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The Bone Microenvironment: A Fertile Soil for Tumor Growth

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Abstract

Bone metastatic disease remains a significant and frequent problem for cancer patients that can lead to increased morbidity and mortality. Unfortunately, despite decades of research, bone metastases remain incurable. Current studies have demonstrated that many properties and cell types within the bone and bone marrow microenvironment contribute to tumor induced bone disease. Furthermore, they have pointed to the importance of understanding how tumor cells interact with their microenvironment in order to help improve both the development of new therapeutics and the prediction of response to therapy.

Keywords

Bone microenvironment; Tumor-induced bone disease; Bisphosphonates; Immune Cells; Nervous System; Vasculature

Introduction

Despite improvements in treatment of primary tumors, patients often develop metastatic disease that involves the bone. It is estimated that nearly 70% of breast cancer patients and 90% of prostate cancer patients that die from disease have bone metastases [1, 2]. Other tumors that can induce bone disease include lung and renal cancer as well as bone invasive oral cancer, multiple myeloma, and melanoma. Once tumors have established residence in

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Compliance with Ethics Guidelines

Conflict of Interest

Denise Buenrostro, Patrick L. Mulcrone, Philip Owens and Julie A. Sterling declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human subjects performed by any of the authors. With regard to the authors' research cited in this paper, all institutional and national guidelines for the care and use of laboratory animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

bone, they interact with multiple cell types present in the local microenvironment [3, 4]. These interactions induce tumor growth and can disrupt the function of bone cells leading to increased osteoclast-mediated bone destruction or increased bone formation.

Early studies in tumor-induced bone disease demonstrated a tight interaction between tumor cells and bone cells, which was described as the “Vicious Cycle” [2]. This theory showed that tumor cells secrete factors like parathyroid hormone related protein (PTHrP) and others that stimulate the osteoblast to produce receptor activator of nuclear factor kappa-B ligand (RANKL), which causes osteoclast-mediated bone destruction. In turn, this destruction causes the release of bone matrix-embedded growth factors that further stimulate tumor growth and more bone destruction [2, 5]. Based on these observations, inhibiting osteoclast mediated bone destruction was predicted to both reduce skeletal related events and tumor growth and became the primary focus for therapeutic intervention. Thus, osteoclast inhibitors were and remain the front-line for treating bone metastatic disease [5]. Other standard therapeutics for treatment of the primary malignancy are often used in conjunction with osteoclast inhibitors, but can have side effects that negatively impact bone health. For example, hormone deprivation therapy (anti-androgen or anti-estrogen) or corticosteroids can result in generalized osteoporosis [6].

The most widely used osteoclast inhibitors are the bisphosphonates [6–8]. Bisphosphonates are a class of drugs whose medicinal properties were discovered by Herbert Fleisch in the 1960’s [9]. These drugs bind the hydroxyapatite of bone to ultimately inhibit osteoclast mediated bone destruction, and are widely used to treat osteoporosis. In patients with cancer metastatic to bone, zoledronic acid (ZA) is the most commonly used bisphosphonate and is the standard of care. While its primary function is to inhibit the osteoclast, there is some debate as to whether bisphosphonates also inhibit tumor growth. While basic studies have shown that the nitrogen containing bisphosphonates inhibit tumor growth through blocking prenylation, pre-clinical and clinical studies suggest that they have minimal direct effects on the tumor, in part due to limited uptake at tumoral sites [8]. Thus, other drugs are being investigated that inhibit osteoclasts. Of these, the most developed is the anti-RANKL antibody denosumab (Amgen). Denosumab blocks the stimulation of osteoclasts, thus functioning similar to ZA. However, there is significant debate in the field as to whether it should replace ZA as the standard of care [10].

While osteoclast inhibitors have been incredibly successful in improving quality of care, their use has not increased survival nor have they cured bone metastases. This directly opposes the notion of the vicious cycle that reducing osteoclast mediated bone disease should reduce tumor burden. Despite this, the vicious cycle remains a strong model for describing bone metastatic disease, and newer research has focused on describing other interactions that occur between tumor cells and other aspects of the unique bone microenvironment. These studies have identified numerous other potential pathways that may lead to the development of newer therapeutics to both improve quality of life and reduce tumor burden.

The Bone Microenvironment and Cancer

As described previously, the interactions between the tumor cells and bone cells have been described since the 1990's in part through studies led by Dr. Gregory Mundy's group and the description of the "Vicious Cycle" [4]. The "Vicious Cycle" encompasses some of the earlier studies investigating tumor cell interactions with their environment [4]. While this early work primarily explored the osteoclast-tumor cell interaction, newer studies have focused on the cellular interactions between tumor cells and the other cell-types found in the bone microenvironment.

The Structure and Rigidity of the Bone Microenvironment

The rigidity of the primary tumor microenvironment has been demonstrated to affect the behavior of numerous types of tumor cells [11, 12]. Our group has shown that the rigidity of bone influences tumor-induced bone disease by mediating the Integrin beta 3 and transforming growth factor beta (TGF- β) signaling pathways [13, 14]. Interestingly, the effect of bone rigidity not only influences gene expression within the tumor cells, but also affects gene expression of fibroblasts [15] and mesenchymal stem cells [16]. More research is needed to fully understand how bone rigidity influences bone cells and tumor cells, but many of the studied pathways appear to be similar to those required for the loading response known to be critical for bone healing and turn-over.

Osteoblasts

Osteoblasts, the bone forming cells, have long been known to play an important role in tumor-induced bone disease (TIBD). Not only is osteoblast differentiation from their mesenchymal progenitors often affected by tumors growing in bone, where osteoblast differentiation can be reduced (osteolytic) or enhanced (osteoblastic), osteoblasts also contribute to bone resorption and tumor growth. Specifically, osteoblasts are the main producers of macrophage colony stimulating factor (M-CSF) and RANKL, which are essential for normal osteoclastogenesis [5, 17, 18]. Tumor cells can secrete factors that further stimulate osteoblastic expression of M-CSF and RANKL, leading to further enhancement of bone destruction.

In addition to stimulating osteoclastogenesis, many studies suggest that osteoblasts can increase bone metastasis and tumor growth in bone. Indeed, it has been suggested that even physical contact between osteoblasts and tumor cells promotes growth of metastatic breast cancer cells [19]. On a more molecular level, administration of parathyroid hormone (PTH), a bone anabolic factor, increased prostate tumor cell localization and growth in bone [20]. It is currently unclear whether these effects of osteoblasts on tumor metastasis are direct, or rather result from the recruitment of other cell types. Studies suggest that osteoblasts can enhance the recruitment of hematopoietic stem cells (HSC) [21–25]. Other work suggests that the osteoblast may release proteins that increase the release of cytokines and other factors that stimulate an increase in myeloid-derived suppressor cells (MDSCs), which can promote tumor growth and vasculogenesis [26]. Other groups have also demonstrated that osteoblasts can stimulate vascular endothelial growth factor (VEGF) and angiogenesis within bone microenvironment [27, 28].

Osteoclasts

The role of osteoclasts in tumor induced bone disease (TIBD) has been highly investigated. It has been shown that these multinucleated cells are responsible for the bone destruction associated with tumors residing in the bone. Osteoclasts differentiate from myeloid progenitor cells under the influence of growth factors and cytokines like M-CSF and RANKL [29]. Under normal physiological conditions, bone resorption is a tightly regulated process that involves signals from osteoblasts as well as signals from other cells found in the microenvironment. During normal osteoclast differentiation, maturation and activation are regulated by RANK/RANKL/osteoprotegerin (OPG) signaling [30, 31]. OPG, a soluble decoy receptor for RANKL, is expressed by osteoblasts to negatively regulate osteoclast activation, thus preventing excessive bone resorption [29, 31]. Deregulation of this process can lead to increased risk of fracture as well as other bone-related diseases [32].

Despite years of studying the role of osteoclasts in the vicious cycle, their role in recruiting other cell types has not been fully elucidated. For example, CXCR4, a factor that is known to contribute to invasion and metastasis, is expressed by osteoclast precursors and regulates hematopoietic and tumor cell homing to bone. Interestingly, *Cxcr4*^{-/-} osteoclasts have been shown to have higher resorptive activity and faster differentiation compared to control osteoclasts [33].

Osteocytes

While osteocytes are known to be important in the regulation of bone turnover, little is known about their role in TIBD. Some studies have suggested that osteocytes may contribute to tumor metastasis to bone. Specifically, one group found that adenosine nucleotides released from the osteocyte could promote migration of breast cancer cells [34]. Another group showed that the pressure from tumors growing in the bone stimulates the osteocyte to increase expression of CCL5 and matrix metalloproteinases (MMPs), which further stimulate the growth of bone metastatic prostate cancer [35]. Mechanistic studies have revealed that multiple myeloma cells can alter osteocytes signaling resulting in an inhibition of osteoblast differentiation, thus possibly contributing to the reduction in bone volume observed in these multiple myeloma[36]. These and other findings suggest a role for osteocytes in contributing to TIBD, but more research is required to fully elucidate this role.

Fibroblasts

The fibroblast has become an important cell type in cancer biology as either a promoter or repressor of cancer [37]. Fibroblasts that grow near the tumor cells are commonly referred to as CAFs (Cancer-Associated-Fibroblasts). Whereas CAFs have been widely studied at the site of the primary tumor of many cancer types, little is known about their function at distant metastatic sites including the tumor microenvironment of the bone. It is now known that fibroblasts originating from primary tumors can travel with metastatic cancer cells, but what they do when they get to the site of metastasis is only now beginning to be understood [38].

CAFs have recently been shown to promote tumor invasion, angiogenesis, and matrix stiffening [39]. CAFs are also potent modulators of tumor supportive immune cell populations [40]. Unlike most immune populations that have short life spans, fibroblasts can

persist under normal circumstances, and during cancer progression, they can sustain secretion of inflammatory cytokines and growth factors to assist tumor cell survival and metastasis. While some cytokines are tumor derived, it is known that the cross-talk between cancer cell and other immune cell populations can enhance fibroblast immunosuppression for immuno-editing of the tumor microenvironment[41].

The fibroblast can be directly isolated from the bone marrow compartment. Bone marrow MSC can be corrupted by a newly arrived tumor, thus promoting fibroblast differentiation and the secretion of pro-tumorigenic factors that allow for tumor establishment in bone [42]. It has been shown that both MSCs and CAFs expand prior to tumor colonization in bone [43]. Not only do the bone marrow derived MSCs promote progression and survival of the tumor, they also aid in the resistance to chemotherapy [44]. Bone marrow stromal cells have, for more than two decades, been recognized for their ability to facilitate the growth of bone tropic prostate cancers [45]. Signaling pathways such as TGF- β have been shown to be crucial not only when activating CAFs but also when absent in stromal fibroblasts. When the type II TGF- β receptor is absent from fibroblasts, it can lead to significant increases in chemokines, which in turn enhances the presence of mixed osteoblastic/lytic lesions in prostate cancer [46]. Additionally, research in oral squamous cell carcinoma (OSCC) has shown that stromal cells secreting TGF- β facilitate bone resorption of the tumor cells [47].

Immune Cells

Macrophages (M Φ)

Macrophages are antigen presenting cells that differentiate from the myeloid lineage and have roles in promoting wound healing, regulating adaptive immunity, and eliminating infectious agents [48]. These cells play active roles in initiation, promotion, and progression of several tumor types such as breast, lung, and colon tumor [49–52]. It is important to note that macrophages are plastic cells, and their phenotype is consistently modulated by the local microenvironment. While beneficial for immune responses, this characteristic can be exploited by cancer cells in ways that promote tumor progression [49].

Macrophages that are activated by factors such as interferon-gamma (IFN- γ) have tumoricidal activity and are considered classically activated macrophages, or M1 macrophages. Conversely, macrophages that are activated by factors like IL-4 and typically perform tissue repair and remodeling considered tumor promoting and are referred to as alternatively activated macrophages, or M2 macrophages [53]. Macrophages are comprised of many subpopulations, including tumor-associated macrophages (TAMs) and metastasis-associated macrophages (MAMs), that express a mixed phenotype of both M1 and M2 markers, thus making it difficult to target and classify these cells solely based on their M1 or M2 subtype [54].

Macrophages have the potential to differentiate into osteoclasts with the correct environmental signals, but their exclusive role in TIBD is still unknown [29]. Published work has identified a novel resident macrophage population that is F4/80⁺ and tartrate-resistant acid phosphatase (TRAP) negative in bone called osteomacs [55]. Osteomacs have been shown to have different functions than osteoclasts and to be important for osteoblast

maintenance and functional activity [56]. However, these studies focused on the osteomacs' role in bone marrow homeostasis and bone healing [57]; as such, their role in TIBD remains to be examined.

Myeloid-Derived Suppressor Cells (MDSCs)

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population that includes immature macrophages, granulocytes, dendritic cells, and myeloid progenitor cells [58]. MDSCs can be further divided into two separate cell populations: one that is phenotypically and morphologically similar to monocytes (M-MDSCs) and the other comprised of immature polymorphonuclear (PMN) cells that are both morphologically and phenotypically similar to neutrophils (PMN-MDSCs) [59]. Several factors that have been found to be involved in MDSCs-mediated immune suppression include expression of arginase (ARG1), inducible nitric oxide synthase (iNOS), TGF- β , IL-10, sequestration of cysteine, decreased expression of I-selectin by T-cells, and induction of Tregs [60–65]. In addition to immune suppression, it has been established that MDSCs can aid tumor growth and metastases through four distinct methods: (1) decreasing immune-surveillance through their suppressive function, (2) remodeling of the tumor microenvironment, (3) establishment of a premetastatic niche, and (4) promoting the epithelial-to-mesenchymal transition (EMT) in tumor cells [59].

MDSCs have been found to play an important role in bone metastasis in both breast and prostate cancer [66, 67]. In the prostate cancer model, tumor-derived PTHrP indirectly increased MDSC's angiogenesis potential, thus promoting tumor growth and angiogenesis in bone [66]. Danilin and colleagues demonstrated that MDSCs contributed to bone destruction by activating expression of Gli2 and PTHrP, important factors for stimulating bone destruction [67], in a breast cancer model of metastatic bone disease. Studies also have demonstrated that MDSCs from a tumor-bearing mouse have the potential to differentiate into osteoclasts, therefore contributing to the bone resorption seen during breast cancer metastasis to bone [67, 68].

Natural Killer Cells (NK)

Natural Killer Cells (NK cells) are recognized as cytotoxic cells that secrete cytokines such as IFN- γ and play an important role in altering immune responses [69]. Very little has been published on the role of natural killer cell in regards to TIBD. Most studies have focused on the natural killer cell's role in the primary tumor site and metastasis to organs such as the lung [70]. Liu and colleagues demonstrated that the number of NK cells was decreased in a mouse model of prostate cancer, and associated with a reduction in overall metastasis [71]. Thus, the role of NK cells specifically in bone metastasis still needs to be elucidated.

T and B cells

T cells and B cells play an important role in the adaptive immune response and have been shown to have opposing roles in cancer progression [72]. Both of these cell types are comprised of several subpopulations that have distinct biological functions including both anti- and pro-tumorigenic activity. For example, CD8⁺ T cells are generally recognized as effector or cytotoxic cells and are primarily responsible for tumor killing by mechanisms

such as apoptosis and target cell cytotoxicity [73]. In contrast, T regulatory cells (Tregs) have been implicated in promoting effector T cell tolerance and exhaustion, thereby further contributing to tumor growth and progression [74]. In the case of B cells, mature B cells can differentiate into plasma cells and release antibodies specific for tumor antigens causing an immune response that targets the tumor [75, 76]. However, these cells can also accumulate at tumor sites and secrete immunosuppressive cytokines, such as IL-10, to promote tumor growth and progression [76].

Several publications have recently emerged describing mechanisms that promote TIBD in a T-cell dependent manner. Zhang and colleagues found that stimulating a T-cell response in mice reduced tumor burden and inhibited overall tumor growth in bone [77]. T cells have also been implicated in secreting RANKL and inducing osteoclastogenesis, leading to bone destruction prior to tumor establishment in bone [78]. Together these findings suggest that T cells may have opposing roles in TIBD, one where they can decrease tumor growth in bone and the other being that they can stimulate bone destruction. However, it is important to note that since the majority of published studies have used T cell deficient mice, it is clear that T cells are not necessary for tumor cells to grow and metastasize to bone [58].

The Role of the Nervous System and Vasculature

The Nervous System

Many tumor types, including breast cancer, have been shown to gain a growth advantage due to stress stimuli [79–82]. Clinically, links between stress and breast cancer progression as well as relapse have been reported. Therefore, a suggested link between chronic stress and cancer mortality due, at least in part, to sympathetic nervous system (SNS) effects on bone, seems plausible [83–85]. Bone is richly innervated with both sensory and sympathetic fibers, making the skeleton subject to nervous system control. Severe emotional stress increases SNS activity, inducing the release of norepinephrine and epinephrine. These catecholamines stimulate β 2-adrenergic receptors on osteoblasts, resulting in profound changes in the bone marrow microenvironment. The activated osteoblasts release a myriad of proteins such as RANKL and IL-6 that affect bone processes such as inflammation, cell trafficking, and bone resorption [86]. These processes are also central to bone metastasis and progression of TIBD [87]. Bone pain is one of the most common skeletal-related events for patients dealing with tumor-induced bone disease [88]. Over time, this pain typically intensifies and may ultimately manifest as a pathological fracture in the bone that houses the tumor. The origin of this pain is reportedly due to both the tumor itself as well as the activity of osteoclasts [89, 90]. Groups have attempted to target specific pathways involved in bone pain such as Nerve Growth Factor (NGF) [91], the receptor P2X7R [92], and glutamate release [93] to help improve quality of life. Additionally, commonly prescribed drugs such as denosumab and bisphosphonates have been shown to reduce bone pain in part through the reduction in osteoclast activity [94]. Similarly, anti-cathepsin K treatment can reduce bone pain [95].

The Vasculature

The vasculature has been shown to play a role in promoting the establishment of bone lesions both through increased density and permeability. The bone marrow is a highly

vascularized tissue, and it has been reported that there is a correlation between elevated microvessel density in the bone marrow of breast cancer patients and progression of metastatic disease [96]. This suggests a link between increased vasculature and tumor survival. In addition, the vasculature of the bone marrow is sinusoidal and fenestrated, meaning the endothelial cells are more permeable and are lacking certain supporting cells commonly seen with other vessel types [97]. Indeed, more sinusoidal vessels through which tumor cells could travel would increase the likelihood of successful tumor cell extravasation into the bone marrow.

Another exciting area of research regarding the role of the vasculature in bone metastasis is the role the endothelium plays in waking dormant disseminated tumor cells. A major concern regarding bone metastasis is the existence of dormant micrometastases, which can remain inactive for decades after primary tumor detection. Ghajar et al. revealed recently that stable endothelial cells in the bone marrow, which are located in close proximity to breast cancer cells, secrete thrombospondin-1 (TSP-1) to induce tumor quiescence. If angiogenesis is stimulated, however, these endothelial cells reduce the levels of TSP-1 and instead increase the production of proteins and growth factors that awaken the dormant tumor cells and cause an accelerated rate of growth [98]. Although more investigation is needed to expand on this mechanism of tumor growth and the transition between dormant and active bone metastatic tumor cells, this data reveals how controlling the vasculature may be a key component of treating cancer at many different stages of progression.

Conclusion

Multiple studies over the past decade have provided strong evidence that tumor induced bone disease results from interactions beyond those described in the “vicious cycle” hypothesis that focused on tumor-osteoblast-osteoclast interactions. As we learn more about these interactions, it has become clear that TIBD is a dynamic and complex condition that requires additional study. Moving forward, newer models will need to be developed to allow for study of the relative contributions of each of the cell types present in the bone microenvironment in order to guide the development of future therapeutic strategies. Currently, osteoclast inhibitors remain the most effective therapeutics, but as we better understand these interactions, perhaps combination therapies that target multiple tumor promoting cell types will develop. Additionally, understanding these interactions may help to predict which tumors will metastasize to bone and result in clinically relevant skeletal disease versus those that will remain dormant. Insight into how the bone microenvironment regulates patient outcomes will help direct treatment and may help reduce disease associated morbidity and mortality.

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Figure 1.

Tumor bone microenvironment interactions. Vasculature allows for circulation and extravasation of tumor cells into the bone microenvironment. Tumor cells interact with the cell populations present in the bone marrow. These include cells such as the cancer-associated fibroblasts, osteoblasts, osteoclasts, immune cells, and others as depicted here. **ECM**, extracellular matrix, **MΦ**, macrophage, **MDSC**, myeloid-derived suppressor cell, **MSC**, mesenchymal stem cell, **NK**, natural killer cell, **CAF**, cancer-associated fibroblast, **Ob**, osteoblast, **Oc**, osteoclast, **BP**, bisphosphonate.