Journal of Cellular Biochemistry 97:661–672 (2006)

Role of Wnts in Prostate Cancer Bone Metastases

Christopher L. Hall,[1] Sona Kang,[2] Ormond A. MacDougald,[2] and Evan T. Keller[1]*

[1]Department of Urology, The University of Michigan, Ann Arbor, Michigan
[2]Department of Internal Medicine, The University of Michigan, Ann Arbor, Michigan

Abstract Prostate cancer (CaP) is unique among all cancers in that when it metastasizes to bone, it typically forms osteoblastic lesions (characterized by increased bone production). CaP cells produce many factors, including Wnts that are implicated in tumor-induced osteoblastic activity. In this prospectus, we describe our research on Wnt and the CaP bone phenotype. Wnts are cysteine-rich glycoproteins that mediate bone development in the embryo and promote bone production in the adult. Wnts have been shown to have autocrine tumor effects, such as enhancing proliferation and protecting against apoptosis. In addition, we have recently identified that CaP-produced Wnts act in a paracrine fashion to induce osteoblastic activity in CaP bone metastases. In addition to Wnts, CaP cells express the soluble Wnt inhibitor dickkopf-1 (DKK-1). It appears that DKK-1 production occurs early in the development of skeletal metastases, which results in masking of osteogenic Wnts, thus favoring osteolysis at the metastatic site. As metastases progress, DKK-1 expression decreases allowing for unmasking of Wnt's osteoblastic activity and ultimately resulting in osteosclerosis at the metastatic site. We believe that DKK-1 is one of the switches that transitions the CaP bone metastasis activity from osteolytic to osteoblastic. Wnt/DKK-1 activity fits a model of CaP-induced bone remodeling occurring in a continuum composed of an osteolytic phase, mediated by receptor activator of NFkB ligand (RANKL), parathyroid hormone-related protein (PTHRP) and DKK-1; a transitional phase, where environmental alterations promote expression of osteoblastic factors (Wnts) and decreases osteolytic factors (i.e., DKK-1); and an osteoblastic phase, in which tumor growth-associated hypoxia results in production of vascular endothelial growth factor and endothelin-1, which have osteoblastic activity. This model suggests that targeting both osteolytic activity and osteoblastic activity will provide efficacy for therapy of CaP bone metastases. J. Cell. Biochem. 97: 661–672, 2006.

Key words: prostate cancer; DKK; metastasis; Wnt; osteoblast; bone

Prostate cancer (CaP) is the most frequently diagnosed cancer in men and the second leading cause of cancer-related death among men in the United States. The most common site of CaP metastasis is the bone with up to 84% of patients demonstrating skeletal metastases [Abrams et al., 1950]. The majority of cancers, such as breast and myeloma, produce areas of bone lysis (osteolytic lesions) when they metastasize to bone. Although there is consistently an osteolytic component to CaP bone metastases, they are typically characterized radiographically by areas of increased bone production (i.e., osteoblastic). The mechanisms through which CaP promotes aberrant bone remodeling are not clearly defined. However, understanding this pathophysiology may help design therapies that prevent the establishment of metastases in the bone or that diminish progression of established CaP metastases. Many groups have been exploring the interactions between cancer and bone with promising results. Recently, Wnts have been documented to play an important role in bone development and modulation of bone production in adults. Based on these observations, we explored the role of Wnts as mediators of CaP-induced osteoblastic activity. In this prospectus, we will discuss our work on Wnts and how that integrates with other osteoblastic factors produced by CaP cells.

Wnt SIGNAL TRANSDUCTION

The Wnt proteins are a large family of cysteine-rich glycoproteins currently consisting

Grant sponsor: National Cancer Institute; Grant numbers: P01 CA093900, R01 CA071672.

*Correspondence to: Evan T. Keller, RM 5304 CCGCB, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0940. E-mail: etkeller@umich.edu

Received 26 October 2005; Accepted 27 October 2005
DOI 10.1002/jcb.20735

of 19 members. They function primarily during development to control body axis symmetry and branching morphogenesis [reviewed in Logan and Nusse, 2004]. Wnts exert their biological effects through three signaling pathways, which are separated by their ability to stabilize β-catenin (the canonical pathway). Canonical Wnt proteins bind at the cell surface to a co-receptor consisting of Frizzled (FZD) and low-density lipoprotein receptor-related protein (LRP) (Fig. 1). This union results in the hyperphosphorylation of disheveled (DSH), which blocks the activity of glycogen synthase kinase 3β (GSK3β) through an unknown mechanism [Yanagawa et al., 1995]. However, GSK3-binding protein (GBP) was recently implicated in the inhibition of GSK3β by blocking its ability to bind the scaffold protein Axin [Farr et al., 2000]. Inhibition of GSK activity results in the stabilization and accumulation of β-catenin which, upon translocation into the nucleus, serves as a co-factor for the T-cell factor family of transcription factors (e.g., T-cell factor (TCF) and Lymphoid enhancer factor (LEF) [Van De Wetering et al., 1997]. The closely related histone acetylases, p300 and CBP, act as co-activators of β-catenin activity [Hecht et al., 2000]. In the absence of Wnt signals, cytoplasmic β-catenin is sequestered by a complex consisting of Axin, the tumor suppressor Adenomatous polyposis coli (APC), and GSK3β [Kikuchi, 2000]. Phosphorylation by GSK leads to ubiquitination and the subsequent degradation of β-catenin. Non-canonical Wnts also bind FZD to mobilize Dsh but do not activate β-catenin/TCF [Yang, 2003]. Instead, non-canonical Wnt proteins such as Wnt5a lead to the activation of protein kinase C and calcineurin in the Wnt/Ca^{2+} pathway where Wnt11 activates the small G-protein Rac and Jun end-terminal kinase (JNK) in the planar cell polarity pathway [Kuhl et al., 2000; Veeman et al., 2003].

The activity of Wnt proteins is controlled by soluble extracellular antagonists including secreted FZD-related proteins (sFRP), Wnt inhibitory factor-1 (WIF-1), Cerberus, and Dickkopf (DKK) [Logan and Nusse, 2004] (Fig. 2). sFRP, WIF-1, and Cerberus act as competitive inhibitors of FZD by sequestering Wnt factors and can, therefore, block both canonical and non-canonical Wnt pathways. DKK-1, in contrast, binds the Wnt co-receptors LRP 5 and 6 to block canonical Wnt signaling [Bafico et al., 2001; Mao et al., 2001]. Specifically, DKK-1 bridges LRP with Kremin2 resulting in the removal of LRP from the cell surface by endocytosis [Mao et al., 2002].

Wnts IN BONE BIOLOGY

Wnts in Bone Development

The use of knockout animals has demonstrated an indispensable role for Wnt signaling

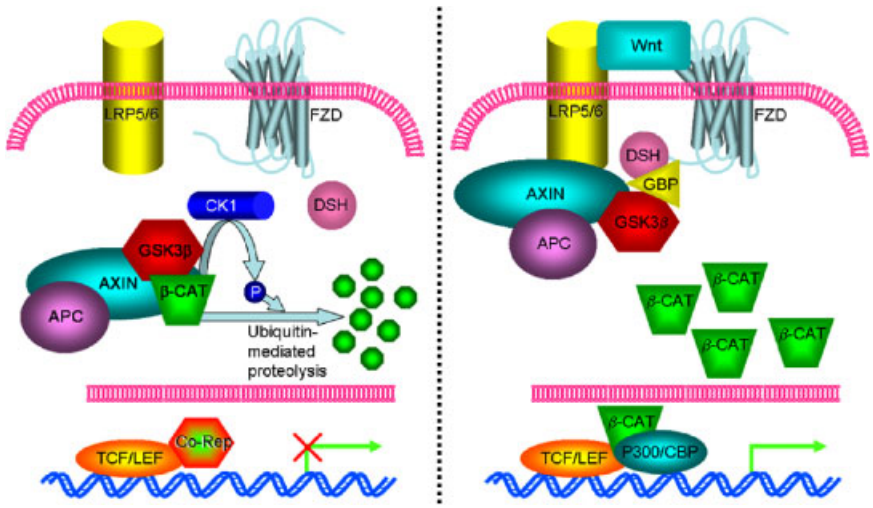

Fig. 1. The canonical Wnt signaling pathway. See text for description of pathway activity. APC, Adenamatous polyposis coli; β-cat, β-catenin; CBP, CREB binding protein; CK, Casein kinase; Co-rep, co-repressor; DSH, Disheveled; FZD, Frizzled; GBP, GSK binding protein; GSK, Glycogen synthase kinase; LEF, lymphoid enhancer factor; P, phosphorus; TCF, T-cell factor. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

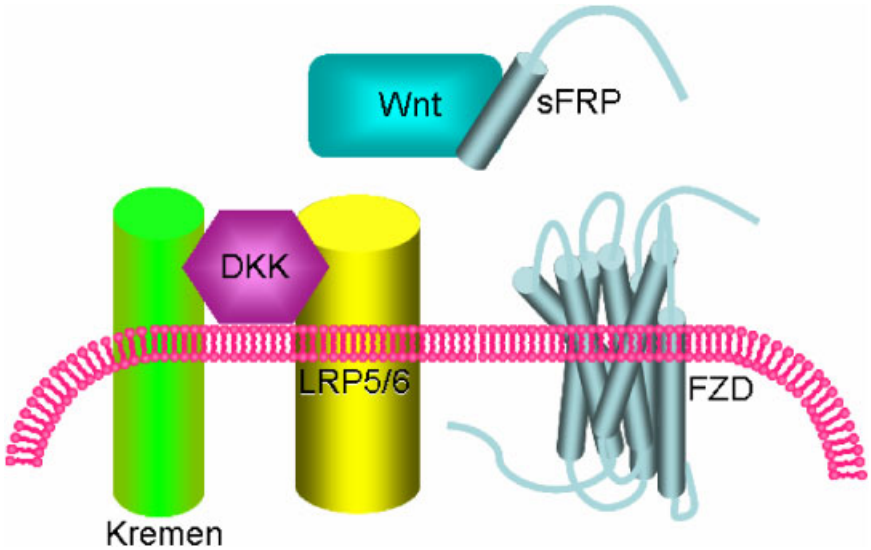

Fig. 2. Endogenous inhibitors of Wnt signaling. Soluble Frizzled related protein (sFRP) can sequester Wnt so that it cannot bind to cell surface Frizzled (FZD). Kremen facilitates the binding of Dickkopf (DKK) to the Low-density lipoprotein receptor-related protein (LRP), which results in blocking Wnt's access to LRP. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

in normal bone development. The spatial and temporal expression of Wnt proteins is required for limb bud initiation, dorsal-ventral patterning, and limb outgrowth [reviewed in Church and Francis-West, 2002; Yang, 2003]. In particular, a requirement for canonical Wnt signaling in limb initiation and patterning has been revealed in animals carrying mutations in Wnt antagonists DKK-1 and sFRP-1. DKK-1 null mice develop fusion and duplication of digits; whereas, overexpression of DKK-1 in the chick results in distal truncation of the limb bud [Mukhopadhyay et al., 2001; Grotewold and Ruther, 2002]. The effect of DKK-1 deficiency in the mouse may relate to a reduction in programmed cell death in the limb [Mukhopadhyay et al., 2001; Grotewold and Ruther, 2002]. Indeed, the overexpression of DKK-1 in the chick results in elevated programmed cell death concomitant with the observed limb truncation [Grotewold and Ruther, 2002]. Viewed together, the data show that disruption of canonical Wnt signaling results in significant limb defects in the developing embryo. Direct evidence that canonical Wnts participate in the bone biology of adults has only recently been elucidated. It was shown that adult mice deficient in the Wnt antagonist sFRP-1 had increased trabecular bone accrual without effects on cortical bone [Bodine et al., 2004]. The demonstration that defects in Wnt antagonists modulate bone remodeling within adults is significant because it defines a mechanism that can be exploited by bone metastatic tumor cells to affect bone biology.

Wnt Signaling and Regulation of Bone Mass

The first indication that Wnt signaling regulates bone mass came from a clinical study in which inactivating mutations in the Wnt co-receptor, LRP5, were linked to the autosomal recessive disorder, osteoporosis pseudoglioma syndrome [Gong et al., 2001], which is characterized by decreased bone mass. Shortly thereafter, it was found that gain-of-function mutations in LRP5 cause a high bone mass phenotype in humans [Boyden et al., 2002; Little et al., 2002; Van Wesenbeeck et al., 2003]. A link between LRP5 and bone formation was supported by the phenotype of mice with gain- or loss-of-function mutations in LRP5 [Kato et al., 2002; Babij et al., 2003]. Analysis of mice containing mutations in LRP5, LRP6, sFRP1, β-catenin, Wnt10b, or DKK2 suggests that Wnt signaling regulates bone mass through multiple mechanisms including osteoblast differentiation, proliferation, function, survival, and coupling to osteoclast formation [Pinson et al., 2000; Boyden et al., 2002; Babij et al., 2003; Bodine et al., 2004; Bennett et al., 2005; Day et al., 2005; Glass et al., 2005; Hill et al., 2005; Holmen et al., 2005; Hu et al., 2005;

Li et al., 2005b] [for in depth reviews, see Johnson, 2004; Westendorf et al., 2004].

Mechanisms by Which Wnt Signaling Increases Bone Mass

Stimulation of osteoblast differentiation. Compelling evidence from humans and mice indicates that Wnt signaling increases bone mass, at least in part through induction of osteoblastogenesis [Bodine et al., 2004]. Analysis of this observation with mesenchymal precursor cells in vitro has resulted in contradictory findings. For example, Wnt1 or Wnt3a increase the early marker of osteoblastogenesis, alkaline phosphatase, in ST2, C3H10T1/2, and C2C12 cells [Gong et al., 2001; Rawadi et al., 2003; Jackson et al., 2005], and expression of Wnt10b or dominant stable β-catenin is sufficient to stimulate the full program of osteoblastogenesis in ST2 cells including mineralization [Bennett et al., 2005]. In contrast, exposure of adult human mesenchymal cells to Wnt3a inhibits nodule formation [Boland et al., 2004], and exposure to Wnt3a or the GSK3 inhibitor lithium chloride inhibits dexamethasone-induced osteogenesis [De Boer et al., 2004]. These seemingly contradictory observations could be due to differences in cell models (the spectrum of receptors expressed, or other unknown factors including differences in mechanism between species) or reflect a temporal importance of Wnt signaling in osteoblast differentiation. Recent evidence suggests that disruption of Wnt signaling through the expression of DKK-1 and 2 blocked osteoblast differentiation in immature osteoblasts but was required to promote terminal differentiation in late stage osteoblasts [Li et al., 2005b; van der Horst et al., 2005].

Mesenchymal stem cells have the potential to differentiate into a number of cell types and considerable evidence now indicates that Wnt signaling stimulates osteoblastogenesis and represses differentiation to alternative cell fates such as the adipocyte [Nuttall and Gimble, 2004]. A negative feedback relationship between osteoblastogenic and adipogenic transcription factors is operative in mesenchymal precursors. For example, activation of PPARγ represses expression and activity of Runx2 [Jeon et al., 2003] and expression of the osteoblastogenic transcription factor Msx2 inhibitsthe ability of C/EBPα to increase expression of PPARγ [Cheng et al., 2003]. Moreover, activation of Wnt signaling suppresses expression of adipogenic transcription factors such as PPARγ and C/EBPα, and increases expression of osteoblastogenic transcription factors such as Runx2, Dlx5, and Osterix [Bennett et al., 2005]. Consistent with this observation, expression of Runx2 increases in the long bone of sFRP1 null mice, and expression of Runx2 is enhanced by several canonical Wnts in vitro, perhaps through direct binding of a β-catenin/TCF complex to the Runx2 promoter [Gaur et al., 2005]. Therefore, it appears that Wnt signaling disrupts the balance between osteoblastogenic and adipogenic transcription factors to induce differentiation of mesenchymal precursors into osteoblasts.

Regulation of osteoblast proliferation and apoptosis. Wnt signaling may also increase number of osteoblasts by regulating their proliferation and/or apoptosis. It is well known that Wnts stimulate proliferation [Reya et al., 2003; Willert et al., 2003; Zechner et al., 2003] of some cell types, including hematopoietic- and neuronal-precursors, and that Wnts inhibit apoptosis of other cell types [Chen et al., 2001; Longo et al., 2002; Hwang et al., 2004; Kennell and MacDougald, 2005] including preadipocytes. Analyses of mice with altered Wnt signaling provide some support for each of these two mechanisms. For example, LRP5 null mice show decreased proliferation of osteoblast and progenitor cells but no obvious differences in apoptotic index [Kato et al., 2002]. However, apoptosis of osteocytes and osteoblasts was reduced in mice with activating LRP5 mutations and in mice devoid of sFRP1 [Babij et al., 2003; Bodine et al., 2004]. These in vivo observations are supported by in vitro studies suggesting that canonical Wnt signaling increases growth rate of undifferentiated and proliferating precursor cell population and inhibits apoptosis [Rawadi et al., 2003; Boland et al., 2004; De Boer et al., 2004; Derfoul et al., 2004]. Although effects of Wnt on proliferation may involve induction of c-myc and cyclin D, this mechanism has not been evaluated specifically in osteoblasts. Similarly, induction of growth factor release inhibits apoptosis in other cell models [Chen et al., 2001; Longo et al., 2002] but has not been evaluated for osteoblasts. Thus, in addition to induction of osteoblastogenesis, Wnt signaling may increase total number of osteoblasts by stimulation of proliferation and survival of osteoblasts.

Induction of osteoblast activity. Wnt signaling may also stimulate bone formation by increasing the mineralizing activity of osteoblasts. Consistent with this hypothesis, activation of β-catenin in osteoblasts specifically increases expression of type I collagen (the major protein component of the bone extracellular matrix), although basal expression of type I collagen is not influenced by loss of β-catenin [Glass et al., 2005]. Furthermore, fatty acid binding protein (FABP)4-Wnt10b transgenic mice have increased mineral apposition rate without changes in surface osteoblast number [unpublished data and Bennett et al., 2005], suggesting that Wnt signaling increases osteoblast activity in this context.

Inhibition of osteoclast differentiation. In addition to increasing bone formation, Wnt signaling could also increase bone mass by decreasing bone resorption. Indeed, there is compelling evidence that Wnt signaling in osteoblasts represses differentiation of bone resorbing osteoclasts [Glass et al., 2005; Holmen et al., 2005]. Although there were no significant difference in number and activity of osteoclasts in LRP5 null or FABP4-Wnt10b mice [unpublished data and Kato et al., 2002] under some conditions, Wnt signaling in osteoblasts can regulate osteoclast differentiation. While conditional deletion of β-catenin in osteoblasts results in increased formation of osteoclasts [Glass et al., 2005; Holmen et al., 2005], increased Wnt signaling due to loss of APC in osteoblasts causes decreased formation of osteoclasts [Holmen et al., 2005]. Wnt signaling inhibits osteoclast differentiation by increasing osteoblast expression of osteoprotegerin, the decoy receptor for receptor activator of NFκB ligand (RANKL), and an inhibitor of osteoclastogenesis [Glass et al., 2005; Holmen et al., 2005; Jackson et al., 2005].

BONE METASTASIS

Osteolysis Versus Osteosclerosis

Bone metastases can be quite debilitating. Outgrowth of tumors within the bone may cause bone pain, fracture, and nerve compression/paralysis each resulting in reduced quality of life [Keller et al., 2001]. The bone is a frequent site of soft tissue tumor metastasis. Typically metastases target the trabecular bone (also called cancellous) of the axial skeleton (e.g., spine, ribs, pelvis). For some tumor types, including breast, prostate, and melanoma, metastasis to the bone occurs with high frequency. In CaP, for example, recent autopsy data suggest that greater than 80% of men who die of CaP have metastatic disease within the bone, typically in the trabecular bone of the pelvis, femur, and vertebral bodies [Bubendorf et al., 2000]. A great deal of research effort has focused on uncovering the mechanisms that lead to bone metastasis [reviewed in Roodman, 2004]. An emerging area is concerned with how tumor cells alter the bone to yield a bone-destroying (osteolytic) or bone-forming (osteoblastic) lesion [Logothetis and Lin, 2005]. A variety of factors are implicated in production of osteolytic lesions. Parathyroid hormone related protein (PTHrP) has been shown in vivo to enhance the formation of osteolytic metastases presumably through induction of osteoclast activity [Boyce et al., 1999]. We and others have shown that osteolytic activity promotes the development of tumor growth in bone and that blocking bone resorption through inhibition of RANKL activity, a requisite factor for osteoclastogenesis, prevents the establishment of osteolytic tumors within the bone [Morony et al., 2001; Zhang et al., 2001, 2003] and that bone turnover due to osteolytic activity promotes the development of tumor growth in bone [Corey et al., 2003; Schneider et al., 2005]. At present, it is unclear if the requirement for osteoclastic activity reflects a need to debulk the bone to permit the seeding of tumor cells or whether bone resorption is needed to liberate growth factors that promote tumor development. It has been suggested that the osteolytic activity releases growth factors from the bone matrix, which then further enhance the cancer cell growth and cancer-induced osteolytic activity in a continuing vicious cycle [Mundy, 1997; Guise, 2000]. CaP is unique in that prostate boney lesions are predominately osteoblastic in nature with underlying osteolytic areas [Keller et al., 2001]. A variety of factors have been implicated in the ability of CaP to induce osteoblastic lesions including insulin-like growth factor [Fizazi et al., 2003], endothelin-1 [Nelson et al., 2003], bone morphogenetic proteins [Dai et al., 2005], adrenomedullin [Chirgwin et al., 2004], and vascular endothelial growth factor [Dai et al., 2004; Rubin et al., 2004]. Most likely cancer-induced dysfunctional bone remodeling involves a variety of osteoblastic factors working in concert. However, prior to

understanding how all these factors integrate at the metastatic site, it may be useful to understand the individual role each factor may play. Accordingly, we sought to explore the role of Wnts in the biology of bone metastasis because of their important role in osteoblast function.

Role of Wnts in Bone Metastasis

Wnts can have both autocrine effects, such as directly influencing tumor growth and survival, and paracrine effects, such as modulating bone cell growth and differentiation. Thus, Wnts may impact the development of metastasis at many steps along the metastatic cascade from primary tumor progression through induction of the metastatic phenotype at the distant site.

Autocrine Wnt signaling and cancer. The role of canonical Wnt signaling in tumor development of colorectal cancer (CRC) has been well established [reviewed in Giles et al., 2003]. The lesions that alter the Wnt signaling pathway are downstream of Wnt itself. In CRC, loss of heterozygosity in APC occurs in over 85% of all sporadic forms of CRC and nearly all familial adenomatosis polyposis (FAP) cases [Giles et al., 2003]. In an additional 10% of CRC cases, mutations in the regulatory region of β-catenin are found [Giles et al., 2003]. Both APC and β-catenin mutations lead to the stabilization of β-catenin and the inappropriate expression of TCF-regulated genes within the tumor cell. These gene products, which have obvious implications to tumor development and progression, include the transcription factor c-Myc [He et al., 1998], the cell cycle regulatory protein cyclin-D1 [Shtutman et al., 1999], the angiogenic chemokine IL-8 [Levy et al., 2002], and the proteases matrilysin and MMP7 [Brabletz et al., 1999; Crawford et al., 1999].

In addition to these classical alterations in the canonical Wnt signaling pathway, there is growing evidence that β-catenin may become activated in tumor cells through cross-talk with non-Wnt signal transduction pathways. These pathways may have relevance to bone metastasis because they have been shown in vitro to become active in bone metastatic tumor cells such as breast and prostate. One example is the receptor tyrosine kinase (RTK) c-Met, which is overexpressed in bone metastases of CaP [Humphrey et al., 1995; Pisters et al., 1995; Hall et al., 2004]. c-Met can activate, in a cell-type-specific manner, invasion, survival, proliferation, morphogenesis, and angiogenesis [reviewed in Jiang et al., 1999]. β-Catenin was shown to physically associate with c-Met in both primary rat hepatocytes and human CRC cells [Herynk et al., 2003]. Hepatocyte growth factor (HGF), the principle ligand for c-Met, was found to induce the nuclear translocation of β-catenin, thus placing it in a position to participate in gene transcription [Monga et al., 2002]. The androgen receptor (AR) has also been shown to directly interact with and mediate nuclear translocation of β-catenin in CaP cells resulting in induction of AR-dependent gene transcription [Mulholland et al., 2002; Cronauer et al., 2005]. A third example of cross talk is oncogenic K-ras that recently was shown to stimulate expression of Wnt target genes through the inhibition of GSK3β by phosphatidylinostiol 3-kinase (PI 3-K) [Li et al., 2005a]. Although c-Met, AR, and K-ras provide novel mechanisms for the nuclear translocation of β-catenin, these novel pathways have yet to be shown to operate in vivo within bone metastases.

In addition to proliferation and survival, canonical Wnt signaling within the metastatic CaP cell may promote osteomimicry. Osteomimicry is a phenomenon described in CaP cells where the tumor cell acquires properties of osteoblasts [Koeneman et al., 1999]. For example, CaP cells have been shown to express the bone matrix protein osteopontin (OPN), the OPN receptor CD44, and the bone-specific transcription factor RUNX2 (Cbfa1/AML3) [Koeneman et al., 1999]. We have also shown that bone metastatic C4-2B CaP cells have the ability to produce mineralized matrix in vitro [Lin et al., 2001]. Wnts may mediate osteomimicry in that both OPN and CD44 are Wnt regulated genes and, as discussed below, canonical Wnts stimulate osteoblast mineralization and differentiation [Wielenga et al., 1999; Muller et al., 2002]. Thus, osteomimicry could represent an autocrine function of canonical Wnt signaling that may contribute to the osteoblastic nature of CaP boney lesions.

Paracrine Wnt signaling in cancer. The previous section outlined how inappropriate Wnt activity within a tumor cell can promote tumor development and metastasis. Whether canonical Wnt signaling within a tumor cell mediates bone metastasis, however, is still under investigation. Accumulating evidence suggests that tumor-derived Wnt factors and antagonists affect bone cell biology that may

determine whether the resulting lesion is osteolytic or osteoblastic in nature.

Wnt signaling has been implicated in limb development, skeletal outgrowth, and in the control of bone mass. However, until recently, direct evidence that Wnt factors contribute to the bone phenotype of metastatic tumor cells in vivo was lacking. cDNA microarray and RT-PCR analysis has demonstrated that both breast and PCa cells express the message for multiple Wnts, including but not limited to Wnt2, Wnt5a, and Wnt7b [Huguet et al., 1994; Lejeune et al., 1995]. To establish a role for Wnts in CaP bone metastasis, we, therefore, chose to modulate canonical Wnt activity by altering the expression of the Wnt antagonist DKK-1, which can regulate the activity of multiple canonical Wnts. The strategy of using overexpression of Wnt antagonists to block Wnt activity in the adult animal has recently been demonstrated and shown to affect both the growth of intestinal epithelial cells and bone mineral density [Bodine et al., 2004; Kuhnert et al., 2004]. Specifically, adenovirus-mediated transfer of DKK-1 inhibited the proliferation in small intestine leading to crypt degeneration where knockout of sFRP-1 prolonged the accrual of trabecular bone in adult mice [Bodine et al., 2004; Kuhnert et al., 2004].

The suggestion that Wnt antagonists may affect bone metastases was first described in patients with multiple myeloma [Tian et al., 2003]. Multiple myeloma is a hematopoietic tumor that metastases to the bone to produce highly osteolytic lesions. In these patients, the expression of DKK-1 was found in osteolytic foci, thus suggesting that blocking canonical Wnt signaling through DKK-1 promoted the osteolytic phenotype. Consistent with this observation, we found that DKK-1 was exclusively and abundantly expressed in human PC-3 CaP cells, which are very osteolytic, compared to other human CaP cell lines (LNCaP, C4-2B, DuCaP, VCaP, LuCaP23.1, and LuCaP 35), which produce mixed lytic/blastic lesions [Hall et al., 2005]. When injected into the marrow space of the tibia (intraosseous injection), PC-3 cells produce highly osteolytic lesions but paradoxically express the RNA for numerous Wnts including Wnts 2, 3a, 5b, 7a, 7b, 10b, and 16. This led us to query if DKK-1 blocked endogenous Wnt activity, thus preventing the formation of osteoblastic metastases.

To test this hypothesis, we inhibited Wnt activity in the mixed osteoblastic/osteolytic C4-2B CaP cell line through stable overexpression of DKK-1. This manipulation resulted in a significant increase in osteolysis (as measured by bone mineral density and percent osteolytic area) following intraosseous injection compared to vector control cells, which produce a mixed osteolytic and osteoblastic lesion similar to that observed in human CaP patients [Hall et al., 2005]. In a corollary study, we inhibited DKK-1 expression in the PC-3 cell line using a shRNA. This resulted in inhibition of PC-3 growth in the bone and an associated decrease of PC3-induced osteolytic lesions (unpublished data). This observation, as discussed above, may reflect a need for initial osteolysis to establish the tumor within the bone. If this is true and yet CaP boney

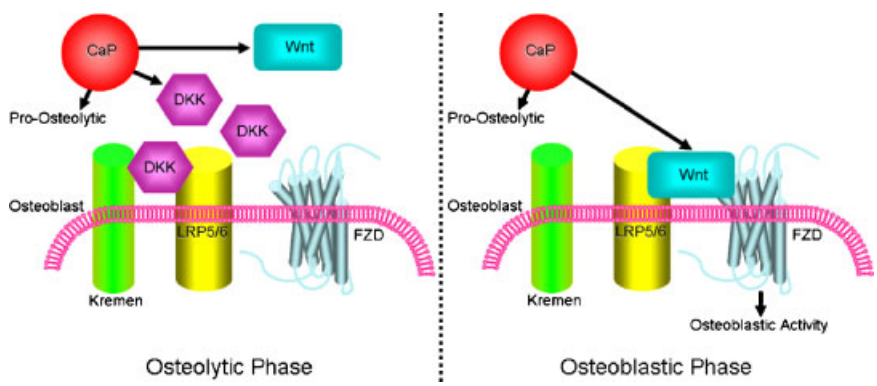

Fig. 3. Model of Wnts paracrine role in prostate cancer (CaP) bone metastases. CaP cells have both osteolytic and osteoblastic potential. Wnts are one of several osteoblastic factors produced by CaP cells. However, early in skeletal metastasis, the CaP cells express DKK-1 which blocks osteoblastic Wnt activity, thus favoring an osteolytic phenotype (left panel). As the metastasis progresses and is exposed to growth factors from the resorbing bone, its phenotype changes such that DKK-1 expression is decreased and unmasks Wnt osteoblastic activity resulting in osteosclerosis (right panel). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

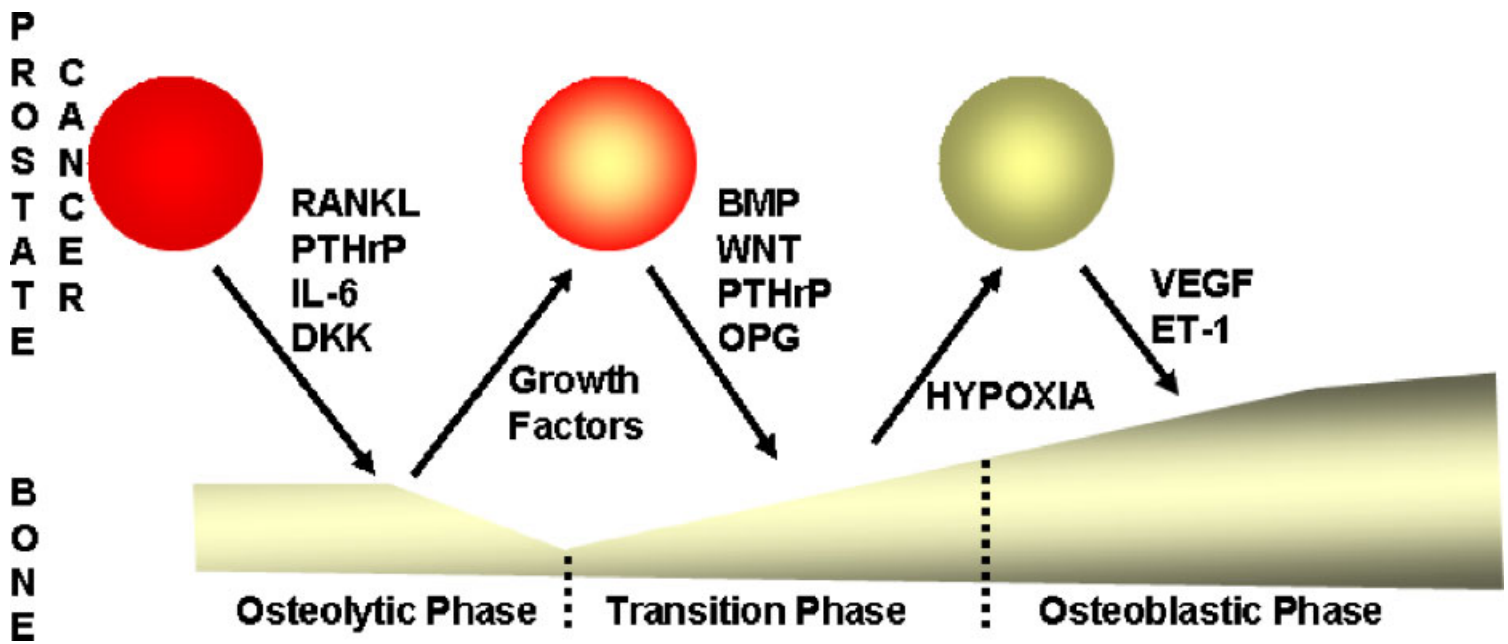

Fig. 4. Integrated model of CaP bone metastasis. Initially when targeting bone, CaP cells produce pro-osteolytic factors such as receptor activator of NFkB ligand (RANKL), interleukin-6 (IL-6), parathyroid hormone-related protein (PTHrP) that stimulate osteoclastogenesis and also produce an inhibitor of osteoblastic activity, dickkopf-1 (DKK-1). The resulting osteolytic activity releases growth factors from the bone and alters the bone microenvironment, which in turn alters the phenotype of the CaP cells. The CaP cells start to produce osteoblastic factors such as bone morphogenetic proteins (BMP), PTHrP (which can act as an anabolic factor) and factors that inhibit osteclastogenic activity, such as, osteoprotegerin (OPG), which blocks RANKL. Additionally, DKK-1 expression is decreased resulting in an unmasking of Wnt osteoblastic activity. This activity effectively transitions the CaP metastasis from an osteolytic to osteoblastic phenotype. As the metastasis continues to grow, it becomes hypoxic, which induces expression of vascular endothelial growth factor (VEGF) and endothelin-1 (ET-1), which both have osteoblastic activity, resulting in a marked induction of bone production and osteosclerosis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

lesions are ultimately osteoblastic, then DKK-1 must at some point be switched off to allow bone formation in CaP metastases (Fig. 3). These data demonstrate, for the first time, that the Wnt pathway contributes to the formation of CaP osteoblastic metastases in vivo and suggest that DKK-1 functions as a molecular switch in CaP that transitions the metastatic lesion from an initial osteolytic to an osteoblastic response.

FINAL THOUGHTS

CaP metastasizes to the bone with high frequency where it produces mixed osteoblastic/osteolytic lesions. Although the precise mechanisms mediating both the organ specificity and bone phenotype of CaP bone metastases are unclear, there is increasing evidence that canonical Wnt signaling could function both in an autocrine and paracrine fashion to facilitate tumor cell growth and osteoblast differentiation. Moreover, the evidence suggests that instead of a vicious cycle of ongoing osteolytic activity, as is observed in breast cancer, that CaP initially has osteolytic activity, which transitions to osteoblastic activity as the metastasis progresses (Fig. 4). We believe the regulation of canonical Wnt signaling by DKK-1 may act as a molecular switch mediating the transition from an osteolytic to an osteoblastic response. Therefore, blocking DKK-1 activity may prove to be a relevant therapeutic target in the prevention of CaP bone metastasis.

ACKNOWLEDGMENTS

Supported in part by National Cancer Institute grants P01 CA093900 and R01 CA071672. We thank Dr. Stuart Aaronson for insightful discussions on Wnts and cancer. We thank many investigators that have contributed to the work on bone metastasis and apologize for work we did not discuss due to length limitations.

REFERENCES

Abrams H, Spiro R, Goldstein N. 1950. Metastases in carcinoma. Cancer 3:74–85.

Babij P, Zhao W, Small C, Kharode Y, Yaworsky PJ, Bouxsein ML, Reddy PS, Bodine PV, Robinson JA, Bhat B, Marzolf J, Moran RA, Bex F. 2003. High bone mass in mice expressing a mutant LRP5 gene. J Bone Miner Res 18:960–974.

Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA. 2001. Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nature Cell Biology 3:683–686.

Bennett CN, Longo KA, Wright WS, Suva LJ, Lane TF, Hankenson KD, MacDougald OA. 2005. Regulation of osteoblastogenesis and bone mass by Wnt10b. Proc Natl Acad Sci USA 102:3324–3329.

Bodine PV, Zhao W, Kharode YP, Bex FJ, Lambert AJ, Goad MB, Gaur T, Stein GS, Lian JB, Komm BS. 2004. The Wnt antagonist secreted frizzled-related protein-1 is a negative regulator of trabecular bone formation in adult mice. Mol Endocrinol 18:1222–1237.

Boland GM, Perkins G, Hall DJ, Tuan RS. 2004. Wnt 3a promotes proliferation and suppresses osteogenic differentiation of adult human mesenchymal stem cells. J Cell Biochem 93:1210–1230.

Boyce BF, Yoneda T, Guise TA. 1999. Factors regulating the growth of metastatic cancer in bone. [Review] [125 refs]. Endocr Relat Cancer 6:333–347.

Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP. 2002. High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 346:1513–1521.

Brabletz T, Jung A, Dag S, Hlubek F, Kirchner T. 1999. beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. Am J Pathol 155:1033–1038.

Bubendorf L, Schopfer A, Wagner U, Sauter G, Moch H, Willi N, Gasser TC, Mihatsch MJ. 2000. Metastatic patterns of prostate cancer: An autopsy study of 1,589 patients. Human Pathology 31:578–583.

Chen S, Guttridge DC, You Z, Zhang Z, Fribley A, Mayo MW, Kitajewski J, Wang CY. 2001. Wnt-1 signaling inhibits apoptosis by activating beta-catenin/T cell factor-mediated transcription. J Cell Biol 152:87–96.

Cheng SL, Shao JS, Charlton-Kachigian N, Loewy AP, Towler DA. 2003. MSX2 promotes osteogenesis and suppresses adipogenic differentiation of multipotent mesenchymal progenitors. J Biol Chem 278:45969–45977.

Chirgwin JM, Mohammad KS, Guise TA. 2004. Tumor-bone cellular interactions in skeletal metastases. J Musculoskelet Neuronal Interact 4:308–318.

Church VL, Francis-West P. 2002. Wnt signalling during limb development. [Review] [119 refs]. Int J Dev Biol 46:927–936.

Corey E, Brown LG, Quinn JE, Poot M, Roudier MP, Higano CS, Vessella RL. 2003. Zoledronic Acid exhibits inhibitory effects on osteoblastic and osteolytic metastases of prostate cancer. Clin Cancer Res 9:295–306.

Crawford HC, Fingleton BM, Rudolph-Owen LA, Goss KJ, Rubinfeld B, Polakis P, Matrisian LM. 1999. The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors. Oncogene 18:2883–2891.

Cronauer MV, Schulz WA, Ackermann R, Burchardt M. 2005. Effects of WNT/beta-catenin pathway activation on signaling through T-cell factor and androgen receptor in prostate cancer cell lines. Int J Oncol 26:1033–1040.

Dai J, Kitagawa Y, Zhang J, Yao Z, Mizokami A, Cheng S, Nor J, McCauley LK, Taichman RS, Keller ET. 2004. Vascular endothelial growth factor contributes to the prostate cancer-induced osteoblast differentiation mediated by bone morphogenetic protein. Cancer Res 64:994–999.

Dai J, Keller J, Zhang J, Lu Y, Yao Z, Keller ET. 2005. Bone morphogenetic protein-6 promotes osteoblastic prostate

cancer bone metastases through a dual mechanism. Cancer Res 65:8274–8285.

Day TF, Guo X, Garrett-Beal L, Yang Y. 2005. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 8:739–750.

De Boer J, Siddappa R, Gaspar C, van Apeldoorn A, Fodde R, van Blitterswijk C. 2004. Wnt signaling inhibits osteogenic differentiation of human mesenchymal stem cells. Bone 34:818–826.

Derfoul A, Carlberg AL, Tuan RS, Hall DJ. 2004. Differential regulation of osteogenic marker gene expression by Wnt-3a in embryonic mesenchymal multipotential progenitor cells. Differentiation 72:209–223.

Farr GH 3rd, Ferkey DM, Yost C, Pierce SB, Weaver C, Kimelman D. 2000. Interaction among GSK-3, GBP, axin, and APC in Xenopus axis specification. J Cell Biol 148:691–702.

Fizazi K, Yang J, Peleg S, Sikes CR, Kreimann EL, Daliani D, Olive M, Raymond KA, Janus TJ, Logothetis CJ, Karsenty G, Navone NM. 2003. Prostate cancer cells-osteoblast interaction shifts expression of growth/survival-related genes in prostate cancer and reduces expression of osteoprotegerin in osteoblasts. Clin Cancer Res 9:2587–2597.

Gaur T, Lengner CJ, Hovhannisyan H, Bhat RA, Bodine PV, Komm BS, Javed A, van Wijnen AJ, Stein JL, Stein GS, Lian JB. 2005. Canonical WNT signaling promotes osteogenesis by directly stimulating RUNX2 gene expression. J Biol Chem 280(39):33132–33140.

Giles RH, van Es JH, Clevers H. 2003. Caught up in a Wnt storm: Wnt signaling in cancer. [Review] [353 refs]. Biochimica et Biophysica Acta 1653:1–24.

Glass DA 2nd, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, Taketo MM, Long F, McMahon AP, Lang RA, Karsenty G. 2005. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell 8:751–764.

Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML. 2001. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 107:513–523.

Grotewold L, Ruther U. 2002. The Wnt antagonist Dickkopf-1 is regulated by Bmp signaling and c-Jun and modulates programmed cell death. EMBO J 21:966–975.

Guise TA. 2000. Molecular mechanisms of osteolytic bone metastases. Cancer 88:2892–2898.

Hall CL, Tsan R, Mugnai G, Mazar A, Radinsky R, Pettaway CA. 2004. Enhanced invasion of hormone refractory prostate cancer cells through hepatocyte growth factor (HGF) induction of urokinase-type plasminogen activator (u-PA). Prostate 59:167–176.

Hall CL, Bafico A, Dai J, Aaronson SA, Keller ET. 2005. Prostate cancer cells promote osteoblastic bone metastases through Wnts. Cancer Res 65:7554–7560.

He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW. 1998. Identification of c-MYC as a target of the APC pathway. [comment]. Science 281:1509–1512.

Hecht A, Vleminckx K, Stemmler MP, van Roy F, Kemler R. 2000. The p300/CBP acetyltransferases function as transcriptional coactivators of beta-catenin in vertebrates. EMBO J 19:1839–1850.

Herynk MH, Tsan R, Radinsky R, Gallick GE. 2003. Activation of c-Met in colorectal carcinoma cells leads to constitutive association of tyrosine-phosphorylated beta-catenin. Clin Exp Metastasis 20:291–300.

Hill TP, Spater D, Taketo MM, Birchmeier W, Hartmann C. 2005. Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell 8:727–738.

Holmen SL, Zylstra CR, Mukherjee A, Sigler RE, Faugere MC, Bouxsein ML, Deng L, Clemens TL, Williams BO. 2005. Essential role of beta-catenin in postnatal bone acquisition. J Biol Chem 280:21162–21168.

Hu H, Hilton MJ, Tu X, Yu K, Ornitz DM, Long F. 2005. Sequential roles of Hedgehog and Wnt signaling in osteoblast development. Development 132:49–60.

Huguet EL, McMahon JA, McMahon AP, Bicknell R, Harris AL. 1994. Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue. Cancer Res 54:2615–2621.

Humphrey PA, Zhu X, Zarnegar R, Swanson PE, Ratliff TL, Vollmer RT, Day ML. 1995. Hepatocyte growth factor and its receptor (c-MET) in prostatic carcinoma. Am J Pathol 147:386–396.

Hwang SG, Ryu JH, Kim IC, Jho EH, Jung HC, Kim K, Kim SJ, Chun JS. 2004. Wnt-7a causes loss of differentiated phenotype and inhibits apoptosis of articular chondrocytes via different mechanisms. J Biol Chem 279:26597–26604.

Jackson A, Vayssiere B, Garcia T, Newell W, Baron R, Roman-Roman S, Rawadi G. 2005. Gene array analysis of Wnt-regulated genes in C3H10T1/2 cells. Bone 36:585–598.

Jeon MJ, Kim JA, Kwon SH, Kim SW, Park KS, Park SW, Kim SY, Shin CS. 2003. Activation of peroxisome proliferator-activated receptor-gamma inhibits the Runx2-mediated transcription of osteocalcin in osteoblasts. J Biol Chem 278:23270–23277.

Jiang W, Hiscox S, Matsumoto K, Nakamura T. 1999. Hepatocyte growth factor/scatter factor, its molecular, cellular and clinical implications in cancer. [Review] [376 refs]. Crit Rev Oncol Hematol 29:209–248.

Johnson ML. 2004. The high bone mass family—The role of Wnt/Lrp5 signaling in the regulation of bone mass. J Musculoskelet Neuronal Interact 4:135–138.

Kato M, Patel MS, Levasseur R, Lobov I, Chang BH, Glass DA 2nd, Hartmann C, Li L, Hwang TH, Brayton CF, Lang RA, Karsenty G, Chan L. 2002. Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. J Cell Biol 157:303–314.

Keller ET, Zhang J, Cooper CR, Smith PC, McCauley LK, Pienta KJ, Taichman RS. 2001. Prostate carcinoma skeletal metastases: Cross talk between tumor and bone. Cancer Metastasis Rev 20:333–349.

Kennell JA, MacDougald OA. 2005. Wnt signaling inhibits adipogenesis through beta-catenin-dependent and -independent mechanisms. J Biol Chem 280:24004–24010.

Kikuchi A. 2000. Regulation of beta-catenin signaling in the Wnt pathway. Biochem Biophys Res Commun 268: 243–248.

Koeneman KS, Yeung F, Chung LW. 1999. Osteomimetic properties of prostate cancer cells: A hypothesis supporting the predilection of prostate cancer metastasis and growth in the bone environment. [Review] [153 refs]. Prostate 39:246–261.

Kuhl M, Sheldahl LC, Park M, Miller JR, Moon RT. 2000. The Wnt/Ca^{2+} pathway: A new vertebrate Wnt signaling pathway takes shape. [Review] [27 refs]. Trends Genet 16:279–283.

Kuhnert F, Davis CR, Wang HT, Chu P, Lee M, Yuan J, Nusse R, Kuo CJ. 2004. Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. Proc Natl Acad Sci USA 101:266–271.

Lejeune S, Huguet EL, Hamby A, Poulsom R, Harris AL. 1995. Wnt5a cloning, expression, and up-regulation in human primary breast cancers. Clin Cancer Res 1:215–222.

Levy L, Neuveut C, Renard CA, Charneau P, Branchereau S, Gauthier F, Van Nhieu JT, Cherqui D, Petit-Bertron AF, Mathieu D, Buendia MA. 2002. Transcriptional activation of interleukin-8 by beta-catenin-Tcf4. J Biol Chem 277:42386–42393.

Li J, Mizukami Y, Zhang X, Jo WS, Chung DC. 2005a. Oncogenic K-ras stimulates Wnt signaling in colon cancer through inhibition of GSK-3beta. Gastroenterology 128:1907–1918.

Li X, Liu P, Liu W, Maye P, Zhang J, Zhang Y, Hurley M, Guo C, Boskey A, Sun L, Harris SE, Rowe DW, Ke HZ, Wu D. 2005b. Dkk2 has a role in terminal osteoblast differentiation and mineralized matrix formation. Nat Genet 37:945–952.

Lin DL, Tarnowski CP, Zhang J, Dai J, Rohn E, Patel AH, Morris MD, Keller ET. 2001. Bone metastatic LNCaP-derivative C4-2B prostate cancer cell line mineralizes in vitro. Prostate 47:212–221.

Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao SC, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, FitzGerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogues X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recker SM, Van Eerdewegh P, Recker RR, Johnson ML. 2002. A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet 70:11–19.

Logan CY, Nusse R. 2004. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 20: 781–810.

Logothetis CJ, Lin SH. 2005. Osteoblasts in prostate cancer metastasis to bone. [Review] [83 refs]. Nat Rev Cancer 5:21–28.

Longo KA, Kennell JA, Ochocinska MJ, Ross SE, Wright WS, MacDougald OA. 2002. Wnt signaling protects 3T3-L1 preadipocytes from apoptosis through induction of insulin-like growth factors. J Biol Chem 277:38239–38244.

Mao B, Wu W, Li Y, Hoppe D, Stannek P, Glinka A, Niehrs C. 2001. LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. [see comment]. Nature 411:321–325.

Mao B, Wu W, Davidson G, Marhold J, Li M, Mechler BM, Delius H, Hoppe D, Stannek P, Walter C, Glinka A, Niehrs C. 2002. Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. Nature 417: 664–667.

Monga SP, Mars WM, Pediaditakis P, Bell A, Mule K, Bowen WC, Wang X, Zarnegar R, Michalopoulos GK. 2002. Hepatocyte growth factor induces Wnt-independent nuclear translocation of beta-catenin after Met-beta-catenin dissociation in hepatocytes. Cancer Res 62:2064–2071.

Morony S, Capparelli C, Sarosi I, Lacey DL, Dunstan CR, Kostenuik PJ. 2001. Osteoprotegerin inhibits osteolysis and decreases skeletal tumor burden in syngeneic and nude mouse models of experimental bone metastasis. Cancer Res 61:4432–4436.

Mukhopadhyay M, Shtrom S, Rodriguez-Esteban C, Chen L, Tsukui T, Gomer L, Dorward DW, Glinka A, Grinberg A, Huang SP, Niehrs C, Belmonte JC, Westphal H. 2001. Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. Developmental Cell 1:423–434.

Mulholland DJ, Cheng H, Reid K, Rennie PS, Nelson CC. 2002. The androgen receptor can promote beta-catenin nuclear translocation independently of adenomatous polyposis coli. J Biol Chem 277:17933–17943.

Muller T, Bain G, Wang X, Papkoff J. 2002. Regulation of epithelial cell migration and tumor formation by beta-catenin signaling. Exp Cell Res 280:119–133.

Mundy GR. 1997. Mechanisms of bone metastasis. Cancer 80:1546–1556.

Nelson JB, Nabulsi AA, Vogelzang NJ, Breul J, Zonnenberg BA, Daliani DD, Schulman CC, Carducci MA. 2003. Suppression of prostate cancer induced bone remodeling by the endothelin receptor A antagonist atrasentan. J Urol 169:1143–1149.

Nuttall ME, Gimble JM. 2004. Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications. Curr Opin Pharmacol 4:290–294.

Pinson KI, Brennan J, Monkley S, Avery BJ, Skarnes WC. 2000. An LDL-receptor-related protein mediates Wnt signalling in mice. Nature 407:535–538.

Pisters LL, Troncoso P, Zhau HE, Li W, von Eschenbach AC, Chung LW. 1995. c-met proto-oncogene expression in benign and malignant human prostate tissues. J Urol 154:293–298.

Rawadi G, Vayssiere B, Dunn F, Baron R, Roman-Roman S. 2003. BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop. J Bone Miner Res 18:1842–1853.

Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, Hintz L, Nusse R, Weissman IL. 2003. A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature 423:409–414.

Roodman GD. 2004. Mechanisms of bone metastasis.[see comment]. [Review] [86 refs]. N Engl J Med 350:1655–1664.

Rubin J, Chung LW, Fan X, Zhu L, Murphy TC, Nanes MS, Rosen CJ. 2004. Prostate carcinoma cells that have resided in bone have an upregulated IGF-I axis. Prostate 58:41–49.

Schneider A, Kalikin LM, Mattos AC, Keller ET, Allen MJ, Pienta KJ, McCauley LK. 2005. Bone turnover mediates preferential localization of prostate cancer in the skeleton. Endocrinology 146:1727–1736.

Shtutman M, Zhurinsky J, Simcha I, Albanese C, D'Amico M, Pestell R, Ben Ze'ev A. 1999. The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. Proc Natl Acad Sci USA 96:5522–5527.

Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy JD Jr. 2003. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma.[see comment]. N Engl J Med 349: 2483–2494.

Van De Wetering M, Cavallo R, Dooijes D, van Beest M, van Es J, Loureiro J, Ypma A, Hursh D, Jones T, Bejsovec A, Peifer M, Mortin M, Clevers H. 1997. Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell 88:789–799.

van der Horst G, van der Werf SM, Farih-Sips H, van Bezooijen RL, Lowik CW, Karperien M. 2005. Downregulation of Wnt Signaling by Increased Expression of Dickkopf-1 and -2 is a Prerequisite for Late-Stage Osteoblast Differentiation of KS483 Cells. J Bone Miner Res 20:1867–1877.

Van Wesenbeeck L, Cleiren E, Gram J, Beals RK, Benichou O, Scopelliti D, Key L, Renton T, Bartels C, Gong Y, Warman ML, De Vernejoul MC, Bollerslev J, Van Hul W. 2003. Six novel missense mutations in the LDL receptor-related protein 5. LRP5 gene in different conditions with an increased bone density. Am J Hum Genet 72:763–771.

Veeman MT, Axelrod JD, Moon RT. 2003. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. [Review] [94 refs]. Dev Cell 5:367–377.

Westendorf JJ, Kahler RA, Schroeder TM. 2004. Wnt signaling in osteoblasts and bone diseases. Gene 341:19–39.

Wielenga VJ, Smits R, Korinek V, Smit L, Kielman M, Fodde R, Clevers H, Pals ST. 1999. Expression of CD44 in Apc and Tcf mutant mice implies regulation by the WNT pathway. Am J Pathol 154:515–523.

Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, Yates JR 3rd, Nusse R. 2003. Wnt proteins are lipid-modified and can act as stem cell growth factors. Nature 423:448–452.

Yanagawa S, van Leeuwen F, Wodarz A, Klingensmith J, Nusse R. 1995. The dishevelled protein is modified by wingless signaling in Drosophila. Genes Dev 9:1087–1097.

Yang Y. 2003. Wnts and wing: Wnt signaling in vertebrate limb development and musculoskeletal morphogenesis. [Review] [139 refs]. Birth Defects Res C Embryo Today 69(4):305–317.

Zechner D, Fujita Y, Hulsken J, Muller T, Walther I, Taketo MM, Crenshaw EB 3rd, Birchmeier W, Birchmeier C. 2003. beta-Catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the nervous system. Dev Biol 258:406–418.

Zhang J, Dai J, Qi Y, Lin DL, Smith P, Strayhorn C, Mizokami A, Fu Z, Westman J, Keller ET. 2001. Osteoprotegerin inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone. J Clin Invest 107:1235–1244.

Zhang J, Dai J, Yao Z, Lu Y, Dougall W, Keller ET. 2003. Soluble receptor activator of nuclear factor kappaB Fc diminishes prostate cancer progression in bone. Cancer Res 63:7883–7890.