signaling and osteoporosis

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Abstract

Major advances in understanding basic bone biology and the cellular and molecular mechanisms responsible for the development of osteoporosis, over the last 20 years, have dramatically altered the management of this disease. The purpose of this mini-review is to highlight the seminal role of Wnt signaling in bone homeostasis and disease and the emergence of novel osteoporosis therapies by targeting Wnt signaling with drugs.

Keywords

Introduction

The mammalian skeleton regenerates throughout life by the removal (resorption) of old bone by osteoclasts and its replacement with new bone by osteoblasts, during a process called remodeling [1]. Osteocytes – former osteoblasts which are entombed within the mineralized matrix – sense the need for regeneration in a particular anatomical site and orchestrate the process by directing the homing of osteoclasts and osteoblasts to the site that is in need of remodeling, by producing and secreting key factors that control osteoclast and osteoblast generation [2, 3]. Under physiologic conditions, bone resorption and formation are balanced with the exact same amount of bone added in the site from which it was previously resorbed. With advancing age, the balance between resorption and formation is disturbed and bone mass declines. In addition bone progressively loses mechanical strength to an extent that is greater than the decline of bone mass because of the deterioration of its microarchitecture and the quality of its matrix and mineral (by mechanisms that are not well understood) and an increase in the number of dead or dysfunctional osteocytes as well as increased cortical

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porosity [4, 5]. Decreased bone mass and strength lead to the bone fragility syndrome known as osteoporosis.

The traditional thinking of osteoporosis as a disease of women starting at menopause is nowadays yielding ground to the recognition that osteoporosis is a multifactorial disease that affects both sexes. The disease process begins as early as the third decade of life and age-related mechanisms intrinsic to bone cells, including oxidative stress, decreased Wnt/β-catenin signaling, increased activation of FoxO transcription factors, oxidized lipids (acting via PPARγ and increasing bone marrow adiposity), declining osteocyte autophagy, and increased osteocyte senescence, play a primary role [6]. Age-dependent changes in other organs and tissues, such the decline of ovarian function at menopause and increased glucocorticoid production and/or responsiveness, contribute to the development of osteoporosis by accelerating the effects of aging.

Wnt signaling

Wnts comprise a large family of secreted signaling glycoproteins that control cell proliferation, differentiation, apoptosis, survival, migration, and polarity in a plethora of cell types [7]. They play a critical role during embryonic development (including skeletal patterning) as well as in postnatal health and diseases, including cancer and degenerative disorders. To date, 19 different Wnt proteins have been found in humans and mice and some of them are specific to certain cells and tissues. Wnt proteins deliver their signal by binding to transmembrane receptor proteins. Like Wnt proteins, there are several Wnt receptors. The list includes 10 members of the Frizzled family and low density lipoprotein receptor-related protein (LRP) 5 as well as LRP6, Ror2, and Ryk. Different Wnts recognize different set of receptors and thereby selectively activate distinct intracellular pathways. Binding of Wnts to their cognate receptors activates at least three different intracellular signaling cascades: the Wnt/β-catenin pathway (also known as the canonical Wnt pathway), the non-canonical Wnt pathway, and the Wnt-calcium pathway. Activation of the Frizzled/LRP5 or Frizzle/LRP6 receptor complexes by Wnts, such as Wnt1 and Wnt3a, leads to the recruitment of Axin – a scaffold protein – to the Wnt/receptor complex on the cell membrane and causes the inactivation of glycogen synthase kinase 3β (GSK-3β) (Figure 1). Inactivation of GSK-3β, in turn, prevents the degradation of β-catenin by the proteasome. This step allows the stabilization of β-catenin in the cytoplasm and its subsequent entry into the nucleus, where it associates with the T-cell factor (TCF) lymphoid-enhancer binding factor (LEF) family of transcription factors and regulates the expression of Wnt target genes [8].

Wnt/β-catenin signaling in bone health and disease

Bone-forming osteoblasts and bone-resorbing osteoclasts are terminally differentiated cells with short lives. Therefore, both need to be continuously replaced with new ones originating from stem cells of the mesenchymal and hematopoietic lineage, respectively [9, 10]. The supply of differentiated cells of either lineage is up- or down-regulated, in order to meet the demand. This is accomplished primarily through an increase or decrease of the replication of lineage-committed descendants of the respective stem cells [11, 12]. Aberrant osteoblast

and/or osteoclast number underlines most acquired metabolic bone diseases, including osteoporosis, and it results from changes in their supply as well as their lifespan [1].

Starting about twelve years ago, genetic studies of four patient families – three with unusually high and one with low bone mass – revealed that activating or deactivating mutations of Wnt signaling were responsible for their high and low bone mass, respectively. In two of the families, the mutations were located on the *LRP5* gene [13, 14]. In the other two, the mutations were located on the *SOST* gene, which encodes for sclerostin – an antagonist of the Wnt signaling that is made and secreted primarily by osteocytes [15, 16]. These discoveries established that lack of sclerostin expression in bone is the cause of Sclerosteosis and Van Buchem disease – two rare bone sclerosing dysplasias; whereas the loss of function mutation of the *LRP5* gene is the cause of the osteoporosis-pseudoglioma syndrome. Following these genetic studies, an intensive research effort has revealed that Wnt signaling is indeed a key regulator of bone health and disease and can, therefore, be targeted to develop new therapies for osteoporosis. For an extensive discussion of the subject, the reader is referred to the excellent review by Baron and Kneissel [17].

Wnt/β-catenin signaling stimulates the generation of osteoblasts by promoting commitment and differentiation of pluripotential mesenchymal stem cells (MSCs) towards the osteoblast lineage, while simultaneously suppressing commitment to the chondrogenic and adipogenic lineage [12]. In particular, Wnt/β-catenin signaling promotes the progression of Osterix1 (Osx1)-expressing cells to bone producing osteoblasts. In addition, Wnts prevent the apoptosis of mature osteoblasts and thereby prolong their lifespan by both β-catenin-dependent and independent pathways [18].

In addition to its effects on osteoblasts, Wnt/β-catenin signaling decreases osteoclast differentiation by stimulating the production and secretion of osteoprotegerin (OPG) [19] – a natural antagonist of the receptor activator of nuclear factor-B ligand (RANKL) [20]. RANKL is indispensable for the differentiation, survival, and function of osteoclasts; thereby critical for bone resorption. RANKL is produced primarily by osteocytes [21]. During the process of osteoclast generation, bone marrow macrophages (BMMs) differentiate into tartrate-resistant acid phosphatase (TRAP)-positive pre-osteoclasts, which then fuse with each other to form mature osteoclasts. RANKL and macrophage colony–stimulating factor, provide the two necessary and sufficient signals for osteoclast differentiation [22]. In addition to their indirect effects on osteoclastogenesis that are mediated by controlling OPG expression and secretion by osteoblasts/osteocytes, Wnts act directly on osteoclasts. However, the biological significance of the direct effects is less clear. In any event, deletion of β-catenin in osteoclasts increases osteoclast number and bone resorption and decreases bone mass [23].

To date, several of the Wnt proteins have been shown to play a role in skeletal development and homeostasis as well as joint formation in humans and mice, including Wnt1, Wnt3a, Wnt4, Wnt5, Wnt5a, Wnt7a, Wnt10b, and Wnt14. Of those, Wnt10b seems to be the most critical positive modulator of bone formation in adult bone [24, 25]. In addition to Wnt proteins, mammals produce enhancers of Wnt/β-catenin signaling, such as the four R-sponding proteins. Recently, missense mutations in the *Wnt1* gene were identified in a form

of autosomal dominant early-onset osteoporosis and a severe form of osteogenesis imperfecta [26].

Wnt signaling, osteocytes, and the mechanical adaptation of the skeleton

Wnt signaling in bone is fine-tuned by several secreted glycoproteins that act as Wnt antagonists [27]. The most potent and best recognized of these are sclerostin, Wise, and the Dickkopf (DKK) proteins 1 and 2. Sclerostin binds to LRP5 and LRP6 and inhibits canonical Wnt signaling by blocking the binding of Wnt proteins to the extracellular regions of LRP5 and LRP6. Interference with the binding of Wnts to LRP6 seems to be functionally most significant in this respect. Sclerostin deficiency, on the other hand, unleashes Wnt signaling and dramatically increase bone mass in mice and humans.

The skeleton adapts to meet mechanical needs. This is best exemplified by the rapid and dramatic loss of bone that occurs with immobilization or weightlessness during space flights. The bone cells that are responsible for both sensing mechanical strains and orchestrating the adaptation of the skeleton to changing strains are the osteocytes [3]. Mechanical stimulation of bone reduces osteocyte expression of SOST-sclerostin [28]. Conversely, sclerostin expression increases during immobilization [29].

Wnt/β-catenin signaling, the FoxO transcription factors, and the pathogenesis of osteoporosis

The hallmarks of age-related osteoporosis are a decrease in bone formation and an increase in bone marrow adiposity [6]. Recent research findings from the mouse model suggest that attenuation of Wnt/β-catenin signaling may be responsible for these changes [30]. The decline of bone mass and increase of marrow adiposity with advancing age is associated with a progressive increase in oxidative stress (OS) [31]. In the last few years, members of the subclass of the forkhead family of transcription factors, called FoxOs, have emerged as an important defense mechanism against OS and growth factor deprivation – another accompaniment of old age. In the setting of OS or growth factor deprivation, FoxOs translocate from the cell cytoplasm to the nucleus where they stimulate the transcription of antioxidant enzymes as well as genes involved in cell cycle, DNA repair, and lifespan [32, 33]. Importantly, β-catenin is an essential co-activator of FoxOs, in addition to its role in TCF/LEF-transcription [34]. In the setting of OS or nutrient depletion, the limited pool of β-catenin in osteoblast progenitors is diverted from Wnt/TCF- to FoxO-mediated transcription [35]. Through this mechanism attenuation of canonical Wnt/β-catenin signaling decreases the progression of Osx1 expressing cells to bone producing osteoblasts, and thereby it decreases bone mass and simultaneously increases adipogenesis [30]. The diversion of β-catenin from TCF- to FoxO-mediated transcription may also contribute to several other age-related pathologies [34]. Thus, similar to several other defense responses against aging, FoxO activation eventually aggravates the effects of aging on bone and becomes a culprit of involutional osteoporosis [36].

Targeting Wnt signaling for the development of a novel bone anabolic therapy for osteoporosis

Heterozygous carriers of the *SOST* mutation have high normal or increased BMD without any of the abnormal traits of the full carriers of the mutation, indicating that decreasing the levels of the gene product sclerostin can modestly unleash Wnt signaling and increase bone formation without undesirable side effects [37]. Importantly, parathyroid hormone (PTH) decreases the production of sclerostin by osteocytes [38]. Intermittent administration of a recombinant form of parathyroid hormone (PTH) is currently the only approved therapy for the treatment of osteoporosis that can increase bone mass de novo. These and similar considerations have paved the way for an attempt by the pharmaceutical industry to develop a novel anabolic therapy for osteoporosis based on antibodies that neutralize sclerostin. Preclinical studies with the sclerostin neutralizing antibody have convincingly shown that sclerostin inhibition leads to increased bone formation, gain of bone mass, and increased bone strength in rodents and monkeys [39, 40]. Moreover, a multicenter, randomized, placebo-control study of post-menopausal women with low BMD have shown that romosozumab, a monoclonal sclerostin-neutralized antibody, increases BMD and bone formation and decreases bone resorption, within a year, to a greater extent (11.3%) than the bisphosphonate alendronate (4.1%) or PTH (teriparatide) (7.1%) [41]. Unexpectedly, nonetheless, the effect of romosozumab on bone turnover markers suggested a rapid, marked, but transient increase in bone formation and a moderate but more sustained decrease in bone resorption. The reason for the unpredictable changes in bone turnover markers is unclear. In any event, denosumab, a neutralizing antibody against RANKL – another osteocyte-derived protein – is an approved and very effective anti-resorptive therapy for osteoporosis [42].

Summary

Appreciation of the critical role of osteocytes in the orchestration of bone remodeling and the discoveries of the seminal role of RANKL and the Wnt/β-catenin signaling and its antagonist sclerostin, have provided a much improved understanding of the pathophysiology of osteoporosis. Furthermore, these developments have paved the way for the development of rational and effective new therapeutic strategies for the treatment of osteoporosis.

Research Agenda

The phase 3 clinical trial with romosozumab is on its way and should provide definitive answers on whether the effect of this therapy on BMD, shown in the phase 2 trial, will translate into anti-fracture efficacy; and whether, of course, romosozumab will be safe for long-term use. Future research aiming to elucidate age-related mechanisms affecting more than one tissue could lead to the development of even more advanced therapies that could simultaneously combat osteoporosis and other degenerative disorders, for example sarcopenia, resulting from shared mechanisms of aging.

Acknowledgments

Reference List

[1]. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev. Apr; 2000 21(2):115–37. [PubMed: 10782361]

[2]. Manolagas SC, Parfitt AM. For whom the bell tolls: Distress signals from long-lived osteocytes and the pathogenesis of metabolic bone diseases. Bone. Sep 23.2012

[3]. Bonewald LF. The amazing osteocyte. J Bone Miner Res. Feb; 2011 26(2):229–38. [PubMed: 21254230]

[4]. Manolagas SC, Parfitt AM. What old means to bone. Trends Endocrinol Metab. Mar 9; 2010 21(6):369–74. [PubMed: 20223679]

[5]. Zebaze RM, Ghasem-Zadeh A, Bohte A, et al. Intracortical remodelling and porosity in the distal radius and post-mortem femurs of women: a cross-sectional study. Lancet. May 15; 2010 375(9727):1729–36. [PubMed: 20472174]

[6]. Manolagas SC. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocr Rev. 2010; 31(3):266–300. [PubMed: 20051526]

[7]. Kikuchi A, Yamamoto H, Sato A. Selective activation mechanisms of Wnt signaling pathways. Trends Cell Biol. Mar; 2009 19(3):119–29. [PubMed: 19208479]

[8]. Clevers H, Nusse R. Wnt/beta-Catenin Signaling and Disease. Cell. Jun 8; 2012 149(6):1192–205. [PubMed: 22682243]

[9]. Long F. Building strong bones: molecular regulation of the osteoblast lineage. Nat Rev Mol Cell Biol. Jan; 2012 13(1):27–38. [PubMed: 22189423]

[10]. Teitelbaum SL, Ross FP. Genetic regulation of osteoclast development and function. Nat Rev Genet. Aug; 2003 4(8):638–49. [PubMed: 12897775]

[11]. Park D, Spencer JA, Koh BI, et al. Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. Cell Stem Cell. Mar 2; 2012 10(3):259–72. [PubMed: 22385654]

[12]. Rodda SJ, McMahon AP. Distinct roles for Hedgehog and canonical Wnt signaling in specification, differentiation and maintenance of osteoblast progenitors. Development. Aug; 2006 133(16):3231–44. [PubMed: 16854976]

[13]. Gong Y, Slee RB, Fukai N, et al. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell. Nov 16; 2001 107(4):513–23. [PubMed: 11719191]

[14]. Boyden LM, Mao J, Belsky J, et al. High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med. 2002; 346(20):1513–21. [PubMed: 12015390]

[15]. Balemans W, Ebeling M, Patel N, et al. Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). Hum Mol Genet. Mar 1; 2001 10(5):537–43. [PubMed: 11181578]

[16]. Balemans W, Patel N, Ebeling M, et al. Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. J Med Genet. Feb; 2002 39(2):91–7. [PubMed: 11836356]

[17]. Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat Med. Feb; 2013 19(2):179–92. [PubMed: 23389618]

[18]. Almeida M, Han L, Bellido T, Manolagas SC, Kousteni S. Wnt proteins prevent apoptosis of both uncommitted osteoblast progenitors and differentiated osteoblasts by beta-catenin-dependent and -independent signaling cascades involving Src/ERK and phosphatidylinositol 3-kinase/AKT. J Biol Chem. Dec 16; 2005 280(50):41342–51. [PubMed: 16251184]

[19]. Glass DA, Bialek P, Ahn JD, et al. Canonical wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell. May; 2005 8(5):751–64. [PubMed: 15866165]

[20]. Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. Apr 17; 1998 93(2):165–76. [PubMed: 9568710]

[21]. Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA. Matrix-embedded cells control osteoclast formation. Nature Medicine. 2011; 17(10):1235–41.

[22]. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. May 15; 2003 423(6937):337–42. [PubMed: 12748652]

[23]. Wei W, Zeve D, Suh JM, et al. Biphasic and dosage-dependent regulation of osteoclastogenesis by beta-catenin. Mol Cell Biol. Dec; 2011 31(23):4706–19. [PubMed: 21876000]

[24]. Bennett CN, Ouyang H, Ma YL, et al. Wnt10b increases postnatal bone formation by enhancing osteoblast differentiation. J Bone Miner Res. Dec; 2007 22(12):1924–32. [PubMed: 17708715]

[25]. Stevens JR, Miranda-Carboni GA, Singer MA, Brugger SM, Lyons KM, Lane TF. Wnt10b deficiency results in age-dependent loss of bone mass and progressive reduction of mesenchymal progenitor cells. J Bone Miner Res. Oct; 2010 25(10):2138–47. [PubMed: 20499361]

[26]. Laine CM, Joeng KS, Campeau PM, et al. WNT1 mutations in early-onset osteoporosis and osteogenesis imperfecta. N Engl J Med. May 9; 2013 368(19):1809–16. [PubMed: 23656646]

[27]. Cruciat CM, Niehrs C. Secreted and transmembrane wnt inhibitors and activators. Cold Spring Harb Perspect Biol. Mar.2013 5(3):a015081. [PubMed: 23085770]

[28]. Robling AG, Niziolek PJ, Baldridge LA, et al. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. J Biol Chem. Feb 29; 2008 283(9):5866–75. [PubMed: 18089564]

[29]. Gaudio A, Pennisi P, Bratengeier C, et al. Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. J Clin Endocrinol Metab. May; 2010 95(5):2248–53. [PubMed: 20305005]

[30]. Iyer S, Ambrogini E, Bartell SM, et al. FoxOs attenuate bone formation by suppressing Wnt signaling. J Clin Invest. 2013; 123(8):3404–19. [PubMed: 23867622]

[31]. Almeida M, Han L, Martin-Millan M, et al. Skeletal involution by age-associated oxidative stress and its acceleration by loss of sex steroids. J Biol Chem. Sep 14; 2007 282(37):27285–97. [PubMed: 17623659]

[32]. van der Horst A, Burgering BM. Stressing the role of FoxO proteins in lifespan and disease. Nat Rev Mol Cell Biol. Jun; 2007 8(6):440–50. [PubMed: 17522590]

[33]. Salih DA, Brunet A. FoxO transcription factors in the maintenance of cellular homeostasis during aging. Curr Opin Cell Biol. Apr; 2008 20(2):126–36. [PubMed: 18394876]

[34]. Manolagas SC, Almeida M. Gone with the Wnts: beta-catenin, T-cell factor, forkhead box O, and oxidative stress in age-dependent diseases of bone, lipid, and glucose metabolism. Mol Endocrinol. Nov; 2007 21(11):2605–14. [PubMed: 17622581]

[35]. Almeida M, Han L, Martin-Millan M, O'Brien CA, Manolagas SC. Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting beta-catenin from T cell factor- to forkhead box O-mediated transcription. J Biol Chem. Sep 14; 2007 282(37):27298–305. [PubMed: 17623658]

[36]. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. Jun 6; 2013 153(6):1194–217. [PubMed: 23746838]

[37]. van Lierop AH, Hamdy NA, Hamersma H, et al. Patients with sclerosteosis and disease carriers: human models of the effect of sclerostin on bone turnover. J Bone Miner Res. Dec; 2011 26(12): 2804–11. [PubMed: 21786318]

[38]. O'Brien CA, Plotkin LI, Galli C, et al. Control of bone mass and remodeling by PTH receptor signaling in osteocytes. PLoS ONE. 2008; 3(8):e2942. [PubMed: 18698360]

[39]. Li X, Ominsky MS, Warmington KS, et al. Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. J Bone Miner Res. Apr; 2009 24(4):578–88. [PubMed: 19049336]

[40]. Ominsky MS, Vlasseros F, Jolette J, et al. Two doses of sclerostin antibody in cynomolgus monkeys increases bone formation, bone mineral density, and bone strength. J Bone Miner Res. May; 2010 25(5):948–59. [PubMed: 20200929]

[41]. McClung MR, Grauer A, Boonen S, et al. Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med. Jan 30; 2014 370(5):412–20. [PubMed: 24382002]

[42]. Cummings SR, San MJ, McClung MR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med. Aug 20; 2009 361(8):756–65. [PubMed: 19671655]
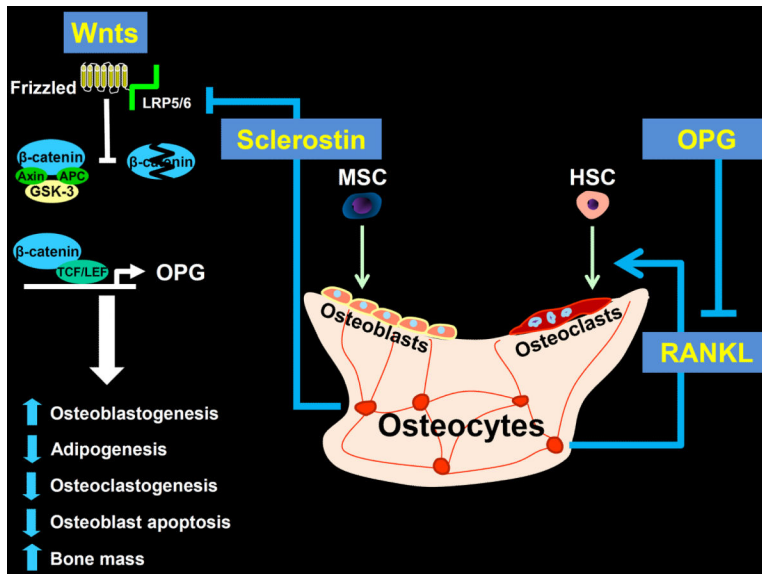
Canonical Wnt/β-catenin signaling, osteocyte-derived sclerostin and RANKL, and the generation of osteoblasts and osteoclasts

Activation of the Frizzled/LRP5/6 receptor complex by Wnt proteins prevents the degradation of β-catenin, which then enters the nucleus and together with TCF/LEF stimulates the transcription of several genes that promote osteoblastogenesis, thereby increasing bone mass. In addition, β-catenin and TCF/LEF stimulate the transcription of the OPG gene, which is the natural antagonist of RANKL – the sine qua non factor for osteoclastogenesis – made primarily by osteocytes. Osteocyte-derived sclerostin blocks the binding of Wnts to their receptor complex. MSC = mesenchymal stem cell; HSC = hematopoietic stem cell.