IBMS BoneKEy. 2008 September;5(9):308-322
http://www.bonekey-ibms.org/cgi/content/full/ibmske;5/9/308
doi: 10.1138/20080335

PERSPECTIVES

Cancer Stem Cells and Bone Metastasis

Gordon J. Strewler

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

Abstract

Cancer stem cells, or tumor-initiating cells, are the progenitors of leukemias and the proposed progenitors of solid tumors that metastasize to bone. Stem cells are a small self-renewing population that gives rise to all the other cell types in a tumor. In some cases cancer stem cells arise by oncogenic mutations in somatic stem cells; in other cases mutations in lineage-committed progenitor cells endow them with the stem-cell properties of self-renewal and multipotency. The property of self-renewal requires asymmetric division in order to give rise to one daughter cell with stem cell properties and one that differentiates. Somatic stem cells divide asymmetrically because they reside in a niche; a niche for cancer stem cells has yet to be characterized but probably exists. Cancer stem cells have a motile, prometastatic phenotype and in some cases have undergone epithelial-mesenchymal transition. As tumor progenitors with invasive properties they are strong candidates to be the pioneer cells that initiate metastases, and it is predicted that what limits bone metastasis is the ability of cancer stem cells to find a niche in bone that will support their dormancy and eventual growth. Cells with stem cell properties can be purified for study from several standard breast cancer cell lines and tools exist to investigate the possibility that metastasis of stem cells explains the genesis, dormancy, and chemotherapy-resistance of bone metastases. *IBMS BoneKEy*. 2008 September;5(9):308-322.

Introduction

Cancer stem cells – some prefer the term tumor-initiating cells – are a small population of self-renewing progenitor cells that are multipotent and give rise to all cells in tumors. This *Perspective* will review the cancer stem cell hypothesis from the perspective of metastasis and explore the hypothesis that bone metastases arise from cancer stem cells that have found their way to bone. We will briefly consider the general properties of adult stem cells, discuss the evidence for cancer stem cells and their niche, using mainly leukemia, brain tumors and breast cancer as examples; present what evidence now exists for a pioneering role of stem cells in bone metastasis; and compare what is known of the cancer stem cell niche to the hematopoietic stem cell (HSC) niche.

Somatic Stem Cells

Self-renewing adult tissues are maintained by somatic stem cells. Among the best examples are the hematopoietic stem cell (1), the intestinal stem cell (2) and the bulge cell of the epidermis (3). Stem cells are defined by the ability to self-renew and by multipotency, the ability to give rise to multiple kinds of differentiated progeny. To maintain a constant number, somatic stem cells self-renew by asymmetric division, each mitosis giving rise to one daughter cell that is committed to differentiate and to a new stem cell. Asymmetric division, in turn, can occur in two ways: either as a cell-autonomous event or because of an asymmetric effect of the stem cell's environment (Fig. 1). Many examples of cell-autonomous divisional asymmetry exist, most of them in lower organisms; the best-known example in mammalian cells is the epidermal stem cell (4). More common in vertebrate tissues is environmental asymmetry, in which the stem cell's niche determines cell fate after mitosis, one daughter cell remaining in the niche, which confers "stemness"; the other being pushed out to initiate differentiation. The best example of the somatic stem cell niche is

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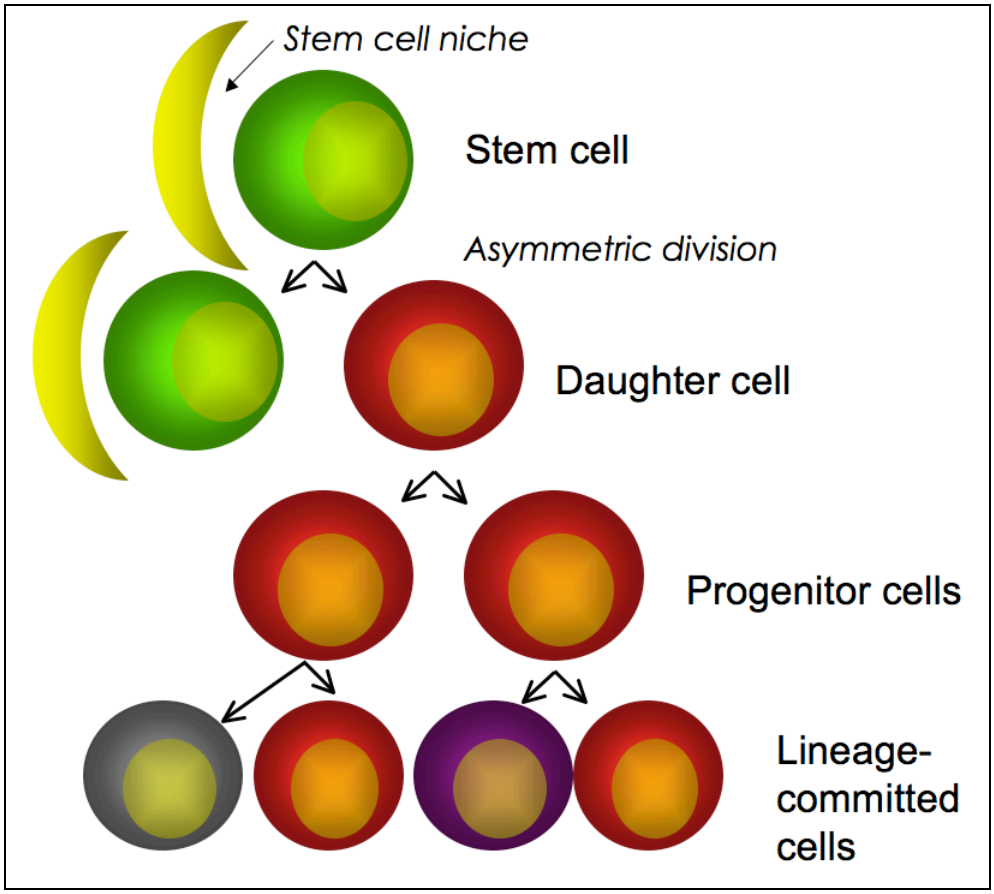

Fig. 1. A stem cell in a niche divides asymmetrically to give rise to another stem cell and a daughter cell that is pushed out of the niche, thus self-renewing and producing progenitors that will proliferate and eventually commit to specific lineages to populate the tissue or the tumor.

the HSC niche in bone (1;5;6), where we have a growing understanding of the cells that constitute the niche and some of the molecular signals that determine the function of the niche. The perivascular neural stem cell niche (3) and the intestinal stem cell niche (2) have also been identified.

Tumor-Initiating Cells

The first and best evidence that tumors are organized hierarchically and initiated by a small number of cells with stem cell properties came from the study of acute myelogenous leukemia. Dick *et al*., in a series of classic papers, showed that only a small fraction of leukemia cells was capable of transmitting leukemia to immunocompromised mice (7-9). These cells gave rise to leukemias in immunocompromised mice that had the same cell types as the original leukemia, demonstrating that the human cells were multipotent. The small fraction of cells that could transmit leukemia did so serially: $CD34^{+}CD38^{-}$ cells could be isolated from human leukemias in mice and injected into fresh mice to grow secondary leukemias, tertiary leukemias, etc. The ability of a leukemia cell to propagate the leukemia serially through generations of mice was taken as experimental evidence for the property of self-renewal, and remains as the best form of evidence for self-renewal in subsequent studies of cancer stem cells. An alternative explanation for this result would be that a small population of tumor cells, rather than being stem cells, is simply well-adapted to grow in the immunocompromised mouse (10). Although estimates of cancer stem cell number are probably affected by the barrier to growth of human cells in xenotransplantation experiments, serial transplantation of putative cancer stem cells has been carried out in syngeneic animal models (11-13) eliminating the possibility in

those cases that tumor stem cells are exclusively an artifact of xenotransplantation. Serial propagation of human tumor cells in the immunocompromised mouse remains an imperfect test of self-renewal, however.

The best-studied examples of solid tumor stem cells are in brain tumors and breast cancer (14). The $CD133^+$ fraction from glioblastomas and medulloblastomas is capable of self-renewal in serial xenotransplantation studies and gives rise to neuronal and glial cell lineages in the resultant tumor (15), thereby fulfilling the criteria of self-renewal and multipotency. The $CD133^+$/$nestin^+$ fraction of glioblastoma cells are capable of anchorage-independent growth as neurospheres, thus defining another common property of tumor-initiating cells, which is taken as additional evidence for self-renewal. Gene expression signatures of glioblastoma-initiating cells also overlap with those of neural stem cells. Glioblastoma stem cells home to a perivascular niche when placed in three-dimensional cultures with endothelial cells, which spontaneously form vascular tubes (16;17). In this perivascular niche, self-renewal and proliferation of tumor cells as well as neural stem cells are maintained by signals from the endothelium (18). $CD133^+$/$nestin^+$ cells in histological sections of brain tumors are also found in a perivascular location, suggesting that glioblastoma-initiating cells may have a native perivascular niche in human tumors (16).

Tumor-initiating cells have been isolated from human breast tumors (19) as $CD44^+CD24^{-/low}Lineage^-$ cells that are capable of giving rise to the full repertoire of breast cancer cells during serial passage in NOD/SCID mice. Tumors were initiated by as few as 200 $CD44^+CD24^{-/low}Lineage^-$ cells whereas no tumors resulted from the injection of 20,000 $CD44^+CD24^+$ cells. (Lineage marker antibodies were anti-CD2, -CD3, -CD10, -CD16, -CD18, -CD31, -CD64, and -CD140b.) Of nine lines that were established, eight were derived from metastatic tumor cells in pleural effusions. Like glioblastoma stem cells and normal mammary stem cells, breast tumor-initiating cells are readily capable of anchorage-independent growth as spherical masses, in this case called mammospheres. As discussed below, cells with a similar phenotype can be isolated from established breast cancer cell lines. CD44 was chosen empirically as a stem cell marker but has recently been shown to be under negative control by p53 and to contribute to tumor initiation by $p53^-$ cells (20).

Breast cancer stem cells express the enzyme aldehyde dehydrogenase, which is characteristic of a number of other kinds of stem cells (21). It is thought that the expression of aldehyde dehydrogenase in stem cells may relate to its role in activating retinoids (22). Aldehyde dehydrogenase activity can be detected in living cells with a commercially available assay (Aldefluor®), and will be a useful marker for cancer cells with stem-like properties in bone and other tissues. The population of $Aldefluor^+$ cells overlaps only slightly with the $CD44^+CD24^{-/low}Lineage^-$ population and cells with overlapping expression of both sets are high enriched in stem-like cells; as many as 1/20 cells in the overlap population is tumorigenic (21), making the $Aldefluor^+$ $CD44^+CD24^{-/low}$ $Lineage^-$ population the most enriched of any putative breast cancer stem cell population derived from tumors. Aldefluor positivity in breast tumors is associated with a poor clinical outcome.

Only a little is known of factors that govern the hierarchical organization of mammary tissue or of breast cancers. Breast carcinomas can be divided into two broad categories, those with basal and luminal cell characteristics (23-25). Basal cells are ER^-/PR^-/$ERBB2^-$ and are keratin 6^+ and/or keratin 17^+. $CD44^+CD24^{-/low}Lineage^-$ breast cancer stem cells have a basal-like phenotype in that they are $CD24^{-/low}$. Mouse mammary stem cells have been conclusively identified and they also have a basal cell phenotype (26;27), but the isolation of human mammary stem cells has been hampered by the lack of a xenotransplantation assay for repopulation of the mammary fat pad and the details of their phenotype are unknown.

Do Tumor-Initiating Cells Arise from Somatic Stem Cells?

In the simplest version of the stem cell hypothesis the somatic stem cell would be the target for transformation to cancer. Available evidence suggests that many leukemias arise by transformation of HSC and some by transformation of early progenitor cells which acquire the ability to self-renew (8;9). Experimentally, the introduction of oncogenes into hematopoietic progenitors confers the ability to self-renew, in some cases without greatly altering their committed phenotype or widespread reprogramming of gene expression (28-30). Spontaneous blast crisis in patients with chronic myelogenous leukemia also appears to result from the acquisition of self-renewal by a clone of granulocyte-macrophase precursors (31). C/EBPα mutations that result in loss of the p42 isoform are associated with acute myelogenous leukemia (AML), and when knocked into the mouse, they cause AML which can be transferred by a leukemia-initiating $Mac1^{+}c\text{-}Kit^{+}$ population (32). It is thus clear in the case of leukemias that lineage-specific progenitor cells can acquire stem cell characteristics. For this reason some prefer the term tumor-initiating cell over cancer stem cell to describe the stem cell phenomenon. The role of somatic stem cells in solid tumor carcinogenesis is less clear, but recent results indicate that medulloblastoma arises from committed progenitor cells rather than neural stem cells (33;34), whereas cutaneous squamous cell carcinomas arise from cells that closely resemble bulge stem cells (13).

Stem Cells in Established Cell Lines

The established tumor cell lines that are widely used in models of metastasis are implicitly considered to be relatively homogeneous. Are they indeed homogeneous or do some or all tumor cell lines contain a distinct population of tumor-initiating cells? If so, can they serve as models for tumor stem cell biology, or should investigators concentrate on isolating stem cell-enriched populations from primary or metastatic tumors? A recent paper used parallel strategies to investigate the properties of breast cancer-initiating cells (35). Taking advantage of the resistance of stem cells to chemotherapy, breast cancer-initiating cells were isolated from breast tumors of patients treated with chemotherapy. Mammosphere-forming cells were enriched by 10-fold and $CD44^{+}CD24^{-/low}$ cells were 9-fold enriched, compared with tumors from women who had not received chemotherapy. To replicate the apparent selection of stem cells by cancer chemotherapy, SKBR3 breast cancer cells were passaged through the mammary fat pad of NOD/SCID mice treated with epirubicin. By the third passage, 93% of freshly isolated SKBR3 cells bore stem cell markers, were multipotent and were markedly enriched in tumor-forming cells. Similar findings have recently been reported for several other human breast cancer cell lines (36). Eight human breast cell lines (human mammary epithelial cells, and MCF10A, MCF7, SUM149, SUM159, SUM1315 and MDA.MB.231) were recently shown, by sorting for CD44+/CD24-/low/ESA+ cells (ESA is epithelial-specific antigen) (37) to contain breast cancer stem cell-like elements which self-renew, reconstitute the parental cell line, and preferentially survive chemotherapy. It thus appears that several established breast cancer cell lines have subpopulations of cells with stem cell-like characteristics which can be expanded for study.

The Cancer Stem Cell Niche

In most somatic stem cells the property of asymmetric division is conferred by the stem cell niche. In turn, asymmetric division can maintain constancy of stem cell number; abrogation of self-renewal by asymmetric division leads to stem cell depletion. Although cancer stem cells may have no homeostatic mechanism to maintain their number, as do normal stem cells, and indeed could be an expanding population during rapid tumor growth, self-renewal requires that at least some of their mitoses be asymmetric. Cancer stem cells could have acquired cell-autonomous asymmetry of division or they may occupy a cancer stem cell niche (38), but very little is known about the putative niche for cancer stem cells. As noted above, there is suggestive

evidence that glioblastoma stem cells occupy a perivascular niche where they are supported by endothelial cells (16). Cutaneous cancer stem cells in a chemical carcinogenesis model have a characteristic location near stromal cells, similar to the location of normal bulge stem cells (13). Leukemia stem cells home to an endothelial niche similar to the HSC niche (39) and their susceptibility to chemotherapy can be modified by matrix and cellular products of the niche (40). Thus in tissues where something is known of the location of somatic stem cells, cancer stem cells appear to occupy a similar physical location, though there is only suggestive evidence for a functional niche that regulates renewal or differentiation of the cancer stem cell (16). Although mouse mammary stem cells have been isolated and a single stem cell shown to reconstitute a mammary gland when implanted in a cleared mammary fat pad (26;27), the location and function of the human mammary stem cell niche has not been identified (41;42).

In general, however, there is a wealth of evidence that the tumor microenvironment instructs tumor cells. Stromal rather than epithelial cells are altered in several hereditary cancer-susceptibility syndromes (43). Stromal fibroblasts are instructive to cancer cells, promoting progression, angiogenesis and metastasis (44;45). Using osteopontin as a signal, aggressive tumors recruit marrow stromal cells to indolent tumors, thereby enhancing their growth and invasiveness (46). Fibroblasts that comprise the tumor cell niche for basal cell carcinomas have a different molecular fingerprint from those in normal stroma (47). This suggests that cancer cells may recruit or activate a stromal cell niche. Whether the supportive functions of fibroblasts, macrophages and endothelial cells (48) are exerted wholly or even partly on stem cells is unknown, however.

Challenges to the Stem Cell Hypothesis

Experimental obstacles make treacherous the interpretation of all the extant data regarding solid tumor stem cells. Progress is greatly hampered by not having true surface markers of stem cells, which, based on xenotransplantation assays, are presumed to make up but a small fraction of the most highly selected cell populations that are available. The surface markers that are used to select cancer stem cells are not always reliable: CD133 is a stem cell marker which has been used to select glioma, pancreatic and colon cancer stem cells, among others, but recent experiments using a CD133 knockin reporter mouse ($CD133^{lacZ/+}$) have shown that CD133 is not stem cell-specific but rather is expressed ubiquitously in colonic epithelial cells, and that aggressive cells in metastases have actually converted from a $CD133^{+}$ to a $CD133^{-}$ phenotype (49). Previous observations that CD133 is a stem cell marker may be explained by cell-specific differences in glycosylation of CD133 that affect its binding to a commercially available antibody.

Assays for self-renewal are highly artificial. Though in a few instances serial transplantation assays for mouse tumor stem cells have been validated in syngeneic mouse strains (11;12), this does not mean that xenotransplantation reliably identifies self-renewing human cancer stem cells, as opposed to selecting for proliferating cells that adapt well to the immunocompromised mouse (10). Tumor-sphere assays of self-renewal are even more artificial. Cells do not survive in mammospheres long enough to be certain that they are indefinitely self-renewing stem cells, rather than proliferating multilineage progenitors which are placed later in the tissue hierarchy. And, if a niche is critical to tumor-initiating cells, what is happening in a mammosphere, where cells are proliferating and giving rise to differentiated offspring without niche elements? In sum, these technical challenges demand a critical and cautious approach to all the extant data on solid tumor stem cells.

A more fundamental challenge is raised by determinations of gene expression patterns by serial analysis of gene expression, which demonstrate that $CD24^{+}$ cells in human breast carcinomas are genetically related to $CD44^{+}$ cells but in some cases have additional mutations (50). These results were interpreted as more consistent with genetic clonal selection, in which tumor

heterogeneity is determined by competition between clones that arise from genetic or epigenetic instability, than with the cancer stem cell hypothesis (14;51). On the other hand, the cancer stem cell hypothesis and the clonal instability hypothesis are not mutually exclusive. It is possible that multiple types of mammary progenitor cells are capable, when transformed, of self-renewal and multipotency (23), and that subsequent genetic or epigenetic events further select their progeny for representation in the final tumor – but in this scenario the notion of a stem cell may no longer be heuristic.

Stem Cells and Metastasis

Because most metastases are clonal outgrowths from a single cell, the pioneer cell that establishes a metastasis would be likely to have the capacity for self-renewal as well as multipotency, and thus be stem cell-like. If the cancer stem cell hypothesis is correct, therefore, it is likely that many or all of the cells that initiate metastases in bone and other tissue sites are cancer stem cells. Although this is an attractive idea, it remains to be proven. There are three lines of evidence to support it.

First, expression of a stem cell gene transcriptional signature in diverse cancers is predictive of metastasis and poor clinical outcomes (50;52-54).

Second, cancer stem cells may express a prometastatic phenotype. Separate lines of work have ascribed two distinct sets of putative properties to the metastatic cell, the stem cell properties of self-renewal and multipotency and the capacity for epithelial-mesenchymal transition. In embryonic life the epithelial-mesenchymal transition (EMT) generates mesodermal cells that give rise to organs and eventually to distinct epithelial populations in these organs, *e.g.*, ovary and kidney, by the reverse process, mesenchymal-epithelial transition (55). A similar EMT program confers a motile and invasive phenotype on cancer cells, thereby enhancing metastasis (56;57).

Mani *et al*. (58) have found that induction of EMT in transformed human mammary epithelial cells results not only in mesenchymal traits but also in stem cell characteristics. Conversely, cells isolated from mammospheres based on stem-like characteristics also express mesenchymal genes, as do stem-like cells isolated from mouse or human mammary epithelial cells. It remains to be shown, however, that mesenchymal properties and “stemness” contribute to metastasis, either separately or in a coordinated fashion. Others have also observed a phenotype of high motility in breast cancer cells with stem cell characteristics (50;59).

A recent report documents the involvement of pancreatic cancer stem cells in liver metastasis (60). Putative pancreatic cancer stem cells are $CD133^{+}$; tumor margins contain a population of $CD133^{+}CXCR4^{+}$ cells. In a pancreatic cancer cell line, $CD133^{+}CXCR4^{+}$ cells have a migratory phenotype and, when tumor cells are injected orthotopically, only $CD133^{+}CXCR4^{+}$ cells are found in portal blood and metastasize to liver. Thus, stem cells metastasize preferentially when they acquire the migratory phenotype that is well-known to be associated with metastasis of breast and prostate cancer (61-63).

Third, it is well known that circulating tumor cells and disseminated tumor cells (DTC) are found in blood or bone marrow, respectively, of patients with diverse cancers, in particular breast and prostate carcinoma, early in the course of the disease at the time of primary tumor resection (64-66). If it is true that pioneer cells in metastases are cancer stem cells, then a population of these DTC should express the stem cell phenotype. One study has found by triple-staining of $cytokeratin^{+}$ DTC from bone marrow of breast cancer patients that all patients had cells with a cancer stem cell phenotype and a mean of 72% of DTC cells were $CD44^{+}CD24^{-/low}$, compared with about 10% of primary tumor cells (67). The prospect of expanding this analysis by isolation of viable circulating or bone marrow cells, *e.g.*, by microfluidic technology (68), and determining their detailed phenotype and stem cell properties is tantalizing and will lead to much more work. Microfluidic technology is reported to

recover an average of 100-200 circulating tumor cells per ml of blood across a wide range of tumor types, with approximately 65% absolute recovery of added tumor cells and about 50% purity (68). The recovered tumor cells can be characterized by immunostaining or by RT-PCR to measure gene expression.

Stem Cells and Dormancy

One of the cardinal features of metastasis is dormancy (69). As discussed in the previous section, tumor cells arrive in bone marrow very early in the course of cancer (64-66). The presence of DTC in blood or bone marrow is predictive of clinical metastasis and is correlated with survival. The long lag between the arrival of tumor cells and the detection of clinical metastasis implies that they undergo a long period of dormancy before awakening to grow and produce clinical disease. Even slow replication would not be compatible with the interval of many years that sometimes passes before metastases are apparent.

In spontaneous and experimental metastasis models, large numbers of solitary tumor cells can be found in distant organs, often in an intravascular location, where they can persist for many weeks (59;69-71). There is little known of the cellular programs associated with long residence in G0/G1 in such cells. Possible mechanisms to explain dormancy in metastasis include a putative angiogenic switch, in which cells or micrometastases are dormant until a blood supply is induced (72;73), immune surveillance and suppression of metastasis (69), or absence of crosstalk with a tumor cell niche (see below).

Quiescence is a property of both somatic stem cells and cancer stem cells, and stem cell quiescence could be the primary mechanism of dormancy in metastasis. Not much is known about mechanisms of quiescence in cancer stem cells. It was noted early on that mouse breast cancers labeled with [3H]-thymidine contained long-term label-retaining cells (74). In a classic experiment, mice exposed to chemical carcinogenesis by painting skin with dimethylbenz(a)anthracene did not manifest skin cancers until they were treated with the tumor promoter TPA one year later (75). Given the rapid turnover of skin epithelial cells, the only plausible candidates as tumor progenitors after this long latency period are cells with stem cell characteristics, either endogenous or acquired. BCR-ABL positive progenitors can persist for many years in CML patients treated with interferon or suppressed with imatinib (76), a kinase inhibitor that blocks the BCR-ABL oncogene. Despite the success of imatinib in CML, the retention of quiescent cells leads to immediate relapse when the drug is stopped, and these relapses are explained by the persistence of stem or progenitor cells, not by drug resistance (although the development of imatinib resistance is an alternative route to relapse). Very little is known of the mechanisms of quiescence in any of these circumstances.

Premetastatic and Metastatic Niches

Several lines of evidence suggest that fibroblasts may serve as components of a premetastatic niche. Mesenchymal stem cells isolated from bone greatly increase proliferation and metastasis of MB-MDA-231 cells (77). A premetastatic niche distant from the primary tumor was defined by experiments in which melanoma cells elaborated factors that upregulate fibronectin expression in resident fibroblasts, which then attract hematopoietic precursor cells that express the receptor for vascular endothelial growth factor, before the arrival of metastatic tumor cells at the site of eventual metastasis (78). This experiment suggests the existence of a complex series of events in which tumor cells prepare a niche for themselves by secreting trophic factors to activate local fibroblasts, then recruit hematopoietic cells from bone marrow. More recent experiments indicate that tumor cells can recruit bone marrow cells to the tumor using osteopontin as a signal (46). It will be important to confirm this complex paradigm in other experimental models.

Bone Metastasis and the Tumor Cell Niche

Metastasis requires tumor cells to invade a basement membrane, intravasate into blood, roll on and adhere to an endothelium, extravasate, and after a period of dormancy, eventually grow as a metastatic tumor. Recovery of circulating tumor cells by microfluidic technology casts these events into perspective by finding hundreds of tumor cells per ml of blood, meaning that 5-10 x 10^5 tumor cells are circulating at any time. Yet despite the large number of tumor cells shed, the development of a metastasis is a rare stochastic event. What then is the rate-limiting step in metastasis? Not invasion or intravasation, considering the number of tumor cells in blood, nor can tumor cells have much trouble finding their way to bone, as they are readily recovered from bone marrow. What remains for the tumor cell is to find a welcoming niche in bone marrow; where might this niche be?

Many parallels between tumor cells and HSC raise the possiblilty that they could occupy a similar niche in bone. HSC occupy an osteoblast-associated niche on the endosteum and in the perivascular space (1). Entry of HSC to their endosteal niche occurs during late fetal life; HSC can also re-enter their niche from the circulation or after bone marrow transplant (79). Among the molecular signposts to the HSC niche are selectins, integrins and the chemokine CXCL12. Selectins constitute a molecular address for the bone marrow; integrins and cytoskeletal controllers such as the GTPase Rac are also essential for engraftment of HSC. Osteoblasts and vascular endothelial cells express the cytokine CXCL12 (SDF1), which is essential to HSC homing: mice lacking either CXCL12 or its receptor CXCR4 on HSC cells have a lethal impairment in hematopoiesis, even though HSC are abundant in the fetal liver. Presumably HSC in transit traverse a perivascular niche to reach their endosteal resting place, but details and molecular signals for this step are unknown. Similarly, prostate cancer cells present an E-selectin ligand in order to roll on bone marrow endothelium (80;81), and both breast and prostate cancer cells use CXCL12 for homing to bone marrow (61;82;83).

The rate of bone remodeling seems to regulate the entry of HSC into the endosteal niche by release of calcium. Fetal mice in which the parathyroid calcium-sensing receptor (CaR) had been inactivated have reduced HSC numbers in bone marrow, and *CaR*(-/-) HSCs display defective homing to the endosteal niche (84). Hence, calcium release from mineralized bone may enhance the engraftment of hematopoietic cells to an osteoblast-associated niche, and this could account for the association of red marrow with sites of active bone remodeling. Breast and prostate cancer cells also express the CaR (85;86) and calcium release could be one mechanism by high bone remodeling states favor bone metastasis (there is good evidence for others, such as release of TGF-β from bone matrix during active remodeling (87)).

Direct imaging of Nalm-6 acute lymphoblastic leukemia cells in the calvarial vasculature has shown that they roll on bone marrow endothelium, attach at specific locations which express E-selectin and CXCL12, and diapedese through the endothelium to a niche that is physically indistinguishable from the location of hematopoietic stem and progenitor cells (39). Other tumor cells – human and murine leukemia cells, multiple myeloma cells and prostate carcinoma cells – behave similarly to Nalm-6 cells. Whether this physical location has the functional properties of a stem/tumor cell niche remains to be shown, but the technique of real time optical sectioning and immunoimaging that was used in these experiments holds great promise for niche characterization.

Summary

The cancer stem cell hypothesis explains many of the properties of human leukemias. It accounts for the fundamental observation that not all leukemia cells can transmit leukemia and explains multilineage leukemia, blast crisis in CML, and resistance to imatinib in CML. The hypothesis that solid tumors are also driven by self-renewing multipotent stem cells has caught the imagination of cancer biologists. Certain well-known properties of solid tumors, such as the long latency of tumor-initiating cells in

animal models, suggest that oncogenic mutations occur within stem cell populations. In numerous instances, cell surface markers can be used to define a population of cancer cells that possesses stem cell properties of self-renewal and multipotency; these populations tend to be highly motile invasive cells with characteristics that have independently been identified as prometastatic. Nonetheless caution is the order of the day. Solid tumor stem cells have yet to be purified; cell surface markers used to identify them are clearly not always reliable; much of what we know about the diversity of tumors such as breast cancer cannot be explained by a single cancer stem cell; and mutations plainly occur in the progeny of putative stem cells, undercutting the notion that all the diverse properties of tumors are determined by their stem cells. As yet the stem cell hypothesis has not generated a fundamental new insight into solid tumor biology.

That is not to deny that the stem cell hypothesis is heuristic. Despite the caveats, it is plainly time to investigate the hypothesis that cancer stem cells are the pioneers that make a bridgehead in bone and, after a long period of latency, give rise to bone metastases. Though they are imperfect, the available tools will allow the preparation of highly enriched stem cell populations for use in standard models of metastasis. Disseminated tumor cells can be isolated from patients for analysis of their stem cell properties. Direct observation of the tumor cell niche, *e.g.*, in mouse calvaria, can identify stem cells in the niche, define the physical and functional properties of the niche, determine whether interactions with the niche confer the stem cell properties of self-renewal and multipotency on cancer cells, and ascertain whether the tumor cell niche overlaps with one or both of the HSC niches in bone. Tumor cell dormancy has been dormant as a topic of study, but models can and should be developed to determine whether the basis of dormancy and reawakening of metastatic cells is derived from a stem cell lineage.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

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