Contributions of the Host Microenvironment to Cancer-Induced Bone Disease

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Abstract
The bone marrow provides a specialized and highly supportive microenvironment for tumor growth and development of the associated bone disease. It is a preferred site for breast and prostate cancer bone metastasis and the hematologic malignancy, multiple myeloma. For many years, researchers have focused upon the interactions between tumor cells and the cells directly responsible for bone remodeling, namely osteoclasts and osteoblasts. However, there is ever-increasing evidence for a multitude of ways in which the bone marrow microenvironment can promote disease pathogenesis, including via cancer-associated fibroblasts, the hematopoietic stem cell niche, myeloid-derived suppressor cells, and the sympathetic nervous system. This review discusses the recent advances in our understanding of the contribution of the host microenvironment to the development of cancer-induced bone disease. Cancer Res; 74(6); 1625–31. ©2014 AACR.

Introduction
As a rich source of signaling and growth factors, the bone microenvironment is favored as a site of metastasis for a number of cancers. Adenocarcinomas originating in the prostate, breast, kidney, or lung often metastasize to the bone in late stages, whereas hematologic malignancies such as multiple myeloma are caused by neoplastic plasma cells that are dependent on the bone microenvironment for survival (1,2). A common feature of all such cancers is the development of a painful and destructive bone disease. Myeloma and breast cancer invasion often causes osteolytic lesions, yet prostate cancers can cause osteoblastic growths or a mixture of the two. Bone metastasis represents a final, incurable stage of these diseases, causing fractures, bone pain, and altered calcium homeostasis (3). The bone also provides a niche in which cancer cells can lay dormant, avoiding chemotherapeutic treatments. The bone microenvironment is not passive and can attract and react to infiltrating cancer cells. The delicate balance between bone mineralization by osteoblasts and resorption by osteoclasts is commonly disrupted by tumor cells, resulting in bone disease. These responses by cancer-associated bone cells can, in turn, aid cancer survival and growth, forming a "vicious cycle" between cancer and bone (Fig. 1; ref. 4). As such, research into therapeutics against bone resorption by osteoclasts is commonly disrupted by tumor cells, resulting in bone disease. The bone marrow provides a specialized and highly supportive microenvironment for tumor growth and development of the associated bone disease. It is a preferred site for breast and prostate cancer bone metastasis and the hematologic malignancy, multiple myeloma. For many years, researchers have focused upon the interactions between tumor cells and the cells directly responsible for bone remodeling, namely osteoclasts and osteoblasts. However, there is ever-increasing evidence for a multitude of ways in which the bone marrow microenvironment can promote disease pathogenesis, including via cancer-associated fibroblasts, the hematopoietic stem cell niche, myeloid-derived suppressor cells, and the sympathetic nervous system. This review discusses the recent advances in our understanding of the contribution of the host microenvironment to the development of cancer-induced bone disease. Cancer Res; 74(6); 1625–31. ©2014 AACR.

Hematopoietic stem cells
Early in development, the bone marrow is colonized by HSCs, from where they self-renew and differentiate along a well-characterized hierarchy of erythroid and myeloid blood cell types. Multipotent HSCs are thought to reside in niches associated with the vascular endothelium (5) and endosteal osteoblasts (6). HSCs are mobilized by the bone marrow in response to granulocyte colony-stimulating factor and other stimuli. They return to the marrow through CXCL12 chemokine signaling from the endosteal niche to the CXCR4 receptor expressed by HSCs and adhesion through integrin α4β1 or αVβ3. Once in location, CXCL12/CXCR4 signaling continues between HSC and the marrow, constraining HSC proliferation (7). Cancers that commonly metastasize to bone, such as those of breast (8, 9), prostate (10), and hematopoietic origins (11), have also been found to use CXCR4 to navigate toward the bone marrow, supporting the theory that metastasizing cells are attracted to the HSC niche itself and alter this site to form a metastatic niche (12). The presence of tumor cells could be expected to displace existing HSCs and accordingly recent evidence shows that hematopoietic progenitor cell numbers are reduced in a mouse model of prostate cancer (12), as well as patients with multiple myeloma (13). It is now thought that HSCs do not reside passively in their niche, but also affect the differentiation potential of nearby mesenchymal cells by...
releasing osteoblast-promoting factors such as bone morphogenetic proteins BMP-2 and -6 (14). Osteoblasts in turn maintain the endosteal HSC niche, creating a positive feedback loop between the osteoblasts and HSCs, which is open to hijacking by osteoblastic prostate cancer cells (15).

**Lymphocytes**

Lymphoid lineage cells also have a complex relationship with invading cancer cells, with many signaling pathways disrupted in a manner similar to that seen in other disorders of bone, such as rheumatoid arthritis. T lymphocytes in general are responsive to myeloma, releasing key osteoclast signaling factor RANKL and suppressing IFN-γ in response to myeloma-derived interleukin (IL)-7, thus promoting osteoclastogenesis (16). Others have identified the T_{H}17 subset of CD4^{+} helper T cells as supporting myeloma and osteoclast growth in the bone through release of IL-17 (17), a similar mechanism to that observed in arthritis (18). Conversely, T_{H}1 and T_{H}2 cells are now thought to act as osteoclast suppressors through release of IFN-γ and several inhibitory interleukins (19), whereas cytotoxic CD8^{+} T cells have a suppressive effect toward melanoma metastasis and growth in bone, which is independent of osteoclast activity (20). B cells are a major source of the RANKL-inhibitor osteoprotegerin (OPG) within the bone and are stimulated by T cells through binding of CD40 ligand to increase OPG release (21), providing a mechanism by which they may contribute to cancer-induced bone disease. In this way, lymphocytes can control the RANKL/OPG balance and consequently cause both anabolic and catabolic bone remodeling. With such apparently contrary roles, it is clear that lymphocytes have the potential to act as regulatory gatekeepers in the bone and as such are targeted by cancer cells to create permissive conditions for bone metastases, particularly at sites of osteolytic lesions but also perhaps at osteoblastic growths. However, our understanding of the in vivo role of lymphocytes in disease pathogenesis is limited by the prevalent use of immune cell–deficient hosts for in vivo models of metastasis.

**Myeloid-derived cells**

Myeloid-derived suppressor cells (MDSC) are a class of myeloid lineage cells that act to quell immune cell activation and have an influence on the bone metastatic niche (22). MDSCs are commonly recruited by cancers to suppress immune system surveillance (23). Within the bone, myeloma cells have been shown to cause increased MDSC activity in
mouse models (24). Recently, it has been revealed that MDSCs can also respond to RANKL to form mature osteoclasts and promote osteolysis in murine models of breast cancer and myeloma (25–27). This suggests an interesting pathway linking cancer-induced osteolysis to immune system avoidance. Invading tumor cells could recruit MDSCs to the metastatic site to suppress lymphocyte activation, with some MDSCs fusing to bolster osteoclast numbers as a side effect. Furthermore, bone marrow myeloid progenitors are also mobilized by cancers to initiate metastatic niches in other organs, such as the lungs (28), through signaling by pathways involving eicosanom and activation of c-MET (29) and hypoxic induction of lysyl oxidases (30). These results underscore the significance of the metastatic bone microenvironment, as developing therapies that target these interactions may also be relevant to cancers that migrate to other favored sites.

Dendritic cells are also reported to contribute to bone metastases, with accumulating evidence to support a role in the development of cancer-induced bone disease. Myeloma cells can stimulate dendritic cells to differentiate into osteoclasts in vitro (31) and depletion of plasmacytoid dendritic cells was found to prevent breast cancer metastasis to bone, associated with the activation of cytotoxic CD8+ T cells and a reduction in MDSCs (32). Megakaryocytes are derived from HSCs, and once fully differentiated are responsible for platelet production. There is increasing evidence to support a role for platelets and platelet-derived lysophosphatidic acid in the development of bone metastases (33–35).

**Bone marrow mesenchymal stem cells and stromal cells**

Although for many years researchers have focused predominately upon the role of the osteoclast, recently the contribution of osteoblasts and bone formation to the pathogenesis of cancer-induced bone disease has also been well studied and has been reviewed elsewhere (36). However, it is becoming increasingly clear that mesenchymal lineage cells control a multitude of aspects of bone metastasis, in addition to the direct regulation of bone formation and bone resorption. The niches occupied by HSCs and their descendants are intimately regulated by the structure of the bone and marrow, which in turn is organized by cells originating from BMSCs. These multipotent cells give rise to a variety of cell types: osteoblasts and osteocytes, adipocytes, chondrocytes, and fibroblasts. Mesenchymal cells are known to be recruited into primary tumors to become cancer-associated fibroblasts, where they encourage epithelial–mesenchymal transition and metastasis of cancer cells (37–40). Therefore, it is no surprise that cancer cells also recruit mesenchymal cells at their secondary sites within the bone marrow.

Cancer-associated fibroblasts are well known to play essential roles in primary tumor growth and metastasis (40), and their pivotal role in tumor growth within bone is emerging. Development of myeloma is dependent, at least in part, upon stromal-derived Dkk1 (41), and expression of TGF-β type II receptor is lost in cancer-associated fibroblasts in prostate cancer bone metastases, promoting development of the associated bone disease (42). Intriguingly, cancer-associated fibroblasts at the primary site have recently been shown to dictate the pattern of metastasis, with expression of CXCL12 and IGF-1 promoting metastasis to bone (43). Gene expression profiles of cancer-associated osteoblasts, fibroblasts and mesenchymal stem cells are altered in skeletal malignancies; however, whether these changes are cause or consequence of tumor growth within bone remains unclear. Expression of XBP1s in myeloma-associated stromal cells has been found to promote tumor growth and bone disease (44), whereas stromal expression of Gfi1 is necessary for bone formation inhibition (45).

Osteocytes are derived from BMSCs and express significant amounts of both OPG and RANKL, acting to control osteoclast formation as well as maintaining the rigidity of the bone extracellular matrix and mobilizing HSCs from their niche (46, 47). These cells are therefore also likely to play a major role in communicating with invading cancer cells. The osteocyte transcription profile is altered by metastasis (48), and their activity should not be discounted in future in vivo studies.

**Adipocytes**

It has long been known that bone marrow has a significant fat content; however, the nature of the fat-containing cells in marrow is more complex than that of a simple energy store. Bone adipocytes follow a similar differentiation pathway as visceral or subcutaneous adipocytes, responding to activation of the C/EBPβ transcription factor and the master adipocyte regulator PPARγ. However, despite not being used for heat generation in humans, they share a profile closer to “brown” fat cells (49). Differentiation cues for BMSCs are thought to often result in mutually exclusive responses, observed particularly in the reciprocal repression between osteoblasts and adipocytes. The relationship between levels of bone fat and bone mass is complex; however, the high proportion of adipocytes within this specialized environment raises the possibility that they may play a functional role in skeletal metastasis. Adipocytes have been shown to support the proliferation and migration of myeloma cells in vitro (50). Furthermore, the adipokine adiponectin has been shown to play a causal role in myeloma pathogenesis and represent a novel therapeutic target, with a loss of host-derived adiponectin increasing myeloma tumor growth and development of the associated osteolytic bone disease in vivo (51).

**Endothelial cells and angiogenesis**

Uncontrolled cell growth in both primary and metastatic tumors means that cancers are frequently challenged with low nutrient conditions. Low oxygen is also a strong driving force for disorganized vascularization within bone and other sites of cancer development and areas of the bone marrow are known to be physiologically hypoxic even before the added burden of tumor metabolism. Hypoxia activates the HIF transcription pathway in both tumor cells and the microenvironment, increasing production and secretion of factors such as VEGF, HGF, and CXCL12, which recruit bone marrow endothelial cells to activate vasculogenesis (52, 53). As with the other cell types of the marrow, the gene expression profile of endothelial cells is altered when associated with cancer cells. Cancer-associated endothelial cells repress regulatory pathways and
contribute a range of chemokines to the microenvironment (54, 55). Targeting HIF is an effective approach to reduce tumor burden and bone disease (56).

**Sympathetic and nociceptive nerves**
Bone is known to be innervated, and there has been recent interest in the role of the sympathetic nervous system (SNS) in providing bone homeostatic signals. For example, leptin has been shown to influence SNS signaling through the hypothalamus to cause a wide variety of effects, such as decreased osteoblast proliferation and increased RANKL and osteoclast activity (57). Leptin serum levels do not seem to correlate with breast cancer or myeloma; however, stress and anxiety are also thought to be able to cause bone loss through the SNS (58), and have been shown to have an effect on not only the osteolytic effect of breast cancer, but also the metastatic infiltration of bone (59). SNS signaling to β-adrenergic receptors on osteoblasts has also been implicated in potentiating other signals, such as parathyroid hormone, osteopontin and IG F-1, and release of HSCs from their niche, which may also have implications for invading cancers (60). Bone pain and neuropathy is a frequent result of bone metastasis, resulting from a variety of mechanisms such as aberrant sprouting and activation of nociceptive fibers, in turn caused by secreted factors from both cancer cells and cancer-associated cells (61). Blocking NGF has been shown to reduce the bone pain associated with prostate cancer bone metastasis, despite not affecting tumor burden or osteolytic bone disease (62).

**The extracellular matrix**
The cells of the bone microenvironment provide many of the signals that provoke a response from metastases; however, the extracellular matrix itself may also regulate cancer cell behavior. Bone and marrow matrices are very rigid, and the stiffness of this substrate onto which cells adhere is known to have an influence on gene transcription, for instance, in selecting between BMSC differentiation pathways (63). While the matrix is known to become stiffer in solid tumors (64), migrating cancer cells are able to move by either releasing matrix metalloproteinases (MMP) or by deforming the matrix in a Rho-associated, coiled-coil containing protein kinase 1 (ROCK)- and myosin-dependent fashion (65). Once within the rigid bone microenvironment, cancer cells are able to respond to the stiffness of the matrix by upregulating osteolytic gene expression, once again using a ROCK- and myosin-dependent mechanism (66).

The MMP family of proteins is frequently dysregulated by cancer cells, causing not only collagen proteolysis and reorganization of the extracellular matrix but also activation of other prosignaling factors and pro-MMPs by cleavage. Host-derived MMP9 has been found to promote myeloma and breast cancer osteolysis (67, 68), whereas osteoclast-derived MMP-7 promotes osteolytic bone disease in murine models of breast and prostate cancer, via solubilization of RANKL (69, 70). The mineralized extracellular matrix is also a considerable store of growth factors (71), and MMPs can release and activate TGF-β, an integral factor in cancer-induced bone disease (72).

**Targeting the bone microenvironment**
The first drugs targeted toward the bone microenvironment in metastatic disease were aimed at cancer-associated osteoclasts, and the success of bisphosphonates against cancer-induced bone disease has been reviewed elsewhere (73). An alternative approach toward osteoclasts has been to target RANKL signaling, by preventing receptor binding using an anti-RANKL antibody. The humanized anti-RANKL antibody Denosumab has progressed through clinical trials against bone metastases arising from multiple myeloma, prostate, lung and breast cancers, as well as non–cancer-related diseases such as osteoporosis and rheumatoid arthritis (74). Targeting TGF-β has proven effective in murine models of skeletal tumors (75, 76). As osteoblasts are now being recognized as major regulators of the bone microenvironment, treatments have also been developed, which encourage osteoblast formation to restore homeostasis and combat osteolytic metastases. Anti-Dkk1 and anti-activin A increase osteoblast differentiation in osteolytic myeloma and breast cancer metastases (77–79), whereas anti–IL-6 offers a pathway to reduce the cancer-supportive signaling responses of myeloma-associated osteoblasts (80). Metastatic cancer cells are attracted to the bone marrow through HSC homing signals such as CXCL12. Therefore, blockade of HSC signals may be able to prevent metastasis and also may increase effectiveness of other therapies when used in combination (81). AMD3100 (now known as Plerixafor) is a CXCR4 antagonist and is effective in preclinical models of myeloma and breast cancer bone metastasis (9, 82). Broad-spectrum MMP inhibitors are no longer considered viable for development due to side effects; however, a specific inhibitor of MMP-13 has improved preclinical effect against breast cancer bone metastasis, indicating that these targets are still worth pursuing (83). The role of the SNS in bone remodeling also suggest that existing β-blockers such as propranolol may be repurposed to stimulate osteoblast activity and inhibit resorption in bone metastases (59).

**Conclusions and Future Directions**
It has long been recognized that the bone marrow provides a hospitable environment allowing many types of tumor to grow and survive. Importantly, this is not a passive environment and reciprocal interactions occur between tumor cells and host cells of the bone marrow. These interactions are critical to multiple stages in disease pathogenesis, from initial homing to the endosteal HSC niche and escape from normal immune suppression, to support of tumor growth and development of cancer-induced bone disease. The delicate balance between the numerous marrow cell types is difficult to recreate in vitro, necessitating the use of in vivo models that both replicate the clinical features of human disease and distinct steps in disease development. The rapidly developing field of mathematical modeling offers an intriguing approach to this problem. Initial studies are encouraging, as discrete interactions within the bone have been modeled, with the ultimate goal of creating a mathematical model of the complete tumor–bone marrow microenvironment (84–86). The progression of research, from studying interactions between tumor cells, osteoclasts, and osteoblasts, to including all components of the specialized...
bone marrow microenvironment has dramatically advanced our understanding of disease pathogenesis. It is exciting to predict that this enhanced knowledge will ultimately reveal novel and effective therapeutic targets, with many encouraging results already arising from preclinical models. Furthermore, there is great enthusiasm for those studies that identify the potential for repurposing drugs currently in clinical use for other conditions, which have effects on the host microenvironment to modulate either tumor growth or osteolytic bone disease. Because of the inextricable nature of the relationship between tumor cells and host cells, the effective treatments of the future will likely utilize a combination of therapeutics targeting both the tumor and the bone marrow microenvironment, to ultimately eradicate this final fatal step in tumor progression.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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