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Contributions of the host microenvironment to cancer-induced bone disease

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Abstract

The bone marrow provides a specialised and highly supportive microenvironment for tumour growth and development of the associated bone disease. It is a preferred site for breast and prostate cancer bone metastasis and the haematological malignancy, multiple myeloma. For many years, researchers have focused upon the interactions between tumour cells and the cells directly responsible for bone remodelling, 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.

Keywords

bone metastasis; myeloma; bone microenvironment; niche; breast cancer; prostate cancer

Introduction

As a rich source of signalling and growth factors, the bone microenvironment is favoured as a site of metastasis for a number of cancers. Adenocarcinomas originating in the prostate, breast, kidney or lung often metastasise to the bone in late stages, while haematological 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 mineralisation by osteoblasts and resorption by osteoclasts is commonly disrupted by tumour 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 (4). As such, research into therapeutics against bone metastases is often aimed at breaking this cycle, historically focussing on osteoclast activity. An improved understanding of the normal physiology of

bone has informed research into the discovery of novel therapeutics, much of which aims to temper the response of the host bone microenvironment to the cancer, rather than attacking the cancer directly. The bone marrow microenvironment contains a highly heterogeneous population of cells, including those derived from haematopoietic stem cells (HSCs), bone mesenchymal stem cells (BMSCs), vascular endothelial cells and nerve fibres. The roles of osteoclasts and osteoblasts in cancer-induced bone disease are well documented, and this review will focus upon the contributions of the other cells within the bone marrow microenvironment.

Haematopoietic Stem Cells

Early in development the bone marrow is colonised by HSCs, from where they self-renew and differentiate along a well-characterised 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 mobilised by the bone marrow in response to G-CSF and other stimuli. They return to the marrow through CXCL12 chemokine signalling from the endosteal niche to the CXCR4 receptor expressed by HSCs, and adhesion through integrin $\alpha 4\beta 1$ or $\alpha V\beta 3$. Once in location, CXCL12/CXCR4 signalling continues between HSC and the marrow, constraining HSC proliferation (7). Cancers which commonly metastasise to bone, such as those of breast (8,9), prostate (10) and haematopoietic origins (11), have also been found to use CXCR4 to navigate towards 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 tumour cells could be expected to displace existing HSCs, and accordingly recent evidence shows that haematopoietic 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 morphogenic 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 signalling 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 signalling factor RANKL and suppressing IFN γ in response to myeloma-derived IL-7, thus promoting osteoclastogenesis (16). Others have identified the T_H17 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_H1 and T_H2 cells are now thought to act as osteoclast suppressors through release of IFN γ and several inhibitory interleukins (19), while cytotoxic CD8⁺ T cells have a suppressive effect towards 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 consequentially cause both anabolic and catabolic bone remodelling. 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 in order 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 (MDSCs) are a class of myeloid-lineage cells which 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 tumour 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 mobilised by cancers to initiate metastatic niches in other organs, such as the lungs (28), through signalling by pathways involving exosomes 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 favoured 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 MDSC cells (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 predominantly 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 organised 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 tumours 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 tumour growth and metastasis (40), and their pivotal role in tumour growth within bone is emerging. Development of myeloma is dependent, at least in part, upon stromal-derived DKK-1 (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 tumour growth within bone remains unclear. Expression of XBP1s in myeloma-associated stromal cells has been found to promote tumour 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 mobilising 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 sub-cutaneous adipocytes, responding to activation of the C/EBP α transcription factor and the master adipocyte regulator PPAR γ . 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 specialised 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 tumour growth and development of the associated osteolytic bone disease *in vivo* (51).

Endothelial Cells and Angiogenesis

Uncontrolled cell growth in both primary and metastatic tumours means that cancers are frequently challenged with low nutrient conditions. Low oxygen is also a strong driving force for disorganised vascularisation 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 tumour metabolism. Hypoxia activates the HIF transcription pathway in both tumour 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 tumour 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 signalling 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 signalling to β -adrenergic receptors on osteoblasts has also been implicated in potentiating other signals, such as parathyroid hormone, osteopontin and IGF1, 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 fibres, 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 tumour burden or osteolytic bone disease (62).

The Extracellular Matrix

The cells of the bone microenvironment provide many of the signals which provoke a response from metastases, however the extracellular matrix itself may also regulate cancer cell behaviour. Bone and bone 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 tumours (64), migrating cancer cells are able to move by either releasing matrix metalloproteinases (MMPs) 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 reorganisation of the extracellular matrix but also activation of other pro-signalling 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 mineralised 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 towards 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 towards osteoclasts has been to target RANKL signalling, by preventing receptor binding using an anti-RANKL antibody. The humanised 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 tumours (75,76). As osteoblasts now being recognised as major regulators of the bone microenvironment, treatments have also been developed which encourage osteoblast formation in order 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), while anti-IL-6 offers a pathway to reduce the cancer-supportive signalling 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 pre-clinical 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 pre-clinical effect against breast cancer bone metastasis, indicating that these targets are still worth pursuing (83). The role of the sympathetic nervous system in bone remodelling 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 recognised that the bone marrow provides a hospitable environment allowing many types of tumour to grow and survive. Importantly, this is not a passive environment, and reciprocal interactions occur between tumour 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 tumour 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 modelling offers an intriguing approach to this problem. Initial studies are encouraging, as discrete interactions within the bone have been modelled, with the ultimate goal of creating a mathematical model of the complete tumour-bone marrow microenvironment (84–86). The progression of research, from studying interactions between tumour cells, osteoclasts and osteoblasts, to including all components of the specialised 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 tumour growth or osteolytic bone disease. Due to the inextricable nature of the relationship between tumour cells and host cells, the effective treatments of the future will likely utilise a combination of therapeutics targeting both the tumour and the bone marrow microenvironment, in order to ultimately eradicate this final fatal step in tumour progression.

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Figure 1. Host:tumour interactions within the bone marrow microenvironment

The specialised cell types within the bone marrow can influence tumour growth and cancer-induced bone disease in a multitude of ways. (1) Regulation of the immune system. Immune cells have been shown to have both positive and negative effects on osteoclast formation and activity, and to suppress tumour growth, whereas MDSCs are increased in bone metastases and contribute to both immune suppression and osteoclastic bone disease. (2) Activation of osteoclastogenesis. Immune cells, dendritic cells and MDSCs all contribute to osteolytic bone disease, at least *in vitro*, with major factors implicated including RANKL, IL-17 and TNF- α . (3) Matrix and stromal-derived growth factors. The host microenvironment is an essential source of tumour-growth factors, including TGF- β , IGF-1 and IL-6. (4) Regulation of osteoblastogenesis. Osteoblast formation in cancer-induced bone disease is tightly controlled by cells including tumour cells and stromal cells, and mediated by factors including Dkk1, activin A, BMPs and noggin. (5) Hematopoietic stem cell niche. The endosteal HSC niche is maintained by osteoblasts and stromal cells, and controlled by factors including GCSF and CXCL12.