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REVIEW ARTICLE

The dynamic microenvironment associated with metastatic bone disease: Current concepts

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Abstract

Patients with bone metastases may experience debilitating pain, neurological conditions, increased risk of pathological fractures, and death. A deeper understanding of the bone microenvironment, the molecular biology of cancer types prone to metastasis, and how bone physiology promotes cancer growth, may help to uncover targeted treatment options. The purpose of this paper is to outline the current concepts relevant to topics including bone remodeling, angiogenesis, and immunomodulation as it relates to metastatic bone disease.

KEYWORDS
metastatic bone disease, pathophysiology, tumor microenvironment

1 | INTRODUCTION

Metastatic bone disease (MBD) is the primary cause of morbidity and mortality in patients with cancer.[1-5] While metastasis may occur to different organs, bone is the most common site of metastasis in certain cancers. The most common origins of primary cancers associated with bone metastases are prostate (34%), breast (22%), and lung (20%).[6] Patients with MBD may subsequently develop debilitating bone pain, possible nerve root or spinal cord impingement, potentially lethal hypercalcemia, and are at increased risk for pathological fractures.[3,7] Table 1 demonstrates the 5-year survival rates of patients with metastatic disease.[6]

Given the profound impact that MBD can have on patients' quality of life and life expectancy, it is crucial for healthcare providers to have a thorough understanding of the molecular biology implicated in the development of bone metastases.[3,9,10] Knowledge of the microarchitecture and related molecular physiology of bones and cancer cells may function as a foundation for the development of potential therapeutic options.[3,7] The microenvironment of bones involves a dynamic interplay of several different types of cells including osteocytes, osteoblasts, osteoclasts, hematopoietic and immune cells, stromal cells, adipocytes, fibroblasts, and endothelial cells.[9] The extracellular matrix (ECM) helps to facilitate cell attachment and communication along with promoting cell growth, movement, and other functions. These cells and their support structures have crucial functions including remodeling, hematopoiesis, immune modulation, tissue regeneration, and disease pathogenesis. Figure 1 illustrates the pathways of the normal physiological functions that occur in the bone microenvironment as compared to the pathways for MBD executed by cancer cells.

The purpose of this review is to outline current concepts in the existing literature regarding the unique microenvironment of bone and how MBD interacts with this environment to lead to cancer progression. The secondary goal of this paper is to potentially generate ideas regarding future therapeutic options by analyzing this pathway.

2 | BONE REMODELING

Bone remodeling occurs throughout life in response to physical or biomechanical stress and metabolic demands.[11,12] Osteoblasts produce the organic ECM of bones that consists of type I collagen and non-collagenous proteins.[12] The inorganic ECM consists of bone mineral hydroxyapatite, which is obtained from diet and synthesized by molecules secreted by bone cells.[12] By contrast, osteoclasts are multinucleated cells that are responsible for the resorption of bone.[12] In healthy individuals, the interplay of these cell types involves a

balance of bone resorption and formation and is regulated by many different signaling pathways including the receptor activator of NF-kB ligand (RANKL), osteoprotegerin (OPG), Pth, Wnt, and Bmp signaling.[13] Parathyroid hormone stimulates osteoblasts to secrete RANKL, which then subsequently activates the osteoclast precursor.[14–16] OPG acts as a competitive inhibitor of RANKL, and thus blocks the activation of osteoclasts.[17,18] Osteocytes are terminally differentiated bone cells embedded in the mineralized matrix, and the most abundant cell type in the bone. These cell types play a critical role in mechanosensing and regulation of bone metabolism in response to physical cues. For example, under high load, the expression of sclerostin (SOST) and Dkk1, two WNT signaling antagonists is repressed, eliciting an anabolic response. Reciprocally, when osteocytes express high levels of SOST, Wnt signaling is inhibited, thereby suppressing bone formation[19] (Figure 2).

In general, for some cancers, such as breast cancer, metastasis to the bone can be exacerbated by osteoporosis, and strong clinical data exists in support of osteoporosis as a risk factor for cancer bone metastases.[20] Towards this end, antiosteoporosis drugs have been used to blunt the bone loss in patients with cancer bone metastasis.

TABLE 1 Average 5-year survival estimates after diagnosis of metastatic disease by primary cancer type.[8]

Primary cancer type	5-year survival (%)
Colon	14
Rectum	17
Lung	7
Melanoma	30
Breast	29
Prostate	31
Kidney	14
Bladder	6

For example, bisphosphonates, have been shown to reduce osteolysis, improve bone microarchitecture, and inhibit the progression of cancer bone metastasis, but these results have been met with limited success in clinical trials.[21] Similarly, denosumab, an antibody targeting RANKL, has been shown to slow down the progression of MBD[22]; however, no treatment to date has been shown to directly eliminate cancer cells in focal bone lesions.

What continues to puzzle scientists is how the cancer-bone microenvironment dynamically interacts. While it is clear that the function of osteoblasts has significant implications with regard to tumor-induced bone disease (TIBD), what remains to be determined is whether the bone microenvironment before metastasis drives this process, or whether the microenvironment rapidly shifts to a new molecular state once it is colonized by tumor cells. Furthermore, clinical data suggest that osteoblastic activity is reduced in lytic pathologies or increased in blastic pathologies, but phenotypes can also be compounded by the complementary osteoclast activity.[23] In particular since, osteoblasts are directly involved in regulating osteoclast activity through the production of macrophage colony-stimulating factor (M-CSF) and RANKL. Tumor cells can also secrete factors that further stimulate osteoblastic expression of M-CSF and RANKL. This leads to a vicious cycle of increased generation and activation of osteoclasts and therefore increased bone destruction.[24,25] In order for tumor cells to evolve the ability to participate in this pathway, there must be physical contact between osteoblasts and tumor cells to promote growth of metastatic cancer cells.[24]

Osteoclasts also have a role in TIBD through their lytic destruction of bone.[26] In normal conditions, bone resorption is a tightly regulated process that involves a balance of signals from osteoblasts with regulation from the RANK/RANKL/OPG pathways.[27] This balance may be disrupted in TIBD, which can lead to an hyperactivation of osteoclasts. Unregulated osteoclastic activity then results in lytic bone lesions with weakened biomechanical strength. The clinical sequelae elicit an increased risk of fracture with even physiologic loads at the sites of disease in bone.[28] In contrast to
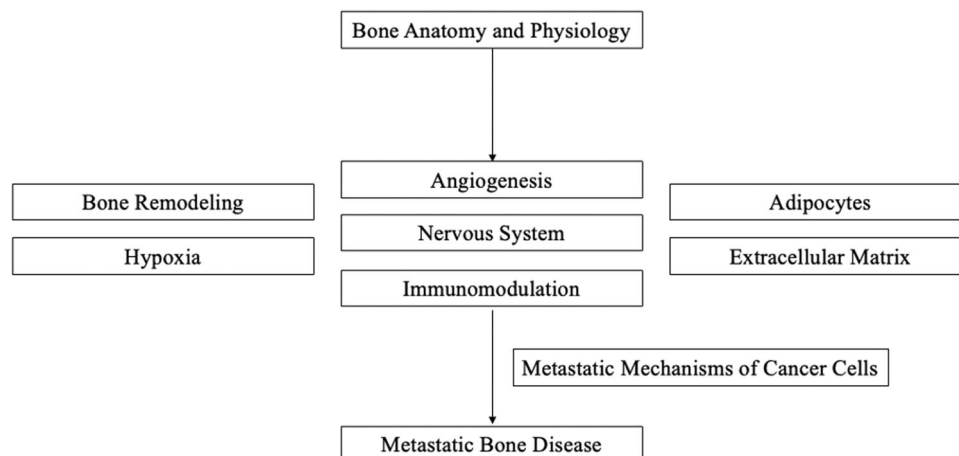

FIGURE 1 Outline of key anatomical and physiological functions of bones along the pathway of metastatic mechanisms of cancer cells that lead to metastatic bone disease.
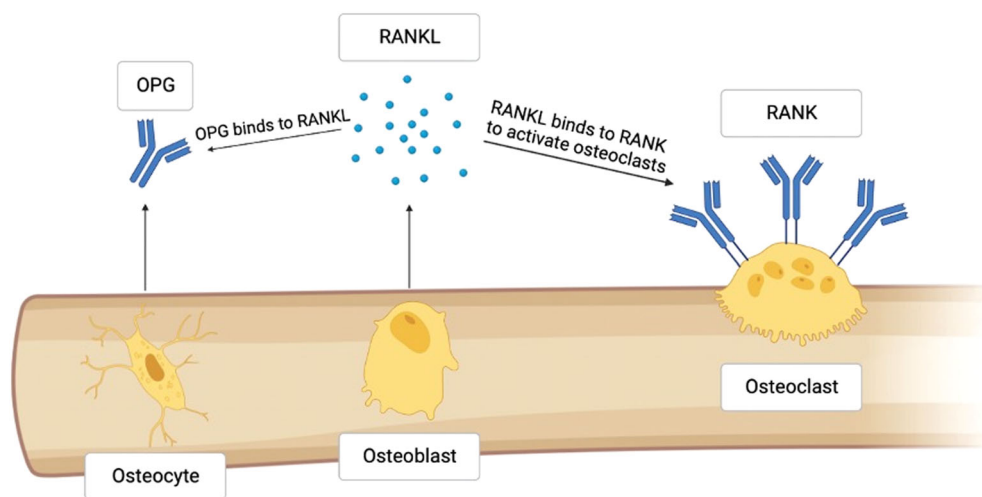
FIGURE 2 Bone remodeling pathway involving osteocytes, osteoblasts, and osteoclasts regulated by RANKL and OPG. NF-κB, nuclear factor kappa B; OPG, osteoprotegerin; RANKL, receptor activator of NF-kB ligand.

TABLE 2 Types of metastatic bone disease with associated osteoblastic and/or osteolytic lesions.

Primary cancer type	Prevalent lesions in humans	Xenograft models
Lung	Osteolytic, Osteoblastic[29,30]	Osteolytic, Osteoblastic[30]
Breast	Osteolytic, Osteoblastic[31,32]	Osteolytic, Osteoblastic[33]
Thyroid	Osteolytic[34,35]	Osteolytic[36]
Colorectal	Osteolytic[37,38]	Osteolytic[39]
Renal	Osteolytic[40,41]	Osteolytic[42]
Prostate	Osteoblastic[43,44]	Osteolytic, Osteoblastic[45]

the formation of lytic lesions, pancreatic carcinoma bone metastases and sometimes breast carcinoma metastases are associated with the development of blastic bone lesions. Russo et al.[27] described that in these diseases the cancer cells primarily utilize their ability to promote osteoclast activation to enter the bone microenvironment. Table 2 outlines types of MBD with commonly exhibited osteoblastic and/or osteolytic lesions.

The role of osteocytes in MBD has been less defined in the literature.[46] It has been shown, however, that the release and expression of adenosine nucleotides, CCL5, and matrix metallo-proteinases from osteocytes can stimulate the growth of metastatic prostate carcinoma bone lesions.[47,48] Furthermore, SOST, a Wnt antagonist discussed above, that is primarily expressed by osteocytes, when genetically deleted in an immune-deficient mouse model of early multiple myeloma (MM) it prevented MM-induced bone disease.[49] However, the role of SOST in TIBD remains unresolved, as other studies have shown that SOST deficient osteoblasts promote invasion of prostate cancer cells[50]

and also that a high bone mass bone microenvironment is more prone to tumorigenesis.

A recent study by Sun et al.[51] found that upregulated SOST expression was associated with breast cancer bone metastases and worse survival of breast cancer patients. When they silenced SOST expression in a mouse cell line (SCP2) with high bone metastatic potential, they observed a significant reduction in metastases presented to the bone, which is consistent with the results observed with MM mentioned above.[49] Similarly, when they treated SCP2 tumor-bearing mice with a small-molecule compound (S6) targeting SOST, the treatment reduced the rates of bone metastasis, suggesting that SOST inhibition has the potential to blunt bone metastasis and should be further explored as a potential therapeutic for the treatment of bone metastasis in breast cancer.[51]

Pharmaceutical companies have developed some therapeutics that target the pathways for activation of bone remodeling in MBD. For example, the humanized monoclonal antibody denosumab, which is commonly used for preventing osteoporosis, has the ability to inhibit osteoclast function via the neutralization of RANKL. Gul et al.[52] concluded that denosumab significantly reduced the risk of fractures by 50% in breast cancer patients and 62% in prostate cancer patients.[52]

Bisphosphonates are part of the management of patients with MBD by harnessing their anti-resorptive activity, impairing the outgrowth of bone metastases, and immunomodulatory effects.[21,53] Namely, bisphosphonates can be directly incorporated into non-hydrolyzable analogs of ATP, which promotes osteoclast apoptosis, while also providing a promising immunomodulatory therapy to further treat MBD.[54,55] Diel et al.[56] conducted a randomized controlled trial evaluating patients with primary breast carcinoma with disease in the bone marrow who were chosen to either receive clodronate or standard follow-up. They concluded that the incidence of both osseous and visceral metastases was significantly lower in the clodronate group compared with the control group.[56]

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Treatment with radium-223, a radioisotope emitting α-particles, targets osteoblastic lesions.[57,58] This mechanism works by using exogenous radium-223 which is chemically related to calcium and is deposited by activated osteoblasts adjacent to cancer cells.[59,60] This proximity results in selective cytotoxicity to cancer cells. This allows the delivery of high-energy radiation to bone metastases while minimizing toxicity to other cells within the bone microenvironment.[61] Parker et al.[62] demonstrated that radium-223 yielded an improved overall survival versus the placebo for patients with metastatic prostate cancer (14.0 vs. 11.2 months, $p = 0.002$).

3 | ANGIOGENESIS

There is a complex vascular system linking bone to the bone marrow and greater circulatory system, which has significant implications with regard to promoting MBD. H-type endothelial cells, which express high levels of the platelet endothelial cell adhesion molecule CD31 and the type I integral membrane glycoprotein endomucin, form blood vessels within the bone. L-type endothelial cells in the bone marrow have low levels of CD31 and endomucin expression and these form the sinusoids that connect to the central vein.[63,64] The microenvironment of L-type vessels is hypoxic relative to that of H-type vessels.[64] While hypoxia in general promotes high bone mass.[64] It is believed that this hypoxic microenvironment promotes cancer growth and suboptimal bone remodeling.[65,66]

In metastatic bone cancer, angiogenesis begins as cancer cells activate pericytes locally to increase production of vascular endothelial growth factor (VEGF), which promotes neo angiogenesis locally.[63] These initiate a stem cell-like population of tumor cells that have the potential for de novo angiogenesis. The histone deacetylase inhibitor entinostat is currently being evaluated in models of breast cancer to target this mechanism in primary tumors.[67] In addition to vasculogenic mimicry, tumor cells may utilize vessel co-option to access the blood supply within the bone microenvironment.[68,69] Vessel co-option involves the cancer cells targeting existing blood vessels for their own supply. These mechanisms disrupt the normal pathways of angiogenesis to the advantage of the tumor cells. Increasing their vascular capacity increases the metabolic capacity of the tumor and promotes tumor growth.

Studies are currently investigating the role of vasculature in bone metastasis in waking dormant disseminated tumor cells.[70] Dormant micrometastases can remain inactive for decades after primary tumor detection, possibly linked to senescence. Ghajar et al.[70] demonstrated that stable endothelial cells in the bone marrow, located in close proximity to breast cancer cells, secrete thrombospondin-1 to induce tumor quiescence. They discovered that once angiogenesis is stimulated, these endothelial cells reduce the levels of TSP-1 and rather increase the production of proteins to awaken the dormant tumor cells and cause growth.[70] This suggests that these tumor cells remain dormant until they have created a microenvironment advantageous to their continued growth and success via their control of angiogenesis. Acknowledging and addressing these microvascular

pathways will shed light on potential therapeutic targets to reduce the ability of tumor cells to take advantage of the body's natural pathways.

3.1 | The contribution of hypoxia

Bone is a hypoxic microenvironment with low oxygen partial pressures (7–29 mmHg).[71] Hypoxia regulates bone remodeling via the HIF transcription factors, along with producing factors including RANKL, VEGF, and CSF-1 to promote osteoclast formation.[72,73] Hypoxia also facilitates a malignant phenotype of cancer cells in a HIF-1α-dependent manner to promote the production of VEGF and angiopoietin-2. The hypoxic microenvironment also regulates the immunosuppressive functions of tumor-associated macrophages, myeloid-derived suppressor cells and regulatory T cells, which promote cancer growth.[65,66] Moreover, HIF-1α activation augments the suppression of effector and cytotoxic T cells and mediates the upregulation of programmed death-ligand 1 (PD-L1) expression in myeloid-derived suppressor cells, which leads to T cell tolerance and exhaustion.[74,75] This, in turn, helps tumor cells evade the immune response and regulation.

3.2 | Bone marrow adipocytes (BMAs) and cancer metabolism

The presence of BMAs increases with aging and obesity.[76] They help to regulate fatty acid responses[77] and secrete cytokines and protein signals which regulate bone remodeling. The presence of BMAs has been linked to formation of bone metastases by providing energy for tumor cells, enhancing tumor cell proliferation, and promoting resistance to chemotherapy and radiotherapy.[78] Cancer cells and adipocytes have a dynamic interaction. Cancer cells are able to alter adipocyte phenotype and certain adipokines, which further impacts cancer cell molecular biology.[79] For example, leptin has been shown to promote bone resorption, which promotes cancer cell growth in the bone marrow, whereas adiponectin has a negative effect on tumor growth.[80] BMAs may also secrete IL-6 which enhances the growth of bone metastases by promoting osteoblastic production of RANKL, leading to osteoclast formation. BMAs are also thought to promote cancer growth via VEGF secretion and subsequent enhanced angiogenesis.[81] The expression of lipid transporters has been shown to be upregulated in prostate cancer cells which may be reflective of changes in the glycolytic activity of cancer cells[82] (Figure 3).

3.3 | Tumor-induced alterations of the ECM

The ECM of the bone is essential for cellular functions including cell differentiation and signaling pathways.[83] This involves a network of collagen, noncollagenous proteins, and hydroxyapatite crystals.[84–87]

Tumor cells alter the structure of the normal ECM and transform it into a disorganized network via the production of thick fibronectin fibrils.[88,89]

Osteopontin, a protein found in the ECM of bone, functions as a regulator of tissue regeneration, inflammation and bone mineralization.[90] It closely interacts with osteoblasts, osteoclasts, macrophages, endothelial cells, and fibroblasts. Tumor cells facilitate bone metastases by disrupting this protein. This concept has been demonstrated in a preclinical model that tested a knockdown of osteopontin in breast cancer cells, leading to a reduction of proliferation, invasiveness, and bone metastases. Unfortunately, efforts to inhibit osteopontin therapeutically have resulted in unwarranted side effects such as promoting atherosclerosis, so further research is needed.[90] Similarly, metastatic cancer cells may secrete the matrix metalloproteinases (MMPs) MMP-2 and MMP-9 which play a role in altering bone turnover and promoting metastatic growth.[91] Dong et al.[92] detected that MMP-9 activity peaked 2 weeks after the colonization of bone by prostate cancer cells, which
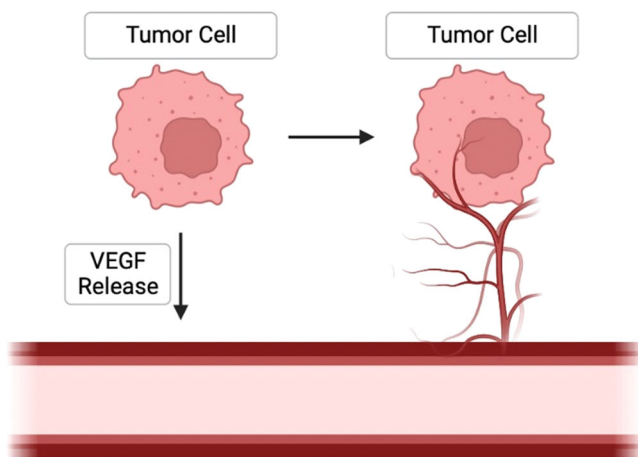

FIGURE 3 Pathway by which tumor cells release VEGF to promote angiogenesis and create their own blood supply. VEGF, vascular endothelial growth factor.

was then linked with a wave of osteoclast recruitment. MMP-9-specific inhibitors may, therefore, yield promising therapeutic targets in bone metastasis.

The proteoglycan heparan sulfate is also important for the structure and function of the ECM and therefore has been targeted by tumor cells in MBD. These proteoglycans have the ability to bind to bone-related proteins through specific heparin binding domains. Tumor cells may target this function to promote bone metastases. Other important proteoglycans include, perlecan and glypicans, which function to modulate the homing, colonization and migration of tumor cells.[93]

3.4 | Immunomodulation in bone metastases

Another important process in MBD is immunomodulation. As with other important processes, tumor cells have developed ways to take advantage of this key physiologic response to promote tumor growth and bone mestastases.[94] The specific cells involved in immunomodulation in bone metastases are described below and in Table 3.

CD8+ T cells are effector or cytotoxic cells primarily responsible for directly killing infected host cells, producing cytokines, regulating the immune response, and killing tumors by mechanisms including apoptosis and cell cytotoxicity. Regulatory T cells, however, promote effector T cell tolerance and exhaustion, stimulate tumor growth.[94,95] Programmed cell death protein 1 (PD-1) is located on T cells and functions to block T cells from killing other cells, including tumor cells, when bound to programmed death-ligand 1 (PD-L1). Tumor cells take advantage of this system to avoid T cell targeting.[102]

Mature B cells may differentiate into plasma cells and release antibodies specific for pathogenic or tumor antigens causing an immune response that targets the tumor. However, if B cells accumulate at tumor sites, they may secrete immunosuppressive cytokines such as IL-10 which will promote tumor growth.[96,97]

Macrophages promote wound healing, regulate adaptive immunity, and eliminate infectious agents. Classically activated macrophages, M1, are activated by factors such as interferon-gamma

TABLE 3 Normal function of immune cells in the bone and the impact of cancer.

Cell type	Normal function	Role in cancer
T cells[94,95]	Cytotoxic properties, produce cytokines and regulate the immune response	Regulatory T cells cause effector T cell tolerance and exhaustion, promoting tumor growth
B cells[96,97]	Differentiate into plasma cells and release antibodies	Can accumulate in tumor cells and secrete immunosuppressive cytokines like IL-10 that promote tumor growth
Macrophages[96,98]	Activated by interferon-gamma that are cytotoxic role in innate immunity	Tumor-associated macrophages and metastasis-associated macrophages promote tumor growth
Myeloid-derived suppressor cells[99,100]	Premature immune cells that suppress both innate and adaptive immune responses	Decreasing immune surveillance, remodeling the tumor microenvironment, establishing a premetastatic niche, and promoting the epithelial-to-mesenchymal transition in tumor cells
Natural killer[101]	Cytotoxic cells that secrete cytokines such as IFN-γ	Number of NK cells is decreased in prostate cancer.

Abbreviations: IFN, interferon; IL, interleukin.

(IFN-γ) and have tumoricidal activity. However, alternatively activated macrophages, M2 are associated with tumor-associated macrophages (TAMs) and metastasis-associated macrophages (MAMs), which express both M1 and M2 markers.[96,98]

Myeloid-derived suppressor cells (MDSCs) includes immature macrophages, granulocytes, dendritic cells, and myeloid progenitor cells. These cells can be divided into those that are morphologically similar to monocytes or polymorphonuclear cells. It is believed that MDSCs can aid tumor growth and metastases by decreasing immune surveillance, remodeling the tumor microenvironment, establishing a premetastatic niche, and promoting the epithelial-to-mesenchymal transition in tumor cells.[99,100]

Natural Killer cells (NK cells) are cytotoxic cells that secrete cytokines such as IFN-γ that can alter the immune response. The number of NK cells was decreased in a mouse model of prostate cancer and associated with an overall reduction in metastasis.[101]

Of note, Robertson et al.[103] demonstrated the role of the ECM with regard to immunomodulation of tumor cells. It was demonstrated that Collagen 4 induces both massive overgrowth and suppression of immune mediated tumor clearance, which was not linked with an upregulation of regulatory T cells or T cell exhaustion. This presented a model to reverse the immune-cold tumor types that can evade immunotherapeutics.[103]

4 | THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM

The health of the microarchitecture of bones has connections with the sympathetic nervous system. Pathologic fractures and pain from bone lesions can lead to direct activation of the sympathetic nervous system.[104,105] Furthermore, many types of tumors have been shown to have a growth advantage secondary to stress stimuli via activation of the sympathetic nervous system.[106] Severe emotional stress from the experience of pain increases SNS activity, which causes the release of adrenergic compounds like norepinephrine and epinephrine. This results in activated osteoblasts to release RANKL and IL-6 which affect bone processes such as inflammation, cell trafficking, and bone resorption, which are important for the development of MBD.[106–108] Treatments for bone pain include denosumab and bisphosphonates which have been shown to reduce bone pain in part through the reduction in osteoclast activity. Similarly, anti-cathepsin K treatment can reduce bone pain.[109]

5 | METASTATIC MECHANISMS OF CANCER CELLS

One of the main prognostic factors that affects the management of cancer is the presence of metastatic dissemination of tumors.[110,111] Traditionally, evidence of tumor dissemination is detected with clinical exam, laboratory abnormalities and imaging modalities. Additionally, providers may detect occult or impending metastases by analyzing the presence of disseminated tumor cells (DTCs) in lymph nodes or bone marrow or circulating tumor cells (CTCs) in the peripheral blood.[111,112] Past research has demonstrated that patients who have undergone complete removal of the primary tumor, but also have detectable CTCs or DTCs may have overt metastases in the future, which may indicate the need for maintained systemic therapies.

Epithelial–mesenchymal transition (EMT) seems to promote the release of cancer cells into the bloodstream, but complete EMT is not mandatory given that CTCs from patients with breast or prostate cancer readily express epithelial markers, such as EpCAM and keratins.[113]

DTCs are typically detected in the bone marrow even in the absence of metastases to lymph nodes or visceral organs. However, it remains unclear if the bone marrow acts as a reservoir for DTCs to mature and then disseminate or alternatively, if the presence of DTCs in the bone marrow indicates that cancer cells can already disseminate to other organs.[114] Conventional Ficoll gradient centrifugation is typically the method of choice for the isolation of DTCs from bone marrow.[115] The long latency periods between cancer diagnosis and metastatic relapse in patients with early-stage breast cancer probably reflect DTC dormancy and resistance to adjuvant chemotherapy.[116]

In comparison, CTCs are found more commonly in peripheral blood, acting as a vector for the dissemination of tumor cells to other sites.[117] The detection of these cells is useful in prognostication and their presence indicates possible distant, overt metastases. Developing a blood test for multiple sampling has been described as a method of monitoring therapeutic success.[118] This, however, has associated challenges because CTCs are relatively rare and existing techniques lack the sensitivity to isolate CTCs for further analysis. Even if one were able to isolate the CTCs, it is difficult to differentiate these from nontarget hematopoietic cells therefore, leading to an increased risk of false positives.[119]

Analyses of CTCs present in peripheral blood and DTCs that have extravasated into the circulation from the bone marrow, have provided important mechanistic insights into the early steps of metastatic progression in humans.[120] DTCs can target the bone marrow early during the development of the primary tumor, as demonstrated by the presence of bone marrow DTCs even in patients with small breast or prostate tumors without detectable metastases years before metastatic relapse.[121]

6 | CONCLUSION

Cancer metastasis has serious implications with regard to patients' prognosis and treatment options, along with contribution to higher morbidity and mortality rates. MBD is a subset in which primary cancers, typically originating from the prostate (34%), breast (22%), and lung (20%), spread to the bone. Afflicted patients present with progressive bone pain with an increased risk of pathological fractures and a diminished life expectancy. The microenvironment of bones,

involving dynamic bone remodeling, angiogenesis, and immunomodulation, for example, makes for a particularly suitable setting for cancer to grow and spread. The purpose of this paper was to outline current concepts relevant to the microenvironment of bones and MBD, along with how therapeutic options may be designed to address this microbiology. Future studies are warranted to further identify hereditary or epigenetic factors in preclinical and clinical studies that can act as targets for personalized treatment of cancer.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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