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REVIEW

The Wnt inhibitor dickkopf-1: a link between breast cancer and bone metastases

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Abstract Breast cancer is the second leading cause of cancer death in women and metastasizes to bone in greater than 80 % of advanced-disease patients. Once breast cancer bone metastases are established, the disease is incurable and drives numerous complications that increase morbidity and diminish patients' quality of life. Many mechanisms have been implicated in bone metastases of breast cancer. The critical role of Wnt signalling pathway inhibition in initiating bone lesions has been demonstrated in a variety of bone diseases and tumours. Overexpression of dickkopf-1 (Dkk1) protein, a negative regulator of the Wnt/β-catenin pathway, has been found in breast cancer cell lines that form osteolytic metastases preferentially and in serum from breast cancer patients with osteolytic bone metastases. Further understanding of the mechanistic role of Dkk1 as a link between primary breast tumours and secondary osteolytic bone metastases may facilitate development of anti-Dkk1 antibody therapeutic tools.

Keywords Breast cancer · Wnt signalling · Dickkopf-1 · Bone metastases · Osteolysis

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Background

Breast cancer continues to be the most frequently diagnosed malignancy among women, with an estimated 1.38 million new cases being diagnosed worldwide each year. There are 458,000 deaths per year from breast cancer, making it the most common cause of female cancer death [1]. Bone is the preferred site of metastatic recurrence, arising in greater than 80 % of patients with advanced breast cancer [2]. Complications resulting from bone metastases include pain, reduced mobility, and reduced quality of life. In addition, patients are at considerable risk of skeletal related events (SREs), such as hypercalcemia, fracture, and spinal cord compression, and often require surgery, radiotherapy or both [3]. Metastatic sequelae account for approximately two-thirds of the costs associated with breast cancer treatment [4].

Elucidation of the fundamental mechanisms responsible for breast cancer metastasis to bone, including identifying specific biomarkers of inter-/intra-tumour spatial and metastatic potential, is required to improve patient risk stratification such that the best current therapies for particular patients can be selected. Moreover, a better mechanistic understanding may reveal promising new therapeutic targets. This review discusses the available evidence for the role of the Dkk1 protein, a Wnt signalling inhibitor, in breast cancer-induced bone metastasis and the potential therapeutic benefits of Dkk1 antibody therapy as a new strategy for decreasing breast cancer burden in osteolytic bone metastases.

Pathophysiology of bone metastases

Physiologic bone homeostasis is the result of the coordinated activities of three separate cell lineages: the haematopoietic stem cell lineage, which leads to the

formation of bone-resorbing osteoclasts, the mesenchymal stem cell lineage, which leads to the formation of bone-forming osteoblasts, and the bone-maintaining osteocytes [5]. Osteoblasts and their precursors express several mediators that regulate osteoclastogenesis and osteoclast activity. The receptor activator of nuclear factor kappa B ligand (RANKL), a member of the tumor necrosis factor (TNF) receptor superfamily, is produced both in membrane-bound and soluble forms. Binding of RANKL to transmembrane receptor activator of nuclear factor kappa B (RANK) on the cell surface of osteoclasts and osteoclast precursors promotes their proliferation and maturation. Osteoprotegerin (OPG), another member of the TNF receptor superfamily, functions as a potent anti-osteoclastogenic cytokine by acting as a competitive decoy receptor for RANKL, thereby inhibiting RANK-RANKL interaction [6, 7]. The OPG/RANKL/RANK triad of proteins has been shown in genetic and pharmacologic studies to play a critical role in bone resorption [8]. Furthermore, macrophage colony stimulating factor, which is produced by several cell types including osteoblasts and stromal cells, activates an intracellular cascade upon binding its receptor c-fms, which is expressed on the surface of osteoclastic cells. This binding leads to proliferation and differentiation of osteoclast precursors and survival of mature osteoclasts [9]. Other osteoblast-produced cytokines including TNF, interleukin (IL)-1, IL-6 and IL-7 have been shown to play important roles in amplifying osteoclastogenesis and intensifying osteoclastic resorption [10].

The coupling of bone resorption to bone formation is essential for the correct function and maintenance of the skeletal system. The arrival of cancer cells within the bone microenvironment perturbs the resorption-formation balance, leading to excess bone loss or formation. The ability of cancer cells to metastasize is characteristic of advanced disease and occurs only after the gradual accumulation of a necessary set of pro-metastatic mutations [11]. Primary breast tumours are heterogeneous in nature, and cancer cells with vastly distinct capacities can exist within a single tumour. Large scale gene expression analyses and microarrays have identified several gene signatures that can distinguish between non-metastatic and metastatic cells derived from the same primary tumour [12, 13], and have identified genes that cooperate in breast cancer metastasis to bone [14, 15]. Cancer cell metastasis is a multistep process consisting of local invasion and intravasation at the primary site, survival in systemic circulation, and extravasation and colonization at the distant sites [16]. Once distant from the primary tumour site and resident in the bone marrow, cancer cells and other cell types within the bone microenvironment establish tumour cell micrometastatic foci within the marrow or the so-called bone metastatic niche [17]. Resident metastatic cells secrete various factors that promote the release of growth factors from the bone matrix, creating a vicious cycle that renders bone metastases incurable [18].

Bone metastases in breast cancer are characterized primarily by increased osteoclast activity and bone destruction [19]. Osteolytic bone metastases are present in 80 % of patients with stage IV disease [20]. Metastatic breast cells secrete factors that are capable of both RANKL-dependent and -independent activation of osteoclast formation, leading to bone resorption. Tumour-derived Jagged1 engages Notch pathway receptors in pre-osteoclasts directly, promoting their differentiation into mature, multinucleated osteoclasts [21]. On the other hand, parathyroid hormone-related protein (PTHrP) increases bone resorption indirectly by stimulating RANKL expression and inhibiting OPG expression by osteoblasts and bone stromal cells [22]. Inhibition of PTHrP with neutralizing antibodies has been shown to reduce osteolytic lesions produced by MDA-MB-231, a subpopulation of breast cancer cells that have the potential to induce osteolytic bone, in mouse models [23]. Elevated expression of both PTHrP and CXCR4A (a member of the chemokine superfamily that regulates cell migration and targeting) were identified in a study of breast cancer patients who developed skeletal metastases [24]. Li et al. 23–25 demonstrated that PTHrP ablation not only delays breast cancer initiation and primary tumour progression, but also inhibits expression of the metastasis marker CXCR4 in primary breast tumours. IL-6 also increases osteoclast formation and activity via the RANK ligand pathway, whereas IL-8 acts directly and indirectly on osteoclasts [26, 27].

As bone is resorbed, growth factors that are stored in bone matrix, such as insulin like-growth factor-I and transforming growth factor-β (TGF-β), are released and stimulate the proliferation of breast cancer cells. TGF-β is released in its active form during osteoclastic resorption and stimulates PTHrP production by tumour cells [28]. In addition, TGF-β regulates several genes that are responsible for enhanced bone metastases such as IL-11 and connective tissue growth factor [29]. Bone loss can result from increased bone resorption as well as decreased bone formation. Wnt proteins and bone morphogenetic proteins (BMPs) are important regulators of osteoblast activity and proliferation [30, 31]. Gregory et al. proved that the injection of the MDA-MB-231 human breast cancer cells into bone tissue of immunodeficient mice caused a significant down-regulation of osteoblast activity in the bone remodeling cycle. mRNA expression of dickkopf-1 (Dkk1) and Noggin, inhibitors of Wnt signalling and BMP signalling, respectively, was confirmed in the MDA-MB-231 cell line [32].

Although ongoing clinical trials targeting breast cancer bone metastases may identify effective treatments, further

study of the molecular interactions between invading tumour cells and host bone cells is required to inform the development of new effective treatments for this challenging clinical problem. This review is focused on the role of Wnt signalling in breast cancer-induced bone metastases with special attention being given to its inhibitor, the Dkk1 protein.

Wnt signalling in breast cancer and bone metastases

Wnt signalling

The Wnt family consists of 19 members that share a signal sequence of approximately 350 amino acids with a conserved pattern of 23–24 cysteine residues [33]. They act in a variety of cellular processes in both the adult organism and the developing embryo, including cell growth, cell proliferation and motility, generation of cell polarity, and apoptosis [34, 35]. Historically, Wnt proteins have been grouped into two classes, canonical and noncanonical, based on their activity in cell lines or in vivo assays. Noncanonical Wnts activate the planar cell polarity pathway (PCP) and the Wnt/Ca+2 pathway [36]. Canonical Wnts stabilize β-catenin, thereby activating transcription of T cell factor/lymphoid enhancing factor (TCF/LEF). In the absence of Wnts, glycogen synthase kinase (GSK3β), axin, adenomatous polyposis coli (APC), and casein kinase I (CKI) form the β-catenin destruction complex, which phosphorylates β-catenin, enabling it to be degraded by proteasomes. In the 'off state', extracellular Wnt ligands can interact with various secreted antagonists and cells maintain low cytoplasmic and nuclear levels of β-catenin. When Wnt concentrations exceed the buffering capacity of Wnt inhibitors, Wnt signalling is initiated by binding of the Wnt protein to one of the 10 members of the frizzled (FZD) receptor family; Wnt-FZD then binds low-density lipoprotein-related protein-5 (LRP5) or LRP6. The resultant complex activates Dishevelled (Dvl), a protein that draws Axin away from the destruction complex and antagonizes its ability to phosphorylate β-catenin, thereby preventing β-catenin destruction. Thus the 'on state' involves increasing the post-translational stability of β-catenin. As β-catenin levels rise, the protein accumulates and translocates to the nucleus, where it interacts with TCF/LEF transcription factors and enhances expression of their target genes (Fig. 1) [37]. The relative activation of the canonical or noncanonical signalling pathways depends on the receptor complement [38, 39].

Wnt pathway activation is modulated by several secreted protein families that can be divided into two functional classes. The first class includes the frizzled related protein (sFRP) family and Wnt inhibitory protein (WIF) family, which bind directly to Wnts, thereby functioning as Wnt antagonists for both canonical and noncanonical signalling [40]. The second class includes the dickkopf (Dkk) family and the sclerostin (SOST) family that bind to the LRP5/LRP6 component of the Wnt receptor complex, inhibiting Wnt/β-catenin signalling [41].

Wnt signalling and bone homeostasis

Wnts play a central role in controlling embryonic bone development and bone mass [42]. They are also essential in postnatal bone regenerative processes, such as ectopic bone formation and fracture repair [43]. Osteoblasts are the main cellular targets of Wnt actions in bone. Canonical Wnt signalling controls osteoblasts on several levels. Firstly, Wnt signalling can affect osteoblast commitment by blocking adipogenesis and directing mesenchymal progenitors to become either osteoblasts or chondrocytes [44]. Secondly, Wnt signalling modulates osteoblast proliferation. Specifically, the Wnt pathway components, Dkk1 and sFRPs, are upregulated markedly during the late phase of osteoblast differentiation, suggesting that a negative Wnt feedback loop may control the last steps of osteoblast maturation [45]. Thirdly, Wnt signalling affects osteoblast function. LRP5-deficient mice display a decrease in bone matrix deposition [46], and osteoblasts overexpressing a constitutively active mutant of β-catenin show an increase expression of the collagens type I α1 and α2 genes. Moreover, Wnt signalling increases the expression of OPG in osteoblasts and stromal cells, whereas β-catenin-deficient osteoblasts exhibit elevated expression of RANKL and diminished expression of OPG [47]. Thus, osteoblast-selective deficiency of β-catenin affects bone resorption as well as bone formation.

The Wnt/β-catenin pathway has previously been shown to be critically involved in other forms of bone malignancy, including multiple myeloma (MM) [48] and prostate cancer bone metastasis [49]. Hence, it is reasonable to suspect that this pathway may be important in breast cancer-bone metastasis as well.

Wnt signalling in breast cancer and its metastasis into bone

Wnt signalling has emerged as a critical mediator of cell–cell singling events during both embryogenesis and adult tissue maintenance, and the association of deregulated Wnt/β-catenin signalling with cancer has been well documented. β-catenin is a multifunctional protein involved in both cell–cell adhesion and signal transduction [50]. Constitutive activation of β-catenin signalling leads to excessive stem cell renewal/proliferation that predisposes cells

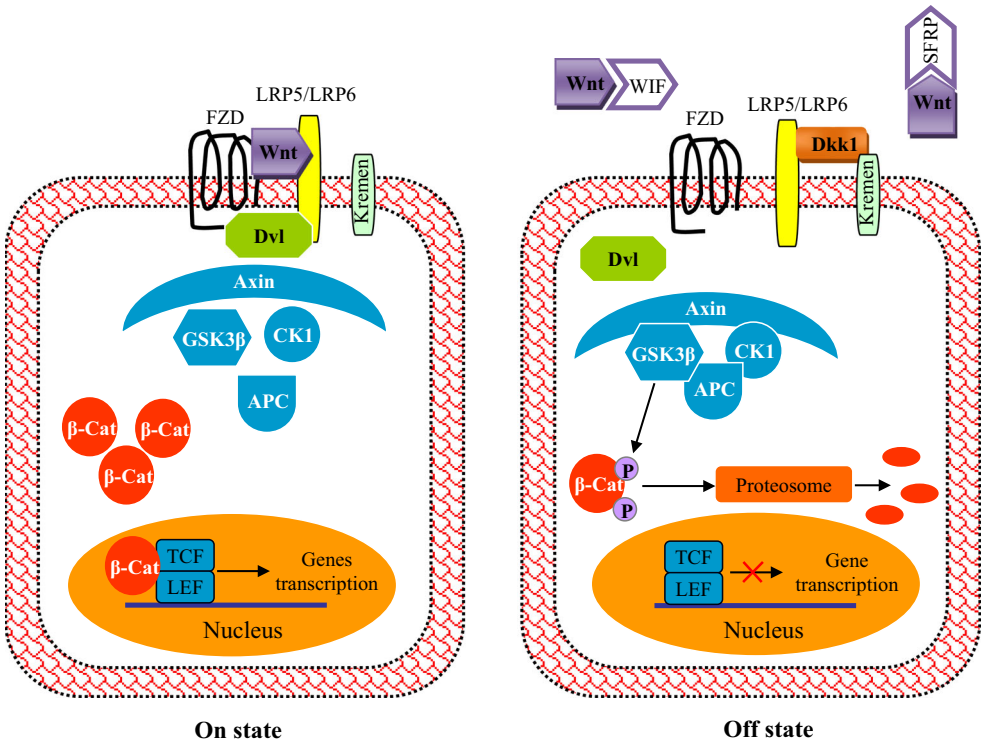

Fig. 1 Canonical Wnt/β-catenin signalling pathway. In the on state, canonical Wnt signalling is activated. Wnt protein binds to one of the 10 members of the frizzled receptor family; Wnt-FZD then binds LRP5 or LRP6. The resultant complex activates Dvl, a protein that draws axin away from the destruction complex and antagonizes its ability to phosphorylate β-catenin, thereby preventing β-catenin degredation. If β-catenin is not degraded, it accumulates and translocates to the nucleus where it binds to the TCF/LEF transcription factor and enhances target gene expression. In the off state, canonical Wnt signalling is inactivated. In the presence of Wnt antagonists (Dkk1, SFRP or WIF), the phosphorylation complex (GSK3β, CK1, Axin and APC kinases) becomes active and phosphorylates β-catenin

to tumorigenesis [51]. Wnt signalling appears to be involved in numerous aspects of mammary development, including cell fate determination, maintenance of mammary progenitor cell populations, branching morphogenesis and alveolar differentiation [52, 53].

The identification of Wnt1 as a key oncogene in naturally occurring mouse mammary tumours [54] led to intense investigation of Wnts and the potential involvement of their signalling in breast cancer over the last three decades. Mutation of APC and overexpression of a stabilized mutant of β-catenin induced mammary tumorigenesis in a mouse model [55]. Wnt/β-catenin pathway dysregulation, as evidenced by abnormal Wnt expression, Wnt antagonist secretion, and APC inactivation, has been observed in human breast cancer [56–58]. Moreover, with respect to β-catenin itself, nuclear β-catenin has been observed in as many as 63 % of breast cancers [59]. Nuclear staining of β-catenin and overexpression of its downstream target cyclin D1 have been associated with a worse prognosis of breast cancer and with metastasis [60, 61]. Moreover, Chen et al. showed that TM40D-MB breast cancer cells, which have a high potential for bone metastasis, exhibit significantly higher endogenous β-catenin signalling activity than TM40D cells, which do not metastasize to bone. In addition, they observed that inactivation of the β-catenin pathway inhibited osteoblast differentiation in a tumour-bone co-culture system, as indicated by decreased alkaline phosphatase activity [62].

Although there is strong evidence, reviewed above, indicating that Wnt signalling in cancerous breast tissue can drive tumour cell growth and invasiveness, the underlying mechanisms mediating these effects remain unclear. Johnson et al. showed that induction of Wnt/β-catenin signalling in highly metastatic breast cancer cells significantly increased Gli2 and PTHrP gene expression and promoter activity [63]. PTHrP promotes the release of TGF-β, which in turn upregulates tumour-derived Gli2 and PTHrP expression and stimulates tumour cell proliferation in bone [64].

Epithelial-mesenchymal transition (EMT) is an essential developmental process that enables reprogramming of polarized epithelial cells towards a motile, mesenchymal phenotype. Aberrant EMT activation can endow cancer cells with the migratory and invasive capabilities associated with metastatic competence [65]. Moreover, tumour progression is driven by a small subpopulation of cancer cells termed cancer stem cells (CSCs) that exhibit the

ability to self-renew and to regenerate the phenotypic heterogeneity of the parental tumour [66]. Mani et al. found that induction of EMT also generates cells with stem-cell-like properties [67]. In addition, studies suggest that Wnt signalling contributes to the induction and maintenance of CSC states activated by the EMT program [68]. Lamb et al. found that Wnt pathway gene expression was increased in malignant breast tissue compared to normal breast tissue, and this expression was predictive for recurrence within subtypes of breast cancer. Furthermore, activation of Wnt signalling was significantly higher in breast cancer stem cell-enriched populations than in normal breast stem cell-enriched populations [69]. These findings suggest that Wnt activation might be limited to a subpopulation of cancer initiating stem cells.

Conversely, other studies failed to find evidence for Wnt pathway activation in human breast tumours [60, 70], or even an association between β-catenin expression and outcome or metastasis [71, 72]. This discrepancy between findings may be due to tumours not being classified into molecular subtypes. Understanding the molecular mechanisms by which Wnt/β-catenin signalling components can act in the bone-tumour microenvironment is important, biologically as well as clinically, for the future development of anti metastatic strategies.

Dkk1 in breast cancer and bone metastases

Dkk1 and bone homeostasis

The Dkk family consists primarily of four secreted proteins in vertebrates (Dkk1, 2, 3, 4) [73]. The most studied member of the family is Dkk1 protein which was discovered due to its ability of blocking Wnt signalling required for head induction during early Xenopus embryogenesis [74]. Dkk1 prevents activation of Wnt signalling by binding to the Wnt co-receptor LRP5/6 [75]. Dkk1 also interacts with the single-pass transmembrane receptor proteins Kremen1 and Kremen2 [76] (Fig. 1). Thus, Dkk1 forms a ternary Dkk1/LRP6/Kremen complex, which promotes endocytosis of LRP, making it unavailable for interaction with Wnt. This Wnt signalling modulation can be achieved by Dkk1, 2, and 4 proteins which bind the same effectors (LRPs or Kremens), but not Dkk3, which does not block canonical Wnt signalling. Dkk4 appears to be functionally indistinguishable from Dkk1, whereas Dkk2 appears to be a poor inhibitor of Wnt signalling [77], perhaps in part, because Dkk2 cannot be expressed at the same high levels achieved by Dkk1. In addition, Dkk2 seems to activate Wnt/β-catenin signalling in Xenopus embryos [78, 79]. Therefore, we will focus on the involvement of Dkk1 in breast cancer-bone metastasis.

Binding of Dkk1 to LRP5 is a key regulator for bone mass. In humans, LRP5 gain-of-function mutations are associated with high bone mass [80], whereas loss-of-function mutations lead to osteoporosis pseudoglioma syndrome, which is characterised by low bone density [81]. Reduced expression of Dkk1 in mice haploinsufficient for the Dkk1 gene results in a high-bone-mass phenotype, whereas transgenic mice overexpressing Dkk1 exhibit osteopenia [82, 83]. Finally, diverse evidence indicates that Dkk1 is also a major determinant of bone and joint pathology in inflammatory arthritis. Dkk1 neutralisation in TNF transgenic mice was found to provide complete protection from inflammatory bone loss by preventing TNF-mediated functional impairment of osteoblast and enhanced osteoclast activity [84]. Weng et al. showed that attenuation of Dkk1 expression in cartilage and subchondral bone tissue promotes expression of β-catenin and survival of chondrocytes and osteoblasts in the osteoarthritic joint microenvironment [85].

Several lines of evidence demonstrate that Dkk1 counteracts Wnt-mediated effects on bone via stimulation of osteoclast activity and inhibition of osteoblast formation and differentiation (Fig. 2). Constitutive expression of Dkk1 has been shown to promote adipogenic differentiation in 3T3-L1 preadipocytes [86]. In vitro examination of

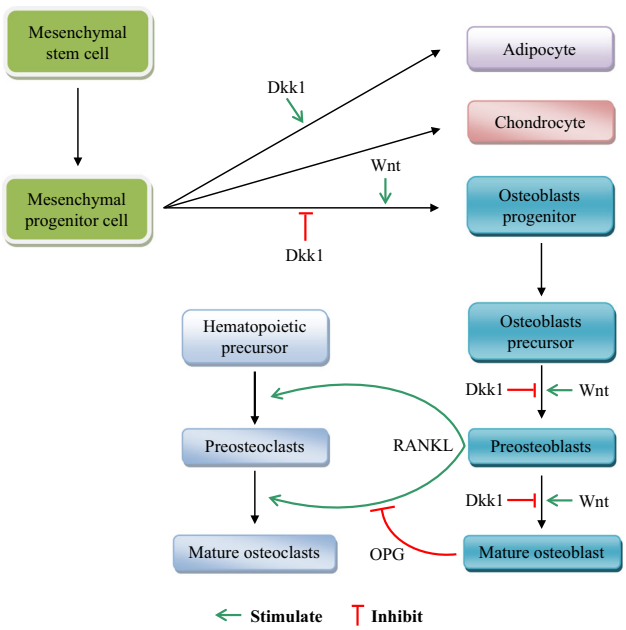

Fig. 2 Wnt signalling and Dkk1 in bone development. Wnt signalling enhances bone formation by directing the developmental program of mesenchymal stem cells toward osteoblast formation. Mature osteoblasts upregulate OPG, which blocks RANKL-induced osteoclastogenesis, resulting in inhibition of bone resorption. Dkk1 inhibits osteoblast formation and differentiation by diverting progenitors toward adipogenesis. Preosteoblasts enhance bone resorption by boosting RANKL-induced osteoclastogenesis

C3H10T1/2 osteoprogenitor cells revealed that Msx2, a homeodomain transcription factor first identified in osteoblasts, inhibited Dkk1 promotor activity and reduced RNA polymerase association with Dkk1 chromatin [87]. In addition, Dkk1-mediated inhibition of Wnt signalling was found to limit OPG expression, thereby shifting the OPG:RANKL ratio in favour of bone resorption [88].

Dkk1 in breast cancer and its metastasis into bone

Dkk1 overexpression in solid tumours is associated with worse survival [89]. However, the significance of Dkk1 expression in breast cancer progression and prognosis remains inconclusive. Some studies have shown that Dkk1 acts as a putative tumour suppressor in breast cancer cells via the suppression of Wnt signalling [90, 91] or via mechanisms independent of β-catenin-dependent transcription [92]. As mentioned above, the Wnt pathway influences self-renewal in the context of stem cells and cancer. Agur et al. showed that high concentrations of Dkk1 decreased mammosphere formation in both primary breast cancer cells and breast cancer cell lines by diverting proliferating cancer stem cells toward differentiation. Consequently, Dkk1 represents a potential target for ablation in differentiation therapy [93]. On the other hand, Sato et al. observed elevated expression of Dkk1 in four out of six human breast cancer types and found that 65.1 % (110/169) of breast cancer patients examined in their study had Dkk1 positive serum [94]. Forget et al. showed that Dkk1 is preferentially expressed in ER and PR-negative tumours, in tumours from women with a family history of breast cancer, and in primary tumours from patients with axillary lymph node invasion [95]. In addition, increased expression of Dkk1 was confirmed in hormone-resistant breast cancer cell lines, and Dkk1 expression in triple negative cancers was associated with poor outcome in these patients [96]. Moreover, elevated Dkk1 levels in the serum of breast cancer patients have been associated with shorter overall survival and relapse-free survival [97]. These contradicting observations may be due to differences in tumour type, tumour stage, tissue origin (epithelial or mesenchymal), or cellular subtypes.

Bone is the most frequent site of metastasis for several forms of cancer, including breast cancer. Evidence suggests that Wnt signalling and Dkk1 are involved in bone metastasis. Breast cancer-induced bone metastases are typically osteolytic, but occasionally osteoblastic lesions can occur. The most extensive data suggesting that Dkk1 promotes osteolytic metastasis come from studies of MM-associated bone disease [98, 99]. Few studies have investigated the role of Dkk1 in bone metastasis secondary to breast cancer showed that Dkk1 serum levels in women with breast cancer Voorzanger-Rousselot et al. and bone metastasis were higher than those in healthy age-matched controls and in women with metastases at sites other than bone [100]. Dkk1 was expressed by osteolytic breast cancer cell lines but not by osteoblastic lines. A subpopulation of breast cancer MDA-MB-231 cells known as the MDA-MB-231/bone cell line (MDA-231-BO) metastasize exclusively in bone and produce larger osteolytic lesions than the parental line [101]. Mice inoculated with MDA-231-BO cells, which developed radiologic and histologic evidence of skeletal lesions, had six-fold higher bone marrow levels of Dkk1 than control non-inoculated mice [102]. Moreover, Bu et al. demonstrated that MDA- MDA-231-BO cells exhibit increased levels of Wnt/β-catenin signalling and Dkk1 expression compared with MDA-MB-231 cells, and these changes were associated with inhibition of osteoblast differentiation and OPG expression. These effects could be neutralized by a specific anti-Dkk1 antibody [103]. Taken together, these observations indicate that Dkk1 may have a pathophysiological role in skeletal metastasis of breast cancer.

Some studies have demonstrated that Dkk1 is a downstream target of β-catenin-mediated transcriptional activity in several cell lines [104]. Nevertheless, because Dkk1 is relatively recently discovered inhibitor of Wnt/β-catenin signalling, the mechanisms by which breast cancer cells can avoid Dkk1 inhibition have to be identified. Menezes et al. postulated that excessive Dkk1 may accumulate in cancer cells but be unable to regulate the Wnt pathway due to malfunction of some component downstream of Dkk1. However, in surrounding bone cells with intact Wnt/β-catenin signalling, Dkk1 appears to function normally when it is up-regulated in a paracrine fashion [105]. If this hypothesis is confirmed experimentally, new therapeutic strategies to neutralize Dkk1 might be used in the treatment of breast cancer-induced bone metastasis.

The specific targeting of Wnt activation in bone may be achievable by targeting Dkk1 or SOST. Like Dkk1, SOST inhibits osteoblast differentiation by binding the Wnt co-receptors LRP5/6 on the surface of osteoblasts [106]. Indeed, SOST-neutralizing antibodies have been shown to have a strong anabolic effect in osteoporotic patients [109]. Mendoza-Villanueva et al. 109 showed that the Runt-related transcription factor 2, contributes to the formation of osteolytic bone metastases in breast cancer through induction of SOST expression [83], which is restricted to osteocytes [107]. Likewise, although Dkk1 is expressed widely during development, it is relatively restricted to bone (osteoblasts and osteocytes) in adult mice [108]. Thus, systemic administration of Dkk1 or SOST antagonists, may affect bone tissue selectively, favouring endogenous Wnt signalling-mediated increase in bone formation without affecting Wnt signalling in other tissues. There have not yet been clinical trials testing SOST antibody effects in cancer-

induced bone diseases. Therefore, further studies are required to determine whether SOST contributes to the development of bone metastases in vivo.

Therapeutic approaches

Currently, approved pharmaceutical approaches for targeting bone metastases are limited to agents that interfere with osteoclasts-mediated bone resorption, including bisphosphonates and anti-RANKL antibody denosumab [110]. However, the discovering that osteolytic lesions result not only from enhanced osteoclast-mediated bone resorption, but also from inhibition of bone formation led to the development of therapeutic strategies aimed at restoring osteoblast function. A number of preclinical in vitro and in vivo studies have defined the role of Dkk1 antibodies as a potential therapy for MM-associated bone disease [111]. In addition, two different anti-Dkk1 antibodies are being tested clinically in phase 1 and 2 trials, respectively, in patients with MM. Although, final results from these studies have not been published yet, preliminarily, they indicate a favourable safety profile and proof of anabolic activity [112, 113]. In contrast to myeloma, the role of Dkk1 antibodies in the treatment of breast cancer bone metastasis is less well characterised.

In a study by Rachner et al., treatment of breast cancer cells with zoledronic acid regulated alkaline phosphatase and OPG production arising from Dkk1 suppression via inhibition of protein geranylgeranylation. In line with the in vitro data, breast cancer patients receiving adjuvant zoledronic acid exhibited a 60 % decrease in serum Dkk1 levels after 12 months of treatment [114]. Another recent study showed that postmenopausal breast cancer patients treated with aromatase inhibition had a modest decrease in Dkk1 serum levels which correlated with increased bone mineral density of the femoral neck and the total hip [115]. Experimental stimulation of bone turnover has been shown to increase skeletal metastases in several animal models, suggesting that high bone turnover should be countered with anti-resorptive drugs [116]. Taken together, the literature suggests that Dkk1 is a mediator of malignant bone disease and that further studies concerning its potential as a novel therapeutic target are warranted.

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Compliance with ethical standards

Conflict of interest Erich-Franz Solomayer holds a consultancy position at Novartis and Amgen and received compensation from Novartis, Amgen and Roche. Mariz Kasoha, Ingolf Juhasz-Boess, Daniel Herr and Jasmin Teresa Ney declare that they have no conflict of interests.

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