Cell Stem Cell
Minireview

Location, Location, Location: The Cancer Stem Cell Niche

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The existence of a stem cell niche, or physiological microenvironment, consisting of specialized cells that directly and indirectly participate in stem cell regulation has been verified for mammalian adult stem cells in the intestinal, neural, epidermal, and hematopoietic systems. In light of these findings, it has been proposed that a "cancer stem cell niche" also exists and that interactions with this tumor niche may specify a self-renewing population of tumor cells. We discuss emerging data that support the idea of a veritable cancer stem cell niche and propose several models for the relationship between cancer cells and their niches.

The Concept of the Normal Stem Cell Niche

In normal adult tissues, stem cells depend on the integration of both cell-intrinsic and cell-extrinsic factors for proper, homeostatic tissue maintenance. The two cardinal characteristics of a stem cell are the capacity both to self-renew, or make more stem cells, and to differentiate, or give rise to the full repertoire of specialized cells that comprise the tissue in question. Achieving a delicate balance between these two opposing processes is critical in the adult organism for maintaining proper tissue homeostasis and for repair and regeneration of tissues after injury. Excessive differentiation at the expense of self-renewal, for instance, can deplete the stem cell pool, whereas excessive self-renewal could lead to aberrant expansion and even tumorigenesis. In 1978, Schofield proposed the existence of a niche, or specialized location, for hematopoietic stem cells (HSCs) that would serve a key regulator of these two distinct processes (Schofield, 1978). The stem cell niche, then, was envisaged to be a physiological microenvironment consisting of specialized cells that would physically anchor the stem cell and provide the necessary factors to maintain its stemness.

Subsequent studies have shed light on the prominent role of the niche in specifying adult stem cell fate determination. Anchoring stem cells to the niche through cell-cell contacts is critical for physically sequestering stem cells such that they remain both close to niche factors that specify self-renewal and far from differentiation stimuli.

In the *Drosophila* adult testis and ovary, in particular, the anatomical structure of the germline stem cell (GSC) niche is now well-defined. In the *Drosophila* adult testis, for instance, GSCs are positioned directly adjacent to a cluster of postmitotic somatic cells termed the hub (Li and Xie, 2005). The hub secretes proteins that activate JAK-STAT- and BMP-related signaling pathways critical for GSC self-renewal and maintenance. When a male GSC divides, it gives rise to one daughter cell that remains tethered via adherens junctions to the niche, where it receives local signals supporting self-renewal, while the other daughter cell is displaced away from the hub and subsequently initiates differentiation. As in the male GSC niche, the *Drosophila* ovary GSC niche is comprised of inner germarial sheath cells and cap cells that contact GSCs via E-cadherin-mediated cell adhesion. This physical docking of stem cells to the niche is essential for GSC maintenance.

In recent years, niches have also been identified for mammalian stem cells in the intestinal, neural, epidermal, and hematopoietic systems (Li and Xie, 2005). As with *Drosophila* GSCs, mammalian adult stem cell niches regulate cell fate by providing cues in the forms of both cell-cell contacts and secreted factors. Numerous signal molecules have been implicated in niche control of cell fate, including Hedgehog, Wnts, BMPs, fibroblast growth factor (FGF), and Notch. In the skin epidermis, for instance, hair follicle stem cells (HFSCs) responsible for hair follicle and sebaceous gland regeneration are located in a region called the bulge. During the process of hair follicle morphogenesis, HFSCs in the bulge are regulated through spatially and temporally dynamic interactions with a specialized mesenchymal structure called the dermal papilla. The dermal papilla is the source of important signals that regulate the HFSC activity, such as inhibitors of the Wnt and BMP pathways (Moore and Lemischka, 2006). Similarly, the modulation of stem cell activity in the intestine is subject to cues derived from underlying mesenchymal cells that surround the crypt. As in the HFSC niche, the intestinal stem cell niche is comprised in part by mesenchymal cells, in this case pericryptal fibroblasts, that secrete modifiers of the Wnt and BMP signaling pathways.

Importantly, recent work has revealed that the interactions between stem cells and their niches may be more

dynamic than originally believed. Concerted efforts from multiple groups have contributed to a more complete understanding of how the balance between self-renewal and differentiation is maintained for adult HSCs. Some reports have suggested that, rather than being statically associated with one niche, HSCs may occupy two anatomically and physiologically distinct niches, an osteoblast niche and a vascular niche, and shuttle between them (reviewed in Kaplan et al., 2007; Li and Xie, 2005). Furthermore, osteoblasts lining the endosteal surface of the bone (at the bone-hematopoietic interface) may function as the "quiescent niche," whereas the endothelial cells lining bone marrow and spleen sinusoids may comprise the "activated niche" inducing HSC expansion and differentiation. The possible promiscuity and mobilization of HSCs to multiple niches suggests that niches in general may be highly dynamic in nature, which may have important ramifications in the search for niches in cancer, as well.

The Cancer Stem Cell Hypothesis

It has been proposed that tumors arise from a rare population of cells with stem cell properties, often termed cancer stem cells (CaSCs) (Al-Hajj et al., 2003; Lapidot et al., 1994). According to this hypothesis, only a small fraction of cells within certain tumors are tumorigenic—that is, only the CaSCs can produce all of the cells necessary to repopulate a tumor. The bulk of the tumor is comprised of cells that are differentiated and do not harbor tumorigenic potential. In this nascent field, some confusion has arisen due to the semantics involved; to many, the use of the term cancer "stem cell" carries the implication that cells in the tumorigenic fraction harbor all properties of normal stem cells. Yet, true multipotency and asymmetric division of CaSCs has yet to be rigorously demonstrated in most solid tissues. Therefore, some investigators have advocated the use of a different term, such as "tumor-initiating cell" rather than cancer stem cell, to describe the subset of cells with tumorigenic potential (Hill and Perris, 2007).

Like normal stem cells, CaSCs would be marked by their ability both to self-renew and to differentiate to specialized cell types with limited proliferative potential. Both properties of CaSCs have been tested with limiting dilution and serial transplantation experiments. Evidence for the existence of CaSCs began in the hematopoietic system with the 1994 demonstration that only a subset of cells from human acute myeloid leukemia (AML) patients were able to engraft in severe combined immunodeficiency disease (SCID) recipient mice (Lapidot et al., 1994). These presumptive leukemic stem cells (LSCs) were prospectively isolated and determined to have a $CD34^+CD38^-$ phenotype. Their frequency was rare (approximately 1 in 250,000 cells). Later studies used lineage-tracing to show that a single LSC could give rise to the repertoire of populations of leukemia cells (Hope et al., 2004).

The isolation of CaSCs has also been reported from various human solid tumors. The subpopulation of breast cancer cells that were $CD44^+CD24^{-/low}$ were described as breast CaSCs based on the ability to regenerate tumors serially from eight out of nine patients when transplanted into the mammary fat pads of nonobese diabetic/SCID mice (Al-Hajj et al., 2003). While tens of thousands of the non-CaSC fraction were unable to propagate tumors in this system, as few as 100 of the breast CaSCs could give rise to tumors that phenotypically resembled the original tumor. The prospective isolation of brain CaSCs was suggested when only $CD133^+$ brain tumor cells were able to propagate xenografted tumors in NOD/SCID mice that closely resembled the original (Singh et al., 2004). Although initiated by cells that were $CD133^+$, the resulting tumors contained a mixture of cells of both $CD133^+$ and $CD133^-$, indicating that the CaSCs may be able to give rise to differentiated cells. In colon cancer, $CD133^+$ cells (approximately 2.5% of the population), but not $CD133^-$ cells, were able to give rise to tumors when transplanted subcutaneously into NOD/SCID mice or in renal capsule xenografts (Ricci-Vitiani et al., 2007). It is important to note that, while markers such as CD44 or CD133 may enrich for tumor-initiating cells in breast or colon cancer, these molecules are not expressed exclusively by tumor cells but also by various normal cells in the tissue. Thus, the true cancer-initiating cells in a given organ are only a subset of the sorted cells. Additional markers should allow increased specificity for improved identification and separation. Another important issue to address will be the phenotypic stability of this tumor-initiating subset over time. How does CaSCs gene expression change over the course of tumorigenesis or during the shift from growth in vivo to experimentation in vitro?

A bewildering issue for stem cells, whether in normal tissues or in cancer, is their frequency. Is the stem cell number fixed, or is it dependent on isolation methods and the microenvironment in which cells find themselves? A case in point is the number of mammary stem cells. Anything from 200,000 cells to as few as 50 unsorted cells (when transplanted in Matrigel [Moraes et al., 2007]) and even one cell from a stem cell-enriched population can reconstitute a complete mammary ductal tree, depending on the detailed method of transplant. This has led to wildly diverging estimates of 1/5000–1/50 for the frequency of mammary stem cells. Is it context and environment? Can any cell be converted to a stem cell if the microenvironment or niche is right, or is a special phenotype required? If the same is true of tumors, then the need for the usual inoculum of 500,000 cells in transplantation experiments may represent our inability to create the niche required for tumor take, rather than the presence of a rare tumor-repopulating cell.

A Cancer Stem Cell Niche?

In light of the significant role of the normal stem cell niche in controlling fate determination, it has been proposed that a "CaSC niche" exists and that interactions with this tumor niche may have a similar role in specifying a self-renewing population of tumor cells. Increasing evidence has emerged that factors derived from the tumor microenvironment serve to regulate cancer cells. Genetic studies

of inherited cancer-susceptibility syndromes have shown that stromal cells are altered in a subset of disorders (Howe et al., 1998). Sustained expression in vivo in the mammary gland of stromelysin-1/matrix metalloproteinase-3 (MMP3), a stromal enzyme that destroys basement membrane, can lead to epithelial tumorigenesis (Sternlicht et al., 1999). In addition, damage to surrounding stromal cells can influence the corresponding epithelial cells toward a neoplastic state. Irradiation of mammary stroma, for instance, promotes tumorigenesis of unirradiated epithelial cells (Barcellos-Hoff and Ravani, 2000). Similarly, carcinoma-derived (but not normal) prostate fibroblasts stimulate tumor progression in prostate epithelial cells (Olumi et al., 1999).

Similarities between the normal stem cell niche and the tumor microenvironment continue to be uncovered. In basal cell carcinoma of the skin and in diverse other solid tumors, fibroblasts that comprise the tumor cell niche are, indeed, molecularly distinct from those that comprise the normal stroma (Sneddon et al., 2006). In a striking parallel to normal stem cell biology, cells that comprise the tumor niche produce some of the same molecular factors (e.g., BMP antagonists) that are produced by the normal stem cell niche to maintain the stem cell pool. Genomic profiling revealed that, unlike their normal counterparts, tumor-associated dermal fibroblasts express high levels of the secreted BMP antagonist *GREMLIN 1*. In contrast, the basal cell carcinoma cells themselves express BMP2 and BMP4. Gremlin 1 protein supports the basal cell carcinoma cells in a less differentiated, more expansive state ex vivo, suggesting that expression of secreted BMP antagonists by tumor-associated stromal cells may promote self-renewal of tumor cells in vivo.

While evidence for an instructive role of the tumor microenvironment has been promulgated for years, however, evidence for an anatomically and/or physiologically specialized environment that constitutes a true CaSC niche is still in its infancy. Nevertheless, data have begun to emerge that support the idea of a veritable CaSC niche. Recently, for instance, brain cancer stem cells were visualized to live in a vascular niche that secretes factors that promote their long-term growth and self-renewal (Calabrese et al., 2007). Increasing the number of endothelial cells in brain tumor xenografts expands the proportion of self-renewing cells in the tumor and also hastens tumor initiation and growth. Disrupting this niche impairs brain cancer stem cell self-renewal, thereby significantly inhibiting tumor growth—providing some support for the theory that targeting the unique aberrant microenvironment of CaSCs may be a critical aspect of effective cancer therapy. As a cautionary note, however, evidence from HIF-1α-deficient astrocytomas has suggested that when brain tumors are unable to induce angiogenesis, they successfully adapt to this disadvantage by migrating along existing normal blood vessels to propagate (Blouw et al., 2003).

Work in the hematopoietic system suggests a possible role for the niche in regulating CaSC maintenance. Specialized microenvironments of bone marrow endothelial cells appear to be required for the homing and engraftment of both normal HSCs and leukemic cells (Sipkins et al., 2005). Moreover, both extracellular matrix (ECM) components and signaling molecules in the HSC microenvironment can promote cell survival in AML, providing resistance to chemotherapeutic treatments (De Toni et al., 2006). An important unresolved question about CaSC niches, if they exist, is whether there are the equivalents of both the quiescent and active niches, as is the case for normal HSCs. If so, then it may be the relative time spent in one versus the other, or alternatively the availability of the quiescent versus activated niches, that distinguishes cancer from the normal case. Interestingly, loss of the quiescent niche for normal HSC results in myeloproliferative disease. The possibility of multiple CaSC niches should be kept in mind for future studies. Indeed, if there are distinct CaSC niches that specify dormancy versus expansion, the molecular cues that distinguish the two will be critical to our understanding of how to therapeutically target CaSCs. If cues from the microenvironment do indeed regulate CaSC activity, this information will be crucial in deciphering results from experimentation of CaSCs in vitro. For instance, if stemness is a function of microenvironment, then the properties of CaSCs may vary significantly depending on the in vitro context in which we interrogate them; this would have important implications for establishing appropriate ex vivo model systems for studying CaSCs.

There are several possible models for CaSC-niche interactions (Figure 1). The CaSC may not require a distinct niche for expansion and may instead be capable of surviving in the normal stem cell niche (Figure 1A). Alternatively, a distinct CaSC niche may be necessary for activation. CaSCs may be dependent on the pre-existence of a favorable niche for expansion (Figure 1B). Just as with normal stem cells, the niche may be important for maintaining asymmetric division of CaSCs and for tethering CaSCs close to signals that maintain stem-like properties. Instead, CaSCs may be capable of providing signals that instruct an otherwise quiescent niche to become activated, effectively hijacking the niche (Figure 1C). Signals from the CaSCs could result in amplification of an activated niche that already exists (possibly at low frequency), permitting further expansion of the tumor (Figure 1D). Alternatively, CaSCs may be niche independent (Figure 1E). That is, they may have acquired the ability to provide themselves with the necessary factors for expansion and self-renewal—processes that would otherwise normally be restricted by the niche. Lastly, there may be a discrete niche that is inhibitory for CaSCs, providing factors that induce differentiation or death (Figure 1F).

The Metastatic Niche

For metastatic spread to occur, tumor cells must reduce cell-cell contacts and gain migration to distant sites. Accordingly, a number of the same signals appear to underlie activation and mobilization of normal HSC as well as cancer cell invasion and metastasis (Kaplan et al., 2005). For instance, chemokines and their receptors are important

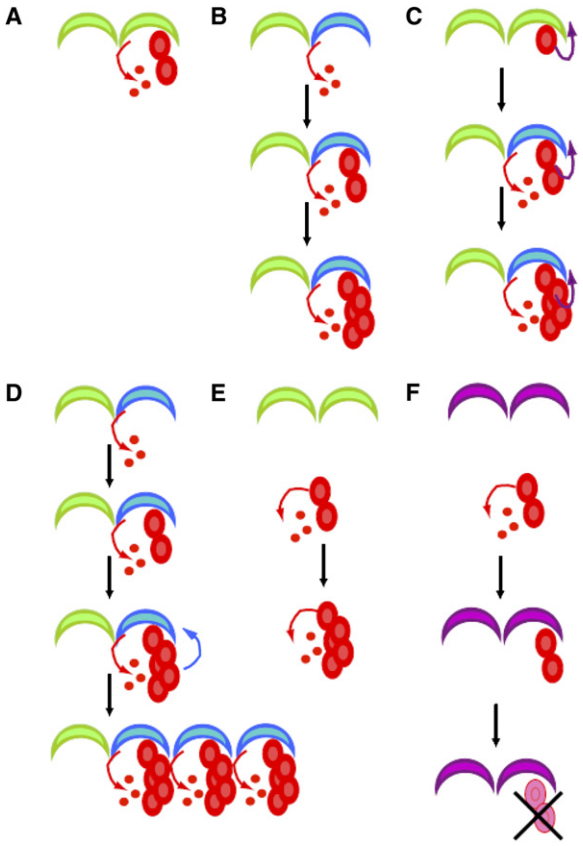

Figure 1. Models for the Relationship between Cancer Stem Cells and Their Niches

An activated niche providing necessary factors (shown as red circles) for expansion and self-renewal is depicted in blue, whereas a quiescent niche lacking such factors is depicted in green.

(A) Normal and CaSC niches may be one and the same. CaSCs may be activated in response to cues from the normal stem cell niche.

(B) An activated niche may precede the advent of the CaSC. CaSCs may be dependent on the pre-existence of a favorable niche for expansion.

(C) CaSCs may provide signals (purple arrow) that instruct an otherwise quiescent niche to become activated.

(D) Signals from the CaSCs (blue arrow) may result in amplification of the activated niche, permitting further expansion of the tumor.

(E) CaSCs may be niche independent. That is, they may acquire the ability to cell-autonomously provide the necessary factors for expansion and self-renewal that are normally restricted by the niche.

(F) An inhibitory niche, shown in purple, may exist as distinct from the normal or CaSC-activating niches. In this case, signals from the niche shut down the CaSCs, inducing differentiation or death.

in mediating leukocyte trafficking, but they may also have a role in specifying the metastatic destination of breast tumor cells. The chemokine receptors CXCR4 and CCR7 are expressed at high levels in malignant breast tumors and in metastatic lesions, and their cognate ligands CXCL12/SDF-1α and CCL21 are expressed in organs that are the first sites of breast cancer metastasis. In the bone marrow, MMP9 is important for the recruitment of and mobilization of hematopoietic stem and progenitor cells from the quiescent bone marrow niche to the proliferative niche. Similarly, in tumors, there is mounting evidence that various MMPs play roles in tumor invasion and metastasis. MMP9 is induced in clusters of premetastatic lung endothelial cells through VEGF Receptor 1 (VEGFR1) signaling from distant primary tumors (reviewed in Kaplan et al., 2007).

As noted earlier, adhesion molecules are necessary for anchorage of stem cells to the niche; they also mediate homing of circulating hematopoietic progenitor cells. The ligands fibronectin and VCAM are expressed on newly forming blood vessels and are recognized via integrin $\alpha4\beta1$ expressed on progenitor cells (Jin et al., 2006). Thus, adhesion molecules facilitate homing of normal hematopoietic progenitor cells to neovasculature. In addition, however, they are involved in processes that are necessary for invasion of metastatic tumor cells, such as loss of cell-cell adhesion and gain of cell motility (Christofori, 2006). Similarly, integrins are required for migration of normal HSCs and have also been associated with migration of tumor cells.

The ability of a tumor to metastasize may depend first on the tumor cells (possibly the CaSCs) acquiring the propensity of stem cells to wander from niche to niche and second on the ability of the cancer cells to establish distant niches that are hospitable for local occupancy. The concept of a "premetastatic niche" is supported by work demonstrating that VEGFR1-expressing bone marrow-derived hematopoietic progenitor cells are directed to sites of future metastasis by factors secreted by the primary tumor cells (Kaplan et al., 2005). Thus, in response to signals from the tumor cells, bone marrow-derived cells (BMDCs) colonize the premetastatic niche before metastatic tumor cells have arrived. Interestingly, these niches, like normal niches, are marked by ECM components such as fibronectin. When niche components are disturbed (by treating with blocking antibodies against VEGFR1, for instance, or by depleting VEGFR1+ cells from the bone marrow), tumor metastasis can be prevented, pointing to potential functional significance of the niche in creating a permissive environment for successful dissemination. Together with the phenomenon of tissue tropisms during metastasis, these lines of evidence point to a possible role for cancer cell-niche interactions in guiding metastasis.

Therapeutic Implications and Future Work

The feasibility of targeting the CaSC niche therapeutically will depend, in part, on the degree of similarity between the normal and CaSC niches. If the factors that promote survival and proliferation are redundant in both contexts, then targeting niche-derived signals could also affect normal stem cell pools. As key distinguishing features are identified, however, therapies aimed at leveraging the differences between the normal and CaSC niches will be vital for therapeutic purposes. The successful depletion of leukemia-initiating cells, but not normal HSCs, in a mouse model of myeloproliferative disease has provided some support for the notion that selective targeting of CaSCs may be possible (Yilmaz et al., 2006).

Toward this end, an important step in the CaSC field will be the ongoing identification of additional markers that provide even more specific isolation and characterization of CaSCs, particularly in solid tissues. Of particular utility

will be the development of markers that can be used for localization and visualization of CaSCs in situ, as this is bound to facilitate anatomical localization of the niche as well. Ideally, markers that allow for sorting niche cells will also be developed, although this will likely be a significant task given the complexity of the niche—comprising fibroblastic cells, myeloid and other inflammatory cells, endothelial and perivascular cells (or their progenitors), and ECM components.

As CaSCs continue to be better characterized and the components of their niche identified, functional studies will be crucial for understanding the contribution of defined molecular constituents to CaSC physiology. In vivo models and ex vivo systems should prove useful in systematically characterizing the intricate molecular language of cell-cell communication in the cancer niche.

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