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Review Series

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Exploiting bone niches: progression of disseminated tumor cells to metastasis

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Many solid cancers metastasize to the bone and bone marrow (BM). This process may occur even before the diagnosis of primary tumors, as evidenced by the discovery of disseminated tumor cells (DTCs) in patients without occult malignancies. The cellular fates and metastatic progression of DTCs are determined by complicated interactions between cancer cells and BM niches. Not surprisingly, these niches also play important roles in normal biology, including homeostasis and turnover of skeletal and hematopoiesis systems. In this Review, we summarize recent findings on functions of BM niches in bone metastasis (BoMet), particularly during the early stage of colonization. In light of the rich knowledge of hematopoiesis and osteogenesis, we highlight how DTCs may progress into overt BoMet by taking advantage of niche cells and their activities in tissue turnover, especially those related to immunomodulation and bone repair.

Introduction

The bone is a common site of metastasis for many tumor types, particularly those of breast, prostate, lung, and multiple myeloma (1–3). Breast and prostate cancers are the most common types of solid tumors in women and men, respectively. As many solid tumors can be surgically removed, metastasis is the real risk to these patients, causing over 90% of cancer-related deaths (4, 5). However, effective therapies for metastases, including bone metastases, are still lacking (6).

Metastases and primary tumors often occur asynchronously. The interval between primary tumor removal and diagnosis of metastases is referred to as metastasis dormancy (7, 8). During metastasis dormancy, microscopic metastases or single disseminated tumor cells (DTCs) survive adjuvant therapies. A fraction of them finally outgrow and become overt metastases (9, 10). A significant proportion of patients have DTCs detected in the bone marrow (BM) at the time of diagnosis (11–13), and even after tumor resection (14, 15), indicating that distribution of these seeds to different organs occurs earlier than diagnosis (16, 17), and therefore may be inevitable with current detection limits. However, targeting the ability of DTCs and micrometastases to adapt to and colonize secondary sites may represent a viable approach to eliminate metastases (18).

Metastatic cancer cells that make it to the bloodstream are subject to immune attack, physical shearing, and other forces (19–21). However, some of these cells are able to travel around the body and extravasate though the endothelial wall into the tissue (22). The BM is a particularly accessible and nurturing nest for DTCs, as sinusoidal capillary beds with fenestrated endothelium are permissive for cancer cell extravasation (23). The BM microenvironment, as the cradle of hematopoiesis, evolved to enrich growth factors (e.g., IGFs and FGFs) and cytokines that normally support stem cell activities of both hematopoietic and mesenchymal lineages (24). Therefore, it is not surprising that the BM niches of DTCs resemble those of hematopoietic and mesenchymal stem cells. Indeed, cancer cells may hijack unique homeostatic and regenerative functions of these niches to facilitate their own survival and progression. In this article, we review recent findings on the roles of BM niches in metastatic colonization. We focus on how cancer cells exploit activities of the niche components to promote metastatic colonization. These activities may be related to their normal functions such as establishing an immune-privileged environment, maintaining tissue homeostasis, and healing wounds.

Bone homeostasis

Bone is a remarkable organ that plays complex physiological roles, such as providing structural support and facilitating hematopoiesis (25–27). The unique bones of the body are diverse in size and shape, with differing amounts of cortical bone (compact and dense) as well as trabecular (or spongy) bone. Bone is maintained by cells of the osteoblast and osteoclast lineages (28, 29). Mesenchymal stem cells develop into preosteoblasts, then mature osteoblasts (30, 31). These cells produce both the mineralized portion of bone, hydroxylapatite, and organic components: collagen, osteocalcin, and osteopontin (32). Mature osteoblasts line the mineral surface within the bone (33). The osteoid, or organic, components are deposited first; as the tissue becomes mineralized and new bone is formed, osteoblasts become trapped within and further transform into osteocytes (34, 35). The osteocytes form long membrane protrusions that connect to each other through thin channels called canaliculi (36), communicating through gap junction channels at their tips (37). Osteoclasts, responsible for breaking down the mineralized bone tissue, develop from the monocyte/

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macrophage lineage upon stimulation by NF-κB/RANKL as well as macrophage colony-stimulating factor (38, 39). Osteoclasts are large, multinucleated cells that become strongly polarized toward the bone surface for resorption, and their ruffled cell edges attach to bone (40). Bone resorption is accomplished by secretion of proteases to dismantle organic bone components and protons to break down inorganic mineral (41).

Hematopoietic stem cells and their BM niches

Hematopoietic stem cells (HSCs) originate during embryogenesis (42, 43), expand in fetal tissues (44), and ultimately home to the BM (45), where they maintain lifelong hematopoiesis. Instead of randomly distributing in the BM, HSCs reside in specialized BM niches. Two types of BM niches harbor HSCs and tightly regulate their fates: the perivascular niche and the endosteal niche (46). The perivascular niche is subdivided into a sinusoidal and an arteriolar portion. The majority of SLAM family markers that label HSCs (Lin^-, $CD48^-$, $CD41^-$, $CD150^+$) are localized in the sinusoidal perivascular niche (47, 48), which comprises endothelial cells and adjacent perivascular stromal cells, including leptin receptor–expressing (LEPR-expressing) cells (49, 50), nestin-expressing cells (50, 51), and CXCL12-abundant reticular cells (52). On the other hand, a small fraction of HSCs are found adjacent to the arteriolar perivascular niche (53, 54), where they come into close contact with $LEPR^+$ perivascular cells, non-myelinating Schwann cells (55), and NG2-expressing stromal cells. These perivascular cells secrete important cytokines such as CXCL12 (also known as SDF-1), stem cell factor (SCF), and TGF-β (55), which make distinct contributions to HSC maintenance (50). Although active HSCs are localized to the perivascular niche, some studies showed that quiescent HSCs mainly reside in the endosteal niche with arterioles (47, 56–58), which is composed mainly of osteogenic cells. HSCs may depart from one niche and enter another niche in response to specific signals (53, 59, 60). Interestingly, some studies suggested that HSCs prefer to engraft at the endosteal region after transplantation (61, 62). Although the perivascular niche and the endosteal niche are cellularly distinct niches and may harbor distinct subpopulations of HSCs, a part of these two niches may be spatially close to each other in the endosteum region, since blood vessels are also rich near the bone. However, a cross section of the tibia shows that HSCs are modestly enriched in the central BM and reduced toward the endosteum in the diaphysis (48). Interestingly, evidence reveals that type H endosteal vessels can regulate osteoprogenitors, thereby coupling angiogenesis to bone development, indicating complex crosstalk among BM niche cells (63, 64).

In addition to the endosteal niche and perivascular niche, other studies also revealed that megakaryocytes, a terminally differentiated hematopoietic cell, serve as a niche component that directly or indirectly regulates HSC fate (65–68). Megakaryocytes predominantly occupy the sinusoidal perivascular niche and contribute to maintaining HSC quiescence by producing CXCL4 and TGF-β (69).

The cellular fates of HSCs, namely dormancy, self-renewal, and differentiation, are regulated by microenvironmental signals emitted from specific niches. During homeostasis, niche signals keep a proportion of HSCs dormant to preserve their long-term self-renewal capacity (57, 58, 70), while other homeostatic HSCs are activated to drive normal hematopoiesis. The current model of hematopoiesis states that the differentiation of HSCs follows a strict hierarchy (71). Long-term reconstituting HSCs possess multilineage differentiation potential and differentiate into short-term reconstituting HSCs (ST-HSCs), which exhibit more-limited self-renewal potential. ST-HSCs further generate multipotent progenitors (MPPs) with distinct differentiation potential. These MPPs rapidly divide into oligopotent progenitors, including common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). CMPs then produce granulocyte-monocyte progenitors (GMPs), megakaryocyte-erythrocyte progenitors (MEPs), and dendritic cell (DC) progenitors. These oligopotent progenitors advance to their lineage-restricted precursors and ultimately give rise to all terminally differentiated blood cells. Specifically, MEPs differentiate into erythrocytes and platelet-producing megakaryocytes. GMPs generate neutrophils, basophils, eosinophils, and monocytes (which can further differentiate into macrophages in the BM and peripheral tissues). CLPs are precursors of DCs, B cells, T cells, and NK cells. However, HSCs may also be heterogeneous and can be organized into a cellular hierarchy with distinct cell differentiation routes, some of which may skip intermediate steps. For instance, the platelet-producing megakaryocytes can skip the intermediate oligopotent progenitors and directly generate from megakaryocyte-primed HSCs (72, 73).

Taken together, all the above-mentioned skeletal and hematopoietic cells constitute an intricate environment for metastatic cancer cells. It should be noted that these cells are not static but are rather highly dynamic in proliferation, differentiation, and relocation. How these dynamics impact seeding and colonization of DTCs remains elusive.

BM niches in metastasis

With the above in mind, we will review the roles of various bone-resident cells throughout the metastatic colonization process. Since a large proportion of literature in this area is based on breast cancer (BCa) models, we will focus on this cancer type, and summarize prominent features of other cancer types in separate sections.

The pre-metastatic niches. The impact of BCa on the BM niches starts even before DTCs arrive in the bone, a notion known as the "pre-metastatic niche." There, BM-derived hematopoietic cells were shown to "prepare the soil" for subsequent metastatic seeding, and subsequent studies supported this finding (74), including some suggesting that the pre-metastatic niche's impact on metastasis can be bidirectional (75, 76). Although BM-derived cells play major roles in modeling the pre-metastatic niche in other organs, the effects of primary tumors on BM itself were not studied until recently. Cox et al. showed that xenograft mammary tumors secreted lysyl oxidase (LOX), which generates systemic effects and activates osteoclastogenesis, thereby priming the formation of osteolytic lesions once cancer cells have seeded (77). Hoshino et al. reported that the metastatic site can receive exosomes derived from primary tumors, which facilitate subsequent colonization in an organ-specific fashion (78). Although bone-tropic exosomes were not identified in this study, lung-tropic exosomes diverted metastatic seeding of bone-targeting cancer cells from bone to lungs, supporting the notion that exosomes help determine organotropism of metastasis through effects on the pre-metastatic niche. Lung cancers also crosstalk with bone before metastasis

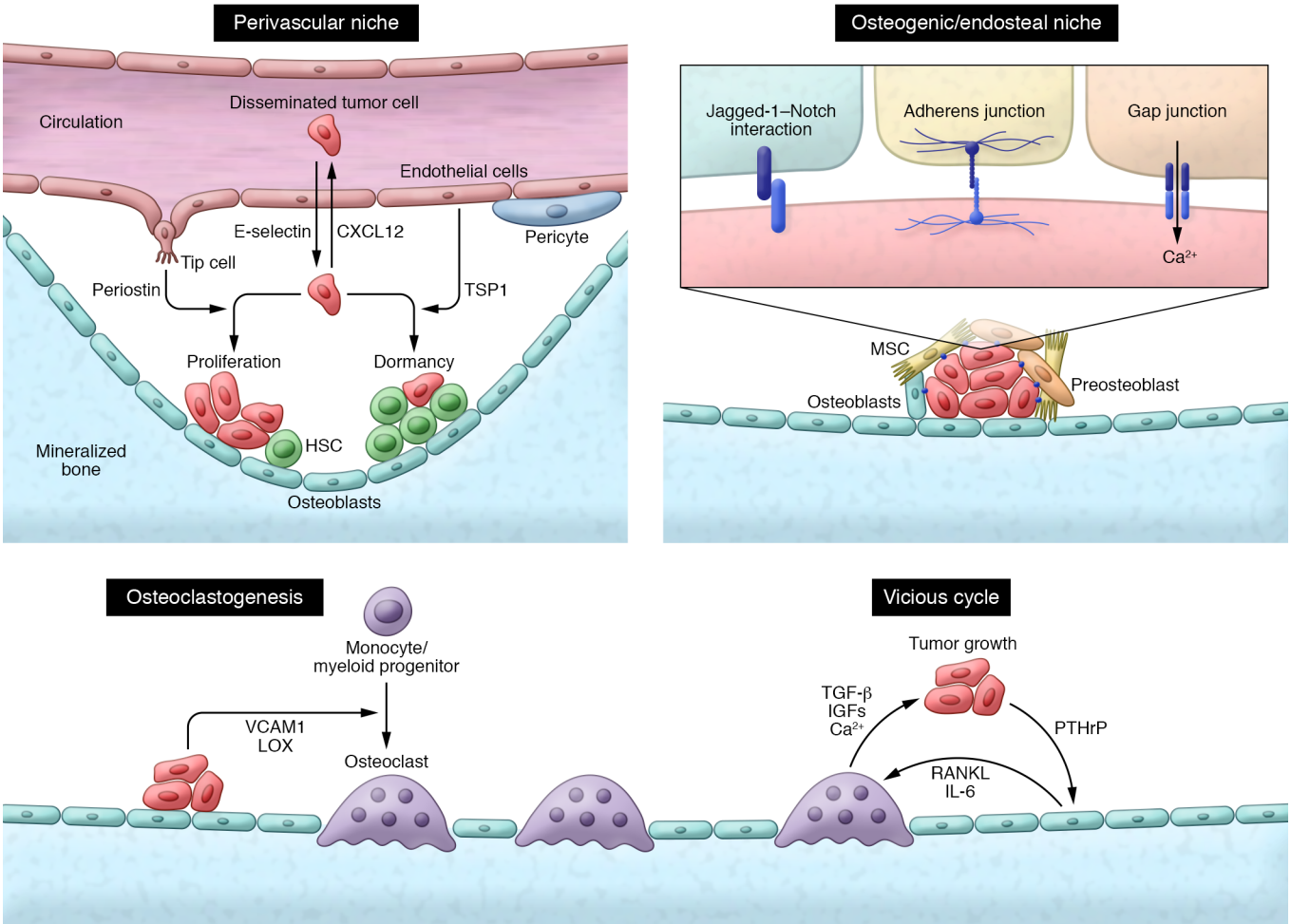

Figure 1. Disseminated tumor cells utilize bone marrow niches to survive and colonize. Disseminated tumor cells (DTCs) occupy many niches in the bone after they arrive in the organ via the bloodstream. When cells first extravasate from the vessels, they may reside in the perivascular niche. DTCs occupy and compete with the HSC niche through recruitment by CXCR4/CXCL12 signaling to the perivascular niche. Several signaling pathways and molecules govern the dormancy/proliferation fate of cancer cells in this niche, including pericyte-derived thrombospondin-1 (TSP1). Cancer cells can also interact with cells of the osteoblast lineage, namely mesenchymal stem cells, preosteoblasts, and osteoblasts, which constitute the osteogenic niche. Here they use heterotypic cellular junctions as well as Jagged-1–mediated Notch signaling to crosstalk with the niche. Gap junctions formed between the cancer cells and osteogenic cells facilitate calcium transfer into the cancer cells. These interactions collectively promote DTC proliferation and chemoresistance. Osteoclastogenesis may be activated by multiple mechanisms, which leads to a feed-forward loop known as the vicious cycle. In this cycle, tumor cells produce both pro-osteoblastic and pro-osteolytic factors, stimulating both osteoblasts and osteoclast activity. The destruction of the bone releases embedded growth factors that act on tumor cells, further stimulating their growth. HSC, hematopoietic stem cell; LOX, lysyl oxidase; MSC, mesenchymal stem cell; PTHrP, parathyroid hormone–related protein.

occurs. While the presence of tumors increases bone mass, osteoblasts return the favor by supplying a specific subset of neutrophils that promote tumor progression (79). Taken together, it has become increasingly clear that BM niches already begin to change under the influence of primary tumors before metastasis occurs. Further studies are needed to characterize these changes thoroughly to determine their functional impact on subsequent metastatic seeding and colonization.

The perivascular niche. When DTCs arrive via the bloodstream to the bone, they extravasate by interacting with the endothelium, which is particularly permeable in the BM (80) (Figure 1). Here, the cells reside in the perivascular niche (81, 82), which harbors HSCs as described above. This location also contains various immune cells and pericytes that possess activities of mesenchymal stem cells (MSCs) (83–85). There is some debate as to whether MSCs and pericytes are distinct cell types (86); nevertheless, these endothelium-adjacent cells have significant impact on DTCs when they arrive. Multiple signaling pathways may mediate the interactions of cancer cells with the perivascular niche and determine their fate accordingly. For example, the balance between the adhesion molecule E-selectin and CXCL12 may be critical for DTCs to remain in the perivascular niche (82), whereas the counteracting forces between thrombospondin-1 (TSP1) and TGF-β/periostin determine quiescence versus proliferation fate (81). Furthermore, vascular E-selectin may also trigger mesenchymal-to-epithelial transition and the acquisition of stemness traits, thereby fueling further progression (87). Interactions with the perivascular niche also render cancer cells resistant to chemotherapies (88). Overall,

it is intriguing to note that the perivascular niche is a common site for DTCs and HSCs, both of which may alternate between quiescence and proliferation states. Interestingly, the perivascular region has the highest degree of hypoxia in the bone, an already hypoxic organ in general (89). Hypoxia plays both direct and indirect roles in the promotion of metastatic progression and is expounded upon in further reading (90, 91). Given the BM's complicated and heterogeneous vascular network, further work will be needed to compare the exact DTC and HSC distribution and the molecular compositions in the corresponding niches.

The osteogenic/endosteal niche. The endosteal niche is located, as the name suggests, adjacent to the endosteum, or the lining of the BM cavity in adult bone. This niche is composed of osteoclasts, osteoblasts (and their progenitors), and fibroblasts in multiple stages of development (92) (Figure 1). As described above, the endosteal niche also plays an important role in harboring and sustaining HSCs. HSC-DTC competition for the endosteal niche was first reported in 2011 (93), thereby connecting the distinct fields of metastasis and hematopoiesis. Subsequently, an overlapping osteogenic niche has been described that promotes proliferation and early-stage bone colonization of BCa DTCs (94–96). In the osteogenic niche, luminal BCa DTCs form heterotypic adherens junctions with MSCs and preosteoblast and osteoblast cells, with E-cadherin on the cancer cell side and N-cadherin on the mesenchymal cell side (96). In fact, as the niche develops and the lesion progresses, the DTCs become completely surrounded by the osteoblast-lineage cells. These junctions facilitate Notch signaling, which in turn renders metastasis resistant to chemotherapies (97). The junctions also lead to the formation of functional gap junctions between the cells through which cancer cells receive a calcium flux from osteogenic cells (95). The tumor cells are inefficient at absorbing calcium from the matrix and therefore depend on osteogenic cells in the niche (95). The DTCs receive a growth advantage in the form of mTOR signaling activation in this niche and are able to progress from single cells to multicellular lesions (96). Perturbation of cadherins, gap junctions, or mTOR signaling decreases luminal cell colonization. Importantly, bone histomorphometry revealed active osteogenesis within the niche (96), suggesting ongoing tissue hemostasis.

The perivascular and osteogenic niches are not completely separated. Some niche components are linked by lineage. For instance, a subset of pericytes have MSC activities and can differentiate into osteoprogenitor cells (83), raising the possibility of conversion from the perivascular niche into the osteogenic niche. In fact, a specific subtype of vessels has been shown to couple angiogenesis and osteogenesis in the BM (63). These vessels, termed type H, express endomucin and CD31, often localize to the growth plate and endosteum, and are closely associated with osteogenic cells and promote their progression via Noggin secretion (63, 64). Interestingly, in prostate cancer, endothelial cells can transdifferentiate into osteoblasts, thereby facilitating the formation of osteoblastic lesions (98). Bone turnover and remodeling may change the dynamics of both niches and strengthen their crosstalk. Therefore, it is possible that DTCs may affect their neighborhoods and alter their cellular fates accordingly. This will be elaborated in a subsequent section.

The vicious cycle. While DTCs and bone micrometastases predominantly interact with perivascular and osteogenic niches where the roles of osteoclasts are limited, the advanced pathogenic bone metastases are often characterized by a feed-forward loop of bone cells and osteoclasts. This feed-forward loop has been described as the "vicious cycle" (3, 99–101) (Figure 1). Tumor cells can secrete parathyroid hormone–related protein (PTHrP) to stimulate osteoblasts, which in turn produce osteolytic factors such as IL-11 and RANKL and promote osteoclast proliferation and activation (102, 103). The resultant increase in osteolytic activity causes lesions to form in the mineralized bone tissue, releasing factors that have been sequestered in the bone, including tumor cell–stimulating growth factors such as IGFs and TGF-β (104–107), thereby fueling the feed-forward loop.

Recent studies uncovered additional molecular players involved in the feed-forward loop. Jagged-1, a TGF-β target gene in cancer cells, activates Notch signaling in osteoblasts and enhances the expression of IL-6, which consequently stimulates osteoclastogenesis (105). IL-6 can also be generated during senescence of osteoblasts (108). Integrin β_3 represents another TGF-β target gene that is specifically induced in the bone microenvironment and confers proliferative advantages on cancer cells (109). Cancer cells and osteoclasts sometimes depend on the same signaling pathways, such as the RON kinase (110). Taken together, these new findings provide additional therapeutic targets for treatment of advanced osteolytic metastases.

Little is known about the transition from the early niches predominantly composed of endothelial and osteogenic cells to the osteoclast-driven vicious cycle that is a hallmark of advanced bone metastases. One possible mechanism is that the development of the osteogenic niche may alter the secretome of both cancer cells and osteoblasts. Indeed, it was shown that some dormant cancer cells in the BM may recruit osteoclast progenitors by secreting a soluble form of VCAM1, of which integrin $\alpha_4\beta_1$ is the cognate receptor (111) (Figure 1).

Prostate cancer. Prostate cancer bone metastases also activate a vicious cycle similar to that seen in BCa that can be both osteoblastic and osteolytic (112, 113). However, these metastases tend to be more osteoblastic in nature, as they express high levels of alkaline phosphatase (ALP) and osteocalcin (114). The increased bone formation is poorly regulated and dysfunctional, often leading to very dense lesions and rigid zones of bone with decreased overall bone strength (115, 116). Endothelin-1, a stimulator of osteoblasts, is overexpressed by prostate cancer cells, and thereby increases osteogenesis, ALP expression, and osteoblastic bone metastases (115, 117, 118). PTHrP produced by the tumor stimulates osteoblasts to produce the osteoclastogenesis-promoting factors MCP-1 and RANKL (119). Paradoxically, PTHrP-expressing tumor foci have areas of both increased osteoblastogenesis and osteclastogenesis adjacent to them in the bone, indicating a more complex role for PTHrP (120, 121). Prostate-specific antigen can also tip the balance toward osteoblastic lesions by cleaving PTHrP (119, 121). To avoid detection and modulate bone homeostasis, prostate cancer cells may use osteomimicry, in which cells undergo osteoblastic-like differentiation, including expression of the osteogenesis markers BMP-2, RUNX2, and others, even to the point of actual mineral deposition (122).

Multiple myeloma and other cancers. Multiple myeloma, a cancer of plasma cells in the BM, can also cause lesions to form in the

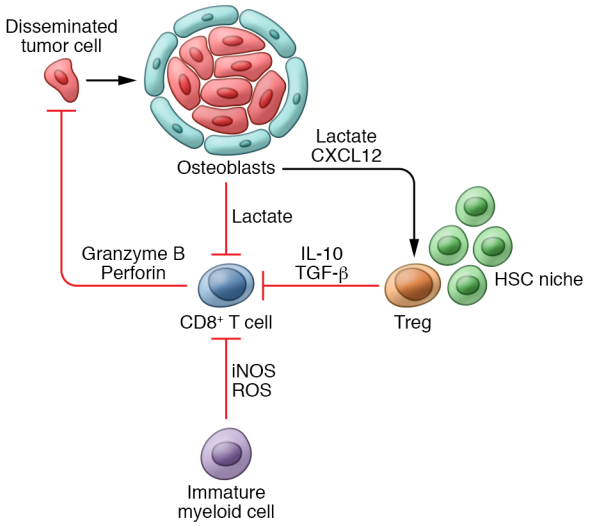

Figure 2. The immunosuppressive microenvironment in the bone and bone marrow. DTCs residing in the bone marrow niches may produce many immunosuppressive factors that also interfere with cytotoxic CD8⁺ T cells. Among these factors are regulatory T cells (Tregs), immature myeloid cells, and osteoblasts. DTCs occupy and compete with the HSC niche to occupy the immune-privileged environment maintained by Tregs. Tregs secrete immunosuppressive cytokines (IL-10, TGF-β) and traffic to the HSC niche via the chemokine CXCL12. Immature myeloid cells develop in the bone marrow and generate immunosuppressive molecules (iNOS, ROS). Osteoblasts are a major source of the chemokine CXCL12 that attract Tregs and produce lactate as a metabolic by-product. Lactate is utilized by cancer cells as an intermediate in the production of ATP. Lactate has also been shown to inhibit cytotoxic CD8⁺ T cells, while Tregs maintain immunosuppressive function. Taken together, these factors within the bone marrow niches maintain an immune-privileged environment for DTCs to colonize.

bone itself (123, 124). Patients can develop myeloma bone disease, when bony lesions cause fractures, pain, and other defects (125). In myeloma bone disease, cancer cells secrete osteoclastogenesis-promoting factors, including RANKL, IL-3, and IL-6 (126, 127), and also interact with BM stromal cells to produce such factors (128). This increased level of osteoclastogenesis is coupled with decreased or halted osteoblastogenesis, which is stimulated by IL-7, secreted frizzled-related protein, and other factors (125, 129). This results in characteristic lytic lesions in the bone that cause pain and fractures, and eventually patients can develop even more severe outcomes such as renal failure and anemia (130). More rarely, kidney, lung, skin, and thyroid cancers can also form osteolytic bone metastases (131).

Are the bone metastasis niches immune-privileged?

An often overlooked fact in bone metastasis (BoMet) research is that the bone is a major immunological organ. The hematopoietic niche within the bone is responsible for the production of erythrocytes and leukocytes, which requires a special environment that reduces aberrant immune activation. It was previously shown that the IRF7 pathway could facilitate escape of BoMet from immunosurveillance (132), which represents one of the earliest findings on the immunosuppressive milieu in BoMet. Given the complexity of BM niches, there might be multiple profound mechanisms contributing to the unique immune microenvironment. In this section, we summarize three likely cell types/pathways that may be responsible for immunosuppression: regulatory T cells, immature myeloid cells, and osteoblasts. Together, these mechanisms provide directions for further investigations into the evolving interactions between BoMet and the BM niches from the perspectives of tumor immunology and immunotherapies.

Regulatory T cells. As previously described, hematopoiesis occurs in the BM, leading to the formation of erythrocytes and leukocytes. Leukocytes can be further divided into two populations: myeloid and lymphoid cells. Among leukocytes, T cells are cells that mature in the thymus. Although maturation occurs in the thymus, mature T cell subsets can be found in the bone. A large portion of bone CD4⁺ T cells are regulatory T cells (Tregs) that contribute to immune suppression (133). FoxP3⁺ Tregs suppress the activity of proinflammatory cells, such as CD8⁺ T cells and Th1 CD4⁺ T cells. This immunosuppression occurs via Treg secretion of antiinflammatory cytokines such as IL-10, IL-35, and TGF-β (134, 135) (Figure 2). These cytokines may play a dichotomous role in promoting dormancy of metastatic seeds and increasing tumor cell proliferation.

The reduction of proinflammatory signaling near the perivascular niche may offer opportunity for dormant cancer cells to evade immune surveillance. As previously stated, bone is a privileged immunological organ that benefits from a reduction in immune activity; therefore, the further reduction of an inherently low baseline of immune activity may create a favorable location for dormant cell immune evasion (136). Additionally, TGF-β production increases the proliferation of metastatic seeds and further perpetuates the vicious cycle of macrometastasis (102, 104). Tregs within the bone have also been shown to affect metastasis through attraction by chemokines. Previous studies showed the importance of CXCL12/CXCR4 signaling both in trafficking of Tregs to the bone and in the attracting of metastatic seeds in prostate cancer and BCa (93, 137). Patients with prostate cancer displayed an increase in the number of Tregs within the bone through an increase in the expansion of Tregs by RANK signaling on DCs (138).

Metabolic effects may also enhance Treg function in the bone microenvironment. Micrometastatic lesions interact with osteoblasts that are found in the endosteum where Tregs localize and may lead to an increase in proliferation to catalyze the formation of the vicious cycle (96). These osteoblasts, as will be discussed in a subsequent section, prefer aerobic glycolysis that results in the accumulation of lactate, thereby creating an environment suitable for Treg function while inhibiting cytolytic T cell function. The presence of Tregs in the HSC niche and adjacent regions of the bone may provide metastatic seeds with an evasive environment for dormancy and proliferation.

Immature myeloid cells. Along with the production of lymphocytes, HSCs give rise to myeloid cell populations including neutrophils, monocytes, and macrophages. However, during development, a group of myeloid cells remains immature and can exert immunosuppressive function. Therefore, this subset of immature myeloid cells is often referred to as myeloid-derived suppressor cells (MDSCs). Immature myeloid cells (IMCs) are among the most abundant immune cell types found within the bone, because they are directly produced within the HSC niche. IMCs are a het-

erogeneous population of cells containing polymorphonuclear (PMN) and monocytic (M) MDSCs based on their morphology. Functionally, PMN-MDSCs contain higher levels of reactive oxygen species (ROS), while M-MDSCs contain higher levels of nitric oxide (NO) and inducible NO synthase (iNOS/NOS2) (139) (Figure 2). Previous studies have shown that there is an increase in the production of IMCs, including MDSCs, in cancer patients (140). This increase in abundance of immunosuppressive IMCs could further promote metastatic seeding, similar to the effects of Tregs. MDSCs have also been shown to produce TSP1, a factor that promotes dormancy, which may aid the early DTCs in remaining undetected in an immunosuppressive environment (75). The overlap of the metastatic niche with the location of IMC production may allow micrometastatic lesions to skew myeloid cell development down an immunosuppressive path. As explained by the model of immunoediting, cancer cells must evade the initial immune surveillance to progress to a state of immune evasion by recruiting immunosuppressive cells and promoting their development (141). Therefore, outgrowth of these micrometastatic lesions may lead to secretion of many antiinflammatory cytokines along with factors to skew development of HSCs toward myelopoiesis.

Osteoblast metabolism. The endosteal region of the bone undergoes constant remodeling as bone resorption and bone formation occur on the endosteal surface. Osteoblasts are responsible for bone formation and develop from MSCs/osteoprogenitors that differentiate into preosteoblasts (31), all of which play a major role in early-stage bone colonization as previously described in *The osteogenic/endosteal niche* section above.

The influence of osteoblasts during early-stage metastasis may also involve suppression of the immune system due to aerobic glycolysis, the preferred form of osteoblast metabolism. Under conditions of aerobic glycolysis, osteoblasts convert glucose into lactate in the presence of oxygen (142). This lactate-rich environment has been shown to be beneficial to metastatic seeds, and the inhibition of lactate dehydrogenase (LDH) reduces metastasis (143). Cancer cells have the ability to uptake and metabolize lactate, allowing for proliferative capacity in lactate-rich environments (144, 145). Lactate accumulation has been shown to have an inhibitory effect on cytotoxic $CD8^+$ T cells. However, the transcription factor FoxP3 induces oxidative phosphorylation, giving $FoxP3^+$ Tregs an advantage over cytotoxic $CD8^+$ T cells in high-lactate environments (146) (Figure 2). Therefore, the interaction between osteoblasts and metastatic seeds may not be limited to increased proliferation, but could also aid in the evasion of immune surveillance by providing an environment unsuitable for cytotoxic interactions while preserving immunosuppressive function. Osteoblasts express CXCL12, which allows for the homing of HSCs, Tregs, and metastatic seeds to the endosteum (Figure 2). Recruiting these cell types into an environment rich in lactate may further enhance metastatic targeting to the immune-privileged HSC niche.

In summary, multiple cell types in the bone microenvironment may cooperate to establish an immunosuppressive environment. This environment is evolutionarily conserved due to the need for hematopoiesis, but may coincidentally facilitate the survival of DTCs and micrometastases. Future studies are needed to dissect the molecular mechanisms underlying DTC–immune system crosstalk and to identify actionable targets for therapeutic interventions.

Bone remodeling and repair impact metastatic colonization

In addition to their unique immune properties, bone and BM are also remarkable in their quick turnover. Bone is remodeled constantly, with old bones removed by osteoclasts and new bones deposited by osteoblasts, resulting in roughly 5%–25% of a total skeleton being replenished every year (147). Hematopoiesis is also a perpetual process generating new blood and immune cells, and 1% of hematopoietic cells are replaced every day. These activities accelerate during tissue repair and may also perturb the seeding and colonization of metastatic seeds. In this section, we summarize knowledge related to bone remodeling and repair and illustrate the potential link to metastasis.

Bone remodeling and repair. Adult bone is continually remodeling to respond to the changing mechanical needs of the body due to aging and environmental perturbations, and to address stress-induced microscopic damages (26, 148, 149). This complex process is regulated by local paracrine and systemic factors, such as calcium and the hormonal regulators PTH, calcitonin, and estrogen (148, 150). Local factors influence bone remodeling by acting on osteoblasts and osteoclasts, including BMPs, TGF-β, and WNTs (151, 152). When bones fracture, the first event that occurs is the formation of a hematoma by clotting blood (153, 154). This is followed by chondrocytes, the cartilage-producing cells of the bone, infiltrating the hematoma and laying down a callus between the broken bone surfaces (154, 155). Osteoblasts and chondrocytes lay down cartilage and hydroxyapatite, respectively, that slowly harden this callus (154, 156). Over the following weeks, both osteoclasts and osteoblasts remove dead bone and debris from the area, and lay down even more new mineral. These osteoblasts are derived from marrow MSCs and migrate into the callus in order to accomplish this repair (157). MSCs respond to chemotactic signals during the callus formation, causing them to migrate toward the sites of repair and undergo osteogenic differentiation (158). Once the callus is totally ossified, it is replaced with trabecular bone, and repair is complete, with new cortical bone forming on the exterior surface (152).

Exploitation of bone homeostasis by cancer cells. DTCs and fully pathogenic metastases may exploit bone homeostasis, remodeling, and repair in a multitude of ways. As noted earlier, DTCs residing in the perivascular niche after extravasation are nearby or in contact with perivascular MSCs and are held in quiescence by these pericytes/$NG2^+$ MSCs as well as by endothelial cells (159–162). Therefore, relocation from the perivascular niche may be advantageous for the DTCs to progress to colonization. Fractures and subsequent remodeling stimulate the chemotaxis of MSCs toward the injured or remodeling site, where local factors subsequently promote MSC osteogenesis (158, 163). This process may coincide with migration of DTCs from the perivascular niche, where they are kept dormant, to the osteogenic niche, which supports their proliferation (Figure 3). New evidence suggests that BCa cells can attach to and migrate with osteogenic cells via specialized protrusions, and this collective migration phenomenon may facilitate such a transition (164).

Thus, osteogenic activity in bone remodeling or in the soft callus, and later hard callus, stage of repairs is an opportunity for BCa DTCs to encounter and be supported by the osteogenic niche. The same process may also provide access to active osteoclasts

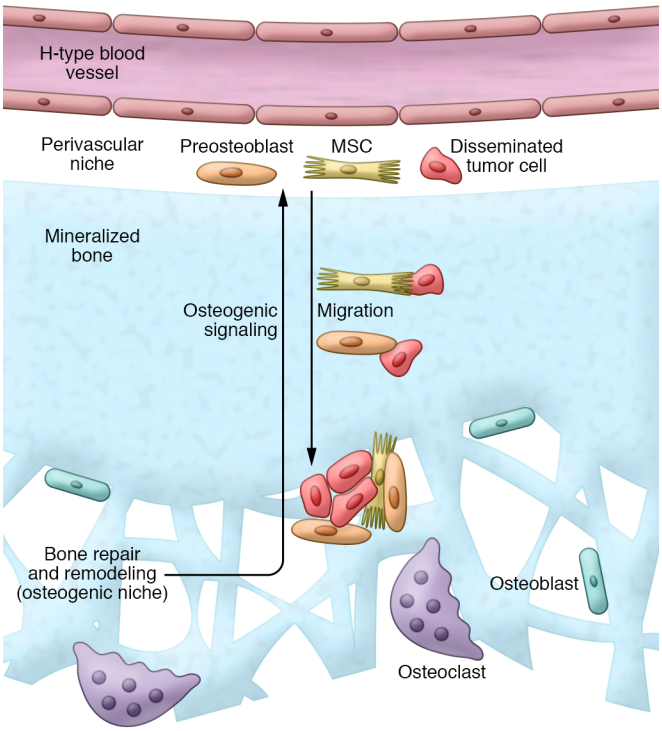

Figure 3. A potential connection between bone repair/remodeling and bone colonization of DTCs. One process that bridges different bone marrow niches is bone repair/remodeling. In this process, MSCs migrate from their perivascular origin to areas of bone repair or turnover. Recent evidence suggests that adhesion and collective migration with osteogenic cells enable DTCs to "ride" MSCs and traffic from the perivascular niche to the osteogenic niche in response to osteogenic signals. This may couple bone homeostasis and metastatic colonization.

and therefore facilitate the transition toward late-stage osteolytic vicious cycle as osteoclasts are recruited to clear the soft callus and resorb the unneeded bone in normal remodeling.

Clinical interventions

A majority of BoMet treatments are palliative, with limited ability to eradicate the bone lesions entirely (165). However, much progress has been made in recent years. BoMet treatments, like many cancer treatments, can be chemical or radiological in nature. Below, we discuss some of the current BoMet treatments.

Antiresorption therapies. Bisphosphonates are a class of drugs originally developed for the treatment of osteoporosis (166). These drugs are chemically similar to inorganic phosphate and thus have high affinity for the calcium ions in hydroxyapatite (167). As calcium is released during bone resorption, bisphosphonates are especially targeted to areas of high osteoclast activity/bone resorption (168). These drugs act by disrupting the key osteoclast enzymes, farnesyl diphosphate synthases (169), thereby impairing their recruitment and differentiation and promoting osteoclast apoptosis (170). Zoledronic acid is the most potent bisphosphonate and was more recently developed to be more potent than earlier-generation bisphosphonates by modifying side chain functional groups (171, 172). Zoledronic acid improves outcomes compared with palliative maintenance of BoMet, with studies showing significant decreases in skeletal-related events and increased overall survival in BCa patients (173). Interestingly, in postmenopausal women, bisphosphonates exhibit significant efficacy in the adjuvant setting in terms of bone recurrence as well as overall survival (174). This observation cannot be explained by the inhibition of osteoclasts, as the vicious cycle has not started during adjuvant therapies. However, it is consistent with the fact that bisphosphonates reduce bone turnover (175), a potential driving force of DTCs to relocate between BM niches as previously discussed.

More recently, denosumab, a RANKL inhibitor/neutralizing antibody, has also been demonstrated to prevent osteoclast differentiation (176). This activity decreases osteolytic metastases in BCa patients (177). A reduction in skeletal-related events and clinical fracture risk was also seen in prostate cancer patients (178) as well as some other solid cancer types (179).

Radiation. External beam radiation treatments can be problematic for treatment of bone metastases because it is difficult to penetrate the mineralized bone tissue. However, radiation treatment is used in palliative care/bone-pain relief and the prevention of skeletal complications (180, 181). Ionizing beam radiation is able to both kill tumor cells and decrease osteoclast activation, both of which can alleviate metastasis-associated pain (182, 183). Other options include radiopharmaceuticals that produce β or α particles like samarium and radium-223 (Rad-223), respectively (184–187). Samarium and strontium have been used for prostate cancer lesions, again for pain management and palliative care, but survival benefits are minimal (188, 189). However, Rad-223 therapy has been shown to prolong survival and increase quality of life in patients with advanced cancer (184, 190). As radium localizes to the mineralized bone tissue, recent work shows it to be much more effective when the initial cell load is smaller, and a higher proportion of tumor cells are physically closer to the bone surface. These data suggest that Rad-223 treatment may be more effective when given to patients at an earlier stage (191, 192).

Immunotherapy. The use of immunotherapies aims to provide patients with more targeted approaches to treat cancer. Cancer cells or immune cells can express checkpoint inhibitor molecules that inhibit $CD8^+$ cytotoxic T cells. A study looking at the effects of immune checkpoint blockade in combination with chemotherapy in triple-negative BCa demonstrated the ability of immune checkpoint blockade to prolong progression-free survival (193). However, in patients with BoMet, this prolonged survival was abrogated. Although this may be due to these patients suffering from additional metastatic burden in other organs, it may also indicate that the BM microenvironment influences patient responses to immunotherapy. TGF-β has been implicated in the effectiveness of immune checkpoint blockade therapy in metastatic urothelial cancer, where a high TGF-β signature resulted in a lower response to immune checkpoint blockade (194). This may have implications in the BM, where TGF-β is produced at various stages of metastasis.

Conclusions

When DTCs arrive in the bone and BM, they encounter a new environment composed of residential cells that are actively maintaining homeostasis of the skeletal and hematopoietic systems. These cells are highly organized and constitute distinctive niches that together fulfill diverse functions. Interactions with different

niches may dictate the cellular fates and therapeutic responses of DTCs and microscopic metastases. Cancer cells may exploit the niches' normal roles, including protection from aberrant immune activation and remodeling/repair of bones, to facilitate metastatic progression. Therefore, identification of the cancer-niche crosstalk pathways, especially those involved in immunosurveillance and tissue repair, may lead to novel mechanistic insights and therapeutic targets.

There are challenges and opportunities in our further investigations of BM niches in metastatic colonization. Bone-resident cells apparently of the same type may actually be heterogeneous with regard to the location, lineage, and functions. This is exemplified by pericytes/MSCs (195, 196) and endothelial cells (197), which may reconcile the seemingly contradictory findings (e.g., refs. 81, 87). Future studies are needed for more precise definition of various niches. To this end, single-cell transcriptomic or proteomic analyses that preserve the spatial information may be highly valuable. It should be noted that fast progress has been made in dissecting and targeting BM niches in blood cancers (198). Systematic comparisons of BM niches between metastatic solid cancers and hematological malignancies will likely provide interesting mechanistic insights. In particular, therapies targeting the niches of the latter may inform future clinical studies of the former.

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