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REVIEW

Signaling Between Tumor Cells and the Host Bone Marrow Microenvironment

Natasa Kovacic · Peter I. Croucher · Michelle M. McDonald

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Abstract Tumor cells with high skeletal homing affinity express numerous cell surface receptors that bind ligands produced in bone. Upon arrival, these cells survive in the host environment, encompassed in close proximity to bone marrow cells. Interactions between tumor cells and cells of the host microenvironment are essential to not only tumor cell survival but also their activation and proliferation into environment-modifying tumors. Through the production of RANKL, PTHrP, cytokines, and integrins, activated tumor cells stimulate osteoclastogenesis, enhance bone resorption, and subsequently release matrix-bound proteins that further promote tumor growth and bone resorption. In addition, alterations in the TGF-β/BMP and Wnt signaling pathways via tumor cell growth can either stimulate or suppress osteoblastic bone formation and function, leading to sclerotic or lytic bone disease, respectively. Hence, the presence of tumor cells in bone dysregulates bone remodeling, dramatically impairing skeletal integrity. Furthermore, through complex mechanisms, cells of the immune system interact with tumor cells to further impact bone remodeling. Lastly, with alterations in bone cell activity, the environment is permissive to promoting tumor growth further, suggesting an interdependence between tumor cells and bone cells in metastatic bone disease and multiple myeloma.

Keywords Myeloma · Breast cancer · Prostate cancer · Osteoblast · Osteoclast · Bone marrow

Introduction

The skeleton provides a highly vascular environment rich in nutrients, growth factors, and cellular niches that both attract and promote the survival and growth of tumors. Upon arrival in the bone marrow environment, tumor cells engraft in specific niches containing a multitude of bone marrow cells, upon which they are dependent for survival. Once activated, these tumor cells proliferate rapidly, forming self-perpetuating tumors that are able to modify the environment upon which they once depended. Skeletal tumors exhibit devastating effects on bone through dysregulation of bone cells, altered activation of immune and endothelial cells, and modifications of stromal elements, all of which feed back to further promote tumor growth.

This review discusses many of the processes involved in the arrival and engraftment of tumor cells in the bone marrow microenvironment and the complex cellular and molecular interactions that occur between tumor cells and bone marrow cells, with a particular focus on the dysregulation of bone remodeling. Although osteoclast and osteoblast interactions are interconnected in this context, this review focuses on these lineages separately, to assist with clarity.

Tumor Cell Homing and Engraftment to Bone

The high affinity for bone demonstrated by disseminated breast and prostate cancer cells results in high rates of bone metastases. Up to 70 % of patients with advanced disease

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N. Kovacic · P. I. Croucher · M. M. McDonald (✉)
Bone Biology Group, Musculoskeletal Division, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia
e-mail: m.mcdonald@garvan.org.au

develop bone metastasis, increasing mortality and reducing the likelihood for remission [1]. Upon dissociation from the primary tumor, metastatic cells circulate, populating the highly vascularized environment of the bone marrow. Here, the cells extravasate and enter the bone marrow compartment, where they may start to grow immediately or survive in a dormant state until activated to initiate tumor formation [2]. Due to the devastating consequences of tumor cells metastasizing to bone, a body of research has focused on defining the mechanisms governing this homing and survival of tumor cells in bone.

Skeletal homing of metastatic cells is a multistep process. The invasive potential of cancer cells is proposed to be initiated by epithelial to mesenchymal transition, although functional data to support this notion are limited. This phenotypic shift leads to the production of proteolytic enzymes, including matrix metalloproteinases (MMPs), which aid the migration of cancer cells into the local vasculature, escaping from the primary tumor. Chemotaxis to bone is heightened by the expression of a number of homing receptors by cancer cells. These include C-X-C chemokine receptor type 4 (CXCR4), CXCR7, CD44, very late antigen 4 (VLA-4), receptor for annexin 2 (Anxa2). Further high levels of the ligands for these receptors are detected in the bone marrow compartment [3–11]. The most widely explored of these ligand–receptor interactions is that of stromal cell–derived factor 1 (SDF-1, or chemokine C-X-C motif ligand 12, CXCL12), which binds to CXCR4 and CXCR7 receptors. Produced by bone marrow stromal cells, endothelial cells, and osteoblasts lining the endosteal surface, SDF-1 attracts tumor cells into the bone marrow compartment. Clinical samples demonstrated CXCR4 expression by metastatic tumor cells [3]. Furthermore, metastatic human prostate cell lines including DU145, LNCAP, and PC3 express functional CXCR4 receptor; and SDF-1 stimulation enhances their ability to migrate [4]. Antibodies to the CXCR4 receptor suppress skeletal metastasis formation in murine models of prostate cancer, confirming a key role of CXCR4 in the formation of metastatic bone disease [5]. In addition to CXCR4, CXCR7 has been identified as a receptor for SDF-1, with increased expression associated with an increase in the aggressive nature of metastatic bone disease in prostate cancer patients [6]. CXCR4 is also expressed by breast cancer cell lines and malignant breast tumors [7]. Overexpression of CXCR4 by MDA-MB-231 breast cancer cells enhances skeletal metastases [8], whereas antibodies to both CXCR4 and CXCR7 reduce skeletal metastases [9]. Expression of CXCR4 and its interaction with SDF-1 expressed by the bone marrow stroma, osteoblasts, and their progenitors is also important for homing of myeloma cells to the bone marrow [10]. Clinical samples and cell lines confirmed that the SDF-1/CXCR4 axis is strongly associated with progressive myeloma bone disease [11]. Further, inhibition of CXCR4 significantly reduced invasion of both 5TMM and 5T33M murine myeloma cell lines into the bone marrow compartment, confirming a pivotal role for this interaction in myeloma cell homing [12].

Once the tumor cells arrive in bone, successful engraftment in the host environment is vital to tumor cell survival. It is at this stage that tumor cells are dependent on the bone environment for survival signals, although the nature of these signals remains poorly defined. In addition to the SDF-1/CXCR4 axis, a number of cell adhesion molecules actively promote homing of cancer cells to bone as well as their engraftment. Anxa2 is a cell surface protein expressed by endothelial cells, myeloid precursors, and osteoblasts [13]. In the cancer setting, Anxa2 is associated with a number of invasive cancers, including prostate cancer, and blocking Anxa2 receptors reduces bone metastases in murine prostate cancer models [14]. Cadherin-11 is another cell adhesion molecule expressed by tumor cells and associated with both breast and prostate cancer cell migration and skeletal invasion [15]. Recently, inhibition of cadherin-11-mediated adhesion of prostate cancer cells with a monoclonal antibody reduced skeletal metastases in the murine PC-3 model [16].

Osteopontin (OPN) is a bone-specific glycoprotein expressed by osteoblasts that is involved in cell-matrix adhesion and binds to CD44 expressed by tumor cells. It has been demonstrated that breast and prostate cancers cells also express OPN, with elevated levels associated with poorer outcomes in breast cancer metastatic disease and prostate cancer [17, 18]. Interestingly, OPN knockout mice demonstrate a severe hematopoietic stem cell (HSC) deficiency as HSCs are unable to engraft in the HSC niche [19]. In addition to OPN, a number of integrins known to be involved in HSC niche engraftment, such as VLA-4, which is discussed later, are also associated with bone metastatic disease. There are a number of common features shared between HSC and tumor cell interaction with the bone microenvironment. This has led to the hypothesis that the tumor cell niche is similar to the HSC niche [20]. Indeed, prostate cancer cells have been reported to compete for the HSC niche in mouse models, highlighting the potential for the HSC niche to support tumor cell engraftment, survival, and the ability of tumor cells to reside in a dormant state within the bone marrow environment [21]. However, it remains contentious whether or not tumor cells compete with HSCs for the HSC niche or reside in a unique microenvironment. As such, further work in this area is ongoing, with the aim to elucidate the role of the local microenvironment in the colonization and activation of tumor cells in bone. It is, however, clear that the initiation of tumor cell proliferation and growth within bone switches the cells from environment-dependent to environment-modifying, altering bone mass and structure by disrupting bone remodeling.

Tumor-Induced Dysregulation of Bone Remodeling

Metastatic bone tumors are characterized radiographically by lytic, sclerotic, or mixed lesions. Lytic lesions are associated with enhanced osteoclast activity resulting from the interaction between tumor cells and osteoclast-lineage cells. However, it is the effect of tumor cells on cells of the osteoblast lineage that determines the extent of lytic or sclerotic disease. In addition to regulating bone cell activity directly, tumor cells interact with other bone-residing cells, such as mesenchymal cells and cells of the hematopoietic lineage, as well as those of the innate and adoptive immune system. These cells directly affect the behavior of bone cells, and hence, tumors may also indirectly alter bone remodeling.

Interactions Between Tumor Cells and Osteoclasts

Osteolytic lesions are caused by increased osteoclast recruitment, differentiation, and function, which can be mediated by multiple signaling pathways (Fig. 1). These include soluble and membrane-bound proteins with the receptor activator of NF-κB ligand (RANKL) and parathyroid hormone-related protein (PTHrP) playing a pivotal role, as well as numerous cytokines and chemokines, integrins, and matrix proteases. All of these molecules can be produced by tumors themselves or induced in the local bone marrow microenvironment by the presence of tumor cells.

RANK/RANKL/Osteoprotegerin System

The RANK/RANKL/osteoprotegerin (OPG) system plays a central role in regulating skeletal homeostasis through coupling of bone formation and resorption. Interaction of RANKL with RANK expressed on the osteoclast progenitors stimulates osteoclast differentiation through activation of NF-κB and the Jun N-terminal kinase pathway [22–24]. RANKL, which is produced by osteoblast-lineage cells and activated lymphocytes, also binds OPG, which acts as a decoy receptor to inhibit RANK/RANKL signaling [25]. In metastatic bone disease, this system is dysregulated,

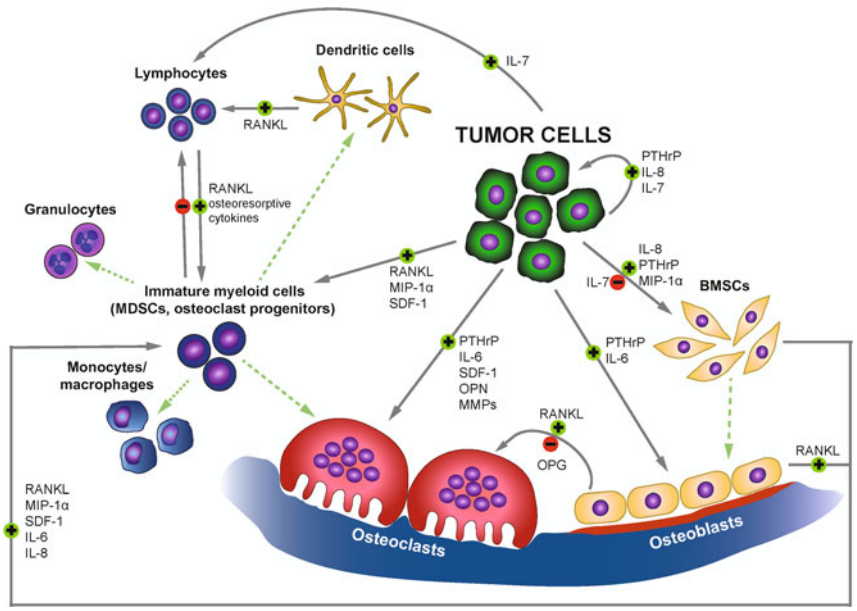

Fig. 1 Interactions of bone tumors with osteoclasts and cells of the immune system. Tumors with affinity to bone often induce osteolytic lesions by increasing osteoclast differentiation and activation. Tumor cells express receptor activator of NF-κB ligand (RANKL) and macrophage inflammatory protein-1α (MIP-1α, CCL3), which stimulate differentiation of osteoclast progenitors in the bone marrow into mature osteoclasts. In addition, parathyroid hormone–related protein (PTHrP), IL-6, stromal cell–derived factor-1 (SDF-1 or CXCL12), osteopontin, and matrix metalloproteinase 13 (MMP13) secreted by various osteolytic tumor cells stimulate bone resorption by mature osteoclasts. PTHrP and IL-6 also stimulate expression of RANKL in osteoblasts, while MIP-1α, PTHrP, and IL-8 upregulate RANKL in stromal cells, thus stimulating osteoclastogenesis indirectly. IL-7 from multiple myeloma cells may additionally suppress osteoblast differentiation from mesenchymal stromal progenitors and contribute to osteolytic process. Besides RANKL, tumor cells upregulate expression of pro-osteoclastogenic factors in bone marrow stromal cells such as MIP-1α and SDF-1. Tumors often induce accumulation of immature myeloid cells, which are able to suppress antitumor immune response (myeloid-derived suppressor cells, MDSCs), by inhibiting cytotoxic T-lymphocyte responses. Differentiation of myeloid-derived macrophages and dendritic cells is also diverted toward the immune-suppressive phenotypes, promoting tumor growth and invasion. On the other hand, tumor cells can stimulate lymphocytic production of osteoresorptive cytokines such as RANKL. *Grey arrows*, effects of stimulatory (*green circles*) or inhibitory (*red circle*) molecules; *green dashed arrows*, differentiation paths

leading to enhanced bone resorption, promoting tumor-induced osteolysis. Breast cancers and breast cancer cell lines have been shown to express RANK and OPG; but not RANKL; thus, their effect on osteoclastogenesis is indirect, through stimulation of osteoblast expression of RANKL [26]. Expression of RANK, OPG, and RANKL has been documented in prostate cancers and prostate cancer cell lines [27], with soluble RANKL produced by prostate cancer cells contributing to the development of bone metastasis [28]. In addition, local production of RANKL, induced by the presence of prostate cancer cells, is crucial for the development of bone disease as blocking of tumor-derived RANKL was not able to abolish the osteolytic response in the C4-2 human prostate cancer xenograft model [29]. Myeloma cells have also been shown to stimulate osteoclastogenesis both directly through RANKL production [30–32] and indirectly through increasing RANKL and decreasing OPG production by primary human bone marrow stromal cells (BMSCs) and osteoblasts [33–35]. The implication for RANK/RANKL in the pathology of the bone disease associated with tumors has led to therapeutic approaches targeting the RANKL/OPG system to prevent development of osteolytic lesions. Recombinant OPG, OPG peptidomimetic, and soluble RANK constructs have all been shown to prevent myeloma-induced bone loss and the formation of bone lesions in murine models of myeloma bone disease, through suppressing osteoclastogenesis [30, 33, 36, 37]. Similar strategies prevented the development of osteolytic lesions induced by MDA-MB-231 breast cancer and decreased metastatic tumor burden [38, 39]. In addition, OPG-Fc suppressed osteolytic lesions, decreased tumor burden, and increased the chemotherapeutic efficacy of docetaxel in the murine model of PC3 osteolytic prostate cancer metastasis [40]. Lastly, direct targeting of RANKL with denosumab, a human monoclonal antibody that specifically inhibits RANKL, has been shown to suppress breast cancer–induced osteolysis in clinical trials [41, 42].

PTHrP

PTHrP is produced by many tumors with affinity for bone and plays an important role in the development of bone metastasis [43, 44]. PTHrP is a potent stimulator of bone resorption. It has been frequently identified in primary breast cancers and in more than 90 % of osteolytic breast cancer bone metastases [45]. Neutralization of PTHrP using a monoclonal antibody in the MDA-MB-231 murine model of osteolytic disease significantly suppressed bone loss through reducing osteoclast numbers as well as tumor area in affected bones [46]. TGF-β from the tumor environment may additionally increase PTHrP production by breast cancer cells. The transcription factor Gli2 plays an important role in this process as its inhibition decreases tumor PTHrP expression and reduces osteolysis [47]. Osteoclastogenic effects of PTHrP appear to be mediated via the upregulation of RANKL in osteoblasts [48]. Prostate cancer cells also express PTHrP, which may contribute to the lytic component of prostate cancer bone metastases. Its receptor, PTH-R1, is often coexpressed on prostate cancer cells, suggesting an additional autocrine role of this PTHrP in supporting tumor invasion [49].

Cytokines and Chemokines

A number of cytokines and chemokines produced by tumor cells also act in both a paracrine and an autocrine fashion to alter osteoclastogenesis as well as to stimulate tumor growth. These include interleukin (IL)-6, IL-7, IL-8, macrophage inflammatory protein-1α (MIP-1α, C-C motif ligand 3, CCL3), and SDF-1.

IL-6 is a cytokine capable of promoting tumor growth as well as modifying bone remodeling. The role of IL-6 in the pathology of multiple myeloma is well established and has led to phase II clinical trials of IL6 antibodies for therapeutic applications in myeloma [50]. IL-6 acts on a variety of hematopoietic, embryonic, and neural cells. The IL-6 receptor is expressed on mature osteoclasts and osteoclast progenitors, suggesting a direct effect on osteoclastogenesis [51]. However, the majority of functional studies point to greater importance of indirect stimulation of osteoclastogenesis, through binding to IL-6 receptors on osteoblast-lineage cells, stimulating RANKL production and, hence, osteoclastogenesis [52]. In addition to its role in multiple myeloma, IL-6 is detected in the glandular tissue of prostate cancer specimens; PC3, DU145, and LNCaP prostate cancer cell lines [53, 54]; as well as breast cancer cells [55]. Bone metastases of breast cancer are able to initiate osteoblastic secretion of IL-6 and other inflammatory cytokines and, hence, promote osteolysis [56]. It has recently been shown that breast cancer-induced upregulation of IL-6 in osteoblasts is mediated by the Notch ligand Jagged1, emphasizing a critical role of Jagged1 in breast cancer metastasis [57]. Jagged1 expression is also suggested to be stimulated by TGF-β, which is released from the matrix during osteolysis, thus supporting the vicious circle of tumor-induced osteolysis [57]. Hence, IL-6 may also contribute, although indirectly, to the osteolytic process during prostate and breast cancer bone metastasis.

IL-7 has also been implicated in multiple myeloma (MM) bone disease, with increased levels detected in bone marrow plasma of patients [58]. MM tumor cells were reported to express IL-7, which may enhance osteoclastogenesis via stimulation of RANKL production by T lymphocytes. Further, with receptors for IL-7 demonstrated on BMSCs, cocultures of osteoblast precursors with myeloma

cells showed reduced colony formation and osteoblastic differentiation, which was partially rescued by inhibition of IL-7 [59]. Hence, IL-7 expression in myeloma is associated directly with suppressed osteoblast activity as well as indirectly with enhanced osteoclastogenesis.

IL-8 (CCL8) is a proinflammatory chemokine, which is highly expressed in breast cancer cell lines as well as in prostate cancer cell lines with high metastatic potential [60]. IL-8 stimulates expression of RANKL mRNA in osteoblasts and induces osteoclastogenesis directly via binding to receptor CXCR1 on osteoclast progenitors [60]. BMSCs from patients with myeloma have enhanced production of IL-8, which is involved in NF-κB-mediated resistance of myeloma cells to the proteasome inhibitor bortezomib [61]. Therefore, IL-8 production in myeloma promotes tumor growth and may also contribute to the enhanced bone resorption.

SDF-1, as mentioned above, is a strong chemo-attracting cytokine produced by multiple cells in the bone environment to promote homing and engraftment of tumor cells in the bone microenvironment. Its receptor, CXCR4, is also expressed by human monocytes, through which SDF-1 induces their differentiation into osteoclasts [62]. Both SDF-1 and CXCR4 are expressed by breast cancer cells and may contribute to breast cancer invasion [7]. In MM, tumor cells are reported to produce SDF-1, and elevated levels of serum SDF-1 are associated with osteolytic bone lesions [11]. Further, in vitro studies revealed that recombinant human SDF-1 increases the resorptive activity of human peripheral blood–derived osteoclasts and that an inhibitor of CXCR4 partially abrogates the increased resorption induced by myeloma-cell conditioned medium [11].

MIP-1α is a chemokine produced by MM cells, capable of promoting osteoclastogenesis through binding to CCR1 and CCR5 receptors on osteoclast precursors [63]. Stromal cells also express CCR5, whose activation by recombinant MIP-1α stimulates the expression of RANKL, indirectly stimulating osteoclast differentiation [64]. As recombinant MIP-1α was unable to induce osteoclastogenesis in RANK-deficient mice, its effect in myeloma appears to require RANK signaling [65]. Specific inhibition of CCR1 and CCR5 signaling revealed differential roles for these receptors in myeloma disease. CCR5 is specifically involved in homing of myeloma cells to bone, whereas both receptors play a role in stimulating osteoclastogenesis and the development of osteolytic lesions [12]. Furthermore, production of MIP-1α has been shown in 4T1 mammary carcinoma cells, where this chemokine is responsible for myeloid cell infiltrations of both primary and metastatic tumors [66]. As myeloid cells contain osteoclast progenitors, this pathway may contribute to the attraction of osteoclast progenitors to the tumor site and promote the development of osteolytic lesions.

Integrins, Matrix Proteins, and Matrix-Degrading Enzymes

In addition to soluble proteins and cytokines, interactions of integrins and extracellular matrix proteins play a critical role in tumor homing, progression, and metastasis, as well as in the regulation of bone cell trafficking and activity. Regulation of osteoclast activity, specifically their cytoskeletal organization, polarization, and intracellular trafficking, is related to downstream signaling of $\alpha_v\beta_3$ [67]. This integrin may be activated by various tumor- or microenvironment-derived signals, for example, OPN, which is often expressed in bone metastatic tumors [17]. Breast cancer cells with strong bone-metastatic ability express a vascular cell adhesion molecule 1 (VCAM-1), which, through the interaction with integrin $\alpha_4\beta_1$ (VLA-4), recruits osteoclast progenitors and is responsible for osteolysis and metastasis progression [68]. Matrix-degrading enzymes produced by cancer cells support dissemination of metastatic cells from the primary tumors but also promote bone destruction directly via matrix proteins or indirectly via osteoclast activation. For example, MMP-13 has been shown to have an important role in osteoclast activation in breast cancer metastasis [69], whereas membrane type 1 (MT1)-MMP, expressed in prostate cancer bone metastases, increases the shedding of RANKL from the tumor cell surface and indirectly stimulates osteoclasts [70]. In addition, MMP-7 derived from host osteoclasts has been shown to play an important role in promoting tumor growth and tumor-induced osteolysis in breast and prostate cancers via solubilization of RANKL [71, 72].

Interactions Between Tumor Cells and Osteoblasts

The effect of tumor cells on osteoblasts plays an important role in determining the nature of tumor-induced bone disease; increased osteoblast activity will lead to osteosclerotic disease, whereas suppression of osteoblast activity can result in osteolytic disease (Fig. 2a–c). A number of pro-osteoblastic, tumor-derived factors have been identified, with profound sclerotic disease exhibited in cancers that produce bone morphogenetic proteins (BMPs) or members of the Wnt family. Interestingly, when antagonists of these pathways, such as Noggin, Dickkopf-1 (DKK1), and secreted frizzled-related protein 1 (SFRP1), are also produced by tumor cells, lytic disease predominates. In addition to BMPs and Wnts, a number of tumor-secreted proteins and growth factors have been implicated in regulating osteoblast activity and promoting stromal cell migration in response to tumor presence. These include endothelin 1 (ET-1), ephrins (Eph), platelet-derived growth

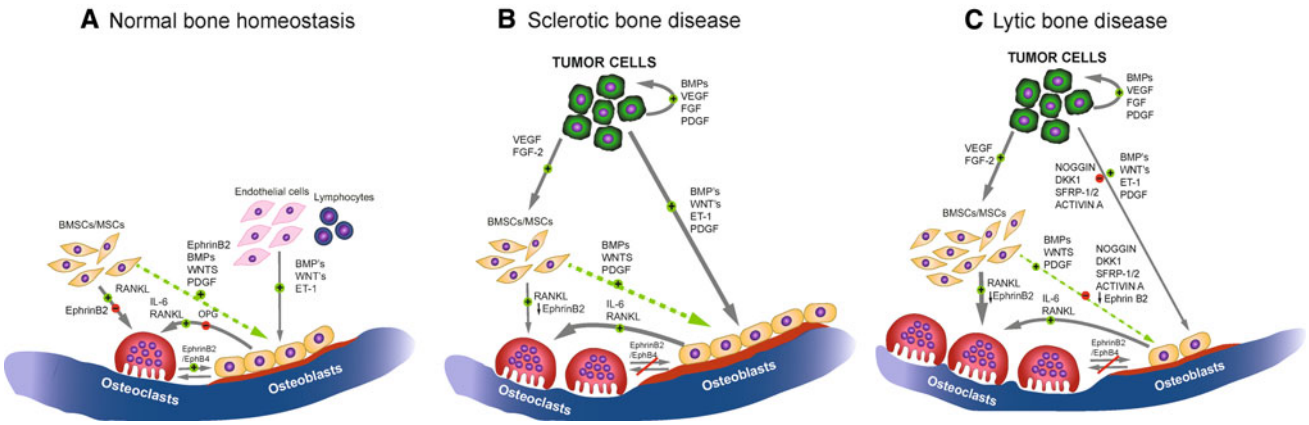

Fig. 2 Interactions of bone tumors with osteoblasts. **a** Illustration of the molecular pathways discussed in this review in the context of normal bone homeostasis. Bone marrow stromal cells, vascular endothelial cells, and cells of the immune system produce a number of proteins and growth factors that promote both osteoblastogenesis and osteoclastogenesis. Coupling between osteoblasts and osteoclasts also regulates bone mass via the RANK/RANKL/OPG system and EphrinB2/EphB4 signaling. Further, the release of growth factors such as PDGF and VEGF through bone matrix resorption stimulates further osteoblastogenesis. **b** Illustration depicting dysregulation through tumor cell–osteoblast/preosteoblast interactions in the sclerotic disease state. Tumor cells produce BMPs, WNTs, ET-1, and PDGF, which enhance osteoblast proliferation and differentiation from MSCs, increasing bone formation. A number of these factors also act to further promote tumor growth. VEGF and FGF-2 production by tumor cells also drives tumor growth in addition to promoting the migration and proliferation of BMSCs. The increased osteoblast activity also acts to promote osteoclast activity through IL-6 and RANKL. Further, EphrinB2/EphB4 signaling between osteoblasts and osteoclasts is dysregulated, while reduced ephrin B2 expression by MSCs may stimulate osteoclast formation. **c** Illustration depicting tumor cell–osteoblast/preosteoblast interactions in the lytic disease state. Tumor cells that lead to lytic disease also produce Noggin, Dkk1, and Sfrp1/2, which oppose the BMP and Wnt stimulation of osteoblast differentiation and proliferation, suppressing bone formation. Activin A is also produced by tumor cells to suppress bone formation in lytic disease. Further, these inhibitors reduce MSC differentiation, leading to accumulation of immature preosteoblasts, which promote osteoclast formation through expressing RANKL. Ephrin production by BMSCs is also reduced in the presence of tumor cells, suppressing bone formation and driving osteoclast formation. *Grey arrows*, effects of stimulatory (*green circles*) or inhibitory (*red circles*) molecules; *green dashed arrows*, differentiation paths

factor (PDGF), fibroblast growth factors (FGFs), and vascular endothelial growth factor (VEGF).

TGF-β Superfamily

TGF-β has an established role in both bone homeostasis and tumor growth and progression, with elevated circulating levels related to poorer prognosis in cancer patients [73]. Locally in bone, TGF-β is abundant in the bone matrix, where, among other roles, upon release after bone resorption it stimulates the production of PTHrP by tumor cells. As discussed above, PTHrP drives bone resorption through inducing RANKL and suppressing OPG production by osteoblasts, stimulating osteoclastogenesis [45]. Further, TGF-β stabilizes hypoxia-inducible transcription factor-1 (HIF-1) by preventing its degradation. HIF-1 has been shown to support osteolytic bone metastases through stimulating angiogenesis and osteoclastogenesis and suppressing osteoblast differentiation [74]. Inhibitors of TGF-β have been explored for their antitumor potential with promising outcomes in a variety of models. Intravenous administration of an adenovirus targeting TGF-β signaling inhibited growth of established bone metastases in the 4T1 mouse mammary tumor model in an immunocompetent syngeneic host [75].

BMPs are members of the TGF-β family with a pleiotropic role in the differentiation of chondrocytes, osteoclasts, and, importantly, osteoblasts. Targeted stimulation of bone formation by BMPs has led to their clinical use to enhance bone repair. BMPs have also been implicated in the uncoupling of bone remodeling in skeletal metastases. For example, expression of BMPs and their receptors has been reported in prostate cancer cells [76]. Further, increased expression of BMP-6 in prostate cancer cells and osteosclerotic tumors correlates with the aggressiveness of the tumor [77]. BMP-4 produced by MDA-PCa-118b prostate cancer xenografts was reported to drive the highly osteogenic nature of this model [78]. Functional evidence was obtained by treating these mice with a small-molecule inhibitor of BMP receptor 1, which reduced bone formation and tumor growth. In addition, neutralizing antibodies to BMP-6 diminished prostate cancer conditioned medium–induced mineralization of preosteoblasts in vitro. In vivo, anti-BMP-6 treatment in the LuCaP23.1 prostate cancer model suppressed the osteoblastic response, without altering the osteolytic component [79]. Myeloma cells also produce BMP-6; however, proliferating myeloma cells and myeloma cell lines demonstrate reduced levels of BMP-6. In myeloma patients undergoing chemotherapy, increased BMP-6

levels are reported to correlate with improved survival [80].

In addition to osteoblastic lesions, BMPs have been implicated in the development of osteolytic disease in both prostate and breast cancer bone metastases, in which bone formation is suppressed. In cells derived from lytic disease, the BMP antagonist Noggin is highly expressed, repressing BMP signaling and, hence, osteoblast activity [81, 82]. Overexpression of Noggin reduced osteoblastic lesions in mice bearing prostate cancer tumors [76]. Further, silencing of Noggin in the osteolytic PC3 cell line led to restoration of osteoblast number and activity within bone lesions [83]. Thus, inhibition of BMP activity by the antagonist Noggin can determine the extent of sclerotic or lytic disease in this setting. Furthermore, increased recruitment of MSCs to tumor sites can promote osteoblastogenesis in sclerotic disease by providing a pool of osteoblast progenitor cells [84]. These progenitors are then responsive to the tumor-produced BMP and/or Noggin, contributing to osteoblast promotion or suppression, respectively.

Activin A also belongs to the TGF-β superfamily. Levels of circulating activin A are elevated in breast and prostate cancer patients suffering from bone metastases as well as MM patients with bone disease [85–87]. Activin A is released from the bone matrix to regulate normal bone turnover but is also a product of tumor cells residing in bone, inducing osteoclast formation and suppressing osteoblast activity [88]. Although the effect on osteoblasts is not well explored in metastatic bone disease, suppression of osteoblast activity by activin A is strongly implicated in the lytic disease exhibited in MM patients [87]. Certainly, inhibition of activin A with a soluble ActRIIA construct showed robust prevention of osteoblast suppression and prevented the development of osteolytic bone lesions, despite having no effect on osteoclasts in preclinical myeloma models [89].

Wnts and Wnt Antagonists

In a similar manner to BMP and Noggin coregulation, the expression of both Wnts and their antagonists by tumor cells can also influence the development of lytic or sclerotic bone disease. Wnts are a group of glycoproteins that activate canonical β-catenin signaling, promoting osteoblastic differentiation and thereby robustly regulating bone formation. A role for Wnt regulation of skeletal tumors was first highlighted by the discovery that myeloma cells expressed high levels of the Wnt antagonist Dkk1 [90]. Subsequently, this led to the implication of Wnt regulation in numerous cancers that grow in bone. For instance, human prostate cancer skeletal metastases demonstrate high levels of Wnt1 expression [91] and osteoblastic prostate cancer cell lines produce a number of soluble Wnt proteins [92], all of which activate osteoblast progenitors. Conditioned media from the MDA-PCa-2b osteoblastic prostate cancer cell line failed to promote osteoblastogenesis in cells from mice lacking the LRP5 Wnt receptor, confirming mechanistic Wnt signaling [93]. Furthermore, Wnt signaling blockade through addition of the Wnt antagonist DKK1 reduced the osteoblastic stimulation by prostate cancer cells [94]. Taken together, these data suggest that Wnt production by tumor cells promotes osteoblastogenesis, which can lead to osteosclerotic lesions.

Tumors that are primarily osteolytic express a number of Wnt antagonists including DKK1 and SFRP1, which suppress osteoblast activity, promoting osteolysis [95, 96]. The lytic PC3 cell line demonstrated a 50-fold higher DKK1 expression compared to the MDA-PCa-2b osteoblastic prostate cancer cell line [93]. Further, when DKK1 was silenced in osteolytic PC3 cells, sclerotic lesions formed instead, confirming regulation of bone formation by Wnt antagonists in this setting [95]. Thus far, only one study has demonstrated an increase in circulating DKK1 levels in prostate cancer patients with skeletal metastases, but evidence points to a modulating role of Wnt/β catenin signaling in the pathology of this disease [97]. Breast cancer bone disease is predominantly lytic, due to high expression of Wnt antagonists, with elevated circulating levels of DKK1 in patients with bone metastases and increased DKK1 expression at bone metastatic sites [98]. Conditioned media from breast cancer cells high in DKK1 levels blocked Wnt3a-induced osteoblastic differentiation and OPG expression [99]. Hence, in a similar manner to BMP and Noggin expression, the drive to form osteoblastic lesions in response to tumor-produced Wnt is opposed by expression of Wnt antagonists such as DKK1. Under these circumstances the balance favors osteolytic disease, suggesting that the key regulator of the nature of metastatic bone disease is the osteoblast response. Interestingly, it has been proposed that BMP and Wnt signaling pathways may act in concert to alter bone formation in metastatic bone disease [100].

Since DKK1 expression was first demonstrated in myeloma cells in 2003 [90], Wnt regulation of osteolytic bone disease in MM has been widely explored. Circulating levels of DKK1 are high in a proportion of patients, correlating with the presence of osteolysis [101, 102]. Furthermore, bone marrow from MM patients can inhibit osteoblast differentiation in vitro through increasing IL-6 production by osteoblasts, an effect that is neutralized by anti-DKK1 antibody [103]. SFRP2, another Wnt antagonist, is also produced by myeloma cells to suppress bone formation [104]. In addition to direct osteoblast suppression, DKK1 production by myeloma cells can alter osteoblast production of RANKL and OPG, increasing osteoclast activity and, hence, indirectly promoting lytic

disease in these patients [105]. Furthermore, in the 5TGM1 murine model of myeloma bone disease, mesenchymal stromal cell production of DKK1 was implicated in the development of tumors, suggesting a role of cells from the host environment in the progression of bone disease [106]. Wnt antagonists therefore provide a new target for bone anabolic therapies in myeloma bone disease [107]. In support of this, neutralizing antibodies to DKK1 prevent the destructive bone disease in a range of preclinical MM models [103, 108, 109]. With proven therapeutic potential from preclinical studies, clinical trials are under way with the anti-DKK1 antibody in patients with MM. Interestingly, when the Wnt promoting agent lithium chloride was administered in the 5TGM1 myeloma model, both bone destruction and tumor burden within bone were reduced [110]. Extraskeletal tumor burden was, however, enhanced with lithium treatment, confirming the importance of the localization of altered Wnt signaling effects on myeloma growth.

More recently, the discovery of the bone-specific Wnt antagonist protein sclerostin (sost) has led to studies examining its role in metastatic bone disease and myeloma. In patients with MM, increased serum levels of sclerostin have been reported along with evidence for production by myeloma cells [111, 112]; however, this remains to be confirmed, and there are no functional data to support a causal role for sclerostin in the pathology of myeloma bone disease. Sost has also been reported to be expressed in prostate and breast cancer cells [113, 114], but its role in this setting also remains to be determined. Sclerostin-neutralizing antibodies have a potent bone anabolic effect; thus, targeting endogenous sclerostin may provide an avenue for treating osteoblast suppression in metastatic bone disease and MM.

Endothelin-1

ET-1 is a vasoconstrictor protein produced by vascular endothelial cells and has direct effects on the vasculature [115]. ET-1 has been shown to promote osteoblast proliferation through binding the endothelin A and B receptors (ETAR, ETBR) on osteoblasts [116]. ET-1 production has been implicated in the development of metastatic bone disease [117]. Patients with prostate cancer have elevated circulating ET-1 [118], and osteoblastic lesions induced in mice by the ET-1 expressing Zr-75-1 breast cancer cell line were suppressed by ETAR antagonists [119]. These outcomes have led to clinical studies of ETAR antagonists in patients with prostate bone metastasis, significantly reducing lesion progression [120, 121]. In addition to direct osteoblastic effects, ET-1 regulates osteoblastic production of IL-6 [122], which may indirectly affect tumor growth and the development of bone disease.

Ephrins

Ephrins and their receptors are cell surface molecules expressed in multiple tissues, which act in a bidirectional manner to control cell–cell interactions, immune regulation, angiogenesis, and neuronal development. Importantly, Eph signaling has also been implicated in tumor growth and metastasis [123, 124]. In bone, Eph signaling is well characterized in osteoclast–osteoblast cross-talk; hence, it is important to coupling of bone remodeling [125]. MSCs from myeloma patients showed reduced expression of Ephs, in turn uncoupling bone remodeling. The Eph/EphB4 receptor axis is dysregulated in osteoprogenitors from myeloma patients, and activation through EphB4-Fc treatment suppresses myeloma bone disease and tumor growth [126]. Further work exploring Eph antagonism in metastatic bone disease may lead to new targeted treatments in this area.

Growth Factors

A number of local growth factors, such as PDGF, FGF-8, and VEGF, also play a role in osteoblast responses to tumor cells. These growth factors are both released via resorption of bone matrix and secreted by tumor cells. Breast cancer cells produce PDGF, and elevated levels of circulating PDGF correlate with increased metastatic disease, resistance to treatment, and reduced survival [127, 128]. In bone, the PDGF-BB isoform has been associated with regulation of both osteoclast and osteoblast formation and function [129]. Evidence for PDGF regulation of osteoblast proliferation and differentiation is limited, although data suggest that PDGF may promote migration of progenitor cells [130] while impairing bone matrix production by mature osteoblasts [131]. These later effects are consistent with the potential for elevated PDGF levels to impair bone formation in bone metastasis. Furthermore, PDGF exhibits primarily mitogenic effects on cell types that reside in the bone marrow environment, such as fibroblasts, stromal cells, and pericytes [132]. Therefore, in the tumor setting, rather than exhibiting bone-specific effects, PDGF production by tumor cells is likely key to promoting tumor growth. This is achieved by stimulating angiogenesis through regulating local stromal cell production of proangiogenic factors such as erythropoietin, promoting endothelial cell proliferation, migration, and tube formation [133]. Similarly, FGF-8 and VEGF, while also associated with stimulating osteoblast proliferation and differentiation [134, 135], are products of tumor cells that primarily promote tumor growth. Hence, their increased expression by tumor cells may indirectly drive lytic or sclerotic lesions through tumor growth promotion. It should be noted that a number of these tumor-produced growth factors also regulate the attraction and migration of mesenchymal stem

cells to the tumor environment, further supporting neovascularization at the site and hence tumor growth.

Interaction Between Tumor Cells and Cells of the Immune System within the Bone Microenvironment

Although it is important to understand the interactions between tumor cells and bone cells, many other cell types, including those of the immune system, also respond to tumor cells invading the bone marrow. These may either directly or indirectly alter the skeletal consequences of tumor presence.

T Lymphocytes

T lymphocytes play an important role in the regulation of tumor invasion and, according to their function, can be divided into several functional and phenotypic subsets. $CD8^+$ cytotoxic T lymphocytes (CTLs) and Th1-type $CD4^+$ T lymphocytes promote immune surveillance, whereas Th2-type $CD4^+$ T lymphocytes, such as regulatory T lymphocytes (Tregs) and IL-17–secreting (Th17) T lymphocytes, may promote tumor growth via suppression of immune surveillance [136]. Activation and phenotypic changes induced by interactions of T lymphocytes with bone-residing tumor cells can alter the expression of factors involved in bone regulation and contribute to imbalanced bone remodeling. For example, myeloma cells induce expression of RANKL in T lymphocytes and promote bone resorption [58]. Furthermore, a subset of T lymphocytes in the bone marrow and peripheral blood from myeloma patients has a Th17 phenotype and is able to induce osteoclast activation and promote osteolytic lesions [137]. Metastatic prostate cancer mediates expansion of Tregs by upregulation of RANK in bone marrow dendritic cells. These expanded Tregs are able to inhibit osteoclast differentiation in vitro, and depletion of these cells decreased bone mineral density in a prostate cancer model in vivo, suggesting a role for Tregs in the development of osteosclerotic bone lesions [138]. An important role of $CD8^+$ T lymphocytes in counteracting the development of osteolytic bone metastases was demonstrated in a murine model of breast cancer. Depletion of a subset of plasmacytoid dendritic cells responsible for shifting the Th cell responses toward the Th2 regulatory type resulted in decreased tumor burden as well as decreased osteolysis. This was suggested to be due to the activation of cytolytic $CD8^+$ T cells [139].

B Lymphocytes

Activated B lymphocytes have been found to infiltrate tumor tissues and lymph nodes of patients with malignancies and may be involved in humoral antitumor response [140]. On the other hand, B lymphocytes may also participate in tumor promotion as B lymphocyte-deficient mice are resistant to tumors due to increased Th1 and cytotoxic responses [141]. Disturbances in the B-lymphocyte compartment within the bone marrow induced by tumors may contribute to skeletal consequences as activated B lymphocytes have been shown to express both RANKL and OPG [129, 142] and the impaired balance between these two molecules promotes the development of osteolytic bone disease.

Myeloid Cells

Myeloid cells, such as granulocytes, dendritic cells, and macrophages, are elements of tumor stroma and elicit diverse effects. These include enhancement of tumor growth through suppression of antitumor immunity, stimulation of angiogenesis, and producing defense responses against tumor cells. Generally, activation of these cells through a Th1-mediated response stimulates an antitumor effect, while activation via Th2 cytokines may suppress the immune reaction to tumors, thereby promoting their growth via different microenvironment-dependent mechanisms [136]. Among myeloid cells, myeloid-derived suppressor cells (MDSCs) are recognized as suppressors of effector T, NKT, and NK cell-mediated responses to tumors. MDSCs are immature myeloid cells, which accumulate not only in response to tumors but also in other pathologic conditions such as infection or trauma [143]. The role of MDSCs in the development of skeletal osteolytic metastases of breast cancer has been explored in the MDA-MB-231 murine model of metastasis [144]. Tumor cells induced significant expansion of immature myeloid (Gr1+CD11b+) cells in both spleen and bone marrow. Intratibial injection of MDSCs increased tumor burden and osteolytic disease compared to mice injected with immature myeloid cells obtained from non–tumor-bearing mice. It has been suggested that this is mediated by increased TGF-β production by MDSCs, which in turn stimulates production of osteolytic PTHrP in breast cancer cells [144]. Furthermore, MDSCs from tumor-bearing mice generated increased numbers of osteoclasts in comparison to control animals, pointing to a direct involvement in osteoclastogenesis [144]. In support of this, MDSCs are increased in osteolytic disease in the 5TGM1 murine model of myeloma in the blood, spleen, and bone marrow. These MDSCs displayed increased osteoclastogenic potential in comparison with control myeloid cells as well as the ability to increase both tumor burden and osteolysis in vivo [145].

These data emphasize the important role of the immune cell compartment in both the regulation of tumor development in bone and the skeletal consequences of bone-residing tumors. Furthermore, this highlights the need to

better understand the different cellular constituents that are altered when a tumor grows in bone.

Conclusion

In conclusion, upon homing to the bone marrow compartment, a sophisticated network of interactions exists between tumor cells and cells of the host environment. Most importantly, tumor cell presence directly influences bone remodeling, which leads to the development of bone lesions. Through direct induction of osteoclastogenesis, tumor cells drive development of osteolytic bone disease, while their concomitant regulation of osteoblastic cells influences the nature of the disease and determines whether there is an osteolytic or osteosclerotic response. These tumor cell-induced alterations are further enhanced indirectly via locally residing host cells, including stromal cells, endothelial cells, and immune cells, and through release of tumor-promoting growth factors from the bone matrix. This interdependence between tumor cells and the bone microenvironment provides multiple avenues for interventions to prevent tumor growth and the associated destruction. Nevertheless, in order to ensure successful treatment of metastatic bone disease, the complex nature of these overlapping molecular pathways requires further study.

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