Cancer Metastasis Rev (2006) 25:551–558
DOI 10.1007/s10555-006-9022-2

The role of Wnts in bone metastases

Christopher L. Hall · Evan T. Keller

Published online: 9 December 2006

Abstract Wnts are a large family of secreted glycoproteins that mediate bone development in the embryo and promote bone production in the adult. Autocrine Wnt signaling within tumor cells has been shown to promote tumorigenesis by enhancing tumor cell proliferation and survival. We recently demonstrated that prostate cancer cells (CaP) produce Wnts which act in a paracrine fashion to induce osteoblastic activity in CaP bone metastases. The ability of tumor-derived Wnts to influence bone development is regulated by multiple families of secreted antagonists including soluble frizzled related receptors (sFrp) and dickkopfs (DKK). CaP cells appear to produce DKK-1 early in the development of skeletal metastases, which masks osteogenic Wnts and thus favors an osteolytic environment at the metastatic site. As the metastases progresses, DKK-1 expression is lost allowing for a Wnt mediated osteoblastic response which predominates CaP boney lesions. Interestingly, blocking DKK-1 expression early in CaP metastasis prevents tumor establishment within the bone suggesting that osteolysis is a required first step in the development of CaP bone metastases. In this review, we discuss our data on the Wnt inhibitor DKK-1 in CaP bone metastasis in the context of current literature evidence that demonstrate that Wnt inhibitors can function as both tumor suppressors and tumor promoters. We provide a model that the affect of Wnt inhibitors on tumor development is dependent on the tumor micro-environment and suggest that DKK-1 is a switch which transitions CaP bone metastases from osteolytic to osteoblastic.

C. L. Hall · E. T. Keller (✉)
Department of Urology, The University of Michigan,
RM 5304 CCGCB, 1500 East Medical Center Drive,
Ann Arbor, MI 48109-0940, USA
e-mail: etkeller@umich.edu

Keywords Prostate cancer · DKK · Metastasis · Wnt · Bone

1 Introduction

Prostate cancer (CaP) is a significant health problem among men in the United States. In 2005, CaP was the most frequently diagnosed cancer in men and was the second leading cause of cancer-related deaths within this group [1]. Death from CaP follows the metastatic spread of the disease from the prostate to the dura, liver, lung, and bone. The most common site of CaP metastasis is the bone. Recent autopsy data suggests that greater than 80% of men who die of CaP have skeletal metastasis, specifically in the marrow containing trabecular bone of the pelvis, femur, or vertebral bodies [2]. Growth of CaP within these sites results in extensive remodeling of the surrounding bone. The majority of cancers, such as breast and myeloma, produce areas of bone lysis (osteolytic lesion) when they metastasize to bone [3]. CaP is unique in that, in addition an osteolytic component, CaP skeletal metastases are characterized by regions of bone formation (osteoblastic lesion) [4]. The mechanisms through which CaP promotes aberrant bone remodeling are not clearly defined. 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 review, we will discuss our work on Wnts and focus specifically on the role of the Wnt inhibitor DKK-1 in CaP bone metastasis.

2 Wnt signal transduction

The Wnt proteins are a large family of cysteine-rich glycoproteins that function primarily during development

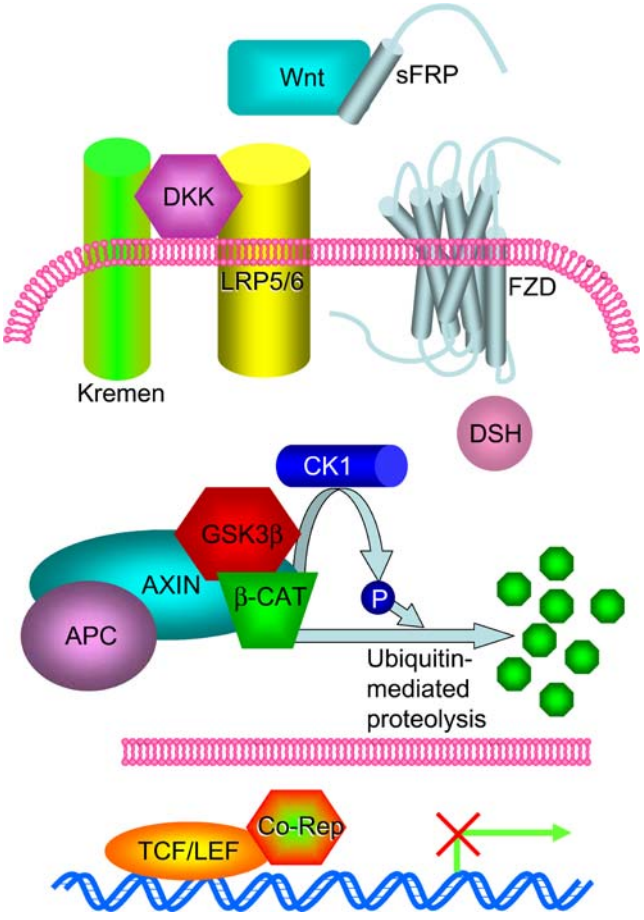

Fig. 1 Secreted inhibitors of Wnt signaling. Soluble Frizzled related protein (*sFRP*) binds directly to Wnt protein and prevents the interaction with Frizzled (*FZD*) receptor. Dickkopf (*DKK*) binds both Kremen and the low-density lipoprotein receptor-related protein (*LRP*) 5/6 which results in the removal of the LRP co-receptor from the cell surface. *APC*, Adenamatous polyposis coli; *b-cat*, b-catenin; *CBP*, CREB binding protein; *CK*, casein kinase; *Co-rep*, corepressor; *DSH*, disheveled; *FZD*, frizzled; *GBP*,GSK binding protein; *GSK*, glycogen synthase kinase; *LEF*, lymphoid enhancer factor; *P*, phosphorus; *TCF*, T-cell factor

to control body axis symmetry and branching morphogenesis [reviewed in [5]]. Wnts are functionally separated into two classes based on their ability to induce duplication of body axis in Xenopus; Wnt8 and Wnt5a, respectively. The differential ability to induce axis duplication suggested that the Wnts in each class use different signal transduction mechanisms. Presently, three separate Wnt signal transduction pathways have been identified, canonical, planar cell polarity, and Wnt/Ca^{2+} pathways. Wnt proteins that activate the canonical pathway signal through β-catenin and, in general, belong to the Wnt8 class. Canonical Wnt proteins bind at the cell surface to a co-receptor consisting of Frizzled (FZD) and low density lipoprotein receptor-related protein 5/6 (LRP). This union results in the hyperphosphorylation of disheveled (DSH) which blocks the activity of glycogen synthase kinase 3β (GSK3β) through an unknown mechanism [6]. 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) [7]. 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 [8]. 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 require lrp co-receptors nor do they activate β-catenin/TCF [9]. 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 [10, 11].

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 Dickkopfs (DKK) [5] (Fig. 1). 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 [12, 13]. Specifically, DKK-1 bridges LRP with Kremin2 resulting in the removal of LRP from the cell surface by endocytosis [14].

3 Wnts in bone development

The bone is in a constant state of remodeling. Under homeostatic conditions, bone remodeling is tightly coupled such that no net loss or gain of bone occurs. Mineralized bone matrix is resorbed by the action of osteoclasts and resulting resorptive pits are filled by osteoblasts recruited to the defect [15]. Osteoclasts differentiate from bone marrow mononuclear cells through the action of receptor activator of NFkB ligand (RANKL) [16]. RANKL is a cell-associated ligand abundantly expressed on mature osteoblasts. The receptor, RANK, is specifically expressed on osteoclast precursors and contributes to the tight coupling of bone remodeling. The activity of RANKL is controlled by a secreted antagonist, osteoprotegerin (OPG), which prevents RANK/RANKL interactions and thus osteoclastogenesis [16]. Osteoblasts, in contrast, differentiate from cells of the mesenchymal lineage, specifically from bone marrow mesenchymal stem cells. Unlike RANKL, no single obligate factor has been shown to mediate osteoblast differentiation but rather a variety of factors contribute to osteoblast formation including insulin-like growth factor,

endothelin-1, and bone morphogenetic proteins [17]. A family of proteins that have recently received a significant amount of attention in the control of osteoblast differentiation are the Wnt proteins.

The use of knockout animals has demonstrated an indispensable role for Wnt signaling 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 [9, 18]]. 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 [19, 20]. The effect of DKK-1 deficiency in the mouse may relate to a reduction in programmed cell death in the limb [19, 20]. Indeed, the over-expression of DKK-1 in the chick results in elevated programmed cell death concomitant with the observed limb truncation [19]. 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 [21]. 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.

4 The role of Wnts in bone metastasis

4.1 Wnt signaling in cancer

Wnts have been implicated in the conversion of normal cells to neoplastic cells (i.e., oncogenesis). Specifically, the role of canonical Wnt signaling in tumor development of colorectal cancer (CRC) has been well established. For review see [22]. In CRC, Wnt proteins do not cause inappropriate signaling but rather loss of APC, part of the β-catenin degrading coplex, occurs in over 85% of all sporadic forms of CRC and nearly all FAP cases [22]. In an additional 10% of CRC cases, mutations in the regulatory region of β-catenin are found [22]. 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, including the transcription factor c-Myc [23], the cell cycle regulatory protein cyclin-D1 [24], the angiogenic factor and chemokine IL-8 [25], and the proteases matrilysin and MMP7 [26, 27] which have obvious implications to tumor development and progression.

There is growing evidence that β-catenin may become activated in tumor cells through cross-talk with non-Wnt signal transduction pathways. For example, the androgen receptor (AR) has been shown to directly interact with and mediate nuclear translocation of β-catenin in CaP cells resulting in induction of AR-dependent gene transcription [28–30]. Alternatively, the AR has been shown to be a target of Wnt signaling [31]. It has been suggested that temporal regulation of GSK3β is associated with the conversion of CaP from an androgen dependent to and androgen independent tumor [32]. This temporal change of GSK3β could be secondary to alteration of Wnt activity, which would then impact GSK3β function. Furthermore, β-catenin and T-cell factor have been implicated to interact with endothelin-1 (ET-1) [33] which can impact bone metastases. The activation of β-catenin has also been associated with anti-apoptotic activity in CaP cells although the mechanism through which it was activated was not defined [34]. In summary, Wnts clearly mediate oncogenesis of certain tumors; however, in the case of CaP there is evidence that Wnts contribute to AR activation, although the methods through which Wnt signaling is increased in CaP are unknown.

Data implicating a direct role of individual Wnt factors in tumorgenesis vs. alterations in Wnt signal transduction pathways are limiting although a role for Wnt1, Wnt2, and Wnt 5a have been suggested. Specifically, Wnt 1 was found to be over-expressed in late stage CaP and metastases [35]. Blockade of Wnt 2 in non-small cell lung cancer induced apoptosis *in vitro* through downregulation of Survivin [36]. Wnt 5a over-expression increased *in vitro* invasion of human melanoma cells [37] and was required for macrophage-induced invasion of breast cancer cells [38]. Despite the limited evidence for Wnt proteins, the importance of outside–in Wnt signaling in cancer is demonstrated by the profound effect that alterations in Wnt inhibitor expression has on both tumor development and progression.

4.2 Wnt inhibitors in cancer

As discussed above, Wnt inhibitors, such as sFRPs, WIF-1, and DKKs, are secreted antagonists that suppress the activity of Wnts proteins. Unlike Wnts, Wnt inhibitors can have both tumor suppressor and tumor promoting activities [39]. Whether a particular inhibitor functions as a suppressor or promoter appears to be dependent on cell type as well as the nature of the surrounding tumor microenvironment. For example, over-expression of the canonical Wnt inhibitor DKK-1 prevented the subcutaneous (sub-Q) growth of DLD-1 colon cancer cells [40] where as its expression in C4-2B CaP cells increased the development of osteolytic lesions with in the bone [41] (Table 1).

Table 1 Wnt inhibitors are both suppressors and promoters of tumor growth

Wnt inhibitor	Tumor suppressor activity	Tumor promoter activity
sFRP-1	42	52
sFRP-2	–	53, 54
sFRP-3	47	–
sFRP-4	–	43, 50
WIF-1	43–46	–
DKK-1	40, 48	51, 55, 41
DKK-3	49	–

Numbers indicate references.

4.2.1 Wnt inhibitors as suppressors of tumor growth

Given the significance of activating mutations of the Wnt pathway in tumorigenesis, it is perhaps not surprising that Wnt inhibitors can function as bona fide tumor suppressor genes. Like many tumor suppressors, Wnt inhibitors are frequently lost or inactivated in carcinomas. For example, the expression of sFRP1 was found to be lost in 80% of clinical samples of invasive breast cancinomas [42]. The expression of WIF-1 was down-regulated in 64% of primary prostate cancers [43] and was frequently found to undergo epigenetic inactivation (methylation) in clinical specimens of gastrointestinal cancers [44], bladder cancer [45], and lung cancer [46]. Direct evidence for a role of Wnt inhibitors as tumor suppressors comes from *in vivo* studies following transfection. Specifically, expression of sFRP3/Frzb, which is frequently deleted in cancer, in PC-3 CaP cells resulted in decreased *in vitro* invasion, soft agar colony formation and Sub-Q tumor growth [47]. The expression of DKK-1 was shown to decrease in 56% of human colorectal cancer (CRC) [48] and was hypermethylated in 17% of primary and 24% of advanced stage cases [40]. Consistent with this observation, expression of DKK-1 in DLD-1 CRC cells suppressed sub-Q tumor growth [40]. DKK-3/REIC is a DKK like molecule that does not block canonical Wnts but that has tumor suppressive action. DKK-3 was found to be down-regulated in half of human hepatoma samples and its expression in Hep3B hepatocellular carcinoma cells suppressed colony formation *in vitro* and reduced tumor growth *in vivo* [49]. Taken together, these data demonstrate that Wnt inhibitors can block tumor development and therefore function as tumor suppressors.

4.2.2 Wnt inhibitors as promoters of tumor growth.

In opposition of a role of Wnts inhibitors as tumor suppressors is the observation that several Wnt antagonists are over-expressed in multiple tumor types. For example, 81% of both human CRC and CaP samples were shown to have increased expression of sFRP4 protein or message compared to normal tissue [43, 50]. The gene for DKK-1 was over-expressed in 81% of human hepatoblastoma samples compared to normal [51] demonstrating that tumor development occurred in the presence of DKK-1. The expression of sFRP1 in BPH1 prostatic epithelial cells increased *in vivo* proliferation (as measured by tumor weight and Ki67 staining) of cells injected under the renal capsule of nude mice [52]. Finally, sFRP2 was shown to be produced by primary multiple myeloma samples [53] and *ex vivo* glioma cells lines where the enforced expression of this gene promoted glioma cell growth and survival *in vivo* [54]. Viewed together, these data clearly demonstrate that Wnt inhibitors can have tumor promoting activity.

4.2.3 Wnt inhibitors and bone metastasis

The discussion to this point has assumed that a given Wnt inhibitor is either a tumor suppressor or promoter due to a direct effect of the Wnt on the developing tumor cell. It is possible that the ability of a Wnt inhibitor to affect tumor development is due to its impact on the tumor micro-environment rather than the tumor cell itself. In this view, the nature of a given organ environment would determine whether a Wnt inhibitor is tumor suppressive or promoting. In the context of the bone micro-environment, Wnt antagonists appear to promote the development of osteolytic lesions. The examples are DKK-1 and sFRP2 in multiple myeloma (MM) [53, 55] and DKK-1 in prostate cancer (CaP) [41]. Multiple myeloma is a hematologic tumor that arises in the bone marrow and metastases to the bone to produce bone destroying or osteolytic lesions [3]. As discussed above, human CaP frequently metastasizes to the bone where it produces bone forming (or osteoblastic) lesions with underlying osteolytic areas (reviewed in [17]).

Wnt signaling has been implicated in limb development, skeletal outgrowth, and in the control of bone mass [17]. However, until recently, direct evidence that Wnt factors contribute to the bone phenotype of metastatic tumor cells *in vivo* was lacking. In their seminole study in MM, Tian et al. observed elevated levels of DKK-1 in both bone marrow plasma and peripheral blood of patients with MM [55]. Further, the expression of DKK-1 was increased in the plasma of patients who had one or more osteolytic lesions. Bone marrow plasma from MM patients inhibited osteoblast differentiation *in vitro* suggesting that DKK-1 contributed to the formation of osteolytic disease [55]. Subsequently, it was shown that primary myeloma cells also express the antagonist sFRP2 and that depleting sFRP2 from the conditioned medium of primary cultures permitted osteoblast differentiation [53] again suggesting that Wnt inhibitors contribute to the formation of osteolytic disease.

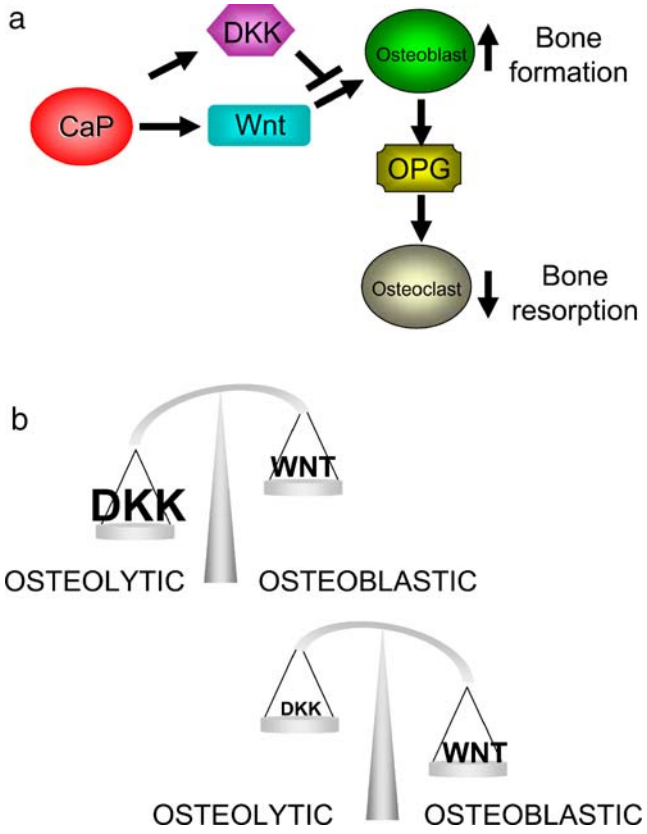

Fig. 2 Model for the role of Wnts in CaP bone metastasis. (a) CaP-derived Wnts stimulate osteoblast differentiation and the formation of an osteoblastic lesion through the activation of b-catenin. Canonical Wnt signaling within the osteoblast in turn suppresses osteoclast formation through the production of osteoprotegerin (*OPG*). (b) In the presence of DKK-1, osteoblast differentiation and Wnt mediated suppression of osteoclast differentiation are blocked allowing osteolysis to predominate the boney lesion

Based on these data we hypothesized that the balance between Wnt and Wnt inhibitors determines the phenotype of bone lesions and therefore sought to test whether canonical Wnts mediate the bone formation in metastatic CaP.

Evaluating Wnt gene expression in human CaP cell lines, we observed 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 [41]. 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 [41]. These data demonstrate, for the first time, that the Wnt pathway contributes to the formation of CaP osteoblastic metastases *in vivo*.

Base on this data, we would anticipate that DKK-1 shRNA transfected PC-3 cells injected into the bone would stimulate significant bone formation. However, when DKK-1 suppressed shRNA cells were injected into the tibias of nude mice, no tumors formed. We concluded from these data that DKK-1 fosters an osteolytic environment which is required for the establishment of CaP tumors within the bone. These data are in keeping with work by Glass et al. that demonstrate that β-catenin signaling within osteoblasts prevents osteoclast formation through the expression of osteoprotegerin [56]. This is significant in that we have previously demonstrated that systemic delivery of OPG prevents the establishment of C4-2B CaP cells within the bone following intratibial injection [57]. Thus, within the bone, the effect of DKK-1 expression may be to block Wnt mediated suppression of osteoclastogenesis that would prevent osteolysis and CaP tumor establishment within the bone (Fig. 2).

An additional role of DKK-1 in bone metastasis is supported by work on bone marrow stem cells/mesenchymal stem cells (MSC). Gregory et al. demonstrated that expression of DKK-1 was required for cell cycle entry of human bone marrow stem cells [58]. If the same require-

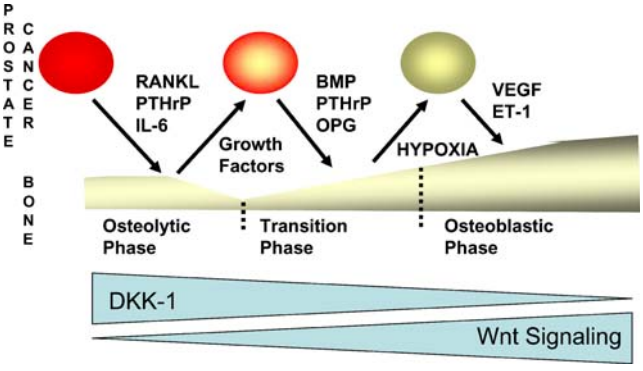

Fig. 3 Integrated model of prostate cancer bone metastasis. Prostate cancer (*CaP*) cells produce pro-osteolytic factors such as receptor activator of NFkB ligand (*RANKL*), interleukin-6 (*IL-6*), parathyroid hormone-related protein (*PTHrP*) that stimulate osteoclastogenesis and also produce an inhibitor of osteoblastic activity, dickkopf-1 (*DKK-1*). The resulting osteolytic activity releases growth factors from the bone which in turn alters the bone microenvironment. As the tumor progresses, DKK-1 expression is decreased resulting in an unmasking of Wnt osteoblastic activity. 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

ment is true of tumor cells, then DKK-1 expression would support tumor cell proliferation and development. Subsequently, DKK-1 expressed by MM cell lines was shown to prevent MSC maturation to maintain a pool of immature stem cells which in turn produced interleukin 6 that stimulated MM proliferation [59]. The maintenance of a stem-cell niche is another example where DKK-1 expression in the context of the bone could promote tumor development.

Our data on Wnt/DKK-1 activity fits a model of CaP-induced bone remodeling occurring in a continuum composed of an osteolytic phase, mediated by osteolytic factors such as RANKL, 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.

5 Final thoughts

Prostate cancer 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. 3). 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.

Acknowledgements This work was supported in part by National Cancer Institute Grants P01 CA093900 and R01 CA071672.

References

1. Logothetis, C. J., & Lin, S. H. (2005). Osteoblasts in prostate cancer metastasis to bone. *Nature Reviews. Cancer, 5*, 21–28.
2. Bubendorf, L., Schopfer, A., Wagner, U., Sauter, G., Moch, H., Willi, N., et al. (2000). Metastatic patterns of prostate cancer: An autopsy study of 1,589 patients. *Human Pathology, 31*, 578–583.
3. Roodman, G. D. (2004). Mechanisms of bone metastasis. *New England Journal of Medicine, 350*, 1655–1664.
4. Keller, E. T., Zhang, J., Cooper, C. R., Smith, P. C., McCauley, L. K., Pienta, K. J., et al. (2001). Prostate carcinoma skeletal metastases: Cross-talk between tumor and bone. Cancer Metastasis Reviews, 20, 333–349.
5. Logan, C. Y., & Nusse, R. (2004). The Wnt signaling pathway in development and disease. *Annual Review of Cell and Developmental Biology, 20*, 781–810.
6. Yanagawa, S., van Leeuwen, F., Wodarz, A., Klingensmith, J., & Nusse, R. (1995). The dishevelled protein is modified by wingless signaling in Drosophila. *Genes & Development, 9*, 1087–1097.
7. van de, W. M., Cavallo, R., Dooijes, D., van Beest, M, van Es, J., Loureiro, J., et al. (1997). Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. *Cell, 88*, 789–799.
8. Kikuchi, A. (2000). Regulation of beta-catenin signaling in the Wnt pathway. [Review] [74 refs]. *Biochemical & Biophysical Research Communications, 268*, 243–248.
9. Yang, Y. (2003). Wnts and wing: Wnt signaling in vertebrate limb development and musculoskeletal morphogenesis. *Birth Defects Research C Embryo Today Part C, Embryo Today, 69*, 305–317.
10. Kuhl, M., Sheldahl, L.C., Park, M., Miller, J.R., Moon, R.T. (2000). The Wnt/Ca2+ pathway: A new vertebrate Wnt signaling pathway takes shape. [Review] [27 refs]. *Trends in Genetics, 16*, 279–283.
11. Veeman, M. T., Axelrod, J. D., & Moon, R. T. (2003). A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. [Review] [94 refs]. *Developments in Cell, 5*, 367–377.
12. Bafico, A., Liu, G., Yaniv, A., Gazit, A., & Aaronson, S. A. (2001). Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. *Nature Cell Biology, 3*, 683–686.
13. Mao, B., Wu, W., Li, Y., Hoppe, D., Stannek, P., Glinka, A., et al. (2001). LDL-receptor-related protein 6 is a receptor for Dickkopf proteins.[see comment]. *Nature, 411*, 321–325.
14. Mao, B., Wu, W., Davidson, G., Marhold, J., Li, M., Mechler, B. M., et al. (2002). Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. *Nature, 417*, 664–667.
15. Buckwalter, J. A., Glimcher, M. J., Cooper, R. R., & Recker, R. (1996). Bone biology. I: Structure, blood supply, cells, matrix, and mineralization. *Instructional Course Lectures, 45*, 371–386.
16. Kostenuik, P. J. (2005). Osteoprotegerin and RANKL regulate bone resorption, density, geometry and strength. *Current Opinion in Pharmacology, 5*, 618–625.
17. Hall, C. L., Kang, S., Macdougald, O. A., & Keller, E. T. (2006). Role of wnts in prostate cancer bone metastases. *Journal of Cellular Biochemistry, 97*, 661–672.
18. Church, V. L., & Francis-West, P. (2002). Wnt signalling during limb development. *Journal of Cellular Biochemistry, 46*, 927–936.
19. Grotewold, L., & Ruther, U. (2002). The Wnt antagonist Dickkopf-1 is regulated by Bmp signaling and c-Jun and modulates programmed cell death. *EMBO Journal, 21*, 966–975.
20. Mukhopadhyay, M., Shtrom, S., Rodriguez-Esteban, C., Chen, L., Tsukui, T., Gomer, L., et al. (2001). Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. *Developments in Cell, 1*, 423–434.
21. Bodine, P. V., Zhao, W., Kharode, Y. P., Bex, F. J., Lambert, A. J., Goad, M. B., et al. (2004). The Wnt antagonist secreted frizzled-related protein-1 is a negative regulator of trabecular bone formation in adult mice. *Molecular Endocrinology, 18*, 1222–1237.
22. Giles, R. H., van Es, J. H., & Clevers, H. (2003). Caught up in a Wnt storm: Wnt signaling in cancer. *Biochimica et Biophysica Acta, 1653*, 1–24.

23. He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., et al. (1998). Identification of c-MYC as a target of the APC pathway.[comment]. *Science, 281*, 1509–1512.
24. Shtutman, M., Zhurinsky, J., Simcha, I., Albanese, C., D'Amico, M., Pestell, R., et al. (1999). The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 5522–5527.
25. Levy, L., Neuveut, C., Renard, C. A., Charneau, P., Branchereau, S., Gauthier, F., et al. (2002). Transcriptional activation of interleukin-8 by beta-catenin-Tcf4. *Journal of Biological Chemistry, 277*, 42386–42393.
26. Crawford, H. C., Fingleton, B. M., Rudolph-Owen, L. A., Goss, K. J., Rubinfeld, B., Polakis, P., et al. (1999). The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors. *Oncogene, 18*, 2883–2891.
27. 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. *American Journal of Pathology, 155*, 1033–1038.
28. Cronauer, M. V., Schulz, W. A., 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. *International Journal of Oncology, 26*, 1033–1040.
29. Mulholland, D. J., Cheng, H., Reid, K., Rennie, P. S., & Nelson, C. C. (2002). The androgen receptor can promote beta-catenin nuclear translocation independently of adenomatous polyposis coli. *Journal of Biological Chemistry, 277*, 17933–17943.
30. Yardy, G. W., & Brewster, S. F. (2005). Wnt signalling and prostate cancer. *Prostate Cancer and Prostatic Diseases, 8*, 119–126.
31. Yang, X., Chen, M. W., Terry, S., Vacherot, F., Bemis, D. L., Capodice, J., et al. (2006). Complex regulation of human androgen receptor expression by Wnt signaling in prostate cancer cells. *Oncogene, 25*, 3436–3444.
32. Mulholland, D. J., Dedhar, S., Wu, H., & Nelson, C. C. (2006). PTEN and GSK3beta: Key regulators of progression to androgen-independent prostate cancer. *Oncogene, 25*, 329–337.
33. Sun, P., Xiong, H., Kim, T. H., Ren, B., & Zhang, Z. (2006). Positive inter-regulation between beta-catenin/T cell factor-4 signaling and endothelin-1 signaling potentiates proliferation and survival of prostate cancer cells. *Molecular Pharmacology, 69*, 520–531.
34. de la Taille, A., Rubin, M. A., Chen, M. W., Vacherot, F., de Medina, S. G., Burchardt, M., et al. (2003). Beta-catenin-related anomalies in apoptosis-resistant and hormone-refractory prostate cancer cells. *Clinical Cancer Research, 9*, 1801–1807.
35. Chen, G., Shukeir, N., Potti, A., Sircar, K., Aprikian, A., Goltzman, D., et al. (2004). Up-regulation of Wnt-1 and beta-catenin production in patients with advanced metastatic prostate carcinoma: Potential pathogenetic and prognostic implications. *Cancer, 101*, 1345–1356.
36. You, L., He, B., Xu, Z., Uematsu, K., Mazieres, J., Mikami, I., et al. (2004). Inhibition of Wnt-2-mediated signaling induces programmed cell death in non-small-cell lung cancer cells. *Oncogene, 23*, 6170–6174.
37. Weeraratna, A. T., Jiang, Y., Hostetter, G., Rosenblatt, K., Duray, P., Bittner, M., et al. (2002). Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell, 1*, 279–288.
38. Pukrop, T., Klemm, F., Hagemann, T., Gradl, D., Schulz, M., Siemes, S., et al. (2006). Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 5454–5459.
39. Rubin, J. S., Barshishat-Kupper, M., Feroze-Merzoug, F., Xi, Z. F. (2006). Secreted WNT antagonists as tumor suppressors: Pro and con. *Frontiers in Bioscience, 11*, 2093–2105.
40. Aguilera, O., Fraga, M. F., Ballestar, E., Paz, M. F., Herranz, M., Espada, J., et al. (2006). Epigenetic inactivation of the Wnt antagonist DICKKOPF-1 (DKK-1) gene in human colorectal cancer. *Oncogene, 25*, 4116–4121.
41. Hall, C. L., Bafico, A., Dai, J., Aaronson, S. A., & Keller, E. T. (2005). Prostate cancer cells promote osteoblastic bone metastases through Wnts. *Cancer Research, 65*, 7554–7560.
42. Ugolini, F., Charafe-Jauffret, E., Bardou, V. J., Geneix, J., Adelaide, J., Labat-Moleur, F., et al. (2001). WNT pathway and mammary carcinogenesis: Loss of expression of candidate tumor suppressor gene SFRP1 in most invasive carcinomas except of the medullary type. *Oncogene, 20*, 5810–5817.
43. Wissmann, C., Wild, P. J., Kaiser, S., Roepcke, S., Stoehr, R., Woenckhaus, M., et al. (2003). WIF1, a component of the Wnt pathway, is down-regulated in prostate, breast, lung, and bladder cancer. *Journal of Pathology, 201*, 204–212.
44. Taniguchi, H., Yamamoto, H., Hirata, T., Miyamoto, N., Oki, M., Nosho, K., et al. (2005). Frequent epigenetic inactivation of Wnt inhibitory factor-1 in human gastrointestinal cancers. *Oncogene, 24*, 7946–7952.
45. Urakami, S., Shiina, H., Enokida, H., Kawakami, T., Tokizane, T., Ogishima, T., et al. (2006). Epigenetic inactivation of Wnt inhibitory factor-1 plays an important role in bladder cancer through aberrant canonical Wnt/beta-catenin signaling pathway. *Clinical Cancer Research, 12*, 383–391.
46. Mazieres, J., He, B., You, L., Xu, Z., Lee, A. Y., Mikami, I., et al. (2004). Wnt inhibitory factor-1 is silenced by promoter hypermethylation in human lung cancer. *Cancer Research, 64*, 4717–4720.
47. Zi, X., Guo, Y., Simoneau, A. R., Hope, C., Xie, J., Holcombe, R. F., et al. (2005). Expression of Frzb/secreted Frizzled-related protein 3, a secreted Wnt antagonist, in human androgen-independent prostate cancer PC-3 cells suppresses tumor growth and cellular invasiveness. *Cancer Research, 65*, 9762–9770.
48. Gonzalez-Sancho, J. M., Aguilera, O., Garcia, J. M., Pendas-Franco, N., Pena, C., Cal, S., et al. (2005). The Wnt antagonist DICKKOPF-1 gene is a downstream target of beta-catenin/TCF and is downregulated in human colon cancer. *Oncogene, 24*, 1098–1103.
49. Hsieh, S. Y., Hsieh, P. S., Chiu, C. T., & Chen, W. Y. (2004). Dickkopf-3/REIC functions as a suppressor gene of tumor growth. *Oncogene, 23*, 9183–9189.
50. Feng Han, Q., Zhao, W., Bentel, J., Shearwood, A. M., Zeps, N., Joseph, D., et al. (2006). Expression of sFRP-4 and beta-catenin in human colorectal carcinoma. *Cancer Letter, 231*, 129–137.
51. Wirths, O., Waha, A., Weggen, S., Schirmacher, P., Kuhne, T., Goodyer, C. G., et al. (2003). Overexpression of human Dickkopf-1, an antagonist of wingless/WNT signaling, in human hepatoblastomas and Wilms' tumors. *Laboratory Investigation, 83*, 429–434.
52. Joesting, M. S., Perrin, S., Elenbaas, B., Fawell, S. E., Rubin, J. S., Franco, O. E., et al. (2005). Identification of SFRP1 as a candidate mediator of stromal-to-epithelial signaling in prostate cancer. *Cancer Research, 65*, 10423–10430.
53. Oshima, T., Abe, M., Asano, J., Hara, T., Kitazoe, K., Sekimoto, E., et al. (2005). Myeloma cells suppress bone formation by secreting a soluble Wnt inhibitor, sFRP-2. *Blood, 106*, 3160–3165.
54. Roth, W., Wild-Bode, C., Platten, M., Grimmel, C., Melkonyan, H. S., Dichgans, J., et al. (2000). Secreted Frizzled-related proteins inhibit motility and promote growth of human malignant glioma cells. *Oncogene, 19*, 4210–4220.
55. Tian, E., Zhan, F., Walker, R., Rasmussen, E., Ma, Y., Barlogie, B., et al. (2003). The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma.[see comment]. *New England Journal of Medicine, 349*, 2483–2494.
56. Glass, D. A., 2nd, Bialek, P., Ahn, J. D., Starbuck, M., Patel, M. S., Clevers, H., et al. (2005). Canonical Wnt signaling in differentiated

osteoblasts controls osteoclast differentiation. *Developments in Cell, 8*, 751–764.
57. Zhang, J., Dai, J., Qi, Y., Lin, D.L., Smith, P., Strayhorn, C., et al. (2001). Osteoprotegerin inhibits prostate cancer-induced osteoclastogenesis and prevents prostate tumor growth in the bone. *Journal of Clinical Investigation, 107*, 1235–1244.
58. Gregory, C. A., Perry, A. S., Reyes, E., Conley, A., Gunn, W. G., & Prockop, D. J. (2005). Dkk-1-derived synthetic peptides and lithium chloride for the control and recovery of adult stem cells from bone marrow. *Journal of Biological Chemistry, 280*, 2309–2323.
59. Gunn, W. G., Conley, A., Deininger, L., Olson, S. D., Prockop, D. J., & Gregory, C. A. (2006). A crosstalk between myeloma cells and marrow stromal cells stimulates production of DKK1 and interleukin-6: A potential role in the development of lytic bone disease and tumor progression in multiple myeloma. *Stem Cells, 24*, 986–991.