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Diseases of Wnt signaling

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Abstract

The Wnt signaling pathways play fundamental roles in the differentiation, proliferation, death and function of many cells and as a result are involved in critical developmental, growth and homeostatic processes in animals. There are four currently known pathways of Wnt signaling; the so-called canonical or Wnt/ β -catenin pathway, the Wnt/ Ca^{+2} pathway involving Protein Kinase A, the planar cell polarity pathway and a pathway involving Protein Kinase C that functions in muscle myogenesis. The best studied of these is the Wnt/ β -catenin pathway. The Wnts are an evolutionarily highly conserved family of genes/proteins. Control of the Wnt pathways is modulated by a number of the proteins that either interact with the Wnt ligands directly, or with the low density lipoprotein-receptor related proteins (LRP) 5 and 6 that along with one of several Frizzled proteins function as co-receptors for the Wnt ligands. Aberrant regulation resulting as a consequence of mutations in any of several components of the Wnt pathway and/or protein modulators of the pathway have been shown to cause a wide spectrum of diseases. This review will briefly touch on various diseases of Wnt signaling including cancer, aortic valve calcification and several bone related phenotypes. Our emerging understanding of Wnt signaling offers great hope that new molecular based screening tests and pharmaceutical agents that selectively target this pathway will be developed to diagnose and treat these diseases in the future.

Keywords

Wnt/ β -catenin pathway; Lrp5; Cancer; Bone; Disease

1 The Wnt signaling pathways

The Wnt genes/proteins are a highly conserved group of secreted proteins that are involved in cell differentiation, proliferation, maturation and activity. They play important roles during embryonic development and in homeostatic mechanisms in adult tissues. Their effects are exerted through autocrine and paracrine mechanisms of action. Nusse and Varmus [1] first described the *int-1* gene as a proto-oncogene that caused mammary tumors when the mouse mammary tumor virus (MMTV) integrated into that locus. Subsequently it was shown that the *Drosophila* homolog of *int-1* was the segment polarity gene *wingless* (*Wg*) and the name Wnt was created as a contraction of *Wg* and *int* [2, 3]. Currently in vertebrates there are 19 known Wnt genes. Basically they can be subdivided into two classes, those that work through the β -catenin signaling pathway and those that utilize other

pathways. Dr. Roel Nusse maintains an excellent website that details the Wnts and various aspects of Wnt biology (<http://www.stanford.edu/~rnusse/>).

Until very recently there were three signaling pathways that were thought to be regulated by Wnt proteins; the Wnt/ β -catenin or canonical pathway [4], a planar cell polarity pathway [5] and a Ca^{+2} /Protein Kinase A pathway [6]. Recently a fourth pathway involving Protein Kinase C has been ascribed to Wnt's that is involved in muscle myogenesis [7]. For the purposes of this review only a brief description of these four known Wnt signaling pathways will be given. Several excellent reviews, including the preceding chapter in this issue, can be found in the literature that details these pathways more fully [4, 8–12]. The best studied of the pathways is the so-called canonical Wnt pathway or the Wnt/ β -catenin signaling pathway. In this pathway binding of Wnt to a co-receptor comprised of Frizzled (Fz) and either Lrp5 or Lrp6 (in vertebrates) or Arrow (in *Drosophila*) leads to the intracellular accumulation of β -catenin (Fig. 1). β -catenin levels inside the cell are tightly regulated and normally kept very low by the action of a degradation complex comprised of a scaffolding protein called Axin that binds the Adenomatous Polyposis Coli protein (APC) and Glycogen Synthase Kinase 3- β (GSK3- β) protein and several other proteins. Binding of Wnt to the co-receptor complex leads to a series of phosphorylation events that ultimately results in an inhibition of GSK3- β , association of Axin with the cytoplasmic tail of the LRP5/6/Arrow protein, and release of β -catenin into the cytoplasm. In the absence of Wnt, GSK3- β phosphorylates β -catenin at critical amino acids, which marks the protein for ubiquitination and degradation by the 26S proteasome complex. When β -catenin accumulates in the cytoplasm, some of it will translocate into the nucleus where it binds to the TCF/Lef family of transcription factors thereby regulating gene expression. Ultimately it is the specific cassette of genes whose expression is regulated by β -catenin that determines the effect of Wnt on that particular target cell.

A key protein in the regulation of canonical Wnt signaling is Dishevelled (Dsh or Dvl). Dvl acts downstream of Fz and is integral to both the canonical Wnt and planar cell polarity pathways. The PCP pathway of Wnt signaling involves the G-protein RhoA and its associated kinase ROCK/Drok and the c-Jun N-terminal kinase (JNK) [5]. How Dvl determines which of these two pathways to activate is partially understood. Capelluto et al. [13] have shown that Dvl localizes to small cytoplasmic vesicles and to actin stress fibers. This binding appears to be mediated by the DIX domain of the Dvl protein. They propose that the formation of the vesicular pool of Dvl is needed for stabilization of β -catenin and commitment to the canonical Wnt signaling pathway. Association of Dvl with actin is postulated to result in signaling through the PCP pathway.

The Wnt/ Ca^{+2} pathway is β -catenin independent and results in intracellular Ca^{+2} release and the activation of Protein Kinase C and CamKII [14, 15]. Evidence supports Fz acting through heterotrimeric G proteins leading to activation of phospholipase C and the production of diacylglycerol (DAG) and inositol triphosphate (IP_3) [16]. Recently, a role for Dvl has also been implicated in this pathway [17]. Wnt 5A and rFz2 have been shown to activate the Wnt/ Ca^{+2} pathway and current models to explain how/when this pathway is activated versus the other Wnt pathways implicates specific Wnt ligands and Fz receptors as being involved in this discrimination [6].

An exciting new development in Wnt biology is the description of a fourth Wnt signaling pathway that has been implicated in the control of myogenesis. Chen et al. have shown that the well known cyclic AMP pathway involving Protein kinase A is downstream of select Wnts [7]. Critical to this pathway is the CREB transcription factor. In embryonic muscle development, Wnt-mediated gene expression requires the activation of adenylate cyclase and Protein kinase A resulting in the phosphorylation of CREB and the subsequent induction

of genes such as *Pax3*, and *Myf5* (#563). Whether this pathway extends beyond muscle development to other developmental pathways is unknown at the present time, but opens up a new area of research and expands the potential role of Wnts in regulating several fundamental processes [18].

2 Wnt signaling and cancer

Perhaps the best studied of the diseases of Wnt signaling involve the role of Wnt in various types of cancer. Several excellent review articles have been previously published in this regard [19–22]. Wnt was originally discovered as a proto-oncogene [1] and of the four known Wnt pathways, the canonical Wnt/ β -catenin signaling pathway has been identified as the main culprit in the cellular events that ultimately result in cancer. This is not to say that the other pathways might not also play a role in the disease process. For example, it has been suggested that Wnt5A expression which can be up-regulated in various types of cancer results in aberrant activation of the Wnt/PCP pathway and more malignant disease [23].

Mutations in the Adenomatous Polyposis Coli (APC) gene/protein account for probably >80% of the sporadic cases of colon cancer and also for the inherited form, Familial Adenomatous Polyposis (FAP). FAP causal APC mutations were first identified by two groups in 1991 [24–26]. A web based registry of the mutations in the APC gene that have been currently identified can be found at <http://p53.free.fr/> [27]. Most of these mutations result in a truncated gene product. There appears to be hotspots for germline mutations in the APC gene at codons 1061 and 1309 whereas somatic mutations tend to be clustered in a region between codons 1250 and 1450 [28, 29]. There are three axin binding sites in the protein critical to APC function and mutations that delete these sites result in increased β -catenin activity and more severe disease. Mutations that retain one or two of these Axin binding sites are associated with milder disease and in some cases of desmoid tumors [30].

Many FAP patients have been found to harbor mutations in β -catenin rather than in APC. In fact only one of these two genes will be mutated in any given individual [19]. The β -catenin protein contains a serine/threonine rich region between serine-29 and lysine-49 and phosphorylation of key residues within this region mark the protein for degradation. Mutations which affect these critical serine and threonine residues lead to constitutively active forms of β -catenin. Downstream targets of Wnt/ β -catenin pathway include various genes that are involved in cell proliferation, apoptosis and cell-cycle regulation and thereby contribute to the pathogenesis of cancer [21]. Within the intestinal epithelium stem cells residing in the crypts produce cells capable of differentiating towards multiple lineages. The proliferative precursors that are produced from these stem cells migrate out of the crypt into the villi epithelium where they undergo further differentiation, lose the ability to proliferate and eventually once they reach the tip of the villi where they undergo apoptosis and are shed [20, 22]. The canonical Wnt signaling pathway is critical in determining cell fate in the crypt-villi axis and is intimately involved with many of these processes of proliferation, differentiation and cell death. Mutations that alter Wnt signaling therefore play a critical role in the development of colon cancer.

Another gene that has been implicated in cancer is Axin. Axin is the scaffold protein that binds several components of the degradation complex responsible for regulating intracellular levels of β -catenin. It was originally called the *Fused* (*Fu*) locus, but later renamed Axin (for Axis inhibition) (*Axin1*) to avoid confusion with the unrelated *Drosophila* gene fused [31]. The human Axin2 gene has been cloned and is homologous to the rat axil and mouse conductin genes [32]. Mutations in Axin1 have been reported in cases of hepatocellular carcinoma [33] and other types of human cancers [34]. Some colorectal cancers associated with defective mismatch DNA repair have been shown to result from Axin2 mutations [35].

Given the overall importance of the Wnt pathway in many developmental, proliferative, cell fate and cell death processes it is not surprising that mutations in genes encoding the various components of this pathway (not limited to those described above) have been identified that contribute to various types of cancer including hepatocellular carcinoma (HCC), pancreatic tumors and ovarian cancer [19], prostate cancer [36] and breast cancer [37]. The complexity of the canonical Wnt signaling pathway presents a large number of potential pharmaceutical targets for developing new drugs to treat these diseases of oncology [38]. At the same time the seemingly ubiquitous nature of the pathway throughout most, if not all cells, poses a huge challenge because of the potential for unwanted side-effects developing as a consequence of manipulation of the pathway by such agents.

3 Wnt signaling in other diseases

The canonical Wnt pathway has also been implicated in other diseases. For example, the Wnt signaling antagonist, Dickkopf-1 (Dkk-1) has been shown to play an important role in the development of osteolytic lesions that occur in multiple myeloma [39, 40]. Axin2 mutations have also been reported to cause cases of Familial Tooth Agenesis and predispose these patients to colorectal cancer [41], but the mechanistic connection between these two seemingly disparate phenotypes is not clear. There is also speculation in the literature about a possible role of Axin [34] and Wnt signaling in the pathology of Alzheimer's disease [42], but direct proof is lacking at this time. A role for Wnt signaling has also been suggested in bipolar disease [43]. New emerging evidence suggests that the Wnt pathway may also play a critical role in regulating cardiac valve formation [44] and in the calcification of aortic valves [45].

Calcific aortic stenosis is the most common cause of aortic valve disease in developed countries. Although calcification in aortic valves has been described in the literature for over 100 years, little is known about the synthesis of bone matrix proteins in the aortic valve. Most research has revolved around descriptive histological and protein expression studies delineating the development of calcification in the aortic valve. Studies have shown that vascular calcification is a process very similar to bone mineralization and many of the key regulators of bone mineralization are active in cardiovascular calcification [46, 47]. Rajamannan et al. have demonstrated by RTPCR analysis and histomorphometry that an osteoblast-like cellular phenotype exists in calcified aortic valves removed at the time of surgical valve replacement [48]. These findings parallel the biology in skeletal bone biology which involves similar processes of bone matrix synthesis and mineralization secondary to binding of calcium to hydroxyapatite [49].

The risk factors for calcific aortic valve disease have been described including male gender, hypertension, elevated levels of LDL, and smoking [50], which overlap with those that promote the development of vascular atherosclerosis [51, 52]. Surgical pathological studies have demonstrated the presence of LDL and atherosclerosis in calcified valves, demonstrating similarities between the genesis of valvular and vascular disease and suggesting a common cellular mechanism [53]. It is known that patients with Familial Hypercholesterolemia develop aggressive peripheral vascular disease, coronary artery disease, as well as aortic valve lesions which calcify with age [54, 55]. Rajamannan et al. have developed an in vivo rabbit experimental model that suggests that hypercholesterolemia induces an atherosclerotic valve lesion which expresses bone matrix markers suggesting that either the myofibroblasts have been induced to change into an osteoblast like cell or there has been invasion of osteoblast precursors [56]. The precise mechanism regulating this mineralization process is unknown. However, it is now known that Lrp5 and the canonical Wnt signaling pathway are key regulators of bone formation and bone mass regulation [57, 58]. Also, there have been extensive studies in the field of bone

biology demonstrating the role of β -catenin in the regulation of osteoblastogenesis [59–61]. Exciting new evidence from two groups have demonstrated that the Wnt signaling pathway is active in cardiovascular calcification [45, 62]. This raises the possibility that the mineralization process in aortic valves is a consequence of abnormal activation of the canonical Wnt pathway and agents that regulate this pathway might be able to prevent this disease.

4 Wnt signaling and bone

The importance of the Wnt/ β -catenin signaling pathway in the regulation of bone mass and its role in many disorders of bone has been firmly established within the past 5 years. Remarkably, what were once thought to be seemingly disparate bone disorders based on their clinical characterization are now known to have an underlying common molecular basis that could not have been predicted when they were first clinically described decades ago [63]. Our molecular dissection of these bone diseases has revealed a complex pattern of regulation of key signaling pathways and interaction between bone cells that can now be explained at an ever increasing finer level of detail. This opens the door to potential new pharmaceutical agents and paradigms for the treatment of these diseases. In this review we will examine several bone disorders that share a common molecular basis in the Wnt/ β -catenin signaling pathway.

The genesis of our current understanding involves an intersection of several key observations from different laboratories. First were the critical observations reported in back-to-back papers published in *Nature* in September 2000 by Tamai et al. [64] and Pinson et al. [65] who demonstrated that Lrp6 was critical for regulation of the Wnt signaling pathway and likely functioned as a co-receptor for Wnt's along with Frizzled (Fz), which was already known to be a receptor and regulator of the pathway. Lrp6 and Lrp5 are homologous members of the low-density lipoprotein receptor-related protein family. They share a high degree of homology to the *Drosophila* protein, Arrow. Next came the initial description of mutations in the human *LRP5* gene that give rise to conditions of extremely low bone mass [58] or extremely high bone mass [57, 66]. These reports were quickly followed by a succession of papers describing other mutations in *LRP5* that resulted in altered bone mass [67–73]. In addition, mutations in *LRP5* have been described that give rise to Familial Exudative Vitreoretinopathy (FEVR) [74, 75], however bone density measurements are not reported for these families. FEVR can be inherited in either an autosomal dominant or recessive fashion. Finally there have been several studies reporting an association between polymorphisms in *LRP5* and normal variation in bone mass or other bone phenotypes although the amount of variation that is explained by *LRP5* polymorphisms varies considerably between studies [76–84]. Collectively these papers clearly demonstrate that LRP5 and the Wnt/ β -catenin signaling pathway that it regulates are key regulators of bone at various levels.

The catalog of mutations in *LRP5* that give rise to the human disease Osteoporosis-Pseudoglioma Syndrome (OPPG) is constantly growing (see reviews [63] and [85]). This disease is characterized by a juvenile onset osteoporosis and congenital or early infancy-onset blindness. OPPG is inherited as an autosomal recessive disorder and the mutations run a spectrum from homozygous due to consanguinity to compound heterozygotes to single heterozygous mutations to some cases where no coding sequence change has been identified. Kato et al. [86] have presented evidence from the *Lrp5*^{-/-} mouse that provides a possible explanation for the eye phenotype in those cases presenting with pseudoglioma. This mouse model recapitulates the human OPPG phenotype and they observed that the hyaloid vessels fail to undergo apoptosis that is responsible for the vessel regression that occurs postnatally. The retention of this vasculature results in the loss of vision. Recently it

has been suggested that within the developing eye, LRP5 may be transducing a Norrin signal rather than a Wnt signal. Ai et al. [85] have shown that FEVR causing mutations in *LRP5* can effect Wnt and Norrin signaling.

The increased bone mass phenotypes resulting from mutations in *LRP5* have been clinically characterized under a number of different names including autosomal dominant osteopetrosis type I, Van Buchem disease, endosteal hyperostosis, osteosclerosis and high bone mass (HBM) [12, 67]. Given the wide range in clinical presentation of these understanding the molecular basis of how mutations in *LRP5* give rise to varied phenotypes is particularly challenging. For example, the original report of the HBM mutation [57] was followed by a report of a second kindred by Boyden et al. [66] with the same G171V mutation, yet the clinical presentation of these two kindreds was different. The kindred presented by Boyden et al. had a noticeable square jaw phenotype and the presence of torus lesions. The HBM kindred presented with neither of these features, although the original proband in that kindred has subsequently developed signs of a more square jaw appearance and a small torus lesion (R.R. Recker, personal communication). Interestingly, the unaffected father of the HBM proband has a square jaw appearance and the affected mother does not, nor does she have any signs of torus lesions. This strongly suggests that other genes are likely interacting with *LRP5* to unknown ways to produce a spectrum of phenotypes. Johnson et al. have suggested that these increased bone mass phenotypes be classified as “craniotubular hyperostoses” [12]. Identifying these possible gene–gene and/or gene–environment interactions that modify the increased bone mass phenotypes is an important area for future investigation.

The role of the Wnt signaling pathway in the genesis of osteosarcoma or in the osteolytic lesions that develop in multiple myeloma has become another area of active research interest. A recent study by Hoang et al. [87] has suggested that osteosarcoma patients negative for LRP5 expression have a higher probability of event-free survival compared to those who are positive for LRP5 expression. In a study of patients with multiple myeloma Tian et al. [39] demonstrated that myeloma cells produce DKK1 an inhibitor of the canonical Wnt signaling pathway. The production of RANKL by these cells, which stimulates osteoclastogenesis, along with the production of DKK1 which inhibits osteoblastogenesis, favors excess osteoclastic resorption of bone and the development of the osteolytic lesion [40]. Thus evidence is now accumulating for the importance of the Wnt/ β -catenin signaling pathway in diseases that directly result during development and maintenance of the skeletal and occur secondary to other disease etiologies and have secondary consequences on bone.

5 What remains?

Although the role of the Wnt/ β -catenin signaling pathway has been extensively studied in a number of diseases, the discovery of its previously unsuspected importance in a number of other disease processes has opened the flood-gates for a whole new series of challenging questions. For example, until the initial reports were published that identified mutations in *LRP5* that affected bone mass the importance of the Wnt/ β -catenin signaling pathway in the regulation of adult bone mass was not appreciated, despite a well know role for Wnt in embryonic skeletal development. Now there appears to be an emerging role for this pathway in aortic valve calcification.

In the preceding review article Bodine and Komm have summarized the role of the Wnt/ β -catenin signaling pathway in the regulation of osteoblastogenesis. We will focus our discussion more on some additional aspects of this pathway in regard to bone biology that remain open questions. First it is important to understand the roles of LRP5 versus LRP6 in

bone. Given that these two genes/proteins are highly homologous, it is natural to ask the question do they have overlapping and/or distinct roles? The *Lrp6*^{-/-} mouse dies at the late embryo stage or shortly after birth and skeletons from these mice have numerous limb patterning defects and are hypomineralized [65, 88]. *Lrp5*^{-/-} mice and humans are viable, but have a hypomineralized skeleton and develop vision problems. In both of these situations, where one gene is non-functional, a normal copy of the other homologous gene is present. This suggests that LRP5 and LRP6 have distinct functions at some level. With regard to the role of these two genes in the mineralization of the skeleton, Holmen et al. [88] have shown that there is a gene dosage effect with regard to *Lrp5* and *Lrp6* and suggest that they have overlapping roles during limb development and bone mass accrual. Studies have shown an important role for *Lrp5* in the response of the adult skeleton to mechanical loading [89–92]. Based on the studies by Sawakami et al. [90] who observed that in the *Lrp5*^{-/-} mouse the response to mechanical loading was essentially eliminated, it appears that *Lrp6* may play little or no role in adult bone response to loading. This suggests that *Lrp5* is the critical determinant of adult bone mass maintenance versus *Lrp6*, but this remains unproven at the present time. Thus there are many unresolved issues regarding the role of *Lrp5* and *Lrp6* in the skeleton.

Another area that remains poorly understood is what interactions exist between the Wnt/ β -catenin signaling pathway and other pathways that are known to be important in bone. For example, interactions between the BMP and prostaglandin signaling pathways need to be more fully explored. Another major question is what is the Wnt ligand that functions in bone? Bennett et al. [93] have shown that Wnt10b production inhibits adipogenesis in favor of osteoblastogenesis. What is the Wnt ligand involved in bone responsiveness to mechanical loading? Is it a Wnt or do other ligands play a role? *Lrp5/6* have a number of other modulators such as the Dkk's [94, 95], sclerostin (the product of the *SOST* gene) [96] and Wise [97]. The role of these and other (unknown?) modulators needs to be fully understood both in terms of their normal role and their possible role in the pathogenesis of disease. *SOST* mutations, for example, have been shown to result in the high bone mass phenotype, sclerosteosis [98, 99]. These are just a few of the many key questions that confront bone researchers regarding the role of Wnt/ β -catenin signaling pathway in bone.

6 The future

Our understanding of the biology of the Wnt pathways has progressed significantly in the last 25 years. However, if the new revelations of the past 5 are any indication, we still have much to learn. The Wnt signaling pathways collectively play important roles in development, growth and maintenance of cells, tissues and organisms. Aberrant regulation of these pathways contributes to the pathophysiology of many diseases. Obviously a thorough understanding of the role of the Wnt signaling pathways in various cells and how these pathways orchestrate cellular behavior offers great hope towards the development of new pharmaceutical agents to treat the myriad of diseases that can arise from malfunction of this pathway. A recent study by Clement-Lacroix et al. [100] demonstrated that lithium treatment of low bone mass *Lrp5*^{-/-} mice was able to restore their bone mass to near normal levels. Lithium activates the canonical Wnt signaling pathway by inhibiting GSK-3 β , thereby bypassing the loss of *Lrp5*. This exciting result suggests that selective Wnt pathway modulators might prove useful in treating disease. At the same time the challenge will be to balance the regulation of the pathway in one cell type/tissue to achieve a desired outcome without simultaneously producing an undesirable change in another tissue because of altered regulation of the pathway in those cells/tissues. If ever there existed a double edged sword, the hope of selectively modulating the Wnt pathway to ameliorate diseases is a very sharp one, but the next 25 years should prove very exciting in this regard.

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Fig. 1.

The Wnt/β-catenin signaling pathway. Wnt binds to the Lrp5 or 6 and Frizzled coreceptor complex which leads to the intracellular accumulation of β-catenin. β-catenin translocates to the nucleus where it binds to TCF/Lef transcription factors and regulates the expression of a number of target genes. Mutations in several components of the pathway have been shown to result in disease. Mutations in *LRP5* can result in diseases of increased or decreased bone mass and vision problems. The Lrp5 receptor and Wnt pathway may also play a key role in aortic valve calcification. Mutations in *APC*, *β-catenin* and *Axin* have been shown to give rise to a number of different types of cancer. Downstream gene targets of β-catenin such as osteoprotegerin (*OPG*) and the secreted frizzled related proteins (*sFRPs*) are known to regulate osteoclastogenesis and may play a role in metastatic bone diseases that result in osteolytic lesions. Mutations in modulators of Wnt signaling such as the *SOST* gene, which produces sclerostin, give rise to the increased bone mass disease, sclerosteosis. These are just a few examples of the variety of diseases that have been attributed to the Wnt/β-catenin signaling pathway.