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PERSPECTIVES

The Stem Cell Niche and Bone Metastasis

Gordon J. Strewler

Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA

Abstract

The hematopoietic stem cell niche has been defined as a microenvironment in bone that supports the stem cell, controls decisions between quiescence and proliferation of stem cells, and provides a mechanism for the self-renewal of stem cells. It is likely that at least two hematopoietic stem cell niches exist in bone: an endosteal niche, in which hematopoietic stem cells are adherent to osteoblasts, and an endothelial niche. Like self-renewing adult tissues, tumors may also be maintained by stem cells. It is likely that a tumor stem cell niche also exists in bone and shares many of the characteristics of the hematopoietic stem cell niche. The principal determinants of bone metastasis may be factors in this microenvironment that control the entry of tumor cells into the niche, permit long-term quiescence of tumor stem cells within the niche and induce their eventual awakening. *BoneKEy-Osteovision*. 2006 May;3(5):19-29.

Stem Cells and the Stem Cell Niche

Self-renewing adult tissues are maintained by stem cells. Stem cells are defined by the ability to self-renew and the capacity to give rise to a population of mature differentiated progeny. If in an adult the number of stem cells is to remain relatively constant, then there must be a balance between self-renewal and the production of differentiated progeny (1). The simplest way to achieve this balance is for an individual stem cell to give rise to two nonidentical daughter cells, one of which maintains the stem-cell identity and the other becomes a differentiated cell. The specification of the daughter cells could occur before cell division (divisional asymmetry) or after (environmental asymmetry).

Divisional asymmetry, a cell-autonomous process in which cell fate determinants distribute unequally during mitosis, is a central feature of stem cell function in *Drosophila* (2-4), but it is not clear how much it operates in vertebrate stem cells – one vertebrate example of divisional asymmetry is the epidermis, where an apical crescent contains a protein complex that dictates the polarity of cell division (5). The alternative mechanism, environmental asymmetry, implies the existence of a stem cell niche, a site in which the stem cell receives cues for "stemness", such that after cell division one daughter cell remains in the niche, retaining exposure to these cues, while the other exits the niche.

Within stem cell populations, most stem cells are quiescent. They are resistant to chemotherapeutic agents that target proliferating cells and can renew the tissue after chemotherapy. The stem cell niche, therefore, must support quiescence, self-renewal, and proliferation of stem cells.

Cancers also contain stem cells – a small population of cells that are capable of both self-renewal and repopulation of the cancer and that, when quiescent, may be resistant to chemotherapy (6-8). Cancer stem cells were first convincingly found in studies of

leukemia, where only a small subpopulation of acute myelocytic leukemia (AML) cells expressed a $CD34^+$ hematopoietic stem cell (HSC) phenotype and could transfer AML to recipient animals (9;10). Deletion of the tumor suppressor PTEN is sufficient to convert HSCs to an AML stem cell phenotype; and, simultaneously, to reduce the normal capacity of HSCs for self-renewal (11). Treatment with rapamycin, a cancer therapeutic whose cellular target, mTor, is downstream in the pathway activated by deletion of PTEN, wipes out the leukemia and simultaneously restores HSC self-renewal. In this model there is a simple relationship between AML stem cells and normal HSCs, illustrating how similar normal and malignant stem cells can be.

Putative cancer stem cells have also been found in solid tumors such as breast, lung and prostate cancer. Breast cancer stem cells were identified as a population of $CD44^+/CD24^-/Lineage^-$ primary human breast cancer cells that are highly tumorigenic compared to the total cell population and capable of generating the full diversity of phenotypes that could be found in the primary tumor (12). These cells can be serially passaged, suggesting that they have the capacity for self-renewal, and at each passage can generate new tumors with a mixed cell population. Their relationship to normal breast epithelial stem cells is unknown.

Stem cells with a $CD44^+$/high $\alpha2\beta1/CD133^+$ phenotype have been identified in normal prostate epithelium, and a population with this phenotype constitutes about 1% of cells in prostate cancers (13). Such prostate cancer cells can self-renew and generate a mixed population of cells that express differentiated cell products such as the androgen receptor and prostatic acid phosphatase, making them putative stem cells. Others have found that $Sca1^+$ prostate cells are enriched for properties including replication quiescence and the potential for multilineage differentiation (14;15) and that perturbations of PTEN/AKT signaling in these prostate-renewing cells initiates tumorigenesis (14). None of these cells, however, have passed the final test, demonstration of their tumorigenicity.

Putative solid tumor stem cells thus have many predicted stem cell properties but have not yet passed every test of the stem cell. Tumor stem cells would not necessarily be derived from normal tissue stem cells, as are leukemia stem cells, but a relationship to tissue stem cells seems likely. Self-renewal of cancer stem cells implies either asymmetric division or environmental asymmetry – a niche to determine the stem cell properties of quiescence, self-renewal and multilineage differentiation – but no stem cell niche has yet been identified within solid tumors.

Stem Cell and Tumor Cell Niches in Bone

The Endosteal Niche

HSC niches in bone can be defined as spaces in which HSCs are supported by their environment so as to maintain the capacity for quiescence and self-renewal. The best-characterized HSC niche in bone exists in close proximity to endosteal osteoblasts. The notion that osteoblasts contribute to the HSC niche arose from the observation that bone mineralization precedes the invasion of the bone marrow by hematopoietic cells and was strengthened by the absence of marrow cells as well as osteoblasts in Runx2 knockout mice (16). Histologically, HSCs are mostly located near the endosteal surface (17). Two classic studies identified an osteoblast-associated stem cell niche (18;19). In one, osteoblast expression of a constitutively active PTH/PTHrP receptor was shown to increase both osteoblast and HSC numbers in bone marrow, and stromal cells treated with PTH were better able to support HSCs *in vitro* than WT cells (20). In the other, mice lacking the bone morphogenetic protein receptor BMPR1A, which is normally expressed on osteoblasts, had increased numbers of immature osteoblasts and of HSCs. Reciprocal transplantation experiments confirmed that the HSC phenotype of BMPR1A(-/-) cells was determined by their environment (21).

In this study, direct contact between HSCs (defined by long-term BrdU retention) and a subset of osteoblasts using homotypic N-cadherin binding was observed.

Further evidence for an endosteal HSC niche comes from ablation experiments. Treatment with gangcyclovir to ablate immature osteoblasts that express thymidine kinase under control of a *Col1a1* promoter leads to a progressive loss of bone marrow HSCs and a reciprocal increase in extramedullary hematopoiesis in spleen and liver (22;23). Ablation of mature osteoblasts using osteocalcin-driven TK has no effect on hematopoiesis, however, consistent with other evidence that the HSC niche is formed by immature osteoblasts (24).

The Endothelial Niche

HSCs are maintained throughout adulthood in liver and spleen, which have no osteoblasts, suggesting that a second stem cell niche exists. A vascular HSC niche has recently been identified in association with the fenestrated endothelium of sinusoids in bone marrow and spleen (25). Its discovery followed the identification of the signaling lymphocytic activation molecule (SLAM) receptor CD150 as a cell surface marker that distinguishes HSCs from multipotent hematopoietic progenitors (MPP) which have lost the capacity for self-renewal. About two-thirds of $CD150^+$ HSCs ($CD150^+CD48^-CD41^-Lineage^-$) in spleen or bone marrow are in contact with sinusoidal endothelial cells; a smaller fraction of bone marrow HSCs are in contact with the endosteum. These imaging results are consistent with previous functional studies showing that endothelial cells from yolk sac, mesonephros and bone marrow express factors that permit maintainance of HSCs (1;26-28). Little is presently known about the vascular HSC niche and its relationship to the endosteal niche: do cells reside permanently in one niche or the other; or is the perivascular niche primarily a site of self-renewing HSCs poised for rapid release in response to stress? And, what molecules define the niche?

Niche Entry

Entry of HSCs to their endosteal niche occurs during late fetal life, during normal homeostatic processes in which HSCs move from their bone marrow niche into the circulation and back, and after bone marrow transplantation (29). In order to enter their niche, HSCs must home to the bone marrow, attach to the endothelium, and migrate across it. Selectins provide a molecular address in the bone marrow and integrins are also critical to homing of HSCs. Cytoskeletal controllers such as the GTPase Rac are essential for engraftment. The cytokine CXCL12 (SDF1) is expressed on osteoblasts and vascular endothelial cells and plays a crucial role in entry; mice lacking either CXCL12 or its HSC receptor CXCR4 have a lethal impairment in hematopoiesis, though HSCs are abundant in their fetal livers (1;29). It is likely that HSCs traverse an endovascular niche to get to their endosteal niche, but very little is known of the architecture of their pathway or the molecular sequence in which chemokines, adhesion molecules and cytoskeletal elements come into play.

Entry of tumor cells into bone also involves selectins and chemokines. Metastatic prostate carcinoma cells present a specific E-selectin ligand and roll on bone marrow endothelial cells that present E-selectin (30;31). Entry into bone may also require an interaction between CXCL12 on endothelial or osteoblast cells and its receptor CXCR4 on tumor cells. Interference with this interaction blocks metastasis of breast cancer cells to soft tissue sites (32). Human prostate carcinoma cells migrate across an endothelial monolayer towards CXCL12 (33) and exposure to antibodies to CXCR4 immediately before and for one day after intracardiac injection inhibits metastasis of prostate carcinoma cells to bone (34). Expression of CXCR4 on a background of interleukin 11 and osteopontin expression markedly increases the bone metastatic potential of MDA-MB-231 human breast carcinoma cells (35). These results do not clarify the microenvironment(s) in which CXCL12 acts: Is it essential for attachment

and transendothelial migration, for entry into an endosteal niche, or both?

A new imaging advance has now allowed investigators to peer directly into an endothelial tumor cell niche, one that overlaps with a hematopoietic stem/progenitor cell (HSPC) niche (36). Real time optical sectioning and immunoimaging using intravenously injected fluorescent antibodies were used to watch tumor cells (Nalm-6 acute lymphoblastic leukemia cells) in the calvarial vasculature. The leukemia cells rolled along the endothelium until they found specific spots to attach and diapedese across it. Other tumor cells – human and murine leukemia cells, multiple myeloma cells and prostate carcinoma cells – behaved similarly to Nalm-6 cells. The vascular "hot spots" where tumor cells attached and diapedesed express E-selectin as well as CXCL12 (SDF1), a ligand for the chemokine receptor CXCR4 on the tumor cells. Blocking CXCR4 function markedly inhibited localization of tumor cells to these domains, while deletion of E-selectin had smaller effects. HSPCs localized to the same domains as tumor cells, and thereafter resided there for up to 70 days, consistent with engraftment into a stem cell pool with slow cell cycling. Thus an endothelial niche accommodates both tumor cells and normal HSPCs and is amenable to further study. It remains to be shown that this niche confers on HSCs or tumor cells the stem-cell properties of quiescence and self-renewal.

Entry into the endosteal niche may be governed by local rates of bone remodeling. The parathyroid calcium-sensing receptor (CaR) is expressed on the surface of HSCs and has recently been shown to regulate entry into the endosteal HSC niche (37). Fetal mice in which the CaR had been inactivated have reduced HSC numbers in bone marrow but increased HSCs in spleen. In bone marrow transplantation experiments CaR(-/-) HSCs display defective homing to the endosteal niche, a trait that is associated with poor adhesion to type I collagen. Calcium release from mineralized bone may enhance the engraftment of hematopoietic cells to an osteoblast-associated niche. Ambient calcium concentrations in actively modeling endosteum may be well above the typical calcium concentration of the ECF; this could account for the association of red marrow with sites of active bone remodeling. Breast and prostate cancer cells also express the CaR (38-40). There is considerable evidence that high bone remodeling states favor bone metastasis (41-43), and it will be important to determine the role of calcium-sensing by tumor cells in bone metastasis, for example by using antagonists of the CaR in model systems.

Quiescence

One of the key properties of the stem cell niche is its ability to regulate exit from and subsequent reentry into the cell cycle. About 75% of HSCs in the endosteal stem cell niche are quiescent. Recent findings have opened a window onto the molecular basis of cell cycle regulation in the endosteal HSC niche. Angiopoietin-1 on osteoblasts signals for quiescence by activating the Tie2 receptor on HSCs (17;44). Tie2-mediated quiescence involves upregulation of the cell cycle inhibitory protein p21. HSCs in mice lacking p21 proliferate abnormally at the expense of self-renewal (1;45). It is not clear whether activation of Tie2 directly blocks entry into the cell cycle or whether the signal is indirect, e.*g*., by enhancing cell adhesion to stromal cells (44). Addition of angiopoietin-1 to HSCs in vivo increases expression of both N-cadherin and β1-integrin and increases adherence of HSCs to the bone surface *in vivo* (17). The transcription factor c-Myc may be downstream of Tie2 in this pathway (44). HSCs that are deficient in c-Myc express abundant N-cadherin and the integrins lymphocyte function-associated antigen-1 (LFA1) and VLA4, overexpress p21, do not differentiate, and pile up in the niche (46). Forced expression of c-Myc has opposite effects.

One component of the quiescence signal from the niche is the extracellular matrix protein osteopontin (47;48). The expression of osteopontin by osteoblasts is restricted to the endosteal surface. Binding of HSPCs to

osteopontin, via β-1 integrin, inhibits their proliferation in vitro, and HSCs in mice deficient in osteopontin display markedly enhanced cycling. This emphasizes that the extracellular matrix is a component of the stem cell niche that is important in the regulation of stem cell quiescence.

Quiescence, or dormancy, is also one of the cardinal features of bone metastasis. Patients with early breast or prostate carcinoma have abundant tumor cells in their blood and bone marrow. In the case of breast carcinoma, early bone micrometastases have been studied extensively and shown to be predictors of prognosis (49;50). Much less is known about prostate cancer, but in one study, prostate cancer cells were detected in the bone marrow of 54% of men before prostatectomy and 33% of men soon after prostatectomy (51). Thus, tumor cells readily escape from the primary tumor and find their way to bone marrow, indicating that mechanisms that target tumor cells to bone, while undoubtedly important, are rarely limiting for bone metastasis. Yet bone metastases often do not make their clinical appearance until many years after the primary tumor was resected. What has happened in the interval? It seems very likely that a fraction of the tumor cells that were already present in bone marrow in large numbers when the primary tumor is removed were lying dormant in a bone marrow niche during the intervening years, eventually to wake up and proliferate as metastatic tumors. Arguably, a key to understanding bone metastasis is to understand how tumor cells find their way to a niche that supports quiescence and what factors lead them to re-enter the cell cycle years later. These considerations and the analogy between tumor cells and HSCs lead to the formulation of several hypotheses.

Hypotheses

A population of the pioneer tumor cells that reach bone early in the course of a cancer are cancer stem cells. They can be defined in the same way as stem cells in self-renewing tissues like the bone marrow, as cells capable of quiescence, self-renewal and the ability to give rise to the mixed cell population of the metastatic tumor. Their stem cell properties are not cell-autonomous but are determined by residence in a cancer stem cell niche, which overlaps with and may be identical to the HSC niche. This niche may be either endothelial or endosteal. Residence in the stem cell niche induces cancer stem cells to become quiescent; in turn, quiescence renders them resistant to standard chemotherapy. The development of clinical bone metastasis requires that quiescent cells re-enter the cell cycle and self-renew as they give rise to the mixed cell population of the metastatic tumor. Re-entry of dormant tumor cells into the cell cycle could be induced by cell-autonomous events such as new mutations in tumor stem cells, but is likely to be determined by events in the cancer stem cell niche. Understanding the factors that determine entry of tumor cells into their niche in bone and the factors that determine re-entry of quiescent tumor cells into the cell cycle will be necessary to produce successful therapies of bone metastasis.

Experimental Approaches

A functional definition of tumor stem cells is required to understand their role in bone. When bone metastasis develops in experimental models, *e.g.*, after intracardiac injection of tumor cells or spontaneous metastasis from an orthotopic or subcutaneous injection of tumor cells, the fraction of cells that colonize bone and go on, after a latent period, to originate metastasis are candidate tumor stem cells. They have undergone a period of quiescence and are capable of producing the diversity of cells present in the bone metastasis. Experimental data suggest that selection of cells from bone metastases may enrich this population (35). If the behavior of such cells in experimental metastasis is determined by the niche they occupy in bone, then they could be regarded as a surrogate of true tumor stem cells. Considerable work would be required to conclude that the fraction of cells that generate bone metastases is a distinct subpopulation, *e.g.,* definable by cell sorting

using cell surface markers; nor it is clear, though it is quite possible, that stem cell subpopulations exist in established tumor cell lines.

Models of dormancy must be developed. Dormancy models could use human cell lines that give rise to bone metastases only many months after intracardiac injection (52). Such cell lines could simply give rise to slow-growing tumors, but it is more likely that the cells undergo a period of dormancy in a bone microenvironment before proliferating as metastases. Can tumor cells that are marked, *e.g.*, with a fluorescent or chemoluminescent marker, be detected during a dormant period? If so, do they exist as single cells or small preangiogenic clusters? Noninvasive imaging methods will ultimately be able to follow the fates of single tumor cells and preangiogenic cell clusters in putative stem cell niches in bone and determine which of them give rise to bone metastases.

New imaging techniques have now visualized HSCs and tumor cells in endothelial and endosteal niches (17;36) and are available to define the functional architecture of tumor cell niches in bone. Real-time immunoimaging with intravenously injected fluorescent antibodies and multiphoton microscopy could be used to visualize tumor cells not only in calvaria but also in other superficial bone sites and in subcutaneously implanted bones. For example, it may be possible to visualize details of niche architecture in implanted vertebral bodies, or “vossicles”, which have been used to introduce bones with mutant phenotypes into athymic mice (53). Experimental metastasis to vossicles would permit genetic approaches to manipulating the bone microenvironment in experimental studies of metastasis. Investigators have also used functional bioluminescent imaging of cells into which a promoter–luciferase cassette has been introduced to visualize gene expression *in situ* in bone metastases (54).

A number of molecules could converse between osteoblasts, HSCs and tumor cells in the tumor stem cell niche. For example, homotypic N-cadherin interactions, which are important for attachment of HSCs to their stem cell niche (21), could also take place between tumor cells and osteoblasts. N-cadherin expression is not a general feature of breast and prostate carcinoma, but N-cadherin in prostate carcinoma cells is upregulated by β1-integrin binding to fibronectin (55). β-catenin could be a key to a decision by tumor cells between a quiescent state in which they are anchored to the niche by cadherin interactions and an activated state in which β-catenin is localized to the nucleus (18), thereby activating cell cycle control genes such as *c-Myc* and *cyclin D1*. Activation of tumor stem cells may involve Wnt signaling. Osteoblast-tumor cell crosstalk in the tumor cell niche could also involve the osteoclast differentiation signal receptor activator of NF-κB (RANK). RANK is a functional signaling molecule in breast and prostate carcinoma and could transmit survival signals to tumor cells from osteoblasts that express RANK ligand (56-58).

The tumor cell niche in bone can be looked at as a special case of the kind of epithelial-stromal cell interactions involving the extracellular matrix that are generally important for progression of epithelial cancers (59;60). Not only are tumor cells themselves influenced by adherence to osteopontin, type I collagen, fibronectin, and other matrix proteins, but osteoblasts, like stromal fibroblasts, may provide the key angiogenic signals required for progression to metastasis (59). HSCs themselves could be a key feature of the tumor cell niche. Surprisingly, HSPCs arrive at sites of soft tissue metastasis before tumor cells do and tell them where to set up shop, possibly by encouraging neoangiogenesis (61). Wilson and Trumpp (1) have likened the HSC niche to a synapse, and the analogy is likely to hold for tumor cells in their niche in bone as well.

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