Roles of Bone Marrow Cells in Skeletal Metastases: No Longer Bystanders

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Abstract Bone serves one of the most congenial metastatic microenvironments for multiple types of solid tumors, but its role in this process remains under-explored. Among many cell populations constituting the bone and bone marrow microenvironment, osteoblasts (originated from mesenchymal stem cells) and osteoclasts (originated from hematopoietic stem cells) have been the main research focus for pro-tumorigenic roles. Recently, increasing evidence further elucidates that hematopoietic lineage cells as well as stromal cells in the bone marrow mediate distinct but critical functions in tumor growth, metastasis, angiogenesis and apoptosis in the bone microenvironment. This review article summarizes the key evidence describing differential roles of bone marrow cells, including hematopoietic stem cells (HSCs), megakaryocytes, macrophages and myeloid-derived suppressor cells in the development of metastatic bone lesions. HSCs promote tumor growth by switching on angiogenesis, but at the same time compete with metastatic tumor cells for occupancy of osteoblastic niche. Megakaryocytes negatively regulate the extravasating tumor cells by inducing apoptosis and suppressing proliferation. Macrophages and myeloid cells have pro-tumorigenic roles in general, suggesting a similar effect in the bone marrow. Hematopoietic and stromal cell populations in the bone marrow, previously considered as simple by-standers in

the context of tumor metastasis, have distinct and active roles in promoting or suppressing tumor growth and metastasis in bone. Further investigation on the extended roles of bone marrow cells will help formulate better approaches to treatment through improved understanding of the metastatic bone microenvironment.

Keywords Bone marrow · Metastasis · Hematopoietic stem cells · Megakaryocytes · Macrophages · Myeloid-derived suppressor cells

Introduction

The majority of cancer patients ultimately develop metastatic lesions, contributing to excessive morbidity and mortality, even though metastasis is a very selective and extremely inefficient process, with less than 0.1% of the intravasated tumor cells surviving cascades of events to form metastatic lesions in distant sites [1, 2]. More importantly, tumor metastasis is determined not by locoregional anatomy of draining vasculature (i.e. hemodynamic factors), but by highly specific interactions between disseminating tumor cells (“seed”) and the microenvironment of the target organ (“soil”) [3]. This seminal concept of “seed and soil” was originally proposed by Stephen Paget in the 19th century [4], but soon challenged by Ewing and many others proposing that mechanical forces and hemodynamic factors determine the metastatic patterns [1, 5, 6]. Later, the “seed and soil” hypothesis was revisited by central evidence that the primary tumor is comprised of biologically heterogeneous cell populations (i.e. subpopulations of different metastatic potentials), and also that metastases selectively develop in congenial microenvironments regardless of hemodynamic trafficking [7, 8]. In addition, Tarin et al. provided clinical

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evidence that the specific organ microenvironment is a critical determinant in metastasis, independent of vascular anatomy, rate of blood flow and the number of tumor cells delivered to the organ [9, 10]. Indeed, the current cancer statistics clearly show that the primary tumors of individual organs have strong preference for their metastatic sites [11, 12]. For example, colon and pancreatic tumors preferentially metastasize to liver; and renal cell carcinoma and bladder cancer frequently spread to lungs. Therefore, tumor metastasis occurs in a predictable manner, tightly regulated by the microenvironment of the recipient organ.

Interestingly, bone is the predominant metastatic soil for a number of human cancers, including prostate, breast and lung cancers as well as multiple myeloma [11, 13, 14]. Skeletal metastasis is the major cause of mortality and morbidity of afflicted patients. For example, approximately 90% of advanced stage prostate cancer patients develop bone lesions, resulting in morbidities such as severe bone pain, immobility, hematopoietic complications and spinal cord compression [12, 15]. Current treatment modalities for bone metastatic lesions are not curative, and the average time from the surgery (for bone lesions, such as pathologic fracture) to death is only 1.5 ± 1.9 years for prostate cancer patients. To overcome this urgent clinical problem, better understanding of the metastatic bone microenvironment is critically important. Bone is an intriguing microenvironment for tumor biology, and still remains largely unexplored [16]. This uniquely complex milieu is due not only to the calcified matrix but also to multiple types of constituting cells, including bone cells (osteocytes, osteoclasts and osteoblasts), hematopoietic cells, immune cells, stromal cells and endothelial cells [17]. Considerable research efforts have been devoted to characterizing this complex microenvironment and also to elucidating differential roles of individual cell types in their contribution to tumor growth and metastasis in bone [13]. Notably, Mundy and colleagues proposed a 'vicious cycle' theory which involves bi-directional interactions between disseminated tumor cells and osteoclasts (as well as osteoblasts) leading to osteolysis and, in turn, tumor growth [13, 18, 19]. For example, parathyroid hormone-related peptide (PTHrP) derived from breast cancer cells promotes osteolytic bone lesions (mediated by activation of osteoclasts), leading to release of transforming growth factor-beta (TGF- β) from the bone matrix to further aggravate tumor growth in the bone [18, 19]. Later, prostate cancer cells were shown to express PTHrP in order to upregulate expression of tumorigenic factors (such as C-C chemokine ligand 2 [CCL2]) in osteoblasts, resulting in destructive cascades in the bone as well as osteoblastic lesions [20–22]. However, the majority of experimental results are from murine models, and the vicious cycle in human breast/prostate cancer skeletal metastases is lacking yet difficult to discern.

Collectively, the current data demonstrate a positive feedback loop of tumor cell interaction with the hard tissue compartment of the bone microenvironment (i.e. osteoclasts, osteoblasts and calcified bone matrix). Additionally, however, current evidence suggests that different hematopoietic lineage cell populations in the bone marrow, previously considered as simple bystanders in the metastatic process, provide distinctive contributions for promoting or suppressing tumor growth and/or metastasis [23, 24]. This review paper will examine the current literature regarding cells in the bone microenvironment, with particular focus on hematopoietic lineage cells in the bone marrow, and their roles in skeletal metastasis.

Cellular Components of the Congenial Soil: Anatomy and Histology of Bone Marrow

The bone marrow is one of the largest organs in the human body, and comprises approximately 5% of body weight in humans and 3% in adult rats [25, 26]. Bone marrow is the primary hematopoietic organ and a primary lymphoid tissue, responsible for the production of the cellular components of blood [27]. It consists of hematopoietic tissue, endosteum, connective tissue and endothelium. The endosteal lining in the marrow cavity contains a single layer of cells, including osteoblasts and osteoclasts, supported by a thin layer of reticular connective tissue. Other connective tissues in the bone marrow include bony trabeculae, adipocytes, fibroblasts and nerves. Of particular note, the bone marrow is extremely well vascularized tissue, served by multiple arteries entering the marrow via nutrient canals of diverse size. Arteries branch and taper down to thin-walled arterioles and capillaries anastomosing with a plexus of venous sinuses. Venous sinuses then merge to form collecting veins and further the central venous sinus draining back via nutrient canals into the systemic circulation. Sinusoidal vessels are thin-walled, consisting of a layer of flat endothelial cells with little to no basement membrane. Bone marrow sinusoids function as an entering point for hematopoietic cells into the systemic circulation. Similarly, metastatic cancer cells are considered to extravasate via sinusoidal barrier. The bone marrow does not have a lymphatic drainage system [27–29].

The hematopoietic compartment of the bone marrow is comprised of stem cells, hematopoietic lineage cells, adventitial reticular cells, adipocytes and macrophages. Hematopoietic cells are not randomly dispersed, but are structured within the microenvironment [30]. More importantly, hematopoiesis occurs as a compartmentalized process, with erythropoiesis occurring in erythroblastic islands; granulopoiesis in less defined areas and megakaryopoiesis adjacent to the sinus endothelium. On demand, the hematopoietic cells transverse the sinusoidal barrier to enter the

systemic circulation, whereas platelets are released directly from the cytoplasm of megakaryocytes into the bloodstream.

During embryonic development, hematopoiesis occurs in the liver, and shortly after birth hematopoietic stem cells (HSCs) migrate and repopulate the bone marrow. This unique feature of bone marrow biology, bone marrow homing, has been extensively exploited clinically to improve the engrafting efficiency of bone marrow transplantation, carried out by simple intravenous injection of marrow cells [31]. Molecular mechanisms of bone marrow homing have been demonstrated primarily by exploring factors inhibiting homing in various mouse models. For example, mice deficient in E- and P-selectins were found to have impaired homing, suggesting that tethering and rolling of bone marrow cells on the sinusoidal endothelium is critical for correct engraftment [32, 33]. More importantly, Peled et al. provide pivotal evidence that stromal-derived factor-1 (SDF-1, also known as CXCL12) expressed by the bone marrow stroma and endothelium interacts with its cognate ligand, CXCR-4 expressed on HSCs, is critical to human HSC engraftment and repopulation in a immune-deficient mouse model [34]. In sum, the bone marrow is structured hierarchically, containing various populations of hematopoietic cells supported by stromal cells, all of which potentially have unique function in skeletal tumor growth and/or metastasis.

Hematopoietic Stem Cells Compete with Metastatic Tumor Cells

Tumor cells frequently usurp physiological mechanisms to promote growth, angiogenesis, invasion and metastasis. For example, most of the so-called tumorigenic molecules (such as vascular endothelial growth factor [VEGF], matrix metalloproteinases [MMPs] and epidermal growth factor [EGF] among myriad others) play critical roles in normal physiology and development. As stated above, liver is the primary hematopoietic organ until birth, and subsequently HSCs migrate into the bone marrow where the microenvironment supports engraftment, repopulation and self-renewal. This phenomenon of physiological HSC homing in the bone marrow led scientists to an interesting hypothesis that bone metastatic cancer cells may mimic the established pathway of HSC homing. Müller et al. for the first time provided pivotal evidence that chemokine receptors (CXCR4 and CCR7, highly expressed by breast cancer cells) and their cognate ligands (expressed in metastatic recipient tissues) play critical roles in organ-specific breast cancer metastasis [35], in the same way that chemokine-chemokine receptor axes mediate HSC homing in the bone marrow during normal development and bone marrow transplantation (BMT). Subsequently, Taichman et

al. demonstrated that CXCL12/SDF-1 (expressed by osteoblasts and endothelial cells) and its receptor (CXCR4, expressed by prostate cancer cells) regulate bone-tropism of prostate cancer cells [36]. In addition to the CXCL12/CXCR4/CXCR7 axis [37], Annexin II, expressed by osteoblasts and endothelium regulates HSC adhesion, homing and engraftment [38]. Interestingly, human prostate cancer cells isolated from the metastatic lesions (PC-3, DU145 and LNCaP) were shown to express receptors for Annexin II, contributing to prostate cancer growth and homing in the bone marrow [39]. Given that data collectively demonstrated that bone metastatic tumor cells (breast and prostate) utilize the chemokine axes of HSC homing, it is reasonable to expect that HSCs may compete with metastatic cancer cells for occupancy in the bone marrow.

Recently, crucial evidence demonstrating that hematopoietic stem cells (HSC) negatively regulate bone metastasis by competing with metastatic cancer cells to preoccupy the HSC endosteal niche came from the works of Shiozawa et al. [40]. The authors demonstrated that increasing the HSC niche size (i.e. expansion of osteoblasts by parathyroid hormone [PTH] treatment) promoted skeletal localization of prostate cancer cells in the systemic circulation, while decreasing the niche size (using a conditional osteoblast-ablation mouse model) reduced tumor cell number localized in the bone marrow. In addition, an experimental treatment to mobilize HSCs (AMD3100, similarly to a clinical regimen used in autologous stem cell transplantation) could mobilize the cancer cells in the niche back into the circulation. Therefore, the HSC endosteal niche serves as a direct target for metastatic prostate cancer cells, and HSCs may function as competitors for metastatic cancer cells with strong bone tropism.

Contrary to the data demonstrating HSCs function as a competitor for niche occupancy, other data shows that HSCs may directly promote tumor growth and/or metastasis. Okamoto et al. demonstrated that HSCs regulate the angiogenic switch and promote tumor growth in the bone [41]. Furthermore, expansion of bone marrow cellularity by treatment with parathyroid hormone (PTH) resulted in significantly increased prostate cancer cell localization and subsequent growth in bone [42]. HSCs are pluripotent cells that can differentiate into any hematopoietic lineage cell types of tumorigenic potential. Accordingly, the direct roles of HSCs in tumor growth, particularly in the context of the bone microenvironment, need further investigation.

Megakaryocytes Attack Extravasating Tumor Cells in the Bone Marrow

As previously mentioned, megakaryocytes reside in parasinusoidal space with cytoplasmic invagination across the vascular barrier. As a result, platelets are released directly

into the sinusoidal venous blood [43]. Because the sinusoidal endothelium is the main entry-exit point between the circulation and bone marrow tissue, bone metastatic cancer cells are thought to utilize the same route to extravasate. Therefore, megakaryocytes are potentially the first cells that tumor cells encounter upon arrival in the bone marrow microenvironment. Interestingly, Li et al. provided the first direct data that megakaryocytes suppress tumor cell proliferation and increase apoptosis in an experimental prostate cancer bone metastasis model [44]. In addition, expansion of the megakaryocyte population (by administering recombinant thrombopoietin) resulted in significantly reduced localization of tumor cells and subsequent growth in the bone *in vivo*. Direct contact between prostate cancer cells and megakaryocytic cells *in vitro* resulted in increased apoptosis as well as decreased proliferation of prostate cancer cells. These results demonstrated novel and specific inhibitory effects of megakaryocytes, a specialized hematopoietic lineage cell residing in the bone marrow, on metastatic cancer growth in the bone.

In parallel to tumor inhibitory effects based on direct cell-to-cell contact, secretory factors from megakaryocytes have been recently demonstrated to suppress osteoclast formation and activation [45–47]. In addition, megakaryocytes promote osteoblast synthesis of type I collagen, osteoprotegerin and receptor activator of nuclear factor kappa-B ligand (RANKL), all of which positively affect bone formation [48]. Reciprocally, osteoblasts directly influence hematopoiesis [49, 50] as well as megakaryopoiesis [51]. Consequently, production and activity of megakaryocytes are tightly coupled with bone remodeling, which in turn affects tumor growth in the bone. Given that the vicious cycle theory integrates activities of osteoblasts and osteoclasts as critical components [13], and that resorption is essential for tumor growth in bone [20, 52], megakaryocytes also alter the bone microenvironment (i.e. suppressing osteoclasts and activating osteoblasts) to affect metastatic tumor growth indirectly. Taken together, the current data suggest that megakaryocytes negatively regulate tumor cells in the bone marrow directly by suppressing tumor cell proliferation and inducing apoptosis, and also indirectly by suppressing osteoclasts and osteoblasts. However, detailed molecular mechanisms and clinical data are required to further characterize the role of megakaryocytes in skeletal metastasis.

Contrary to anti-metastatic functions of megakaryocytes, the end-products, platelets, have been shown to have opposite roles. Firstly, platelet-derived growth factor (PDGF) is one of the first angiogenic factors discovered, and critical to vessel maturation [53]. In addition, aggregation of platelets surrounding tumor cells had been shown to protect tumor cell lysis by natural killer cells [54]. Most notably, Boucharaba et al. provided pivotal evidence supporting the role of platelets in breast cancer skeletal metastasis [55, 56]. Activation of platelets by tumor cells result in production of

lysophosphatidic acid (LPA), which in turn promotes breast cancer growth and skeletal metastasis in mice [55]. However, there is currently no clear evidence supporting clinical benefits of anti-platelet agents (such as aspirin and heparin) in cancer patients [56].

Macrophages Promote Tumor Growth and Metastasis in Bone: More than a Scavenger

Increasing evidence now clearly supports that tumor-associated macrophages (TAMs) are important regulators of tumor progression in multiple types of cancers [57–61]. Clinical studies reveal that the density of TAMs in tumor tissue significantly correlates with poor prognosis in prostate, breast, ovarian and cervical cancers, and with controversial outcomes in stomach and lung cancers [62]. In comparison with classically activated macrophages (M1 macrophages) associated with inflammatory phagocytosis, TAMs are an alternatively activated and polarized population of macrophages (M2 macrophages) with tumorigenic potential [63]. The role of immune cells, particularly macrophages, in tumor progression is not a new idea. The first suggestion of their involvement dates back to 1863 [64]. Recent studies now provide the clinical correlations as well as potential molecular mechanisms of recruitment, activation and function of TAMs (M2 macrophages). In particular, most prominent molecules produced by tumors to affect TAMs include C-C chemokine ligand 2 (CCL2, also known as monocyte chemoattractant protein-1 [MCP-1]), macrophage colony stimulating factor (M-CSF, also known as CSF-1) and VEGF. For example, bone metastatic prostate cancer cells express CCL2 to recruit monocytes to tumor sites, which then differentiate into TAMs (M2 macrophages) and osteoclasts [58, 65–67]. In addition, CCL2 has been seen to increase prostate cancer growth and bone metastasis in an experimental metastasis model, which was accompanied by the recruitment of macrophages and osteoclasts [57, 68]. Lin et al. also demonstrated that macrophages switch on tumor angiogenesis, using the polyoma middle-T antigen mouse mammary tumor (PyMT) spontaneous breast cancer model [69]. Macrophages are highly specialized phagocytic cells, derived from monocytes. In tumor tissue, a wide variety of factors are secreted by tumor cells, including those that function as recruiting factors for monocyte-macrophages. The most prominent and widely investigated functions of TAMs (M2 macrophages) in tumor tissue are increased angiogenesis and tumor growth caused by growth factors and proteinases. Data of Harris et al. showed by immunohistochemical quantification that TAMs cluster in areas of increased angiogenesis in human breast cancer samples [70]. In addition, TAMs (M2 macrophages) produce many pro-angiogenic cytokines such as urokinase-type plasminogen activator (uPA)[71], tumor

necrosis factor- α (TNF- α) [72], IL-1, VEGF [73] and nitric oxide (NO) [74]. Moreover, TAMs express a wide variety of growth factors and proteinases such as MMP-7 and 9; fibroblast growth factor (FGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), all of which have independent pro-tumorigenic functions [75–77].

Recently, interesting data from Pettit and colleagues demonstrated that a discrete population of macrophages, osteal tissue macrophages (termed ‘OsteoMacs’). Later, the authors also showed that OsteoMacs are required for physiological bone remodeling as well as intramembranous bone healing, suggesting that osteal macrophages are critical components in bone physiology [78, 79]. However, there is currently no definitive data showing the tumorigenic function of resident macrophages in tumor growth and/or metastasis in bone. To sum up, clinical and experimental data supports the tumorigenic roles of macrophages in primary tumor tissue, but further investigation is required for the potential roles in the bone microenvironment.

Myeloid-Derived Suppressor Cells and Monocytes: An Elusive Population with Confronting Functions

As frequently portrayed as ‘wounds that never heal’, cancer is comprised of multiple types of immune/inflammatory cells [80]. Clinical data have now accumulated indicating that human tumor samples positively correlate with infiltration of bone marrow-derived immune cells (BMDCs) such as macrophages and neutrophils. In particular, recent evidence collectively shows that bone marrow-derived macrophages and monocytes (collectively termed ‘myeloid lineage cells’) play crucial roles in tumor angiogenesis [76, 81, 82]. However, those pro-angiogenic myeloid cells are yet poorly defined, and show overlapping phenotypes [83]. The most widely accepted population of pro-tumorigenic BMDCs are myeloid-derived suppressor cells (MDSCs), expressing both CD11b (a myeloid cell marker) and Gr1 (a granulocyte marker). MDSCs were originally investigated for their roles in suppressing CD8⁺ T cell immunity, contributing to tumor escape from the host immune-surveillance [84–86]. Yang et al. demonstrated that CD11b⁺Gr1⁺ MDSCs promote vascular density and vascular maturation while decreasing necrosis [87, 88]. In addition, the authors showed that MDSCs express high levels of matrix metalloproteinase (MMP)-9, and also MDSCs acquire endothelial properties to incorporate into endothelium. Similarly, Kim et al. demonstrated that circulating monocytes in tumor-bearing hosts express an endothelial cell marker (CD31) and directly contribute to tumor angiogenesis [89]. However, the idea that MDSCs can differentiate into endothelial cells remains controversial and to be further investigated [90]. Interestingly,

while tumors cannot grow in MMP-9 knockout mice, wild-type bone marrow transplantation can restore tumor growth in the same host, suggesting that BMDCs are the primary source of MMP-9 in tumor angiogenesis. CD11b⁺ myeloid cells, but not endothelial progenitor cells, are the main source of MMP-9 in the tumor tissue [91], which can increase the bioavailability of VEGF and other endothelial growth factors. In addition, neutrophils have been shown to secrete VEGF [92]. Recent data from Yang et al. suggest that MDSCs enhance tumor cell invasion and contribute to TGF- β -mediated breast cancer metastasis [93]. Furthermore, recruitment of CD11b⁺Gr1⁺ cells is mediated by the two chemokine axes, SDF-1/CXCR4 and CXCL5/CXCR2 [93].

Given the roles of MDSCs in tumor angiogenesis and invasion, it is likely that MDSCs promote tumor growth and/or metastasis in any organ site including bone. In addition, the surface markers of MDSCs overlap with those of osteoclast lineage cells, suggesting that MDSCs have potential to differentiate into osteoclasts. However, the role of MDSCs specifically in bone metastasis is not yet clearly understood. Some supporting evidence came from the work of Mundy and colleagues who discovered that MDSCs were increased in the bone marrow and spleen in a syngeneic myeloma mouse model, and the MDSCs from the myeloma-bearing mice had a greater capacity to form osteoclasts, compared to the MDSCs from control mice [88, 94]. Furthermore, these authors presented their preliminary data that MDSCs can be precursors of osteoclasts in myeloma bone lesions [95], and also that an osteoclast inhibitor, zoledronic acid, suppressed the differentiation of MDSCs into osteoclasts [96].

Discussion and Conclusions

Bone marrow is comprised of diverse populations of hematopoietic lineage cells as well as stromal cells. Increasing lines of evidence support pro-tumorigenic roles of individual bone marrow-derived cell populations in such processes as angiogenesis, tumor cell apoptosis, escape from immune-surveillance, etc. However, each cell population mediates distinct and sometimes contradictory (pro- or anti-tumorigenic) roles, and continued research endeavors are required to delineate the complexity. This review article summarized key evidence describing the differential roles of hematopoietic lineage cells, including HSCs, megakaryocytes, macrophages and MDSCs in bone metastasis (see Fig. 1 for a schematic summary of data). Briefly, expansion of bone marrow cellularity has been found to promote prostate cancer skeletal metastasis, suggesting in general that cells in the bone marrow have tumorigenic functions [42]. Indeed, HSCs switch on angiogenesis, promoting tumor growth and potentially metastasis [41]. However, recent data demonstrated

Fig. 1 Interactions between metastatic tumor cells and bone marrow cells are illustrated. Hematopoietic stem cells increase tumor growth by promoting angiogenesis and osteoblastogenesis. Concurrently, hematopoietic stem cells compete with metastatic tumor cells for occupancy of the osteoblastic niche, resulting in negative regulation of skeletal metastasis. Osteoblasts and osteoclasts both contribute to the positive feedback leading to tumor growth in bone (a ‘vicious cycle’). Immune cells (phagocytic cells and dendritic cells) attack tumor cells.

However, a subset of immune precursor cells (e.g. myeloid-derived suppressor cells) is implicated in promoting tumor growth and metastasis. Megakaryocytes induce apoptosis of extravasating tumor cells in bone, and also suppress tumor cell proliferation. (Legend: *blue arrows with circled plus mark* indicate positive regulation; *red arrows with circled minus mark* indicate negative regulation; and *black arrows* indicate differentiation. Cellular components of this cartoon are not proportionate to the actual size)

that tumor cells compete with HSCs for niche occupancy, thus the presence of HSCs can negatively regulate tumor metastasis to bone [40]. More interestingly, HSCs have been shown to increase bone morphogenetic proteins (BMP)-2 and 6 in response to erythropoietin stimuli, potentially contributing to augmented osteoblastogenesis [97]. These data collectively support that even a single cell population entity (i.e. HSCs) can have a dual function in the context of tumor metastasis to bone. For example, data demonstrate that mesenchymal stem cells (MSCs), which give rise to multiple types of stromal cells including adipocytes, muscles, fibroblasts, chondrocytes, etc., contribute to the creation of a favorable tumor microenvironment in general as well as in bone [23]. Contrarily, Naveiras et al. demonstrated that bone marrow adipocytes, which frequently infiltrate red marrow spaces after chemotherapy or radiation, negatively regulate HSCs [98].

Other components of the bone marrow such as megakaryocytes and macrophages also have unique roles in

tumor progression. Megakaryocytes are potentially the first cells that extravasating tumor cells encounter in the bone marrow, and megakaryocytes induce tumor cell apoptosis and decreased proliferation [44]. Despite the lack of definitive experimental results in bone metastasis, macrophages, particularly TAMs, are highly likely to play critical roles in tumor growth and angiogenesis in bone. Similarly, MDSCs are essential components for a favorable tumor microenvironment. Collectively, as the bone marrow is the primary supplying organ of macrophages, monocytes and other immune cells, precursors and the differentiated macrophages and MDSCs surely play essential roles in bone metastasis.

Even with the data described in this article, elucidating the roles of bone marrow cells in the metastatic bone microenvironment remain a rich area of research opportunity. For example, one emerging question is how solid tumors in a primary organ site or in circulation regulate bone marrow cells before the occurrence of bone metastasis. The tumor microenvironment is comprised of primary tumor cells mixed

with multiple types of stromal cells, of which a significant fraction originates from the bone marrow. Increasing evidence supports the critical roles of those bone marrow-derived cells (BMDCs) in tumor progression. As BMDCs are such critical components, it is likely that primary tumors somehow communicate with the cells in the bone marrow to supply the indispensable components to enhance metastatic capacity. In addition, the data demonstrating that tumor cells prime the metastatic soil (termed ‘pre-metastatic niche’) before arrival of tumor cells in the metastatic recipient organ by VEGF-receptor 1-positive bone marrow cells [99, 100], suggest similar mechanisms may occur in the bone marrow before arrival of breast or prostate cancer cells in the bone marrow. Particularly, the unique bone-tropism of metastatic prostate or breast cancer cells may be due to breast or prostate tumor-derived factors modulating bone and bone marrow cells. One potential candidate molecule mediating crosstalk between tumor cells and bone marrow cells is parathyroid hormone-related peptide (PTHrP). PTHrP was first discovered as an etiologic factor for malignancy-induced hypercalcemia, and was later implicated in pro-tumorigenic roles such as cellular proliferation, angiogenesis as well as stimulating osteoblasts and osteoclasts. Similar to parathyroid hormone (PTH), a physiological counterpart, PTHrP promotes bone turnover and anabolic response, which can promote tumor growth in bone. In addition, PTHrP up-regulates cytokine expression from the bone marrow stromal cells (i.e. osteoblasts), including VEGF, IL-6 and C-C chemokine ligand (CCL)-2 (also known as monocyte chemoattractant protein [MCP]-1) all of which have the potential to promote bone marrow cells. Therefore, tumor cells in their primary organ site may secrete PTHrP to prime the cells in the bone marrow indirectly via up-regulating cytokines from osteoblasts, leading to expansion and/or potentiation of fractions of bone marrow cells (e.g. MDSCs). In turn, the primed bone marrow cells either cultivate the metastatic recipient site, and/or travel back to the primary tumor tissue to promote growth, invasion and angiogenesis. However, there is currently no data supporting this potential loop of crosstalk between tumor tissue and the bone marrow. Continued research in this field may yield potential mechanisms that could be targeted for the treatment and prevention of metastasis, thereby providing a means to increase length and quality of life for cancer patients.

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