Hematopoietic Stem Cell Niche Is a Potential Therapeutic Target for Bone Metastatic Tumors

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Abstract

Despite significant improvements in therapy, the prognosis for cancer with bone metastasis is generally poor. Therefore, there is a great need for new therapeutic approaches for metastatic disease. It has been appreciated that tumor cells metastasize to bone using mechanisms similar to those of hematopoietic stem cells (HSC) homing to bone marrow (e.g., CXCL12/CXCR4). It was recently found that prostate cancer cells target the bone marrow microenvironment for HSCs, or the HSC niche, during metastasis. Of importance, these disseminated prostate cancer cells can be mobilized out of the niche with the use of HSC mobilizing agents. These findings suggest that the bone marrow HSC niche is a potential therapeutic target for metastatic disease. Therefore, a hypothesis worth considering is that agents that can disrupt the interactions between tumor cells and the HSC niche may be efficacious when used in conjunction with standard chemotherapeutic agents. Although further understanding of the tumor-niche interactions is needed, the concept of targeting the niche in conjunction with chemotherapy could open up new possibilities to eradicate incurable metastatic diseases. Clin Cancer Res; 17(17); 5553–8. ©2011 AACR.

Background

Bone is a common metastatic site in certain cancers, including breast and prostate cancer (1). Bone metastasis involves a complex process in which cancer cells leave from a primary tumor, intravasate into the blood stream, and extravasate through the endothelium into bone marrow. Although conventional treatments such as surgery, chemotherapy, and radiation have been improved to treat localized cancer, bone metastasis remains a major cause of death in these cancer patients. Current therapies do not always work for metastatic disease, and unfortunately, effective therapeutic strategies for metastatic disease are illusive. In prostate cancer, disseminated tumor cells (DTC) occupy the marrow space, which can lead to replacement of hematopoietic tissues and create both osteolytic and osteoblastic lesions in the metastatic bone site that can lead to pain, fractures, and spinal-cord compression (2). This suggests that interactions with the microenvironment are crucial for establishing bone metastasis. Therefore, recent attention has focused on alternative strategies whereby tumor cells may be eliminated by manipulation of the microenvironment (3). The growth and metastasis of solid tumors are thought to rely on tumor microenvironments (or nontumor host cells), including endothelial cells, cancer-associated fibroblasts, and tumor-associated macrophages (3). The interaction between tumor cells and their microenvironments is referred to as the tumor ecosystem (3). Potential approaches for targeting the tumor ecosystem rather than the tumor cells alone may open up new venues in therapy.

Although multiple factors are thought to be involved in the process of DTCs disseminating to the marrow, growing evidence suggests that DTCs gain access to the bone marrow using homing mechanisms similar to those of hematopoietic stem cells (HSC; refs. 4–10). In the marrow, HSCs reside in a unique microenvironment (or niche) (11). Defining the cells that are responsible for creating the HSC niche is a topic of great debate; however, cells of the osteoblastic and endothelial lineage are believed to be major components of the niche (11). Chemokine gradients in the marrow and adhesion molecules expressed by the HSC niche are believed to be crucial for HSC homing (11). Chemokines are small cytokines that bind to G protein-coupled receptors containing 7 transmembrane domains. Its major role is chemotaxis (or cellular trafficking). Several downstream signaling pathways are also activated to regulate the survival and proliferation of both normal and malignant cells when chemokines bind to their receptors. One of the best-studied molecules involved in HSC homing is chemokine CXCL12 (or SDF-1), which is expressed by several cell types in the marrow (11, 12). The binding of CXCL12 to its receptor, CXCR4, plays an important role in regulating homing, adhesion, and survival of HSCs through key pathways, including phosphatidylinositol
3-kinase (PI3K)/Akt, mitogen-activated protein (MAPK)/extracellular signal-regulated kinase (ERK), and Janus kinase (JAK)/signal transducer and activator of transcription (STAT; Fig. 1; refs. 11–14). In this context, serine-threonine protein phosphatase 2A regulates CXCL12-mediated chemotaxis and adhesion of human cord blood HSCs through the Akt signaling pathway (15). Cross-talk between CXCL12 and TGF-β signaling pathways controls the cell cycling of HSCs by activating the PI3K/Akt/Foxo3a/mammalian target of the rapamycin (mTOR) pathway (16). In addition, by coupling with the Flt3 ligand, which is known to promote HSC homing and proliferation, CXCL12 facilitates the migration of HSCs via the MAPK, cyclic adenosine monophosphate response element binding protein (CREB), and Akt pathway (17). Conversely, CXCL12-mediated cell trafficking of HSCs is prevented by inhibition of JAK2 (18). Consistent with this notion, granulocyte colony-stimulating factor (G-CSF) mobilizes HSCs from the marrow by degrading CXCL12 in the marrow (19). The CXCR4 inhibitor AMD3100 is also known to be an HSC mobilization agent (20). Conversely, by inhibiting the activity of CD26 (DPP IV/dipeptidylpeptidase IV), a glycoprotein that is expressed on the surface of many cell types which cleave CXCL12 [including tumor cells where it has been shown to promote tumor progression (21)], enhanced engraftment of HSCs during bone marrow transplantation can be achieved (22).

DTCs are frequently found in the bone marrow (23). Based on the molecular mechanisms related to the HSC homing, it was recently reported that CXCL12/CXCR4 (or alternative receptor CXCR7) chemokine axis also plays a major role in the bone metastasis of prostate cancer (5, 6, 8–10). CXCL12 signaling through CXCR4 may trigger the dissemination of prostate cancer by activating αvβ3 integrins (cell surface receptors that play a role in adhesion, migration, invasion, growth, and angiogenesis of tumor cells; ref. 9) and CD164 (a sialomucin protein that mediates adhesive function and regulates hematopoiesis; ref. 24) expression in prostate cancer. In addition, CXCL12/CXCR4 signaling participates in both metastasis and the angiogenesis process of prostate cancer by downregulating the expression and secretion of the glycolytic enzyme phosphoglycerate kinase 1 (PGK1) and angiostatin (25). Of importance, CXCL12 regulates the angiogenic phenotype in prostate cancer through CXCR4 (26). VEGF and tissue inhibitor of metalloproteinase 2 (TIMP-2) secretion

Figure 1. Conceptual scheme of a niche target therapy for bone metastatic disease. Bone metastatic DTCs target the osteoblastic niche where HSCs reside and compete for occupancy of the niche with HSCs. Both DTCs and HSCs are believed to use similar mechanisms to gain access to the HSC niche (e.g., CXCL12/CXCR4 axis and VLA-4/VCAM-1). The CXCL12/CXCR4 axis is believed to play major roles in the homing, adhesion, survival, and proliferation of HSCs. Accumulating evidence suggests that, similar to HSCs, the CXCL12/CXCR4 axis is also involved in dissemination, adhesion, survival, and growth of DTCs. CXCL12 activates several key survival signaling pathways (i.e., PI3K/Akt, MAPK/ERK, and JAK/STAT) in both HSCs and DTCs by binding to its receptor, CXCR4. Herein, both HSCs and DTCs undergo growth arrest (also known as quiescence or dormancy) and are prevented from undergoing apoptosis when they bind to the niche. This is why current therapies that target proliferating cells fail to eradicate DTCs. If the cell cycle of dormant DTCs is accelerated by mobilizing them out of the niche with the use of HSC mobilizing agents (e.g., G-CSF and AMD 3100), the currently available chemotherapies can be used to treat metastatic disease.
is induced through the PI3K/Akt pathway, whereas interleukin (IL)-6 and IL-8 secretion is stimulated through the MAPK/ERK pathway (26). In fact, the metastasis and growth of prostate cancer in bone were prevented by blocking the CXCL12/CXCR4 pathway (6, 8, 10). Therefore, this pathway is also likely to be involved in the bone metastasis of other solid tumors (Fig. 1; ref. 4). Additionally, both HSCs and prostate cancer bind to the adhesion molecule annexin II expressed by osteoblasts, and blocking annexin II or the receptor for annexin II prevents homing of both cell types to the marrow (7, 27).

Another major role of the niche is to maintain HSC quiescence (11). Quiescence is important for HSCs to retain their self-renewal ability. It was shown that the endosteal (or osteoblastic) HSC niche maintains quiescent HSCs through the pathway of Tie2 (the cell surface receptor for angiopoietin) and its ligand angiopoietin-1 (Ang-1, a growth factor that regulates angiogenesis) in the marrow (28). Moreover, quiescent HSCs are thought to be localized close to the endosteal region, which is extremely hypoxic (29). Recent studies have shown that hypoxia-inducible factor-1α (HIF-1α) is crucial for HSCs to undergo quiescent in endosteal region (30, 31). Moreover, metastatic tumors can exist in a dormant state within such a hypoxic microenvironment (32). Tumor cells are believed to become dormant to escape from apoptosis and eventually proliferate (33, 34). Several lines of evidence also show that bone marrow cells facilitate the drug resistance of DTCs (35, 36). A recent study revealed that the bone marrow microenvironment facilitates drug resistance in multiple myeloma by enhancing the HIF-1α–mediated STAT3 signaling pathway following adhesion to β1 integrin (37). Once tumors become dormant, they acquire an ability to evade the chemotherapeutic agent or radiation that is currently targeting proliferating (or dividing) cells. Although the mechanisms responsible for this phenomenon remain unknown, these observations indicate that both HSC quiescence and tumor dormancy are regulated by the niche in a similar fashion (Fig. 1).

Using an in vitro micrometastatic model (38), Shiozawa and colleagues (39) showed that disseminated prostate cancer cells compete for occupancy of the osteoblastic HSC niche with HSCs to create metastatic foci. They also showed that disseminated prostate cancer cells competed directly with transplanted HSCs for occupancy of the osteoblastic HSC niche and that HSCs and prostate cancer colocalized to the endosteal bone surfaces (39). A greater number of disseminated prostate cancer cells could be recruited into the vacant niche following the mobilization of HSCs out of the niche (39). In addition, the number of DTCs correlated closely with the number of osteoblastic niches. When the osteoblastic niche was conditionally compromised with skeletal tissues obtained from a transgenic mouse line in which the herpes thymidine kinase gene was fused with the 2.3-kb fragment of the rat type I collagen α1 promoter (40), fewer disseminated prostate cancer cells were observed in micrometastatic assays (39). Conversely, the expansion of osteoblastic niche with parathyroid hormone boosted the dissemination of prostate cancer cells (39). Intriguingly, disseminated prostate cancer cells pushed HSCs outward from the niche, and the cell cycle in HSCs was accelerated, resulting in an increased number of progenitor cells (39). Of more importance, disseminated prostate cancer cells could be mobilized back into the peripheral blood with the use of HSC mobilizing agents such as G-CSF and the CXCR4 inhibitor AMD3100 (39).

These findings suggest that the osteoblastic HSC niche serves as a specific component of the tumor ecosystem in the marrow, and that this niche may be able to support both tumor dormancy and HSC quiescence, and regulate eventual tumor recurrence.

**Clinical-Translational Advances**

It is clear that existing monotherapies are not sufficient to eradicate tumor cells once they metastasize to organs. Tumor cells are thought to acquire drug resistance by interacting with the distant microenvironment. Based on our recent observation (39), tumor cells that favorably metastasize to bone target the bone marrow microenvironment for HSCs (or HSC niche) and may be parasitic on such a microenvironment to survive for an extended period. Therefore, the engagement of the HSC niche by DTCs may induce dormancy, which protects DTCs from the majority of the existing chemotherapeutic agents. Potentially, interfering with adhesion molecules that link tumor cells to the niche could be an attractive target to reverse their drug resistance. A critical implication of the strategy to target the HSC niche is that agents that induce HSCs to leave the niche will also stimulate the cell cycle progression of the released cells. If similar agents can be used to release dormant DTCs from the marrow niche, then they, too, are likely to be susceptible to existing chemotherapeutic agents that target cells in the cell cycle.

CXCL12 and its receptor, CXCR4, are believed to play a major role in HSC mobilization. CXCL12 is known as a molecule associated with HSC homing, and the osteoblastic niche is one of the major sources of CXCL12 in the marrow (11). Hematopoietic growth factors, such as G-CSF, have been widely used to mobilize HSCs into peripheral blood (19, 41). G-CSF is thought to induce HSC mobilization by degrading CXCL12 through the stimulation of protease activities [e.g., neutrophil serine proteases, cathepsins, elastase, matrix metalloproteinases (MMP), and CD26; ref. 41]. G-CSF appears to regulate HSC mobilization through actions on bone remodeling. A recent study showed that G-CSF cleaves CXCL12 in the marrow by enhancing the expression of MMP9 and cathepsin K in osteoclasts (42). It was also shown that G-CSF reduces the levels of CXCL12 in the marrow by directly inhibiting osteoblastic activity (43, 44). The blockade of the receptor for CXCL12 has also been approved as an agent for HSC mobilization. AMD3100, a CXCR4 antagonist, induces HSC mobilization in mice and humans, and it exerts a synergistic effect on mobilization when administered with G-CSF (20). In addition, growing evidence
suggests that the central nervous system participates in HSC mobilization from the osteoblastic niche via the CXCL12/CXCR4 axis (45–47). In addition to blocking the CXCL12/CXCR4 axis, the degradation of other adhesive interactions between HSCs and the niche has been used to mobilize HSCs from the marrow (41). Therefore, a combination of blockade of very late antigen-4 (VLA-4) with G-CSF and/or AMD3100 dramatically augmented the effects of both G-CSF and AMD3100 on HSC mobilization (48, 49). VLA-4 is an integrin family protein that is known to bind to vascular cell adhesion protein 1 (VCAM-1), which appears to play an important role in HSC homing. VLA-4/VCAM-1 interactions seem to be independent of the CXCL12/CXCR4 axis. Like AMD3100, G-CSF mobilized disseminated prostate cancer cells from the niche into the peripheral blood following the induction of MMP2/9 and the enhancement of osteoclastic activity (39). These findings suggest that therapies that target the HSC niche to interfere with tumor-niche interaction are promising.

However, few therapies are available that target the bone marrow microenvironment or HSC niche. AMD3100 enhances the chemosensitivity of acute myeloid leukemia (AML; ref. 50) and multiple myeloma (51). Consistent with this notion and our findings (39), AMD3100 increases the mobilization of niche-engaged leukemia and myeloma cells into the circulation and enhances their sensitivity to chemotherapy (50, 51). Treatment with anti-VLA-4 antibodies can also minimize tumor burden in AML in conjunction with chemotherapy (52). Bone metastases of breast and ovarian cancer were significantly prevented by blocking integrin αvβ3 in an in vivo model (53). Other approaches target osteoblastic IL-6 activity (54, 55), the RANK/RANKL axis (56), and TGF-β signaling in bone marrow stromal cells (57, 58).

Conclusions

The tumor ecosystem is a dynamic, complex, and evolving environment. Interactions between tumor cells and the surrounding host components provide multiple options for therapeutic targets. Because one of the major functions of the HSC niche is to induce growth arrest in its occupants while supporting their self-renewal (11), tumor cells that metastasize to bone may usurp the HSC niche to become dormant and stay that way for years. If this is true, it could partially explain why DTCs are sequestered from current therapies. Although the mechanisms that regulate the induction and release of tumor dormancy are poorly understood, strategies that target the HSC niche to mobilize DTCs using agents that mobilize HSCs would open up new possibilities to eradicate incurable DTCs (Fig. 1).

Once a cancer patient’s tumor spreads to distant organs, such as bone, the survival rate of that patient drastically declines. Although significant progress has been made in the early diagnosis and treatment of localized tumors, we are still losing the battle against metastatic disease. Patients with bone metastases and their families suffer physically, emotionally, and financially for a long time. Simultaneously, the financial burden of health care is growing steadily. Therefore, new approaches to cure cancer with bone metastasis are urgently needed. We believe that the concept of mobilizing DTCs out of the HSC niche using HSC mobilizing agents prior to chemotherapy is highly innovative and directly challenges existing paradigms. However, some critical questions remain unresolved:

1. Why do DTCs target the osteoblastic HSC niche during dissemination?
2. Do all DTCs become dormant?
3. Do specific osteoblastic HSC niche subtypes play a role in tumor dormancy?
4. How do DTCs become dormant?
5. Do DTCs use the same mechanisms to become dormant as HSCs use to become quiescent?
6. Can HSC mobilizing agents accelerate the cell cycling of dormant DTCs?
7. Does DTC mobilization depend on a circadian rhythm?
8. Are there any agents that can mobilize only DTCs and not HSCs?

At present, the role of the bone marrow HSC niche in DTCs is not directly known. However, the answers to these questions would provide new cellular and molecular targets that could be used as a niche-related therapy for bone metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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