Review

Cancer Research

Myeloid-Derived Suppressor Cells as Osteoclast Progenitors: A Novel Target for Controlling Osteolytic Bone Metastasis

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Abstract

Immune cells and their secreted growth factors play major roles in tumor growth and metastasis. Interplay between the growing tumor and infiltrating immune cells determines the nature of immune response and ultimately, tumor fate. Increased infiltration of protumorigenic immune cells promotes tumor growth as well as dissemination to distant sites. These cells induce immunosuppression that inhibits proliferation and functions of cells of antitumor immune response. One population of immunosuppressive cells that is increasingly gaining attention is myeloid-derived suppressor cells (MDSC). MDSCs are immature myeloid progenitors that suppress T-cell effector functions and promote angiogenesis. MDSC numbers are elevated at both the primary tumor and metastatic sites, including bone. In addition to immunosuppressive functions of MDSCs, we and others have recently discovered a novel function for MDSCs as osteoclast progenitors. Osteolysis is a common complication in the carcinomas of breast, lung, prostate, and multiple myeloma with poor prognosis. Therefore, targeting the functions of MDSCs may exert dual therapeutic effects on immunosuppression and bone pathology. *Cancer Res; 73(15); 4606–10. ©2013 AACR.*

Introduction

One of the hallmarks of tumor progression is evading immunosurveillance, which allows tumor cells to escape antitumor immune response and/or to actively suppress it (1). This contributes to the establishment of primary tumors and subsequent metastasis. Innate and adaptive immune cell often infiltrate tumor sites. Different types of these immune cells and the products secreted by them determine the fate of tumor progression. Immune cells also play a major role in establishing metastasis of the primary tumor to various organs, including the bone (2, 3). A protective immune response against tumor is often prevailed by the protumor immune response and eventually, it is the balance or the lack of it between these two processes that determines the fate of tumor growth and metastasis. One of the immuosuppressive populations that is rapidly gaining interest and attention in the tumor biology is myeloid-derived suppressor cells (MDSC). These cells infiltrate various cancers and are often associated with poor prognosis (4). Several reports have shown increased infiltration of MDSCs in breast cancer, lung cancer, and multiple myeloma, both in the primary tumor and metastatic sites, including bone (5–7), which necessitates further understanding of the roles played by this population in cancer progression.

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MDSCs as Immunosuppressive Cells

MDSCs are a heterogeneous population comprising immature myeloid cells (IMC). Under normal conditions, the IMC differentiate into mature macrophages, dendritic cells, and granulocytes. However, in pathologic conditions, including cancer, IMC differentiation is inhibited, resulting in accumulation of immunosuppressive MDSCs (4). In mice, MDSCs are identified mainly by the presence of CD11b and Gr-1. Two main subsets of MDSCs are monocytic MDSCs (M-MDSC; $CD11b^{hi}Gr\text{-}1^{mid}Ly6C^{hi}Ly6G^{lo}$) and granulocytic MDSCs (G-MDSC; $CD11b^{hi}Gr\text{-}1^{mid}Ly6C^{lo}Ly6G^{hi}$). In humans, MDSCs are characterized mainly as $CD11b^{+}CD33^{+}HLA\text{-}DR^{-}$ cells. MDSCs expressing CD14 are M-MDSCs, whereas $CD14^{-}$ cells are G-MDSCs. In both mice and humans, M-MDSCs have high levels of nitric oxide (NO) and very low levels of reactive oxygen species (ROS), whereas the reverse is true for G-MDSCs. However, both of these subtypes have high arginase activity (4, 7, 8).

MDSCs play a pivotal role in cancer progression by suppressing both innate and adaptive immune response. Accumulation of MDSCs has been reported in almost all cancers, both in the preclinical models and human patients. MDSCs are present in abundance within primary and metastatic solid tumors (8). Tumor progression is associated with gradual accumulation of this immunosuppressive population in bone marrow, spleen, peripheral blood, and lymph nodes. MDSCs suppress T-cell effector functions in several ways. MDSCs deplete L-arginine through arginase-1 (ARG)-dependent consumption and by

sequestering L-cysteine and thus suppress the proliferation of T cells. ROS and NO generated by MDSCs suppress T-cell functions by loss of TCR-ζ chain expression, nitration, and desensitization of the T-cell receptor (TCR) and interference with interleukin (IL)-2 receptor signaling. MDSCs express certain surface proteins, such as ADAM 17 (a disintegrin and metalloproteinase domain-containing 17) and Galectin 9, that interfere with trafficking of T cells and induce their apoptosis, respectively. Besides suppressing effector T-cell populations, MDSCs promote the activation and expansion of regulatory T cells (Treg) and thus mediate immunosuppression. MDSCs also produce factors that promote tumor growth by inducing angiogenesis and lymphangiogenesis. MDSCs secrete several proangiogenic markers and can directly incorporate into tumor endothelium (4, 7).

These diverse mechanisms used by MDSCs allow tumor growth and metastasis to multiple organs, including the bone (4, 9). MDSC numbers are elevated in multiple myeloma and as breast cancer disseminate to the bone (5, 10). Bone is one of the major organs for breast, lung, and prostate metastasis, whereas multiple myeloma originates in the bone. Bone metastasis is often associated with poor prognosis and high morbidity. Nearly 80% to 90% of all breast cancer patients with advanced disease have osteolytic pathology as characterized by increased bone damage resulting from enhanced osteoclast activity (11). Bone undergoes a constant remodeling through osteoclast-mediated bone resorption and osteoblast-mediated bone regeneration in a tightly coupled manner to maintain homeostasis. However, during tumor growth in the bone, dysregulation of this process leads to either osteolytic or osteoblastic phenotypes (12). Bone metastases are often associated with increase in osteoclast activation and osteolysis.

Given that MDSCs are progenitors of macrophages, which are osteoclast precursor cells, and MDSC numbers greatly increase during cancer bone metastasis, their role in enhanced osteoclastogenesis is of significant interest.

MDSCs as Novel Osteoclast Progenitors

Osteoclasts are giant, multinucleated, bone-degrading cells. They are characterized by high expression of tartrate-resistant acid phosphatase (TRAP) and cathepsin K. Two critical factors for osteoclast formation are receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). Interactions of RANKL with the receptor RANK and that of M-CSF with its receptor colony-stimulating factor 1 receptor (c-fms) trigger a series of signaling pathways that lead to osteoclastogenesis (12). Stimulation of macrophages *in vitro* with M-CSF and RANKL induces their differentiation into multinucleated osteoclasts (13). Various signaling mechanisms, such as ROS and NO production, are involved in osteoclastogenesis (13).

Aside from being progenitors of macrophages, which differentiate into osteoclasts, MDSCs have recently been identified by ourselves and others in the bone-tumor microenvironment that undergo direct osteoclastic osteoclast differentiation and contribute to enhanced bone destruction and tumor growth. Our data show that MDSCs isolated from the tumor-bearing mice with bone metastasis differentiated into functional bone-resorbing osteoclasts *in vitro* and *in vivo* (5). This indicates that these cells are primed to be osteoclast progenitors (OCP) and the bone microenvironment *in vivo* triggers their differentiation into functional osteoclasts. More importantly, not all MDSC populations were capable of osteoclast differentiation. In our study, we differentiated MDSCs from lung, spleen, blood, and lymph nodes of tumor-challenged mice into osteoclasts. None of these MDSCs differentiated into osteoclasts, which suggested that the bone microenvironment is essential. However, MDSCs isolated from bone showing presence of metastasized tumor underwent osteoclast differentiation, but this did not happen in the ones without tumor or control bone marrow–derived MDSCs (Fig. 1). This clearly shows that for MDSCs to differentiate into osteoclasts, signals from both bone marrow cells and bone metastases are needed. It remains to be determined which factors are involved in polarizing MDSCs for osteoclast differentiation. Breast cancer cells present in the bone secrete a variety of growth factors, including MCP-1 and RANTES, both of which are osteoclastogenic. MDSCs express CCR2, the receptor for MCP-1, and thus are responsive to this cytokine. Ongoing studies in our laboratory are focused on understanding interactive signals between cancer cells, MDSCs, and the bone microenvironment.

Besides our report, Danilin and colleagues reported similar observations using a human breast cancer cell line (9). They showed that MDSCs from tumor-bearing mice upregulated parathyroid hormone-related protein (PTHrP) and Gli2 mRNA levels in cancer cells, both of which are involved in osteoclast activation. When mice were injected with breast cancer cells along with either immature myeloid precursors (iMC) from healthy donors or MDSCs from tumor-bearing mice, the latter showed significant bone metastasis and osteolysis, thus supporting our observation that MDSCs from bone, containing tumor, are primed to be osteoclast precursors. Although iMC underwent osteoclast differentiation, they generated much fewer osteoclasts and reduced bone resorption compared with MDSCs. It is possible that prolonged culture of iMC in RANKL and M-CSF may have induced their differentiation into macrophages and therefore resulted in osteoclast differentiation. Clearly, these findings also can be extended to other osteolytic malignancies such as lung metastases and multiple myeloma. A recent report by Zhuang and colleagues showed a similar function of MDSCs as OCPs in a syngeneic murine multiple myeloma model (10). MDSCs from this model could promote bone resorption both *in vitro* and *in vivo*. This study also showed that MDSCs from tumor-bearing mice differentiated with greater potential into osteoclasts, thus bolstering observations reported in breast cancer. Studies have shown that osteoclast differentiation of macrophages can occur independently of RANK stimulation during certain pathologic states (14, 15). It will be interesting to determine whether any such RANK-independent activation triggers MDSC differentiation into osteoclasts.

Similar to bone metastasis, osteolysis is also observed under other pathologic conditions such as rheumatoid arthritis. Using a murine rheumatoid arthritis model, which is clinically

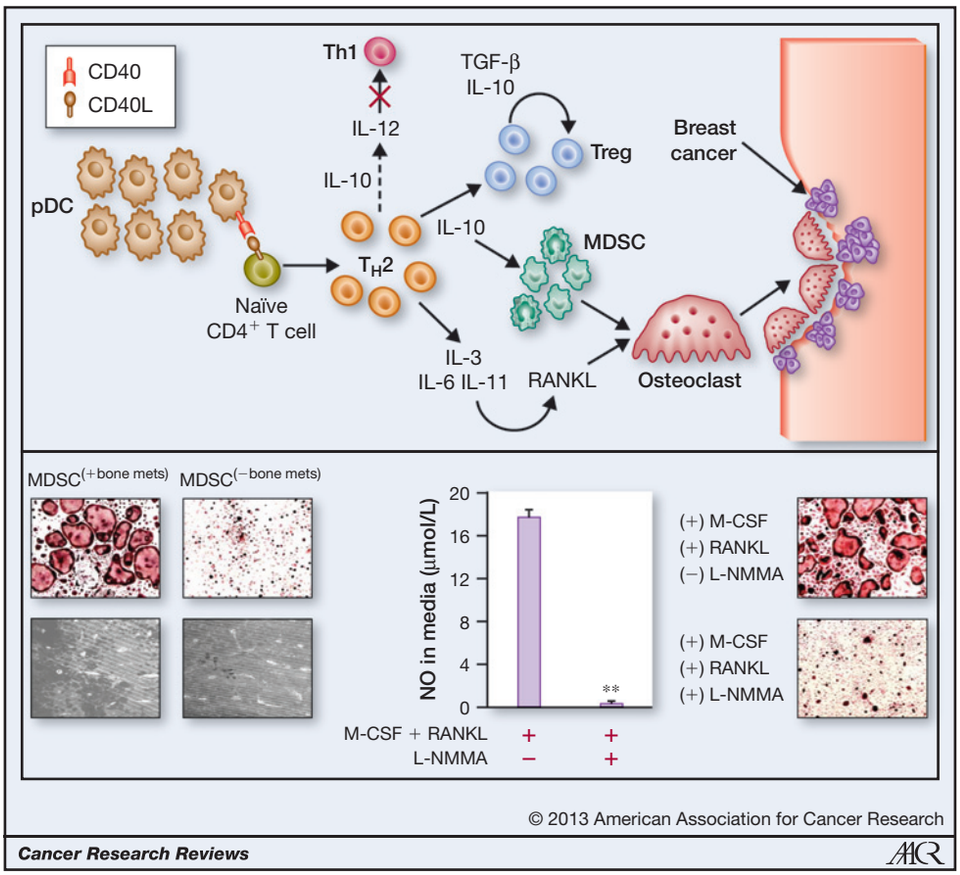

Figure 1. A, with dissemination of breast cancer to the bone, there is a significant elevation in the numbers of pDCs. pDCs interact with naïve $CD4^+$ T cells via CD40/CD40L and induce their differentiation in the immunosuppressive T_H2 cells. The presence of T_H2 cells results in suppression of T_H1 cell response and further induces immunosuppression via Tregs and MDSCs. Cytokines secreted by T_H2 cells induce RANKL. MDSCs in the bone microenvironment undergo osteoclast differentiation and induce osteolysis. This allows growth and establishment of bone metastasis of breast cancer. B, MDSCs isolated from tumor-bearing mice with bone metastasis ($MDSC^{(+bone\ mets)}$) undergo osteoclast differentiation and resorb bone, whereas those from tumor-bearing mice without bone metastasis ($MDSC^{(-bone\ mets)}$) are incapable of differentiating into osteoclasts or inducing osteolysis. Furthermore, NO is crucial for differentiation of MDSCs into osteoclasts. In the presence of L-NMMA, an inhibitor of NO synthesis, $MDSC^{(+bone\ mets)}$ do not differentiate into osteoclasts, as described earlier by Sawant and colleagues (5).

similar to human rheumatoid arthritis, Charles and colleagues showed MDSCs as the primary OCP that is capable of differentiating into functional osteoclasts involving NO signaling (16). Similar to our observation, in the rheumatoid arthritis model OCPs also expressed CD115, the M-CSF receptor. From these recent reports, it is clear that MDSCs are novel OCPs and thus targeting MDSC could help develop new therapeutic strategies for controlling osteolysis.

Role of NO in MDSC-Mediated Osteoclast Differentiation

Both NO and ROS are well-known mediators of osteoclast differentiation. NO is known to induce osteoclast differentiation of macrophages. Mice with knockout of inhibitor of inducible NO synthase (iNOS) show reduced bone loss due to impaired osteoclast function. Stimulation of macrophages via RANKL transiently increases ROS production through TNF receptor–associated factor (TRAF) 6, Rac1, and NADPH oxidase (Nox) 1. Inhibitors that block Nox1 or a deficiency in TRAF6 inhibit response of macrophages to RANKL, thus resulting in reduced osteoclastogenesis. Pretreatment of osteoclasts with antioxidants also reduced RANKL signaling and therefore osteoclast differentiation (13). Because MDSCs mediate immunosuppression via ROS and NO, we sought to find out if any of these pathways are also involved in osteoclast differentiation. Our data showed a critical role of NO synthesis in generation of MDSC-mediated osteoclastogenesis. Inhibition of NO production reduced MDSC-mediated osteolysis both *in vitro* and *in vivo*. Hence, NO production has a dual role in MDSC function. Considering that monocytic MDSCs are major producers of NO, it will be interesting to determine if M-MDSCs are the major source of MDSC-generated osteoclasts.

The question is what leads to increased NO levels in the bone-tumor microenvironment. One of the important features of the bone microenvironment during metastasis is hypoxia, which makes it a fertile ground for homing of metastatic cancer cells. The most common transcription factor that is expressed

under hypoxic conditions is hypoxia inducible factor (HIF)-1α. Breast cancer that has metastasized to the bone expresses higher levels of HIF-1α than the primary tumor, and it was shown, in turn, to increase the bone metastatic potential of these cells. On the other hand, reduced bone metastasis was observed in tumor cells lacking HIF-1α. Overexpression of HIF-1α increased osteoclast formation while inhibiting osteoblast differentiation, thus showing a direct role of this transcription factor in osteoclastogenesis (17).

One of the signaling pathways regulated by HIF-1α is NO production via iNOS (18). Evidence also shows that NO can induce HIF-1α by signaling through the phosphoinositide 3-kinase and mitogen-activated protein kinase (19). Hence, this positive feedback response allows for osteoclast differentiation.

Clinical Significance of MDSCs as Osteoclast Precursors

Metastasis of cancer cells to the bone is a major clinical complication that is associated with pain, bone fracture, hypercalcemia, and decreased mobility and thus affects overall quality of life for the patient. Although current therapies exist for treatment of bone metastases, they are limited and are focused on symptomatic management, thus limiting progression of established disease. Hence, a better understanding of the processes that play a role in therapy of bone metastases may offer new strategies for therapeutic intervention and extended survival. From recent evidence that MDSCs contribute to bone metastases in multiple ways, future treatment options may be developed that target this population of cells. Such approaches may be combined with existing therapies for adjuvant effect.

Gemcitabine is a chemotherapy agent used for carcinomas of the lung, pancreas, bladder, and breast. Interestingly, this drug also specifically inhibits MDSC populations *in vivo* (20). Therefore, gemcitabine may be used not only as an antitumor drug but also for reducing bone destruction. More importantly, use of gemcitabine in patients with bone metastasis may have more impact because it will not only reduce overall tumor burden but also reduce tumor growth in the bone and osteolysis. Our *in vivo* study showed that gemcitabine-treated mice not only had fewer MDSCs but the breast cancer growth in the bone was also reduced (5). Elevation in MDSCs is also observed in lung cancer. A similar approach may be used for treatment of patients with lung cancer to control both tumor growth and bone dissemination. As MDSCs function as osteoclast precursors in rheumatoid arthritis, gemcitabine could be used for controlling MDSC-mediated osteolysis under this pathologic condition. Besides gemcitabine, doxorubicin-cyclophosphamide also has inhibitory effects on MDSCs proliferation. Docetaxel, a common chemotherapy drug for patients with breast and prostate cancer, also suppresses MDSCs by polarizing their differentiation into M1 macrophages (21). Docetaxel is commonly given to patients with bone metastasis, where it delays the onset of osteolytic lesions, which may be the result of docetaxel-mediated inhibition of MDSCs (22). A therapeutic approach using combinations of these chemotherapy drugs may help in controlling bone destruction and tumor growth.

Studies have identified that plasmacytoid dendritic cells (pDC) are elevated in patients with carcinomas of breast and lung, and multiple myeloma, both in the primary cancer and at metastatic sites. Using a syngeneic mouse model of breast cancer, we reported recently that pDCs numbers are significantly elevated during progressive stages of cancer dissemination to the bone (Fig. 1). Elevated pDCs correlated with increased MDSCs infiltration in bone (6). A similar situation is noted in multiple myeloma, where immune dysfunction is caused partially by pDCs and results in high MDSCs numbers in the bone. Therefore, treatments that control pDC function may help not only to control tumor growth and osteolysis but also to reduce MDSC-mediated immunosuppression and osteolysis. Depletion of pDCs in the preclinical murine model, using a specific antibody, resulted in a reduction of breast cancer growth and bone metastasis (6). Depletion of pDCs also reduced MDSCs levels. Therefore, targeting pDCs has a dual effect on tumor growth by (i) resulting in an antitumor immune response that reduces the tumor growth and (ii) decreasing bone dissemination of cancer by reducing MDSC numbers and osteolysis induced by MDSCs. Although these antitumor effects of pDC depletion have been reported in breast cancer, they may be extended to multiple myeloma as well as lung cancer, which also show increased pDC and MDSC numbers with tumor progression. Depletion of pDCs may be a useful therapeutic approach in combination with chemotherapy or bisphosphonate therapy for achieving better antitumor activity.

The common therapy for controlling bone metastasis of cancer is use of bisphosphonates. Bisphosphonates are used for treatment of breast cancer, multiple myeloma, and prostate cancer. Bisphosphonates attach preferentially to calcium and thus are accumulated in the bone at high concentrations. Nitrogen containing bisphosphonates such as zoledronic acid are ingested by osteoclasts, in which they inhibit the enzyme farnesyl pyrophosphate synthase. The bisphosphonates induce osteoclast apoptosis that in turn leads to an antiresorptive effect (23). Such bisphosphonates also reduce proliferation of MDSCs and thus can be used as viable agents for controlling both MDSCs and MDSC-mediated osteolysis. Zhuang and colleagues treated MDSCs from multiple myeloma–challenged mice with zoledronic acid. This resulted in a significant decrease in the number of osteoclasts formed in MDSCs culture and also inhibition of MDSCs and bone lesions *in vivo* (10). Therefore, a combination therapy using zoledronic acid along with gemcitabine may help to greatly control bone loss and tumor growth. In fact, a recent report showed that gemcitabine used along with bisphosphonates was more effective in reducing the number and size of bone metastases compared with gemcitabine alone in an animal model of human breast cancer (24). In addition to drugs that decrease MDSC numbers, inhibitors that can arrest MDSC functions, including ROS and NO inhibitors, may provide significant therapeutic effects not only targeting MDSCs but also targeting other protumorigenic mechanisms in the tumor microenvironment.

Administration of all-*trans* retinoic acid (ATRA), a vitamin A metabolite, also results in substantially decreased MDSC numbers in cancer patients and tumor-bearing mice. ATRA reduces numbers of MDSCs by inducing their differentiation into

dendritic cells and macrophages (4). This function of ATRA will help reduce MDSC-mediated osteolysis when this agent is administered in patients with bone metastasis. Thus, there are multiple therapy options that could be used to target MDSCs. These approaches will not only reduce MDSC-mediated osteolysis but also MDSC-mediated immunosuppression.

Conclusions

MDSCs besides being immunosuppressive, contribute actively to cancer-induced osteolysis by differentiating into functional, bone-resorbing osteoclasts. This displays the plasticity of the MDSC population in regards to their ability to differentiate into osteoclasts. This phenomenon is observed under pathologic conditions of cancer and rheumatoid arthritis, both of which are associated with complications caused by bone destruction. Therefore, therapies that target MDSCs directly may not only reduce its immunosuppressive functions but also MDSC-mediated osteolysis and for controlling disease complications.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Sawant, S. Ponnazhagan
Development of methodology: A. Sawant, S. Ponnazhagan
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Sawant, S. Ponnazhagan
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Sawant, S. Ponnazhagan
Writing, review, and/or revision of the manuscript: A. Sawant, S. Ponnazhagan
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Sawant, S. Ponnazhagan
Study supervision: S. Ponnazhagan

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