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Review

Molecular Regulation of Bone Metastasis Pathogenesis

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Key Words
Bone metastasis • Tumor microenvironment • Osteoclast • Osteoblast • Myeloid-derived suppressor cells

Abstract
Distant metastases are the major cause of mortality in cancer patients. Bone metastases may cause bone fractures, local pain, hypercalcemia, bone marrow aplasia, and spinal cord compression. Therefore, the management of bone metastases is important in cancer treatment. Normal bone remodeling is regulated by osteoprotegerin ligand (OPGL), receptor activator of NF-κB ligand (RANKL), parathyroid hormone-related protein (PTHrP), and other cytokines. In the tumor microenvironment, tumor cells induce a vicious cycle that promotes osteoblastic and osteolytic lesions. Studies support the idea that distant metastases may occur due to the immunosuppressive function of myeloid-derived suppressor cells (MDSCs). These cells inhibit T cells and natural killer (NK) cells and differentiate into tumor-associating macrophages (TAMs), monocytes, and dendritic cells (DCs). In this review, we summarize studies focusing on the role of MDSCs in bone metastasis and provide a strong foundation for developing anticancer immune treatments and anticancer therapies, in general.

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Introduction

Distant metastases are a major cause of mortality in cancer patients, and of several complications, including pulmonary edema, hollow organs obstruction, and tumor embolism. They also significantly impact survival rate and treatment planning. The bone is the third most common site for metastasis, after the lung and liver [1]. Advanced bone metastases commonly cause skeletal-related problems in patients, including pathological fractures, local pain, hypercalcemia, bone marrow aplasia, and spinal cord compression [2]. The prevention and treatment of bone metastasis is an important concern in current oncology. According to many epidemiological studies, bone metastases show an organ-specific pattern of spread, especially in breast and prostate cancer [3-5]. This phenomenon led Paget to propose the "seed and soil" hypothesis in 1889 [1, 6]: the "seed" indicates the dissemination of cancer cells from primary sites and the "soil" refers to the metastatic sites. This hypothesis highlights that the specific organ microenvironment plays a critical role in the development of metastases. In this review article, we summarize the current knowledge regarding the molecular regulation of bone metastasis, focusing on their pathogenesis. The importance of the microenvironment in this context is also discussed, with the purpose of helping develop anticancer immune treatments and anticancer therapies.

Physiological bone remodeling

The bone is a dynamic tissue regulated by a variety of systemic hormones [7] and is composed of two major kind of cells: osteoblasts and osteoclasts. Osteoblasts are specialized bone-forming cells that develop from pluripotent mesenchymal stem cells via the wingless (Wnt)/β-catenin pathway, and express parathyroid hormone (PTH) receptors [8]. The Wnt pathway regulates cell fate determination, cell proliferation and migration, and gene expression through a signal cascade activated by the interaction of secreted glycoproteins with their membrane receptors. Osteoblasts contribute to the expression of osteoclastogenic factors and production of bone matrix [9]. Some osteoblasts become osteocytes, trapped in the bone matrix, that function as mechanosensors, activating osteoblasts and osteoclasts for bone remodeling [10]. Osteoclasts derive from mononuclear precursors and are activated by macrophage colony stimulating factor (M-CSF) and receptor activator of NF-κB ligand *(*RANKL) secreted by stromal cells or osteoblasts [11]. They break down and digest bone tissue, allowing repair and remodeling of the bones. When bone microdamage or mechanical stress occur, osteocyte apoptosis induces bone remodeling through the sequential phases of activation, resorption, formation, and termination (Fig. 1).

Specifically, damage to the bone matrix induces osteocyte apoptosis and release of osteotropic growth factors and cytokines that increase osteoclastogenesis [12, 13]. The apoptosis of the osteocytes decreases the secretion of transforming growth factor β (TGF-β), which inhibits osteoclastogenesis. The mononuclear monocyte-macrophage-osteoclast precursor cells are recruited and activated by osteoblast lineage cells via the osteoclastogenesis-related cytokines RANKL and M-CSF. Systemic hormones such as PTH, 1, 25-dihydroxy vitamin D, estrogen, and calcitonin also play an important role in the regulation of bone remodeling [14-16]. PTH regulates serum calcium (Ca^{2+}) concentration upon interaction with its G-protein-coupled receptor on osteoblasts. After activation, the osteoblasts release the chemokine monocyte chemoattractant protein-1 (MCP-1), inducing osteoclast differentiation and bone resorption via the Ca^{2+} intracellular signaling pathway. The expression of osteoprotegerin (OPG) from osteoblasts, a decoy receptor for RANKL that negatively regulates osteoclastogenesis, is reduced in these conditions [17]. At the same time, the production of MCSF and RANKL is increased to promote osteoclast formation. Osteoblasts secrete matrix metalloproteinases (MMPs), such as MMP-13, to degrade the unmineralized osteoid for osteoclast attachment. During the resorption phase, growth hormones, such as TGF-β, platelet-derived growth factor (PDGF), and insulin-like growth factor I and II (IGF-I

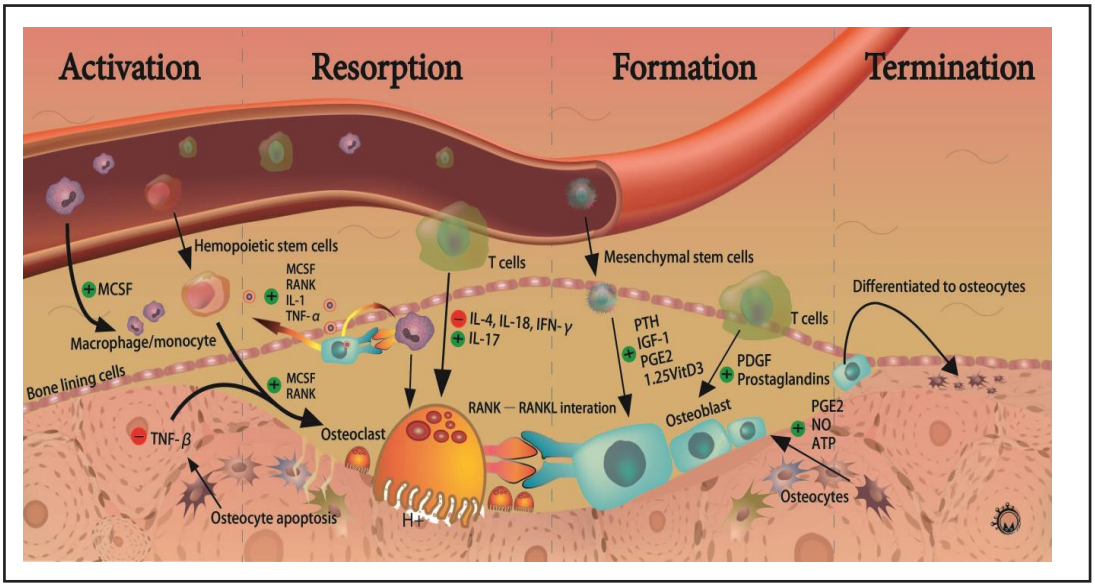

Fig. 1. Mechanism of bone remodeling. Osteoblasts derive from pluripotent mesenchymal stem cells via the wingless (Wnt)/β-catenin pathway, and express parathyroid hormone (PTH) receptors; osteoclasts derive from mononuclear precursors, activated by macrophage colony stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL) to digest bone. In bone damage, osteocytes decrease the secretion of transforming growth factor β (TGF-β) and promote osteoclastogenesis via M-CSF, RANKL, IL-1 and TNF-α. Systemic hormones, such as PTH, 1,25-dihydroxy vitamin D, estrogen, and calcitonin, promote osteoblasts release the chemokine monocyte chemoattractant protein-1 (MCP-1), inducing osteoclast differentiation and bone resorption via the Ca^{2+} intracellular signaling pathway. Osteoblasts secrete matrix metalloproteinases (MMPs), such as MMP-13, to degrade the unmineralized osteoid for osteoclast attachment. During the resorption phase, growth hormones, such as TGF-β, platelet-derived growth factor (PDGF), and insulin-like growth factor I and II (IGF-I and II), regulate the activity of osteoclasts to dissolve the mineral matrix by secreting H^+ ions. After the resorption of damaged bone, osteoclasts undergo apoptosis and activate osteoblasts to synthesize the osteoid matrix to refill the gap with new bone. Osteocytes also regulate the bone formation by producing prostaglandin E_2 (PGE_2), nitric oxide (NO), and adenosine tri-phosphate (ATP) to stimulate osteoblasts activity. During the termination phase, some osteoblasts differentiate to osteocytes, which become lining cells, secreting inhibitory factors that slow the rate of bone formation.

and II), regulate the activity of osteoclasts. After activation, the osteoclasts attach to the bone surface and dissolve the mineral matrix by secreting H^+ ions [18]. When the organic matrix is exposed, proteolytic enzymes are released for resorption and digestion, producing the so-called "Howship's resorption lacunae." After the resorption of damaged bone, reversal cells allow the transition from the "resorption" phase to the "formation" phase [19]. Osteoclasts undergo apoptosis and activate osteoblasts. During the bone formation phase, osteoblasts are recruited and differentiate [20]. Under the regulation of local growth factors and hormones, osteoblasts synthesize the osteoid matrix to refill the gap with new bone. In addition, osteocytes can also regulate the bone formation by producing prostaglandin E_2 (PGE_2), nitric oxide (NO), and adenosine tri-phosphate (ATP) to stimulate osteoblasts activity [21]. During the termination phase, some osteoblasts differentiate to osteocytes, which become lining cells, secreting inhibitory factors that slow the rate of bone formation. Finally, a dynamic balance is reached between osteoblast and osteoclast activity, to maintain slow bone resorption and formation. Under normal biological conditions, the bone microenvironment is a dynamic milieu. However, bone metastases may break the balance of the RANK/OPG system, leading to increased bone resorption and local inflammation.

Pathogenesis of bone metastases

In 1928, James Ewing observed that the liver is a common site of metastasis. He proposed that this happens because the liver is the first organ receiving a large amount of circulating blood and cancer cells from the gastrointestinal tract. This hypothesis was confirmed, and became the so-called "circulation theory" [22]. Current studies indicate that the development of bone metastases is a multi-step process [23, 24]. After the tumor has progressed to locally invade the vessels, few disseminated tumor cells are released and evade the immune system, circulating from the primary tumor site to the bone. The tumor cells start colonizing the bone marrow microenvironment and some adapt to the local environment. The tumor cells that survive may grow immediately or enter a dormancy state upon interaction with the local environment. This phase may last several years. Some dormant cells might, at any point, be reactivated, start dividing, and promote the formation of micrometastases (Fig. 2) [25].

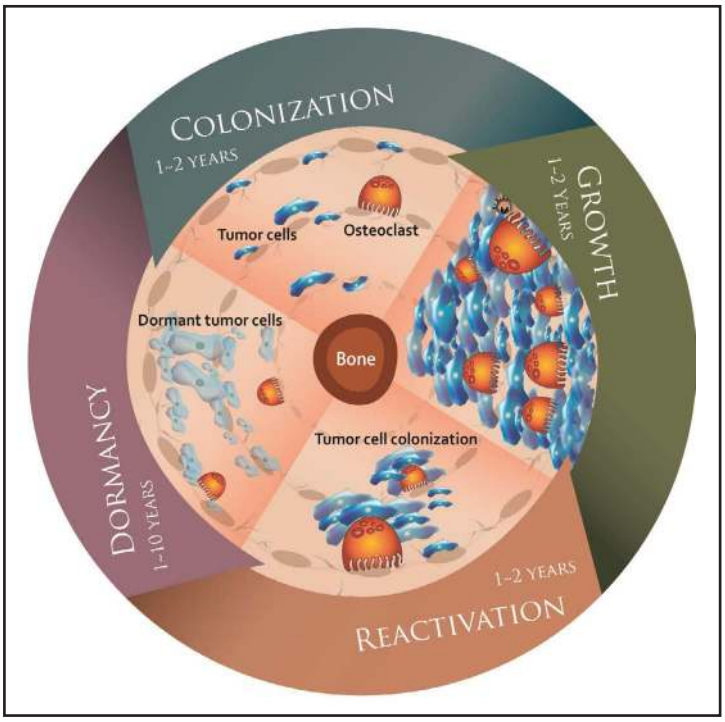

Fig. 2. Process of bone metastasis development. The development of bone metastases is a multi-step process. Initially, few disseminated tumor cells are released and evade the immune system, circulating from the primary tumor site to the bone. The tumor cells start colonizing the bone marrow microenvironment and some adapt to the local environment. The tumor cells that survive may grow immediately or enter a dormancy state upon interaction with the local environment. This phase may last several years. Some dormant cells might, at any point, be reactivated, start dividing, and promote the formation of micrometastases.

The bone is one of the most common sites of cancer metastases. According to published studies, the incidence of bone metastasis in breast, prostate, and thyroid cancer accounts for more than 60% of the metastases [26] (Table 1). Bone metastases are classified as osteolytic or osteoblastic lesions. Breast cancer metastases are predominantly osteolytic lesions, while metastases originating from prostate cancer are predominantly osteoblastic. However, some patients with bone metastases have mixed osteolytic and osteoblastic lesions. The different mechanisms of bone destruction reflect on the radiological appearance of the metastases. The "seed and soil" mechanism discussed above promotes organotropic attraction of cancer cells to different organs. The pathogenesis of both osteolytic and osteoblastic bone metastasis is discussed below.

Mechanisms of osteoblastic metastases

In the bone metastatic microenvironment, the tumor cells express adhesion molecules, such as vascular endothelial molecule-1 (VCAM-1), that bind to the integrins $\alpha4\beta7$ and $\alpha4\beta1$ (also called very late antigen-4 [VLA-4]) on osteoclast precursors, inducing the secretion

of angiogenic and osteoclastogenic factors [27-30]. Cluster of differentiation 44 (CD44), an adhesion molecule expressed by tumor cells, also promotes invasion and adhesion and directly induces bone metastasis [31-33]. In osteoblastic metastases, C-X-C chemokine receptor type 4 (CXCR-4) induces migration and bone metastases by interacting with C-X-C motif ligand 12 (CXCL12) on osteoblasts [34, 35]. After invasion, the tumor cells release several growth factors, inducing a dysfunction in bone remodeling.

Table 1. Incidence of bone metastases according to the study from Coleman R.E. [26]

Cancer type	Incidence of bone metastases
Myeloma	70–95%
Renal	20–25%
Melanoma	14–45%
Bladder	40%
Thyroid	60%
Lung	30–40%
Breast	65–75%
Prostate	65–75%

In prostate cancer, osteoblastic metastases are common at metastatic sites upon activation of osteoblast activity [36-38]. However, the specific mechanisms of osteoblastic metastases are unknown. Several growth factors released by tumor cells, such as endothelin-1 (ET-1), TGF-β, IGF-1, fibroblast growth factor (FGF), PDGF, and Wnt, are involved in bone metastasis by promoting tumor cell invasion and increasing the activity of other growth factors and cytokines [39-43]. Studies indicate that ET-1, which is a mitogenic factor for osteoblasts, is found at high concentrations in metastatic sites and that tumor cells release ET-1 to promote osteoblastic metastases through a signaling initiated by the interaction of ET-1 with endothelin A receptor (ETA) [44, 45]. Specifically, ET-1 inhibits the expression of dick kopf 1 (DKK1), a Wnt-signaling antagonist that suppresses bone formation, therefore inducing osteoblasts differentiation [46]. In this regard, it has been found that interference with ET-1 signaling negatively affects the progression of osteoblastic metastases [47].

Urokinase-type plasminogen activator (uPA) is a protease that acts as a mediator to regulate tumor cell proliferation and activation [48], decreases the secretion of osteoclastogenic factors and inhibits bone resorption [49]. Tumor cells also secrete prostate specific antigen (PSA), a kallikrein serine protease that cleaves PTH-related protein (PTHrP) and inhibits the activity of osteoclasts [50]. PSA also activates osteoblasts by stimulating release of several growth factors in the bone metastases, such as IGF-1 and TGF-β [51]. The interaction between tumor cells, osteoblasts, and osteoclasts establishes a vicious cycle in osteoblastic metastases (Fig. 3). In clinical practice, PSA is a biomarker indicating disease progression and bone metastases in prostate cancer [52].

Members of the Wnt family are mediators that regulate osteoblasts development and bone formation [53]. Wnt proteins initiate a paracrine activity that promotes the formation of osteoblastic metastases. In prostate cancer, specifically, the expression of DKK-1 decreases during bone metastases progression. The activity of Wnt consequently increases and causes osteoblastic lesions in metastatic site [53-56]. At the same time, and as indicated above, ET-1 also promotes Wnt signaling by inhibiting DKK-1. These data highlight the importance of the tumor microenvironment in bone metastasis.

Mechanisms of osteolytic metastases

Breast cancer is the most common cancer inducing osteoclastic metastases. The formation of osteolytic lesions is mediated by osteoclasts via the release of several osteoclastogenic factors from tumor cells, such as interleukin-1 (IL-1), IL-6, IL-11, macrophage inflammatory protein 1a (MIP1a), M-CFS, PDGF, RANKL and PTHrP. PTHrP expression is key to promote osteolytic lesions, especially at metastatic bone sites. The expression of PTHrP is higher in bone metastases compared with that in metastases in soft tissues and the primary tumor site. PTHrP and other osteoclastogenic factors, such as PGE_2, IL-1, and IL-6, increase osteoclast activity by inducing the production of RANKL from osteoblasts and stromal cells and decreasing OPG levels [57]. RANK signaling promotes the differentiation of osteoclast

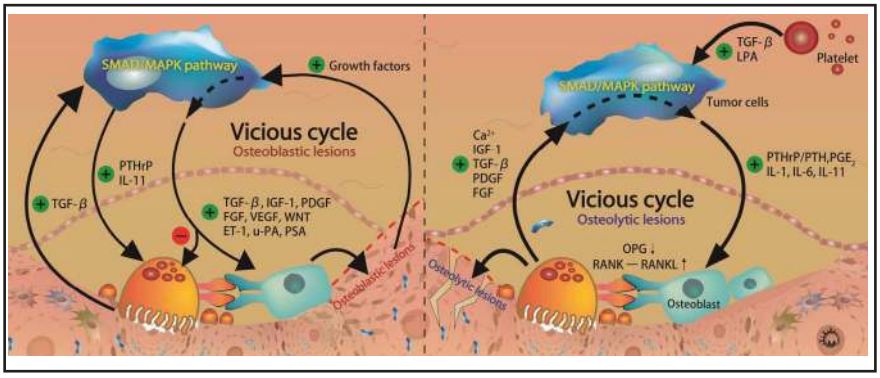

Fig. 3. Role of osteoblasts and osteoclasts in bone remodeling. In osteoclastic metastases. The formation of osteolytic lesions is mediated by osteoclasts via the release of several osteoclastogenic factors from tumor cells, such as interleukin-1 (IL-1), IL-6, IL-11, macrophage inflammatory protein 1a (MIP1a), M-CFS, PDGF, RANKL and PTHrP. PTHrP and other osteoclastogenic factors, such as PGE_2, IL-1, and IL-6, increase osteoclast activity by inducing the production of RANKL from osteoblasts and stromal cells and decreasing OPG levels. RANK signaling promotes the differentiation of osteoclast progenitors and stimulates bone resorption. After bone resorption, several growth factors stored in the bone matrix, such as TGF-β, PDGF, IGF-1, and FGF, are released and establish a vicious cycle in osteolytic metastases. In addition, the platelets release TGF-β and directly interact with tumor cells. This interaction enhances tumor invasion and metastasis. TGF-β and platelet-derived lysophosphatidic acid (LPA), also secreted by platelets, promote osteoclastic activation and bone resorption. In osteoblastic metastases, the tumor cells released several growth factors, such as endothelin-1 (ET-1), TGF-β, IGF-1, fibroblast growth factor (FGF), PDGF, and Wnt, are involved in bone metastasis by promoting tumor cell invasion and increasing the activity of other growth factors and cytokines Bescides, Urokinase-type plasminogen activator (uPA) and prostate specific antigen (PSA) also increase osteoblast activity and inhibit osteoclasts.

progenitors via transcription factors like nuclear factor kappa B (NF-κB) and activator protein 1 (AP1) and by activating Jun N-terminal kinase (JNK), extracellular signal-regulated kinase 1 and 2 (Erk1/2), and P38 mitogen activated protein kinase (MAPK), thus mediating bone resorption [58-60]. After bone resorption, several growth factors stored in the bone matrix, such as TGF-β, PDGF, IGF-1, and FGF [61], are released and establish a vicious cycle in osteolytic metastases (Fig. 3).

In the bone microenvironment, the release of TGF-β from mineralized bone matrix supports tumor and local cell proliferation through the activation of TGF-β type II receptor and the regulatory Smad proteins [62-64]. TGF-β also suppresses the proliferation of T-cells and the activity of natural killer cells to inhibit the immune system. Additionally, TGF-β promotes the vicious cycle of osteolytic metastases by activating its auto-phosphorylation in tumor cells [65]. After activation, tumor cells show increased proliferation and production of PTHrP. IGF-1 and extracellular Ca^{2+} are also involved in this vicious cycle [57, 66, 67]. In addition, the platelets release TGF-β and directly interact with tumor cells. This interaction enhances tumor invasion and metastasis. TGF-β and platelet-derived lysophosphatidic acid (LPA), also secreted by platelets, promote osteoclastic activation and bone resorption (Fig. 3) [68, 69]. The ''vicious cycle'' described offers a strong foundation for therapeutic intervention. Inhibition of the vicious cycle seems a useful way to arrest tumor progression and bone metastasis. Several studies have focused on targeting TGF-β, which plays a key role in metastasis in many tumor types, by using TGF-β receptor I (TGFβRI) inhibitors [70-72]. The results of ongoing clinical trials targeting the TGF-β pathway are eagerly awaited [73].

Roles of the immune system in bone metastases

Bone physiology and the immune system are tightly linked by reciprocal regulation and interaction. Bone cells regulate hematopoietic cells through the expression of surface

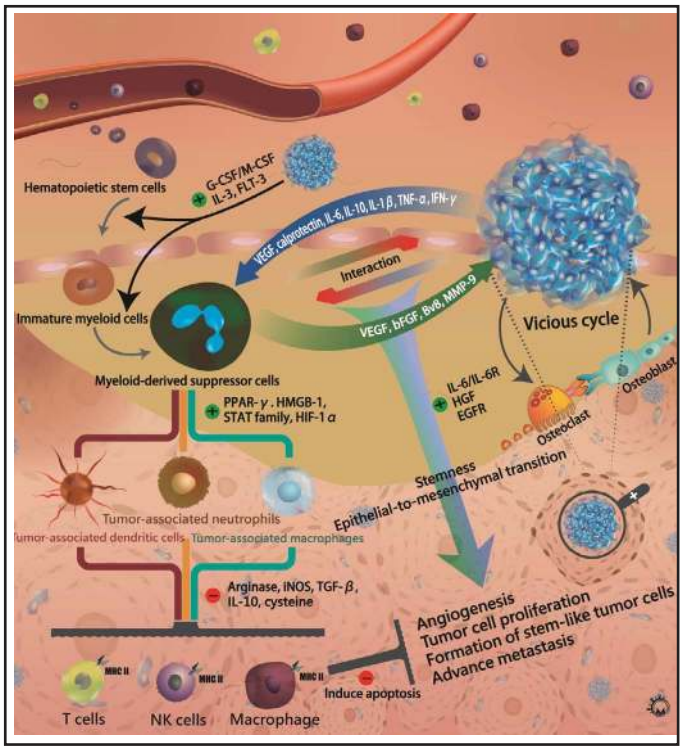

Fig. 4. The role of MDSCs in bone metastases. Initially, hematopoietic stem cells differentiate into common myeloid progenitor cells and immature myeloid cells under the effect of cytokines and growth factors, such as M-CSF, IL-3, and FMS-related tyrosine kinase 3 (FLT-3). In the pathological conditions, a small population of immature myeloid cells are pathologically activated into MDSCs, which is a heterogeneous population of pathologically activated myeloid cells with immunosuppressive function. MDSCs can differentiate into tumor-associated macrophages (TAMs), tumor-associated dendritic cells (TADCs), and tumor-associated neutrophils (TANs), and directly suppress natural killer (NK) cells and $CD8^+$ T cells through the expression of signal mediators as arginase, inducible nitric oxide synthase (iNOS), TGF-β, IL-10, and cysteine. They release NO and cytokines that suppress T cells. MDSCs protect the tumor cells from detection of immune system by releasing vascular endothelial growth factor (VEGF), basic FGF (bFGF), the VEGF analogue Bv8, and MMP-9, causing tumor growth and local angiogenesis. Tumor cells secrete several growth factors and cytokines, such as VEGF, calprotectin, IL-6, and IL-10, that recruit and activate MDSCs at the sites of future metastases. MDSCs interact with tumor cells, inducing the release of IL-6 and hepatocyte growth factor (HGF) and epithelial-to-mesenchymal transition (EMT) via the crosstalk between IL-6 receptor (IL-6R) and EFG receptor (EGFR), to promote the proliferation of stem cell-like tumor cells, which aggressively induce the progression of metastases.

molecules [74]. The bone and the immune system also share some common pathways [75, 76]. Osteoblasts regulate the proliferation of hematopoietic cells and differentiation of B cells [77]. Osteoclasts derive from mononuclear precursors, which generate macrophages and monocytes as well. RANKL, in particular, is a bridge linking the immune system and bone remodeling [78]. RANKL is indeed expressed not only in osteoblasts but also in T and B cells; additionally, RANKL receptors are expressed by dendritic cells (DCs), monocytes, macrophages, and tumor cells, and the bone and the immune system may interact via the RANKL signaling pathway [78-82]. The immune system can identify tumor-specific antigens or stress ligands produced by transformed cells and expressed on antigen-presenting cells and consequently inhibit the proliferation of tumor cells and metastasis. However, through mechanisms not fully clarified, the transformed cells might escape immune control; this causes bone metastases. Therefore, the immune cells have a critical role in controlling tumor proliferation and invasion within the bone microenvironment.

Role of myeloid-derived suppressor cells (MDSCs) in bone metastases

MDSCs are a heterogeneous population of pathologically activated myeloid cells with immunosuppressive function; they commonly express the myeloid marker CD33 and lack normal cell-surface markers of monocytes, macrophages, or DCs and the major histocompatibility complex (MHC) class II molecule HLA-DR [83, 84]. Initially, hematopoietic

stem cells differentiate into common myeloid progenitor cells and immature myeloid cells under the effect of cytokines and growth factors, such as M-CSF, IL-3, and FMS-related tyrosine kinase 3 (FLT-3) [85, 86]. Then, immature myeloid cells differentiate into dendritic cells, monocytes, and macrophages in peripheral organs and tissues. In the pathological conditions existing in the tumor microenvironment, or in the presence of trauma or sepsis, a small population of immature myeloid cells are pathologically activated into MDSCs. MDSCs can differentiate into tumor-associated macrophages (TAMs), tumor-associated dendritic cells (TADCs), and tumor-associated neutrophils (TANs), and directly suppress natural killer (NK) cells and $CD8^+$ T cells through the expression of signal mediators as arginase, inducible nitric oxide synthase (iNOS), TGF-β, IL-10, and cysteine (Fig. 4) [87-94]. TAMs have stronger immune suppressive ability compared to TANs; they release NO and cytokines that suppress T cells. Specifically, NO nitrates T cell receptors and decreases the function of tumor-associated MHC [95].

Interaction between MDSCs and bone metastases

The mechanisms through which MDSCs promote the proliferation and metastasis of tumor cells have been reported. Initially, MDSCs protect the tumor cells from detection by the immune system; they regulate the tumor microenvironment in metastatic sites by releasing vascular endothelial growth factor (VEGF), basic FGF (bFGF), the VEGF analogue Bv8, and MMP-9, causing tumor growth and local angiogenesis [96]. Some studies have suggested that a "pre-metastatic niche" originates under the effect of MDSCs, far from the primary tumor, before the appearance of metastases [97, 98]. According to this hypothesis, primary tumor cells secrete several growth factors and cytokines, such as VEGF, calprotectin, IL-6, and IL-10, that recruit and activate MDSCs at the sites of future metastases [99-101]. After the formation of the pre-metastatic niche, MDSCs interact with tumor cells, inducing the release of IL-6 and hepatocyte growth factor (HGF) and epithelial-to-mesenchymal transition (EMT) via the crosstalk between IL-6 receptor (IL-6R) and EFG receptor (EGFR) [102]. Additionally, the interaction between MDSCs and tumor cells promotes the proliferation of stem cell-like tumor cells, which aggressively induce the progression of metastases (Fig. 4) [103, 104]. Therefore, MDSCs play a critical role in the formation of bone metastases.

Mechanisms of activation of MDSCs in bone metastases

In the tumor microenviroment, MDSCs are mainly activated by M-CSF and granulocyte CSF (G-CSF) secreted from tumor cells and stromal cells. Several proinflammatory cytokines, such as IL-6, IL-1β, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ), stimulate the immunosuppressive abilities of MDSCs. Furthermore, several studies have indicated that members of the signal transducer and activator of transcription (STAT) family and the hypoxia-inducible factor 1α (HIF-1α) play important roles in promoting the differentiation of MDSCs [105-111]. Members of the STAT family, including STAT-3, STAT-5 and STAT-6, mediate the activity of MDSCs. STAT-3, in particular, directly binds to the arginase-I promoter to regulate the production of arginase and some studies suggest that the downregulation of the activity of arginase-I, through the inhibition of STAT-3, could reduce the immunosuppressive activity of MDSCs [112, 113], indicating the importance of STAT-3 in bone metastasis because of its effect on MDSCs. Metastatic sites are hypoxic; in these conditions, HIF-1α is expressed and induces the proliferation and immunosuppressive function of MDSCs [114]. Specifically, after the expression and activation of HIF-1α, MDSCs increase their expression of iNOS and arginase-I, leading to the suppression of T cells. Additionally, HIF-1α regulates expression of immune checkpoint molecules, such as programmed death 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death ligand 1 and 2 (PD-L1 and PD-L2), CD80 and CD86, on MDSCs, to promote their differentiation into TAMs and

their immunosuppressive function [115, 116]. Other mediators, such as high mobility group box 1 (HMGB-1) and peroxisome proliferator-activated receptor gamma (PPAR-γ), have also been reported to regulate MDSCs. HMGB-1 activates MDSCs and promotes their immunosuppressive function, and the induction of PPAR-γ expression increases the suppressive activity of TANs on T cells [103, 104, 117].. However, the detailed mechanisms of these processes are unclear. The full understanding of the role of MDSCs might help clarify the mechanisms of immune suppression in the tumor microenviroment and promote new therapeutic interventions.

Therapeutic and molecular interventions

The concept of the vicious cycle affects the new treatment of bone metastasis. Current stuies support that osteolysis inhibitors might be another treatment to decrease tumour burden [118-120]. Bisphosphonates is a famous bone-targeted agents inhibiting osteoclast activity and decreasing tumour burden [121]. In metastatic site, bisphosphonates tightly bind to the bone matrix and leading osteoclasts apoptosis via higher concentrations. The common side effect include fever, arthralgias, myalgias, bone pain and general malaise. Due to the good effect of controlling activity of osteoclasts, the new-generation bisphosphonates was created, such as pamidronate, zoledronic acid, and ibandronate. New-generation bisphosphonates have different sturcture and known as newer nitrogen-containing bisphosphonates with different mechanism to reguate osteoclastic lesion. Several therapy, such as osteroprotegerin, RANK-Fc, PTHrp antibodies, and Vitamin-D analogues, are under phase I to III studies. Other target therapy, such as anti-MMP-9,anti-IGF-1R, Quercetin, Osthole and activating aromatic hydrocarbon receptor, were reported in rescent [120, 122-126]. Current treatments mainly target the RANK-RANKL pathway and PTHrP signaling. However, control the osteroclastgenic factors seems only to reduce tumor burden.

In recent studies, the role of MDSCs in immune escape is a critical role for preventing and decreasing bone metastasis [127, 128]. The strategies of MDSC inhibition was proposed: deactivation, blocking development, and depletion of MDSCs. Several agents were investigated via inhibition of NO, arginase, ROS, and MDSC migration. *In vitro* studies, the Phosphodiesterase-5 inhibitors degraded the cyclic guanosine monophosphate leading to reduce myeloid-derived suppressor cell function by decreasing the expression of arginase 1 and nitric oxide synthase-2 expression, which mediated the MDSC to induce T cell suppression [129]. The NO production by MDSC may decrease T cell responsiveness via CCL2 and STAT1. The NO-aspirins was promoted to suppress the production of ROS to inhibit nitric oxide synthase-2 expression causing reversal of function of inhibition of T-cell [130-133]. In distant metastasis patients, the colony stimulating factor receptor 1 is an important role to recruit MDSCs to induce angiogenesis. In animal data, the high level of GM-CSF from MDSCs was found in in the spleen and tumor [134, 135]. The inhibitor of colony stimulating factor receptor 1 reduced the recruitment of MDSCs into tumors and inhibited pro-angiogenic and immunosuppressive genes. The monoclonal antibodies targeting colony stimulating factor receptor 1 are undergoing phase I clinical trials. Other potential agents to inhibit MDSC activation and differentiation, including all-trans retinoic acid, Vitamin D3, Vitamin A and N-Bisphosphonates, had a potential effect to control the function of inhibition of T-cell. Although several reported pharmacologic therapies had potential effect in basic studies, the safety and efficacy are necessary to be confirmed by clinical randomized controlled trials in the future.

Conclusion

In this review, we reported the data from studies focusing on the role of MDSCs in the pathogenesis of bone metastases. We highlighted several principles, worth reiterating here. Normal bone remodeling is regulated by OPGL, RANKL, PTHrP, and other cytokines. Bone

metastases (both osteoblastic and osteolytic lesions) follow the phases of colonization, dormancy, reactivation, and growth. The secretion of several growth factors generates a vicious cycle that amplifies the signals that induce metastasis. In the tumor microenvironment, MDSCs play an important role in immune escape; their immunosuppressive function inhibits T and NK cells, leading to the formation of distant metastases. Finally, although the mechanisms associated with immune escape remain unclear, we believe that their understanding will allow the identification of novel targets for therapeutic intervention. This review article provides an overview of the recent reports regarding the molecular regulation of bone metastasis and highlights the role of MDSCs in immune escape, and may help build a strong foundation for developing anticancer immune treatments and anticancer therapies, in general.

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Disclosure Statement

The authors declare no conflict of interest.

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