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REVIEW

Wnt and Wnt inhibitors in bone metastasis

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Bone metastasis is a clinically devastating development of progressive cancers including prostate carcinoma, breast carcinoma and multiple myeloma. Bone metastases are typically painful, lead to adverse skeletal-related events, such as fracture, and are highly resistant to therapy. A major contribution to the ability of cancers to successfully establish bone metastases is their ability to exploit mechanisms of normal bone remodeling. Wnts are a large family of morphogenic proteins that are critical for bone development and contribute to maintaining bone mass in the mature organism. Wnt function is balanced by the presence of a variety of endogenous inhibitors, such as the dickkopf family members, secreted frizzled related proteins and sclerostin. Together, these factors contribute to normal bone homeostasis, allowing for dynamic changes in bone to withstand alterations in physical forces and physiological needs. In this review, we describe the role that Wnts and their inhibitors have in normal bone biology and cancer-related bone pathology. An overview of Wnt signaling pathways is discussed and key bone microenvironment cellular players, as they pertain to Wnt biology, are examined. Finally, we describe clinical trials of several Wnt inhibitor antagonists for patients with tumor-related bone disease. As few options currently exist for the treatment of bone-metastatic disease, Wnt proteins and their inhibitors offer promise for the development of novel therapeutics.

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Introduction

The *Wnt* gene family is a contraction of the Int family, discovered in 1982 by Nusse and Varmus while studying the integration sites of the mouse mammary tumor virus, and the wingless gene (*Wg*) mutation previously observed in *Drosophila melanogaster*. These genes were found to have similar structure and function, and thus the term Wnt has been used since to describe these shared characteristics. To date, there are 19 members of the Wnt family that share a signal sequence of approximately 350 amino acids in length with a conserved pattern of 23–24 cysteine residues.[1] Wnt proteins are of great importance in development, as they are important for axis formation and morphogenesis in a variety of tissues, including bone. For example, Wnt signaling is critical for differentiation of osteoblasts from progenitor cells, the primary bone mineralizing cells.[2] However, Wnts have also been shown to have a role in carcinogenesis and tumorigenesis. In conjunction with bone morphogenetic proteins (BMPs), Wnts contribute to the formation of bone during development, and control of bone homeostasis in the adult. In this review, we will describe the importance of Wnt proteins in bone development and homeostasis, as well as the interactions that favor tumor growth in the bone microenvironment.

Wnt Biology

The *Wnt* gene family encompasses a number of secreted proteins with a highly conserved glycosylation pattern. Wnts can be broadly classified into canonical and non-canonical signaling mediators. Canonical Wnts are characterized by the ability to stabilize β-catenin and induce gene transcription through co-activators TCF/LEF. Non-canonical pathway activation is typically compromised of cGMP-related calcium signaling, Jun kinase activation (JNK) and/or activation of protein kinase A. However, it has recently been suggested that the specific Wnt itself may not confer specific signaling, but interactions with the various receptors may cause alterations in the pathways utilized.[3] Furthermore, there is evidence that the non-canonical Wnt signaling pathways may also inhibit the canonical pathways.[4]

Wnt signaling is complex owing to the various ligands, receptors and signaling pathways involved, and has been reviewed in great detail.[1,5,6] In brief, canonical Wnt signaling is mediated through inhibition of β-catenin degradation (**Figure 1**). In the absence of Wnt, a degradation complex consisting of axin, glycogen synthase kinase 3β (GSK-3β) and adenomatous polyposis coli (APC) phosphorylates and targets β-catenin for

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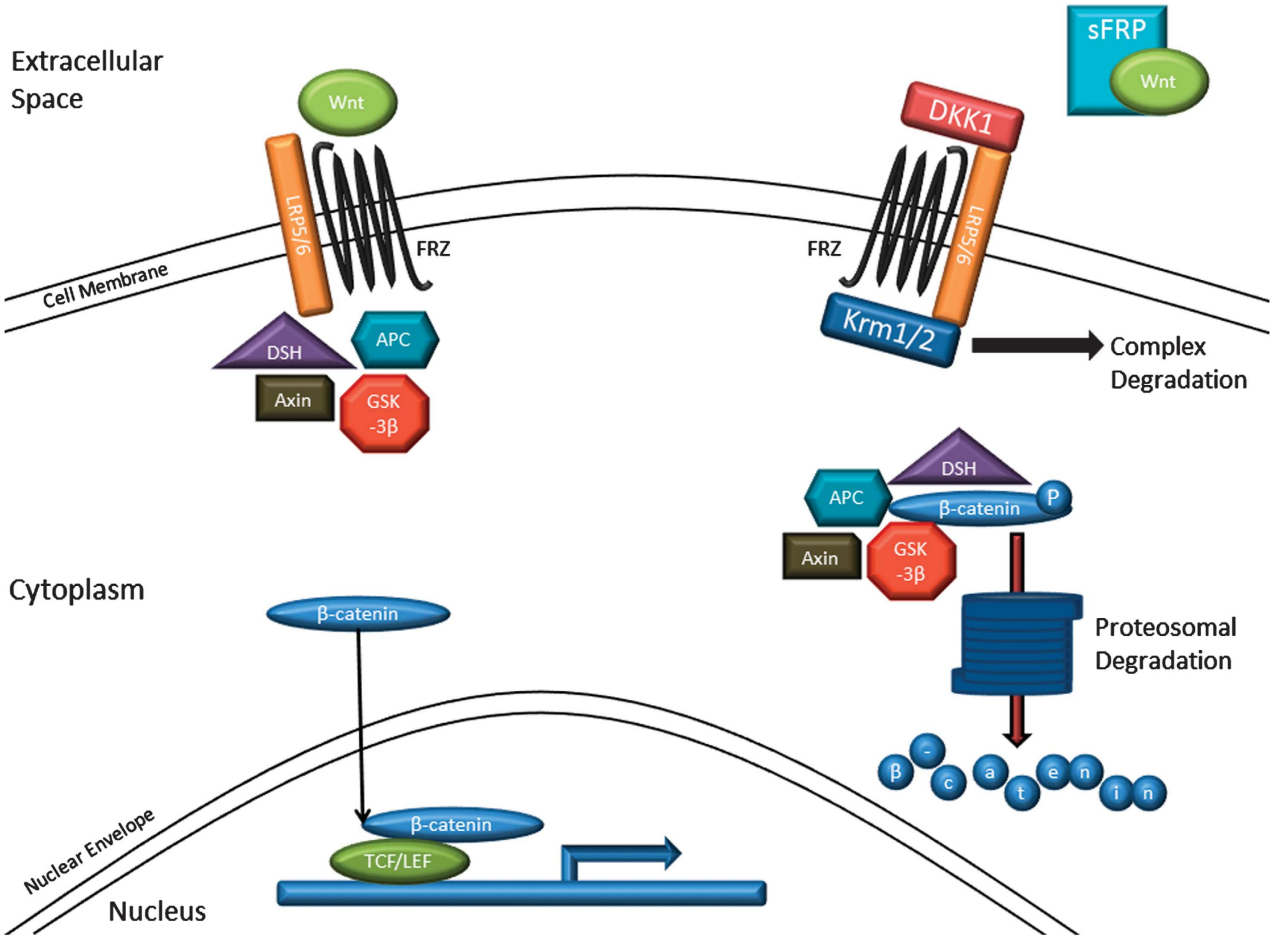

Figure 1 Overview of canonical Wnt signaling. Wnt binding to membrane-bound frizzled (FRZ) receptors and association of low-density LRP5/6 leads to sequestration of the β-catenin phosphorylation complex composed of disheveled (DSH), adenomatous polyposis coli (APC), axin and GSK-3β. Complex formation allows for β-catenin accumulation and translocation to the nucleus, where it binds to the TCF/LEF and initiates transcription. However, Dicckopf-1 (DKK1) can bind to LRP5/6 leading to association of Kremen 1/2 (Krm1/2) leading to complex degradation and inhibition of Wnt binding and signaling initiation. sFRP can also bind Wnt's extracellularly to prevent binding to Frz. Frz-LRP5/6 degradation allows for the axin, DSH, APC and GSK-3β to phosphorylate β-catenin. Phosphorylated β-catenin is subsequently proteosomally degraded.

degradation in the cytoplasm. When Wnt binds to its receptor, typically a member of the frizzled (FRZ) family of membrane-associated proteins on target cells, that leads to complex formation with low-density lipoprotein receptor-related protein (LRP) co-receptor, primarily LRP 5/6 and disheveled (DSH). Promotion of the Wnt/LRP/DSH complex leads to sequestration of the axin/GSK-3β/APC. This promotes β-catenin stabilization and nuclear translocation where it acts as a transcription factor with TCF/LEF.

Wnt signaling is inhibited by two primary gene families, the secreted frizzled-related proteins (sFRP, five members) and the dickkopf family (DKK, four members). The sFRP family is related to the membrane-bound frizzled receptors, and sequesters Wnt from binding to membrane FRZ receptors. sFRP can also interact with FRZ to inhibit the receptor complex directly. sFRPs have been identified as possible tumor promoters in advanced breast tumors, as sFRP1 is downregulated in these cancers, which promotes growth and metastasis conferred by the increased Wnt activity.[7] Increasing sFRP1 expression may be related to osteoblast differentiation, as its expression peaks during the transition of the osteoblast to the osteocyte, this change in sFRP1 may also prevent osteoblast apoptosis.[6] These findings suggest that sFRP1 has a role in forming a negative feedback loop regulating mineralization, as maturing osteoblasts isolate themselves in mineralized ECM to become osteocytes. In addition to sFRP, dickkopfs (DKK) are secreted proteins that inhibit Wnt signaling. DKKs interact with the cell surface membrane component of LRP5/6, and sequester the protein in conjunction with the Kremen proteins, kremen 1–2. The Kremen/LRP/DKK complex leads to internalization, ubiquitination and proteosomal degradation of the complex. Destruction of LRP inhibits the formation of the Wnt/LRP/DSH complex, thereby inhibiting Wnt signaling in the cell. Mutations in LRP5 were originally described in genetic studies of patients with osteoporosis-pseudoglioma syndrome.[8] Knock out of LRP5 in mice is associated with decreased bone mass yet can be rescued through activation of GSK-3β showing the importance of LRP5 in signal initiation.[9] Interestingly, the G171V mutation in LRP5 leads to abnormally increased bone mass and prevents DKK1 binding.[10] LRP5 mutations in mice also produce mice with increased bone mass.[11] Wnt inhibitory factor 1 (WIF-1) has been described as an important mediator of osteoblast maturation, but its role as a mediator of cancer progression in adults has been conflicting.[12,13] Similar to DKK, sclerostin is a related cysteine-rich glycoprotein, which

is predominantly secreted by osteocytes. Sclerostin interaction with LRP5/6 leads to complex formation with kremen and subsequent degradation, therefore leading to inhibition of Wnt signaling. Sclerostin production from osteocytes is inhibited by mechanical loading and parathyroid hormone-related protein (PTHrP) through osterix and Cbfa1, leading to the formation of a negative feedback loop for the catabolic properties of osteoblasts, which are induced by BMPs and Wnts.[14]

Bone development and maintenance is relatively complex considering there are three primary cellular players involved: (1) the osteoclast responsible for mineral degradation and resorption, (2) the osteoblast responsible for bone catabolism and mineralization, and (3) the osteocyte whose primary role is mechanotransduction and coordination of blastic and lytic processes. Wnts have been shown to have a role in promoting osteoblast differentiation through directing bone marrow mesenchymal stem cells into the osteoblast pathway and inhibiting their differentiation into adipocyte and chondrocytes. Additionally, they inhibit the promotion of osteoclast differentiation.[5] *In vitro* evidence suggests that osteoblasts produce Wnts to promote differentiation, allowing for collagen deposition and mineralization of the extracellular matrix. Wnts 1, 2, 3a, 4 and 7b have been found to be osteogenic and produced from calvarial and primary osteoblasts in culture.[6] Induction of alkaline phosphatase, an indicator of osteoblast differentiation and activation, can be readily induced by recombinant Wnt 3a *in vitro*. These effects have been observed to be independent of BMPs, even though the importance of BMPs in bone mineralization cannot be underestimated. Induction of Wnt drives osteoblast differentiation, and as described above, a temporal induction of inhibitors of osteoblastic processes, such as sFRP and DKK. Osteoblasts in turn mature into osteocytes. Osteocytes are primarily responsible for mechanotransduction, the sensation and translation of physical forces into biochemical signals. Increased mechanical loading leads to the induction of osteoblastic processes to strengthen bone, through the induction of Wnt and BMP, while inhibiting Wnt antagonists, such as sclerostin and DKK.[14] However, the reverse is also true, and decreased loading of bone leads to the stabilization and secretion of sclerostin, leading to bone loss.

Canonical Wnt signaling in osteoblasts has been shown to suppress osteoclast function, likely through Wnt-mediated production of osteoprotegerin (OPG).[15,16] OPG acts as a decoy receptor for receptor activator of NFκB ligand (RANKL), a key mediator of osteoclastogenesis, and therefore suppresses bone resorption. Similarly, Wnt may act directly on the osteoclast to inhibit the production of RANKL directly, leading to the same effect of decreased osteoclastic activity and bone resorption.[17] RANKL and OPG are part of the tumor necrosis factor (TNF) superfamily, and it has been suggested, based on a model of rheumatoid arthritis, that inflammatory cell production of TNFα inhibits Wnt activity through the production of DKK1.[18] Similar impacts are plausible in the tumor microenvironment, which contains many inflammatory-related cells and cytokines.

Wnt, DKK and sclerostin, are all critical factors in coordinating bone remodeling and maintenance, but there are numerous other factors, such as RANKL, OPG, TGF-β, PTHrP and others that help mediate these processes. Bone homeostasis is typically controlled through tightly regulated negative feedback loops to control bone growth and resorption to ensure new bone is sufficient for the mechanical loads present. However, the development of tumors in bone, including metastatic involvement, leads to an uncoupling of these processes, and bias towards osteoblastic or osteolytic disease depending on the tumor type present.

Cancer and Bone

Although multiple cancers develop bone metastases, the most commonly studied cancers with bone involvement include multiple myeloma (MM), prostate cancer (PCa) and breast cancer (BrCa). Intriguingly, it appears these cancer share similar mechanisms, as well as unique aspects in terms of bone metastases and the Wnt pathways (summarized in **Figure 2**). In this section, we highlight how Wnt signaling modulates bone metastasis of these cancers.

Multiple Myeloma

MM is a hematological malignancy of plasma cells that produce severe osteolytic lesions, leading to hypercalcemia and renal insufficiency. Even though MM is a relatively rare hematological malignancy (~14% of all hematological malignancies diagnosed), approximately 20 000 new cases of MM are expected to have been diagnosed in 2011, with over 10 000 deaths related to the disease.[19] Males have a higher incidence of disease than females, but the reason for this is unknown. Conventional therapy is highly varied, but typically includes bortezomib, dexamethasone, doxorubicin, thalidomide/lenalidomide and melphalan for induction therapy before bone marrow transplant if possible. Even with advances in recent years, the 5-year survival rate is only 40%. Novel therapeutics are required for this rapidly progressing tumor and recent studies on Wnt biology in MM have led to clinical trials to target the Wnt pathway.

It is believed that an uncoupling of the normal pathways associated for bone remodeling, including Wnt signaling become perturbed in patients with MM. Increased bone resorption and decreased bone formation have been shown to be required for MM progression.[20] The importance of DKK1 expression in MM was first identified by Tian *et al.*[21] in 2003 through microarray profiling of MM patients. In a clinical study of myeloma patients, only DKK1 overexpression was found to correlate with the degree of bone involvement at diagnosis out of 10 osteolytic candidate genes.[22] In an attempt to explain this phenomenon mechanistically, Dun *et al.*[23] have recently shown that stromal cells from patients with MM overexpress LRP5/6 and Krm1/2 in response to increased DKK1 expression from MM cells compared with the stromal cells of healthy volunteers. These data suggest that increased DKK1 expression from MM is potentiated by the bone marrow (BM) microenvironment leading to the production of osteolytic lesions. Qiang *et al.*[24,25] have produced evidence suggesting that Wnt signaling is functional in osteoclasts in MM patients by the presence of FRZ, and that DKK1 overexpression in MM cells leads to increased RANKL expression and diminished OPG production from osteoblasts, thereby increasing the osteoclastic phenotype. Gunn *et al.*[26] originally showed that DKK1 produced from MM cells could inhibit mesenchymal stem cell differentiation into osteoblasts. Promotion of the vicious cycle occurs when undifferentiated mesenchymal stem cell produce interleukin-6 leading to enhanced MM growth.[26] More recently, Fowler *et al.*[27] have shown that co-injection of bone marrow stromal cells with functional DKK1 are necessary for MM growth engraftment and growth in mouse models. Previously, it was thought that patients with the premalignant condition monoclonal gammopathy

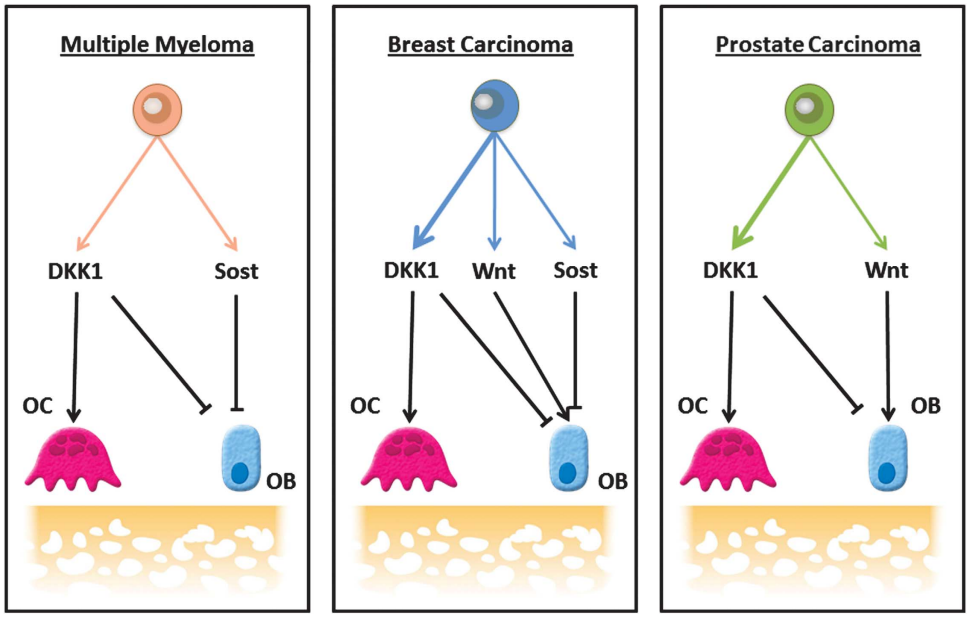

Figure 2 Bone microenvironment interactions of metastatic tumor cells. Wnt signaling between tumor cells and resident bone cells is coerced during metastases, with tumor cells disrupting normal signals to promote tumor growth and invasion. MM, BCa and PCa, have a high incidence of bone metastases. Expression of Dickkopf-1 (DKK1) and sclerostin (Sost) activate osteoclasts (OC) leading to bone resorption (heavier arrows denote changes seen more prominently in more aggressive forms of those diseases). Increased expression of Wnt from tumor cells activates osteoblasts and promotes bone mineralization. Differential expression of Wnt and DKK1 has been associated with aggressiveness of disease in BCa and PCa. Expression of DKK1 is thought to be a primary driving factor of MM progression.

of undetermined significance (MGUS) had significantly lower serum DKK1 concentrations than MM patients. However, Ng *et al.*[28] have recently shown that MGUS patients have increased DKK1 serum levels compared with healthy controls, though still lower than patients with MM. They suggest that increased serum DKK1 and impaired bone formation in MGUS patients shows the importance of DKK1 as a mediator of osteoblast function and progression to MM. Together, these data suggest that MM overexpression of DKK1 leads to osteoblast inhibition, by inhibiting pro-blastic processes in osteoblasts, and promoting osteoclast function, through increased signaling through membrane-bound FRZ receptors on osteoclasts.

The formation of a vicious cycle on the molecular level, MM and stroma promoting DKK1 expression and activity related to DKK1 function, mirrors the vicious cycle of MM growth and invasion with increased osteolytic disease. Even though MM expression of DKK1 is viewed as a primary mechanism leading to Wnt-inhibition that promotes osteolytic progression, some evidence suggests that MM expression of sFRP2 and sclerostin have a role also.[29] The soluble Wnt inhibitor sFRP2 was found to be secreted from patients with advanced lesions.[30] sFRP2 inhibits osteoblast mineralization and ALP expression by neutralizing Wnt mediators before they can bind to membrane-bound frizzled receptors. Sclerostin has been shown to be elevated in MM patients with advanced bone disease, and has been shown to be produced directly from MM cells.[29,31] Expression of sclerostin suggests that MM cells inhibit osteoblast differentiation as well as promote osteoclastogenesis leading to progressive osteolysis. Together, these data suggest that several mediators of Wnt inhibition are key mediators that result in decreased Wnt-mediated osteoblastic activity in MM that contribute to bone loss in MM.

The recent addition of thalidomide, lenalidomide and bortezomib to conventional chemotherapeutics, has led to a significant increase in 2- and 5-year survival rates for patients with MM.[32] Bortezomib is a proteasome inhibitor, which may lead to direct cytotoxicity of MM cells and increase BMD by Runx2 stabilization in the osteoblast, and DKK1 inhibition.[33] The immunomodulators thalidomide and lenalidomide lead to direct cytotoxicity of MM cells, inhibit angiogenesis and inhibit the production of TNFα, a potentiator of DKK1 expression.[33] Thalidomide acts through stimulation of BMP signaling in response to reactive oxygen species, and secondary upregulation of DKK1.[34] However, thalidomide treatment of MM patients resulted in decreased serum concentrations of DKK1,[35] showing the need for further investigation into the mechanistic actions of thalidomide. Novel therapeutics currently targeting the Wnt pathways include the neutralizing anti-DKK1 antibody BHQ880 (Novartis, Cambridge, MA, USA), the neutralizing anti-sclerostin antibody AMG785 (Amgen, Thousand Oaks, CA, USA) and inhibition of GSK-3β.[33] BHQ880 has been shown to inhibit tumor growth, inhibit MM adhesion to BM stromal cell and increase nuclear β-catenin.[36] Another study found that treatment with BHQ880 lead to reversal of osteoblast inhibition and inhibited trabecular bone loss, while not impacting osteoclast numbers.[37] Because of these promising results, two clinical trials are currently underway to determine the safety and efficacy of BHQ880 in combination with conventional chemotherapy and determine the impacts on tumor burden and bone turnover in MM patients. AMG785 was developed for the treatment of osteoporosis, but may have promise for other bone resorptive diseases. Recent completion of a phase I study in 72 healthy volunteers concluded that treatment with AMG785 resulted in a dose-related increase in osteoblastic serum markers (osteocalcin and

bALP), a decrease in the osteoclastic marker sCTx, increase in overall BMD.[33,38]

Inhibition of GSK-3β is another novel mechanism for inhibition of Wnt signaling. Inhibition of GSK-3β leads to inhibition of β-catenin phosphorylation and thus restores β-catenin translocation to the nucleus and activation. One of the oldest GSK-3β inhibitors is lithium chloride (LiCl), whose clinical use was prevalent long before the mechanism of action was understood.[39] Many compounds have impacts on GSK-3β, and less specific compounds may have a greater impact in complex diseases such as cancer owing to inhibition of compensatory pathways.[39] Recent work by Gunn *et al.*[26,40] using the GSK-3β inhibitor 6-bromoindirubin-3′-oxime (BIO) showed inhibition of MM growth through maturation of mesenchymal stem cell into osteoblasts leading to preservation of bone in murine models. These data suggest that GSK-3β inhibition is a plausible method for inhibiting MM, and possibly other tumor types in bone.

Concerns of Wnt signaling promoting tumor growth have inhibited the enthusiasm for some of these treatments.[33,41] The impact of Wnt signaling has been shown to be microenvironmentally dependent, as LiCl treatment inhibits MM growth in bone yet has little effect on subcutaneous tumors.[42] These data may be important for treatment of soft tissue metastases. Conflicting evidence persists concerning the role of Wnt signaling as a survival factor for MM cells.[42,43] Therefore, further work must be performed to detail the importance of Wnt signaling as it relates to the tumors' growth compared with the possible benefits gained from the inhibition of osteolysis.

Prostate Cancer

PCa, like MM, metastasizes to the skeleton with high frequency. Studies of men with progressive castration-resistant PCa indicate that nearly one half of patients will develop skeletal metastases within 2 years; whereas, at autopsy greater than 80% of all men who die of PCa will have metastatic disease within the bone, specifically in the trabecular bone of the pelvis, femur and vertebral bodies.[44,45] However, unlike MM tumors, which promote the formation of osteoclastic or osteolytic lesions, PCa skeletal metastases are predominantly bone forming or osteoblastic in nature with regions of osteolysis. Given the central role of Wnt proteins within bone biology, the involvement of Wnts and Wnt inhibitors in PCa-induced osteoblastic metastases has been investigated.

Human PCa cell lines express mRNA for multiple Wnts, including canonical Wnts 3a, 7b and 10b, which are known mediators of osteoblast differentiation and mineralization.[46,47] Furthermore, aggressive PCa cell lines PC-3 and Du145, which produce osteolytic lesions in murine tumor models, were found to express the Wnt inhibitor DKK-1 compared with less aggressive PCa cell lines, such as LNCaP and MDA PCa 2b, which produce mixed to osteoblastic lesions.[46,47] Within PCa patient tissues, Wnt7b was expressed in 42% of PCa skeletal metastases compared with normal prostate tissue; whereas, DKK-1 was expressed in two-thirds of osteolytic PCa lesions relative to normal prostate.[47] We have shown that DKK-1 was elevated 5-fold in prostatic intraepithelial neoplasia and primary PCa lesions compared with non-neoplastic prostate tissue but was decreased over 50% in skeletal metastases relative to primary lesions.[48] Taken together, these data suggest that the balance between Wnt and Wnt inhibitors determine the osteogenic nature of PCa skeletal metastases and that DKK-1 may serve as a molecular switch between osteolytic and osteoblastic aspects of PCa bone metastases. This hypothesis is supported by studies that manipulated DKK-1 expression in PCa cells to modulate Wnt activity. The knock-down of DKK-1 in osteolytic PC-3 cells was found to stimulate osteoblast mineralization *in vitro* within co-cultured bone marrow stromal cells.[46] Conversely, the overexpression of DKK-1 in human C4-2B or canine Ace-1 PCa cells was shown to transform these osteoblastic cell lines into a highly osteolytic tumor *in vivo*. Taken together, these data support the hypothesis that canonical Wnt proteins contribute to the osteoblastic component of PCa skeletal lesions metastases, whereas Wnt inhibitors promote an osteolytic environment.[46,49]

Within PCa skeletal metastases, an overall reduction in Wnt inhibitors would be required to permit a Wnt-mediated osteoblastic response and is supported by patient data, which demonstrate that DKK-1 expression is decreased in skeletal metastasis compared with primary lesions.[48] It is currently unclear how DKK-1 expression is altered in PCa; however, several factors could account for the changes in DKK-1 expression. For example, endothelin-1 and transforming growth factor-beta-1 were shown to decrease DKK-1 expression in primary murine osteoblasts and human endometrial stromal cells, respectively.[50,51] The molecular mechanism resulting in decreased DKK-1 expression in these models is still under investigation, however, DKK-1 repression has been reported to result from epigenetic silencing[52] and through the activity of miR-335-5p.[53] Additionally, several oncogenic transcription factors have been shown to repress DKK-1 expression, including c-Myc in mammary epithelial cells,[54] NYCN in neuroblastoma,[55] GATA6 in pancreatic cancer[56] and EWS/FL1 in Ewing's sarcoma.[57]

In addition to modulating the nature of PCa bone lesions, DKK-1 may also regulate the formation of tumors both in the bone and at soft tissue sites. Several studies using a DKK-1 neutralizing antibody or shRNA molecules have shown that blocking DKK-1 activity reduced tumor establishment in animal models of MM, PCa and lung cancer.[36,37,58–60] The mechanism for the anti-tumor activity of DKK-1 knock-down appears to result from a Wnt-independent suppression of tumor growth in part through stabilization of the cyclin-dependent kinase inhibitor $p21^{cip\text{-}1/waf\text{–}1}$. On the surface, these studies appear to contradict the established role of canonical Wnt signaling as a driver of tumorigenesis within many solid tumors including colorectal, breast and prostate cancers. However, in advanced PCa, data from five separate studies of over 500 patient samples has shown that cytoplasmic rather than nuclear β-catenin is a poor prognostic marker suggesting that β-catenin signaling within advanced disease may be growth suppressive rather than growth promoting. These data raise the possibility that tumor grade as well as the nature of the tumor microenvironment determines whether Wnt signaling will be a promoter or a suppressor of tumor growth.

Breast Cancer

Like PCa, BrCa metastasizes to the bone with high frequency sharing a similar distribution pattern. The bone is the most frequent site of distant relapse following hormonal therapy, accounting for 30–40% of all first recurrences. However unlike

PCa, human BrCa skeletal metastases are largely osteolytic and therefore are more similar to MM. The vast majority of literature indicates that Wnts drive BrCa tumor development and as such the Wnt inhibitors act as tumor suppressor capable of initiating cell death.[61,62] There are some notable exceptions particularly in the context of the bone, which provides further evidence for the potential influence of the tumor microenvironment on Wnt activity in tumor cells. Among them are data which shows that DKK-1 was overexpressed in ER/PR double negative BrCa samples vs normal[63,64] and that increased levels of DKK-1 were found in the serum of BrCa patients with bone metastases compared to women with soft tissue metastases.[64] Similarly, both BrCa-derived DKK-1 and sclerostin were shown to inhibit osteoblast differentiation *in vitro*.[65,66] Taken together, the data indicate that BrCa cells, in general, overexpress Wnt inhibitors, which promote the formation of osteolytic vs osteoblastic lesions within the skeleton.

Summary/Conclusion

Wnts are important mediators of bone health and homeostasis. The role of Wnts and their inhibitors are important in maintaining healthy bone while performing a careful balancing act between bone resorption and deposition. However, multiple tumor types have been able to deregulate this system, nullifying the necessary negative feedback loops used to maintain bone health, and exacerbating lytic and blastic processes that presumably promote tumor growth. Bone is unique, in that spaces are confined owing to relatively inelastic mineralized structures and poor vasculature. Tumors metastasizing to bone are able to thrive in this location, and co-opt Wnt signaling to promote a beneficial metastatic niche. Subsequent alteration of the blastic and lytic components of bone allow for increased tumor growth, creating great challenges to treat this disseminated disease. Numerous novel therapeutics are being tested (**Figure 3**), but studies are still in relative infancy, and novel targets are necessary to ensure the inhibition of overlapping and convergent pathways that compose Wnt signaling.

Understanding the role that the Wnt pathway has in bone metastases provides a great opportunity to allow for developing clinical therapeutics to achieve clinical progress for established disease. Attention must also be given to the micrometastatic disease and changes occurring before metastases are clinically measurable. Understanding the mechanisms behind the relatively early events leading to the development of bone metastases represents the best chance for inhibiting further growth and dissemination of bone disease and Wnt likely have a role in this activity. Bone metastases are a debilitating and difficult effect of progressive disease, as supported by dismal survival statistics, but a better understanding of the pathways discussed herein is a beginning in solving such problems.

Conflict of Interest

The authors declare no conflict of interest.

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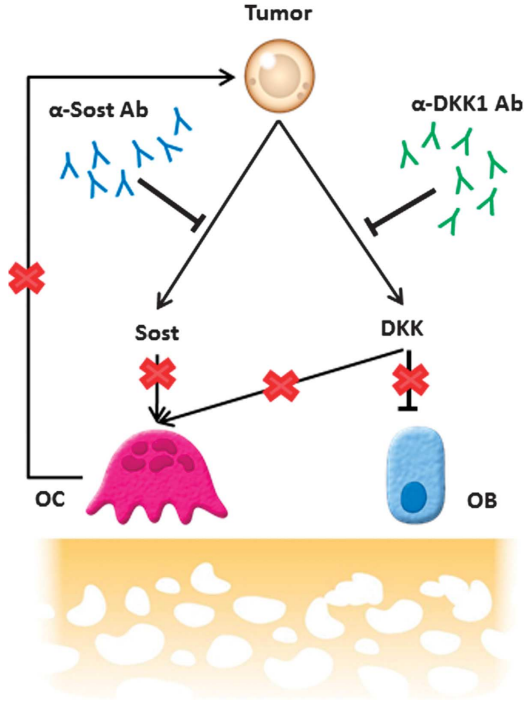

Figure 3 Novel therapeutics to inhibit metastatic induced bone lysis. Tumor cells produce numerous factors, such as Dickkopf-1 (DKK1) and sclerostin (Sost), to inhibit osteoblast function and enhance osteoclastic bone resorption in the tumor microenvironment, facilitating tumor growth and invasion. Novel therapeutics to target DKK1 and Sost are currently undergoing clinical trials. Specifically, fully humanized antibodies against DKK1 (α-DKK1 Ab) and Sost (α-SostAb) are being investigated. These therapeutics inhibit mediators associated with osteoclast (OC) activation established by tumor cells. Inhibition of osteoclasts may further indirectly decrease tumor growth by decreasing the effectiveness of the vicious cycle associated with bone lysis and tumor growth. Further, inhibition of DKK1 may also increase osteoblast differentiation and maturation, thereby restoring bone growth and possibly inhibiting tumor growth.

References

1. Milat F, Ng KW. Is Wnt signalling the final common pathway leading to bone formation? *Mol Cell Endocrinol* 2009;**310**:52–62.
2. Day TF, Yang Y. Wnt and hedgehog signaling pathways in bone development. *J Bone Joint Surg Am* 2008;**90** (Suppl 1): 19–24.
3. van Amerongen R, Mikels A, Nusse R. Alternative wnt signaling is initiated by distinct receptors. *Sci Signal* 2008;**1**:re9.
4. Arnsdorf EJ, Tummala P, Jacobs CR. Non-canonical Wnt signaling and N-cadherin related beta-catenin signaling play a role in mechanically induced osteogenic cell fate. *PLoS One* 2009;**4**:e5388.
5. Glass DA II, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H *et al.* Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell* 2005;**8**:751–764.
6. Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. *Gene* 2004;**341**:19–39.
7. Klopocki E, Kristiansen G, Wild PJ, Klaman I, Castanos-Velez E, Singer G *et al.* Loss of SFRP1 is associated with breast cancer progression and poor prognosis in early stage tumors. *Int J Oncol* 2004;**25**:641–649.
8. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM *et al.* LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 2001;**107**:513–523.
9. Clement-Lacroix P, Ai M, Morvan F, Roman-Roman S, Vayssiere B, Belleville C *et al.* Lrp5-independent activation of Wnt signaling by lithium chloride increases bone formation and bone mass in mice. *Proc Natl Acad Sci USA* 2005;**102**:17406–17411.
10. Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA *et al.* High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med* 2002;**346**:1513–1521.
11. Babij P, Zhao W, Small C, Kharode Y, Yaworsky PJ, Bouxsein ML *et al.* High bone mass in mice expressing a mutant LRP5 gene. *J Bone Miner Res* 2003;**18**:960–974.
12. Krishnan V, MacDougald OA. The silent wif of death. *IBMS BoneKEy* 2009;**6**:339–341.
13. Yee DS, Tang Y, Li X, Liu Z, Guo Y, Ghaffar S *et al.* The Wnt inhibitory factor 1 restoration in prostate cancer cells was associated with reduced tumor growth, decreased capacity of cell migration and invasion and a reversal of epithelial to mesenchymal transition. *Mol Cancer* 2010;**9**:162.
14. Galli C, Passeri G, Macaluso GM. Osteocytes and WNT: the mechanical control of bone formation. *J Dent Res* 2010;**89**:331–343.
15. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R *et al.* Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997;**89**:309–319.

16. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S *et al.* Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci USA* 1998;**95**:3597–3602.
17. Takahashi N, Maeda K, Ishihara A, Uehara S, Kobayashi Y. Regulatory mechanism of osteoclastogenesis by RANKL and Wnt signals. *Front Biosci* 2011;**16**:21–30.
18. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D *et al.* Dickkopf-1 is a master regulator of joint remodeling. *Nat Med* 2007;**13**:156–163.
19. Rajkumar SV. Multiple myeloma: 2012 update on diagnosis, risk-stratification, and management. *Am J Hematol* 2012;**87**:78–88.
20. Esteve FR, Roodman GD. Pathophysiology of myeloma bone disease. *Best Pract Res Clin Haematol* 2007;**20**:613–624.
21. Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B *et al.* The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N Engl J Med* 2003;**349**:2483–2494.
22. Haaber J, Abildgaard N, Knudsen LM, Dahl IM, Lodahl M, Thomassen M *et al.* Myeloma cell expression of 10 candidate genes for osteolytic bone disease. Only overexpression of DKK1 correlates with clinical bone involvement at diagnosis. *Br J Haematol* 2008;**140**:25–35.
23. Dun X, Jiang H, Zou J, Shi J, Zhou L, Zhu R *et al.* Differential expression of DKK-1 binding receptors on stromal cells and myeloma cells results in their distinct response to secreted DKK-1 in myeloma. *Mol Cancer* 2010;**9**:247.
24. Qiang YW, Chen Y, Brown N, Hu B, Epstein J, Barlogie B *et al.* Characterization of Wnt/beta-catenin signalling in osteoclasts in multiple myeloma. *Br J Haematol* 2010;**148**: 726–738.
25. Qiang YW, Chen Y, Stephens O, Brown N, Chen B, Epstein J *et al.* Myeloma-derived Dickkopf-1 disrupts Wnt-regulated osteoprotegerin and RANKL production by osteoblasts: a potential mechanism underlying osteolytic bone lesions in multiple myeloma. *Blood* 2008;**112**:196–207.
26. Gunn WG, Conley A, Deininger L, Olson SD, Prockop DJ, Gregory CA. 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* 2006;**24**:986–991.
27. Fowler JA, Mundy GR, Lwin ST, Edwards CM. Bone marrow stromal cells create a permissive microenvironment for myeloma development: a new stromal role for Wnt inhibitor Dkk1. *Cancer Res* 2012;**72**:2183–2189.
28. Ng AC, Khosla S, Charatcharoenwitthaya N, Kumar SK, Achenbach SJ, Holets MF *et al.* Bone microstructural changes revealed by high-resolution peripheral quantitative computed tomography imaging and elevated DKK1 and MIP-1alpha levels in patients with MGUS. *Blood* 2011;**118**:6529–6534.
29. Brunetti G, Oranger A, Mori G, Specchia G, Rinaldi E, Curci P *et al.* Sclerostin is overexpressed by plasma cells from multiple myeloma patients. *Ann NY Acad Sci* 2011;**1237**:19–23.
30. Oshima T, Abe M, Asano J, Hara T, Kitazoe K, Sekimoto E *et al.* Myeloma cells suppress bone formation by secreting a soluble Wnt inhibitor, sFRP-2. *Blood* 2005;**106**:3160–3165.
31. Terpos E, Christoulas D, Katodritou E, Bratengeier C, Gkotzamanidou M, Michalis E *et al.* Elevated circulating sclerostin correlates with advanced disease features and abnormal bone remodeling in symptomatic myeloma: Reduction post-bortezomib monotherapy. *Int J Cancer* 2011(e-pub ahead of print 21 December 2011; doi:10.1002/ijc.27342).
32. Kumar SK, Rajkumar SV, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK *et al.* Improved survival in multiple myeloma and the impact of novel therapies. *Blood* 2008;**111**:2516–2520.
33. Vallet S, Raje N. Bone anabolic agents for the treatment of multiple myeloma. *Cancer Microenviron* 2011;**4**:339–349.
34. Knobloch J, Shaughnessy JD Jr, Ruther U. Thalidomide induces limb deformities by perturbing the Bmp/Dkk1/Wnt signaling pathway. *FASEB J* 2007;**21**:1410–1421.
35. Heider U, Kaiser M, Mieth M, Lamottke B, Rademacher J, Jakob C *et al.* Serum concentrations of DKK-1 decrease in patients with multiple myeloma responding to anti-myeloma treatment. *Eur J Haematol* 2009;**82**:31–38.
36. Fulciniti M, Tassone P, Hideshima T, Vallet S, Nanjappa P, Ettenberg SA *et al.* Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. *Blood* 2009;**114**:371–379.
37. Heath DJ, Chantry AD, Buckle CH, Coulton L, Shaughnessy Jr JD, Evans HR *et al.* Inhibiting Dickkopf-1 (Dkk1) removes suppression of bone formation and prevents the development of osteolytic bone disease in multiple myeloma. *J Bone Miner Res* 2009;**24**: 425–436.
38. Padhi D, Jang G, Stouch B, Fang L, Posvar E. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. *J Bone Miner Res* 2011;**26**:19–26.
39. Meijer L, Flajolet M, Greengard P. Pharmacological inhibitors of glycogen synthase kinase 3. *Trends Pharmacol Sci* 2004;**25**:471–480.
40. Gunn WG, Krause U, Lee N, Gregory CA. Pharmaceutical inhibition of glycogen synthetase kinase-3beta reduces multiple myeloma-induced bone disease in a novel murine plasmacytoma xenograft model. *Blood* 2011;**117**:1641–1651.
41. Sukhdeo K, Mani M, Zhang Y, Dutta J, Yasui H, Rooney MD *et al.* Targeting the beta-catenin/TCF transcriptional complex in the treatment of multiple myeloma. *Proc Natl Acad Sci USA* 2007; **104**:7516–7521.
42. Edwards CM, Edwards JR, Lwin ST, Esparza J, Oyajobi BO, McCluskey B *et al.* Increasing Wnt signaling in the bone marrow microenvironment inhibits the development of myeloma bone disease and reduces tumor burden in bone in vivo. *Blood* 2008;**111**:2833–2842.
43. Derksen PW, Tjin E, Meijer HP, Klok MD, MacGillavry HD, van Oers MH *et al.* Illegitimate WNT signaling promotes proliferation of multiple myeloma cells. *Proc Natl Acad Sci USA* 2004;**101**:6122–6127.
44. Smith MR, Cook R, Lee KA, Nelson JB. Disease and host characteristics as predictors of time to first bone metastasis and death in men with progressive castration-resistant nonmetastatic prostate cancer. *Cancer* 2011;**117**:2077–2085.
45. Bubendorf L, Schopfer A, Wagner U, Sauter G, Moch H, Willi N *et al.* Metastatic patterns of prostate cancer: an autopsy study of 1,589 patients. *Hum Pathol* 2000;**31**:578–583.
46. Hall CL, Bafico A, Dai J, Aaronson SA, Keller ET. Prostate cancer cells promote osteoblastic bone metastases through Wnts. *Cancer Res* 2005;**65**:7554–7560.
47. Li ZG, Yang J, Vazquez ES, Rose D, Vakar-Lopez F, Mathew P *et al.* Low-density lipoprotein receptor-related protein 5 (LRP5) mediates the prostate cancer-induced formation of new bone. *Oncogene* 2008;**27**:596–603.
48. Hall CL, Daignault SD, Shah RB, Pienta KJ, Keller ET. Dickkopf-1 expression increases early in prostate cancer development and decreases during progression from primary tumor to metastasis. *Prostate* 2008;**68**:1396–1404.
49. Thudi NK, Martin CK, Murahari S, Shu ST, Lanigan LG, Werbeck JL *et al.* Dickkopf-1 (DKK-1) stimulated prostate cancer growth and metastasis and inhibited bone formation in osteoblastic bone metastases. *Prostate* 2011;**71**:615–625.
50. Clines GA, Mohammad KS, Bao Y, Stephens OW, Suva LJ, Shaughnessy JD Jr *et al.* Dickkopf homolog 1 mediates endothelin-1-stimulated new bone formation. *Mol Endocrinol* 2007;**21**: 486–498.
51. Kane N, Jones M, Brosens JJ, Saunders PT, Kelly RW, Critchley HO. Transforming growth factor-beta1 attenuates expression of both the progesterone receptor and Dickkopf in differentiated human endometrial stromal cells. *Mol Endocrinol* 2008;**22**:716–728.
52. Aguilera O, Fraga MF, Ballestar E, Paz MF, Herranz M, Espada J *et al.* Epigenetic inactivation of the Wnt antagonist DICKKOPF-1 (DKK-1) gene in human colorectal cancer. *Oncogene* 2006;**25**:4116–4121.
53. Zhang J, Tu Q, Bonewald LF, He X, Stein GS, Lian J *et al.* Effects of miR-335-5p in modulating osteogenic differentiation by specifically downregulating Wnt antagonist DKK1. *J Bone Miner Res* 2011;**26**:1953–1963.
54. Cowling VH, D'Cruz CM, Chodosh LA, Cole MD. c-Myc transforms human mammary epithelial cells through repression of the Wnt inhibitors DKK1 and SFRP1. *Mol Cell Biol* 2007;**27**: 5135–5146.
55. Koppen A, Ait-Aissa R, Hopman S, Koster J, Haneveld F, Versteeg R *et al.* Dickkopf-1 is down-regulated by MYCN and inhibits neuroblastoma cell proliferation. *Cancer Lett* 2007;**256**: 218–228.
56. Zhong Y, Wang Z, Fu B, Pan F, Yachida S, Dhara M *et al.* GATA6 Activates Wnt Signaling in Pancreatic Cancer by Negatively Regulating the Wnt Antagonist Dickkopf-1. *PLoS* 2011;**6**:e22129.
57. Navarro D, Agra N, Pestana A, Alonso J, Gonzalez-Sancho JM. The EWS/FLI1 oncogenic protein inhibits expression of the Wnt inhibitor DICKKOPF-1 gene and antagonizes beta-catenin/ TCF-mediated transcription. *Carcinogenesis* 2010;**31**:394–401.
58. Hall CL, Zhang H, Baile S, Ljungman M, Kuhstoss S, Keller ET. p21CIP-1/WAF-1 induction is required to inhibit prostate cancer growth elicited by deficient expression of the Wnt inhibitor Dickkopf-1. *Cancer Res* 2010;**70**:9916–9926.
59. Sato N, Yamabuki T, Takano A, Koinuma J, Aragaki M, Masuda K *et al.* Wnt inhibitor Dickkopf-1 as a target for passive cancer immunotherapy. *Cancer Res* 2010;**70**:5326–5336.
60. Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy Jr JD. Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. *Blood* 2007;**109**:2106–2111.
61. Mikheev AM, Mikheeva SA, Maxwell JP, Rivo JV, Rostomily R, Swisshelm K *et al.* Dickkopf-1 mediated tumor suppression in human breast carcinoma cells. *Breast Cancer Res Treat* 2008;**112**:263–273.
62. Veeck J, Geisler C, Noetzel E, Alkaya S, Hartmann A, Knüchel R *et al.* Epigenetic inactivation of the secreted frizzled-related protein-5 (SFRP5) gene in human breast cancer is associated with unfavorable prognosis. *Carcinogenesis* 2008;**29**:991–998.
63. Forget MA, Turcotte S, Beauseigle D, Godin-Ethier J, Pelletier S, Martin J *et al.* The Wnt pathway regulator DKK1 is preferentially expressed in hormone-resistant breast tumours and in some common cancer types. *Br J Cancer* 2007;**96**:646–653.
64. Voorzanger-Rousselot N, Journe F, Doriath V, Body JJ, Garnero P. Assessment of circulating Dickkopf-1 with a new two-site immunoassay in healthy subjects and women with breast cancer and bone metastases. *Calcif Tissue Int* 2009;**84**:348–354.
65. Bu G, Lu W, Liu CC, Selander K, Yoneda T, Hall CL *et al.* Breast cancer-derived Dickkopf1 inhibits osteoblast differentiation and osteoprotegerin expression: implication for breast cancer osteolytic bone metastases. *Int J Cancer* 2008;**123**:1034–1042.
66. Mendoza-Villanueva D, Zeef L, Shore P. Metastatic breast cancer cells inhibit osteoblast differentiation through the Runx2/CBFbeta-dependent expression of the Wnt antagonist, sclerostin. *Breast* 2011;**13**:R106.