



## Research paper

## The unresolved role of systemic factors in bone metastasis

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## ABSTRACT

Systemic factors including cytokines, cell-free nucleic acids, microvesicles, and platelets are appreciated as important regulators of adenocarcinoma progression. Research findings using pre-clinical mouse models have revealed that many such systemically acting factors are either secreted by or responsive to peripheral tumors and impact bone and bone marrow (collectively referred to as the bone micro-environment) to initiate processes that ultimately govern disease progression, even in the absence of detectable bone metastases. In some cases, cancer-driven modulation of the bone microenvironment involves mobilization of bone marrow hematopoietic and mesenchymal cells into the circulation that are subsequently recruited into peripheral tissues and tumors. In other cases, systemic factors alter bone marrow cell (BMC) differentiation and/or gene expression to render the BMCs pro-tumorigenic even prior to their mobilization into the circulation. Given their effect on the bone microenvironment, it stands to reason that such systemic factors might also influence metastases in the bone; however, this hypothesis remains to be comprehensively tested. Here, we briefly review what is known, and not known, about systemic factors that regulate the bone microenvironment and thereby influence bone metastases. We also pose a number of currently unanswered questions in this active area of research. A better understanding of systemic processes that influence bone metastasis should aid discovery of therapeutic approaches that aim to eradicate or reduce disease burden in the bone, which is the cause of significant patient mortality and morbidity and is currently incurable.

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## 1. Is there a role for systemic factors in formation of pre-metastatic niches in the bone?

Bone is a common site for metastatic spread of solid tumors, particularly for patients with metastatic breast and prostate cancers [1]. Here, we specifically focus on the factors impacting the bone microenvironment that thereby influence bone metastasis, which is the cause of significant patient mortality and morbidity and is currently incurable. At present, very little is known about systemic processes that influence bone metastasis. Increasingly, efforts are being directed toward this area of investigation with the notion that a better understanding of systemic processes that influence bone metastasis should aid discovery of therapeutic approaches that aim to eradicate or reduce disease burden in the bone.

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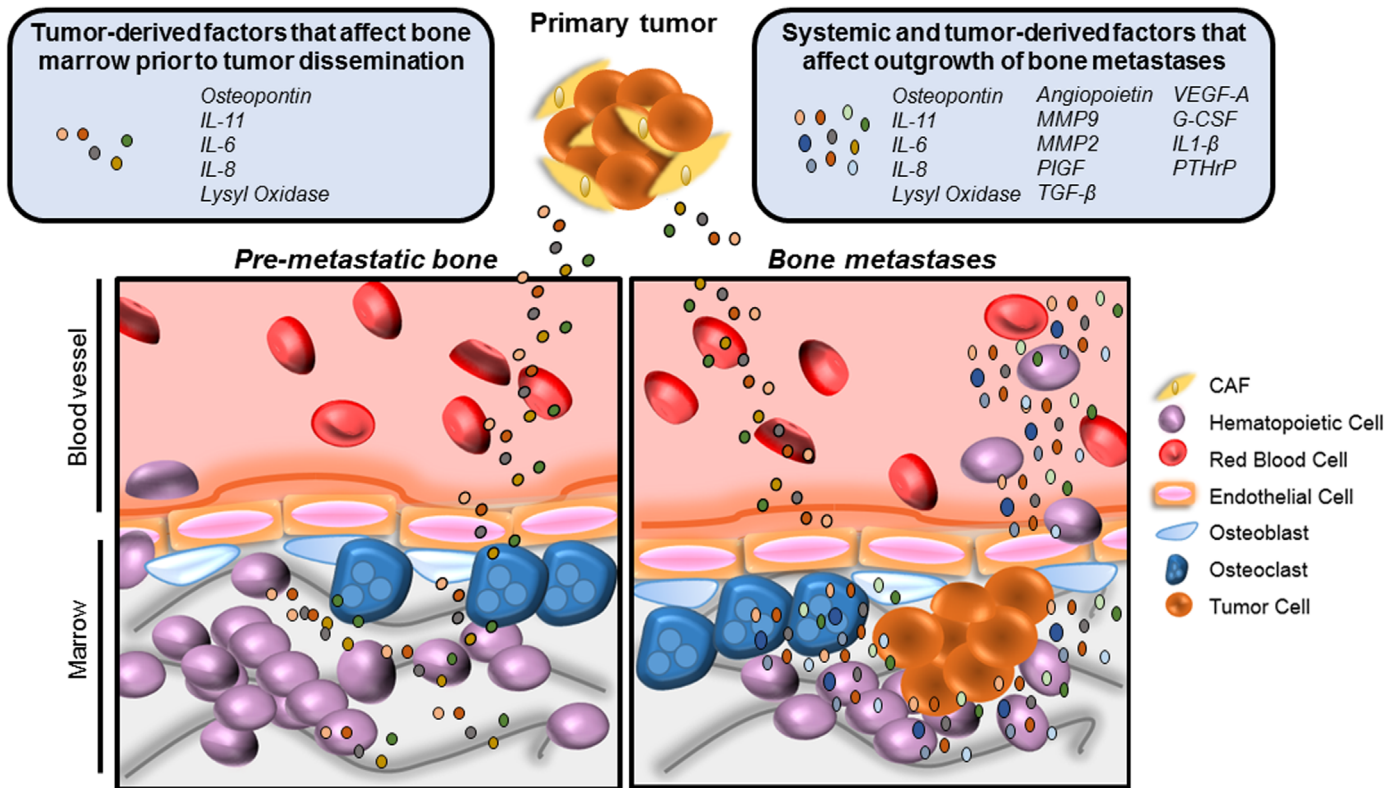
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Results from studies using pre-clinical metastasis models have revealed that primary tumor-derived circulating factors can affect various tissue microenvironments, even in the absence of observable metastases to those tissues, to make them a more hospitable environment for seeding and colonization of tumor cells that eventually disseminate from the primary tumor [2,3]. This process was termed "pre-metastatic niche" formation and most of what is known in this regard was gleaned from pre-clinical studies of lung metastasis. Much less is known about cancer-derived circulating factors that establish pre-metastatic niches in the bone. On one hand, the paucity of information may be due to the fact that there are very few bone metastasis models currently available to researchers. On the other hand, the bone microenvironment may inherently provide a favorable environment for disseminated tumor cells, thus eliminating the need for pre-metastatic modulation.

The fact that disseminated tumor cells are frequently detected in bone marrow aspirates of cancer patients who are tested in this manner [4] favors the idea that disseminating tumor cells find a ready-made niche in the bone microenvironment. Traditionally,



**Fig. 1.** Some of the systemic factors involved in dissemination and outgrowth of bone metastases. In the pre-metastatic bone, primary tumor-derived circulating factors can affect various tissue microdomains (hematopoietic cells, mesenchymal stromal cells, osteoblasts, osteoclasts, and vascular cells) to make them a hospitable environment for seeding of tumor cells that eventually disseminate from the primary tumor. After tumor cells have disseminated to bone, primary tumor-derived factors and other circulating cytokines of unknown origin can influence colonization of those tumor cells. Likewise, the tumor cells within the bone microenvironment secrete factors that disrupt normal bone homeostasis to fuel metastatic progression. Abbreviations: CAF, cancer-associated fibroblast, see Table 1 for growth factors.

bone metastatic niches have been defined as microdomains within the bone that support tumor cell seeding and outgrowth via paracrine interactions, and can be comprised of hematopoietic cells, a variety of mesenchymal stromal cells, osteoblasts, osteoclasts, and/or vascular cells [1] (Fig. 1). Within these microdomains, a number of chemokines and integrins that are endogenously expressed by various bone stromal cells to regulate mobilization and homing of hematopoietic cells are also thought to aid tumor cell recruitment to bone [1]. For example, bone stroma derived CXCL12 (SDF-1 $\alpha$ ) has been demonstrated to recruit CXCR4-expressing neuroblastoma cells [5]. Likewise, differentiating osteoclasts secrete CCL22, which was shown to promote bone metastasis of CCR4-expressing breast cancer cells [6]. Additionally, osteoblasts express a number of factors, including CCL12, which has been correlated with increased tropism of CCR7-expressing metastatic breast cancer cells [7].

More recently, primary tumor driven generation of a bone pre-metastatic niche was observed in a mouse model of estrogen receptor-negative breast cancer metastasis. Specifically, lysyl oxidase (LOX) secreted into the circulation from hypoxic primary breast tumors disrupted bone homeostasis, thereby inducing osteolysis [8]. The osteolytic microdomains within the bone served as niches for subsequent metastatic tumor cells, and bisphosphonate administration in the pre-metastatic setting prevented development of metastatic disease. This work is the first reported observation of breast cancer induced osteolytic action-at-a-distance (breast cancers frequently induce osteolysis following their dissemination to bone, as we discuss later). These findings, if further supported, have important clinical implications and should prompt further investigation into systemic modulation of bone-specific pre-metastatic niches.

## 2. Do primary tumors that impact the bone microenvironment also influence bone metastases?

Interestingly, bone is a conduit during pre-metastatic niche formation in visceral tissues in nearly all reported studies to date [2]. In other words, pre-metastatic niche formation in extra-ossseous organs involves mobilization, modification, and recruitment of bone marrow derived cells (BMDCs) that help create the niche. Even before the discovery of pre-metastatic niches, investigation into the role of BMDCs in primary tumor progression and metastasis was an active area of research, as it still is today.

Numerous studies have shown that tumor-derived systemically acting factors impact the bone microenvironment to expand and mobilize bone marrow cells (BMCs) into the circulation that are subsequently recruited to tumor sites where they instigate various processes that support tumor progression [9,10] (Table 1). For example, tumor-derived granulocyte colony-stimulating factor (G-CSF) and interleukin-1 $\beta$  (IL1 $\beta$ ) mobilize tumor-supportive CD11b+/Gr1+ myeloid cells from the bone marrow into circulation, while vascular endothelial growth factor-A (VEGF-A) and placental growth factor (PIGF) release hemangiogenic BMCs (VEGFR1+ cells) from the bone marrow into circulation [9]. Breast cancer-associated fibroblasts (CAFs) have been demonstrated to secrete CXCL12, which induces the release of pro-angiogenic hematopoietic progenitor cells into the circulation [11].

Tumor-derived microvesicles—membrane-bound particles released from a primary tumor that carry lipids, proteins, mRNAs and miRNAs—could also modulate cells in the bone microenvironment [12]. For example, melanoma exosomes were shown to ‘educate’ bone marrow progenitor cells toward a pro-metastatic phenotype [13]. More recently, OPN carried through the

**Table 1.**  
Some of the bone modulating factors that impact cancer progression.

Factor	Source	Mechanism of action
SDF-1 $\alpha$ /CXCL12	Osteoblasts; CAFs	Provides a supportive stromal microenvironment for CXCR4-expressing cancer cells [5]. CAFs secrete CXCL12, which induces the release of pro-angiogenic hematopoietic progenitor cells into the circulation [11]
CCL22	Osteoclasts	Promotes bone metastasis of CCR4-expressing breast cancer cells [6]
CCL12	Osteoblasts	Promotes bone tropism of CCR7-expressing breast cancer cells [7]
Angiopoietin	DTCs	Promotes metastasis to the bone by enhancing tumor-cell responsiveness to CXCL12 via CXCR4, and potentially other unknown mechanisms [12]
OPN	Primary tumor and microvesicles	Microparticle-derived OPN mobilizes pro-angiogenic cells from the bone marrow in response to chemotherapy [14]; necessary for pro-tumorigenic function of Sca1 + /ckit-CD45 + hematopoietic bone marrow cells that are recruited to the tumor microenvironment [16]
Lysyl Oxidase	Primary tumor and DTCs	Generates pre-metastatic niche in bone through disruption of bone homeostasis [8]
VEGF-A	DTCs	Releases hemangiogenic VEGFR1 + cells from the bone marrow into circulation [9]
G-CSF	DTCs	Mobilizes tumor-supportive myeloid cells from the bone marrow into circulation [9]; upregulates pro-angiogenic factors in myeloid cells [15]
IL1 $\beta$	DTCs	Mobilizes tumor-supportive myeloid and myeloid derived suppressor cells from the bone marrow into circulation [9]
PIGF	DTCs	Releases hemangiogenic VEGFR1 + cells from the bone marrow into circulation [9]
TGF- $\beta$	DTCs, Bone ECM	Mobilizes myeloid cells from the bone marrow [12]; Latent TGF $\beta$ released from bone ECM stimulates cytokine production in DTCs [18]
IL-6, IL-8, IL-11	Primary tumors, DTCs, osteoblasts	Accelerates growth factor release from bone matrix via upregulation of RANKL/RANK, and thus fuels the vicious cycle of tumor progression [1]
PTHrP	DTCs	Promotes osteoclast maturation or stimulates osteoblast secretion of IL-6 and RANK, thus propagating a vicious cycle of tumor outgrowth and bone breakdown [18]
MMP2, MMP9	DTCs	Cause release of TGF- $\beta$ and other factors from the bone marrow microenvironment that induce DTC production of a variety of cytokines, most notably PTHrP [18]
GDF15	CAFs	Promotes metastatic outgrowth in the lung [20]
Pro-angiogenic factors	Cancer-associated platelets	Platelets take up factors secreted by primary tumors to aid angiogenesis in disseminated tumors at distant sites [12]

SDF-1 Stromal cell-derived factor 1; CCL22 Chemokine (C-C motif) ligand 22; CCL12 Chemokine (C-C motif) ligand 12; OPN Osteopontin; VEGF-A vascular endothelial growth factor A; G-CSF Granulocyte colony stimulating factor; PIGF Placental growth factor; TNF- $\alpha$  Tumor necrosis factor  $\alpha$ ; TGF- $\beta$  Transforming growth factor  $\beta$ ; IL-6 interleukin 6; IL-8 Interleukin 8; IL-11 Interleukin 11; PTHrP Parathyroid hormone-related protein; MMP matrix metalloproteinase; ECM extracellular matrix; GDF15 growth/differentiation factor 15; CAFs cancer-associated fibroblasts; DTCs disseminated tumor cells. While many of these factors have pleiotropic effects in various contexts, we limit descriptions to bone-specific effects that are known to affect cancer progression.

circulation by murine mammary carcinoma-derived microparticles in response to chemotherapy, was necessary for mobilizing pro-angiogenic cells from the bone marrow [14].

It is becoming increasingly apparent that tumor-derived factors can also alter certain BMCs by affecting their gene expression even prior to their mobilization into the circulation [12] (Table 1). Murine cancer models revealed that a subset of myeloid cells upregulated pro-angiogenic factors in response to tumor-derived G-CSF [15]. In studies of mouse xenograft models, tumor-derived soluble OPN, which functions as an inflammatory cytokine, rendered Sca1 + /cKit-/CD45 + hematopoietic BMCs pro-tumorigenic by modulating gene expression prior to their mobilization into circulation [16].

Based on the seemingly intimate relationship between primary cancers and the bone microenvironment, and given that certain tumors can act in an endocrine fashion to generate pro-tumorigenic hematopoietic BMCs, it stands to reason that these same BMCs would provide support to tumor cells that land in the bone. However, this hypothesis remains to be tested, thus representing an area where additional research is necessary. In this context, it is likely that many more tumor-derived systemic factors that modify BMCs remain to be identified.

### 3. Do cancer-dependent systemic factors influence tumors that have disseminated to bone?

Despite the frequency with which disseminated tumor cells (DTCs) are found in the bone marrow of cancer patients, the significance of these cells is unknown, as a considerable number of these patients never develop overt metastatic disease [4]. Such findings support the concepts of metastatic inefficiency and tumor dormancy [17] and suggest that while the bone microenvironment

may be conducive to initial dissemination and survival of tumor cells, other processes are required to promote disease progression.

Certainly, dynamic interactions between DTCs and stromal cells within the bone microenvironment disrupt bone homeostasis, which is normally tightly controlled, to fuel metastatic progression [18] (Table 1). For example, it is well established that osteolytic breast cancer DTC-derived factors, such as matrix metalloproteinases, cause the release of latent TGF- $\beta$  from the bone microenvironment. This, in turn, induces DTC production of a variety of cytokines, most notably parathyroid hormone-related protein (PTHrP), which either promotes osteoclast maturation or stimulates osteoblast secretion of IL-6 and RANKL thus propagating a vicious cycle of tumor outgrowth and osteolytic bone breakdown [18]. Tumor cells can also directly secrete interleukins (specifically IL-6, IL-8, and IL-11) to increase osteoclastogenesis, and thus fuel the vicious cycle of tumor progression [1]. Likewise, osteoblastic DTCs, such as those that metastasize from prostate cancers, initiate paracrine interactions within the bone microenvironment that serve to promote their growth [18].

The fact that many of the same tumor-supportive cytokines that are secreted by DTCs within the bone microenvironment are also secreted by the primary tumors that spawned them begs the question of how much influence the primary tumor has on DTCs in the bone. The answer may have clinical relevance, considering that patients who develop metastatic disease after surgery and treatment of their primary cancer clearly had DTCs in the periphery at the time of their initial diagnosis. Indeed, pre-clinical models of the early phases of metastatic disease, when patients harbor indolent DTCs at a time when their primary tumor is present, have provided important insights into systemic cross talk between distantly located tumors. For example, early studies of chemically-induced cancers revealed that some tumors establish an immune-permissive environment for the outgrowth of otherwise

immunogenic tumors at distant sites [19]. In a process that was termed “systemic instigation”, certain primary tumors promote the outgrowth of otherwise indolent lung metastases by secreting factors that cause mobilization and recruitment of BMDCs that aid metastatic outgrowth [12]. In the case of systemic instigation by triple-negative breast cancer, primary tumors secrete OPN into the circulation, which is necessary for rendering BMCs pro-tumorigenic. In systemic instigation models of luminal breast cancer, tumor-derived cytokines and growth factors are taken up by circulating platelets that cooperate with BMDCs to promote angiogenesis in the distant tumors. In addition to tumor cells, cells in the primary tumor microenvironment can impact distant metastasis. For example, cancer activated fibroblasts secrete growth/differentiation factor 15 (GDF15, also known as macrophage inhibitory cytokine-1, MIC-1, a member of the TGF- $\beta$  family of growth factors) to promote metastatic outgrowth in the lung [20].

Although it stands to reason that primary tumor derived systemic factors that render BMCs pro-tumorigenic would almost certainly impact tumor cells that had metastasized to bone, systemic instigation of bone metastasis has not been reported. Research in this area seems crucial for identifying therapeutic avenues designed to restrict growth of DTCs in the bone.

#### 4. Conclusions and questions

The past decade has seen a significant expansion in our knowledge about the intimate, yet long-distance relationship between various cancers and the bone microenvironment (Fig. 1).

Nevertheless, surprisingly little is known about whether or how such systemic endocrinal interactions impact bone metastases. Development of better pre-clinical models of bone metastasis, with validation in clinical studies, should help guide interventions aimed at inhibiting underlying systemic signaling cascades, if they exist, and could offer clinical benefit for cancer patients. Hence, some critical questions in this active area of research remain unanswered:

- Does the bone environment need to be modified in order to establish a hospitable niche for disseminating tumor cells?
- What systemic factors, if any, are necessary and/or sufficient to generate tumor-supportive or tumor-inhibitory niches in the bone marrow?
- How do modulation of osteoblast/osteoclast, hematopoietic, vascular, and/or bone stromal compartments affect bone metastasis?
- What lessons can be learned from studying metastatic niches in other visceral organs to inform bone metastasis?
- Are there primary tumor-derived systemic instigation factors that promote outgrowth of dormant DTCs in the bone?
- Are factors secreted by bone DTCs to influence the bone microenvironment also secreted by primary tumors from a distance to influence bone metastasis?
- Do physiological conditions that impact the bone microenvironment, such as pregnancy, wound healing, bone remodeling, and aging, also influence bone metastases?

- What is the impact of systemic regulation of immune function on metastatic colonization in the bone?
- Do bone-modulating drugs (denosumab, bisphosphonates, filgrastim, plerixafor, etc.) also influence bone metastases?
- What therapeutic interventions could be developed to inhibit systemic signaling mechanisms of cancer metastasis to the bone?
- What patient biomarkers may be used to indicate the tumor-specific systemic environment in the bone, and subsequent response to adjuvant therapies?

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