Cancer Stem Cell Niche: The Place to Be

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Abstract

Tumors are being increasingly perceived as abnormal organs that, in many respects, recapitulate the outgrowth and differentiation patterns of normal tissues. In line with this idea is the observation that only a small fraction of tumor cells is capable of initiating a new tumor. Because of the features that these cells share with somatic stem cells, they have been termed cancer stem cells (CSC). Normal stem cells reside in a "stem cell niche" that maintains them in a stem-like state. Recent data suggest that CSCs also rely on a similar niche, dubbed the "CSC niche," which controls their self-renewal and differentiation. Moreover, CSCs can be generated by the microenvironment through induction of CSC features in more differentiated tumor cells. In addition to a role in CSC maintenance, the microenvironment is hypothesized to be involved in metastasis by induction of the epithelial-mesenchymal transition, leading to dissemination and invasion of tumor cells. The localization of secondary tumors also seems to be orchestrated by the microenvironment, which is suggested to form a premetastatic niche. Thus, the microenvironment seems to be of crucial importance for primary tumor growth as well as metastasis formation. Combined with its role in the protection of CSCs against genotoxic insults, these data strongly put forward the niche as an important target for novel therapies. Cancer Res; 71(3); 1–6. ©2011 AACR.

Introduction

It is becoming increasingly clear that tumors are hierarchically organized heterogeneous populations of cells with the cancer stem cell (CSC) compartment on top. This fraction of tumor cells shares many similarities with normal stem cells, such as self-renewing capacity and multilineage differentiation properties (1). In addition, CSCs are highly tumorigenic and can generate a serially transplantable phenotype of the primary human malignancy in immunocompromised mice (1). From a clinical point of view, the main concern with CSCs is their resistance to conventional treatments, a feature suggested to be the underlying cause of tumor recurrence (2, 3). Thus, it is necessary to define the factors that sustain CSCs in order to develop more efficient therapeutics. Normal stem cells reside in the distinct environment called the "stem cell niche." The niche regulates stemness, proliferation, and apoptosis resistance of stem cells. It has a complex architecture and is composed of diverse stromal cells, such as mesenchymal and immune cells, a vascular network, soluble factors, and extracellular matrix components. Analogously, tumorigenicity not only involves the biology of tumor cells themselves but also results from a rather complex interplay between tumor cells and the nonmalignant cells that make up the tumor environment. Like normal stem cells, CSCs seem to depend on a similar, permissive environment, the CSC niche, to retain their exclusive abilities to self-renew and give rise to more differentiated progenitor cells, while staying in an undifferentiated state themselves (5). Moreover, the CSC niche also has a protective role. By sheltering CSCs from diverse genotoxic insults, the niche contributes to their enhanced therapy resistance (6, 7). Here, we discuss the concept of the stem cell niche in a tumor setting, with special emphasis on brain and colon cancer as examples of malignancies in which CSCs seem to rely on a specialized microenvironment. Furthermore, we review the role of the tumor microenvironment in the progression of primary tumors, focusing on dedifferentiation of non-CSCs and epithelial-mesenchymal transition (EMT) induction by the niche and, finally, the involvement of a premetastatic niche in the formation of metastasis (Fig. 1).

The Perivascular Niche in Glioblastoma Multiforme

In the adult mammalian brain, neural stem cells reside in the hippocampus and subventricular zone, close to the blood vessels. During embryogenesis and early brain development, ventricular neuroectoderm secretes high levels of vascular endothelial growth factor (VEGF), which attracts and stimulates vessel growth in this region of the brain (8). Thus, vascular endothelial cells and neural stem cells come together during early development and stay in close proximity throughout life. Protein ligands found within the neural stem cell niche, such as pigment epithelium–derived factor and stem cell factor, have been implicated in both stem cell self-renewal and regulation of angiogenesis, also suggesting that these two
processes are tightly linked (9–12). More direct evidence for the role of endothelial cells in neural stem cell biology comes from the observation that these cells regulate asymmetrical division of the stem cells in the subventricular zone (13). Furthermore, they maintain neural stem cell self-renewal, in part via Notch signaling, and simultaneously inhibit stem cell differentiation (4).

Glioblastoma multiforme (GBM) is the most aggressive type of primary brain tumor in humans, with high morbidity and median survival of less then a year. Recently, a CSC fraction was identified in these tumors. These glioma-initiating cells seem to have a higher DNA repair rate and resistance to treatment than more differentiated glioma cells, making them the main suspects for tumor regrowth after therapy (2). GBM is a highly vascularized tumor, which led to the speculation that GBM CSCs might depend on a similar niche as neural stem cells. Indeed, recent publications have shown the validity of this concept. Calabrese and colleagues showed the existence of a close relationship between brain CSCs and blood vessels and, furthermore, that the vascular endothelial cells are able to maintain patient-derived brain tumor cells in a stemlike state and promote their tumorigenicity when coinfected in immunocompromised mice (5). A soluble factor mediating this interaction and promoting CSC self-renewal seems to be nitric oxide produced by endothelial cells, which activates the Notch pathway in glioma CSCs (14). However, the interaction between CSCs and tumor vasculature is likely a more complex and bidirectional process. Brain tumor CSCs are able to promote recruitment and formation of blood vessels by secreting VEGF (15). Furthermore, inhibition of angiogenesis and depletion of blood vessels by the VEGF-neutralizing antibody bevacizumab reduced the CSC pool and, subsequently, inhibited tumor growth (5). Moreover, tumor cells protect their niche and, vice versa, the vascular microenvironment contributes to enhanced therapy resistance of GBM CSCs. Accordingly, glioma cells induce upregulation of different survival genes in endothelial cells, protecting them from hypoxia or irradiation-induced apoptosis (16, 17).

Figure 1. The CSC niche in tumor growth and metastasis. The CSC niche is composed of blood vessels (red), stromal cells such as myofibroblasts (orange), and extracellular matrix components. Tumors are organized in such a way that CSCs (purple) reside close to their niche. In addition to maintaining CSCs in a stemlike state, (1), the niche has the ability to dedifferentiate nontumorigenic cells (blue) into tumorigenic CSCs (purple) and, (2), to induce the EMT, leading to dissemination of tumor cells from the primary tumor and, (3), seeding at the metastatic place. Furthermore, tumor cell engraftment in different organs is suggested to be facilitated by the formation of a premetastatic niche that potentially enables the initiation and outgrowth of secondary tumors.
Reciprocally, inhibition of Notch signaling in an explant system of surgical GBM specimens leads to detachment of CSCs from their vascular niche and increased efficacy of radiotherapy on CSCs (6). Similarly, application of antiangiogenic therapy to gliomas and eradication of tumor vasculature results in a higher susceptibility of CSCs to cytotoxic agents (7). Although these data sound promising, they should be viewed with caution as the long-term effect of antiangiogenic therapy on tumor growth and the final outcome of the treatment is still not fully known. A recent publication even suggested that anti-VEGF treatment eventually leads to increased invasiveness and metastasis of the tumor (18). One of the desired consequences of anti-VEGF treatment could be an increase in hypoxic areas, which are described to be refractory to therapy. Being located far from blood vessels, hypoxic tumor cells are usually exposed to relatively low concentrations of chemotherapeutics. Furthermore, cytotoxic drugs mainly target proliferating cells and, therefore, do not harm hypoxic tumor cells that are usually quiescent due to a lack of oxygen and nutrients (19).

In addition, hypoxia also creates problems for radiotherapy, as it reduces the formation of oxygen free radicals that, as a byproduct of radiotherapy, would normally induce DNA damage in tumor cells (20). Hypoxia also has more direct cellular effects, as it has been linked to regulating cell survival, enhanced motility and invasiveness of tumor cells, and tumor angiogenesis. Moreover, hypoxic regions are related to the areas of pseudopallidating necrosis, another hallmark of GBM, and have been proposed to also form a GBM CSC niche, in addition to tumor vasculature (21). GBM CSCs are frequently found to be located at the edges of necrotic regions. Indeed, similar to normal neural stem cells, it has also been shown that hypoxia regulates GBM CSC maintenance (21, 22). The effects of hypoxia are mainly mediated by hypoxia-inducible factors (HIF), of which HIF2α is of particular importance for the GBM CSC pool, increasing their self-renewal and tumorigenic capacity (21). Not surprisingly, HIF2α expression correlates with the poor survival of GBM patients. Combined, these data clearly put forward the role of brain tumor vasculature and hypoxia in the maintenance of CSCs, as well as their therapy resistance; however, further investigations are needed to successfully apply this knowledge in treating GBM patients.

The Stem Cell Niche in Colorectal Cancer

Another example of an extensively studied stem cell niche exists in the intestine. The intestinal crypt is the functional unit of the intestinal tract, including colon and small intestine. Stem cells reside in the bottom region of the crypt, within a stem cell niche composed of epithelial cells and mesenchymal cells of the myofibroblast lineage that line the crypt. Moving upwards, progenitor cells start to differentiate and once they reach the top, they are shed into the lumen of intestine and die. Current data show that the Wnt signaling cascade is a prominent force controlling cell proliferation, differentiation, and apoptosis along the crypt–villus axis and in maintaining stem cell fate (23). The absence of Wnt signaling activity in Tcf4−/− mice or its inhibition by transgenic Dickkopf-1 expression leads to complete loss of crypts in adult mice (23). BMP signaling has opposite effects and drives the cells toward differentiation (24). A fine-tuned balance between these and other signaling pathways maintains the intestinal homeostasis and this is partially directed by the intestinal stem cell niche. Myofibroblasts that line the crypt produce Wnt ligands, together with BMP antagonists such as gremlin 1/2, which, in combination, are involved in preservation of the stem cell pool (25, 26).

A similar model has been suggested to delineate the colon CSC interaction with their microenvironment. Studies of heritable juvenile polyposis syndrome highlighted the importance of tumor stroma for the development of colorectal cancer. One of the features of the gastrointestinal polyps seen in Apc-Smad4 mutant heterozygous mice is increased proliferation of stromal cells. It is also one of the characteristics of juvenile polyps seen in humans and predisposes for development of carcinomas that arise from epithelial cells. In addition, a connection between inflammatory processes and colon cancer is well established. For example, it has been described in models of colitis-associated cancer, as well as in other forms of colorectal cancer, that interleukin 6 secreted mainly by the infiltrating immune cells stimulates proliferation of tumor-initiating intestinal cells (27). Analogously to the normal intestinal stem cell niche, myofibroblasts and mesenchymal stem cells are shown to be components of the colon cancer stroma. Mesenchymal stem cells have the ability to enhance growth and metastasis of colon cancer and have been, furthermore, proposed to give rise to fibroblasts that further promote tumorigenesis (28, 29). Mutations in the APC gene, an inhibitor of Wnt signaling, are early events in the transition of healthy colon mucosa toward colon carcinoma, resulting in stabilization of β-catenin and subsequent translocation to the nucleus. In the nucleus, β-catenin binds to T-cell factor/lymphoid enhancer factor (TCF/LEF) family members and acts as a transcriptional regulator of many genes that control proliferation and differentiation. However, despite the same genetic background, cells within the tumors display differential Wnt activity, judging by the localization of β-catenin, which is referred to as the so-called β-catenin paradox (30, 31). It indicates that, besides APC mutations, additional regulatory mechanisms of Wnt activity are at play in these tumors. One possible explanation could be additional KRAS mutations. According to Phelps and colleagues, APC mutations primarily play a role in stabilizing the levels of β-catenin in the cytoplasm, whereas additional mutations in KRAS are necessary for translocation of β-catenin to the nucleus (32). In addition to these cell-intrinsic events, the Wnt pathway also seems to be regulated by the tumor microenvironment. Accordingly, cells harboring nuclear β-catenin are mainly clustered at the invasive front of the tumor in regions highly populated with myofibroblasts (30, 31). How does this finding relate to colon CSCs? Tumor-associated myofibroblasts (TAF) are a major cellular component of colon cancer stroma. They have higher proliferation rates than normal fibroblasts and are the primary source of type I collagen, shown to promote a stem cell–like phenotype in colorectal carcinoma cell lines (33). The importance of TAFs in tumorigenesis is supported by data showing...
that depletion of TAFs through CD8+ T-cell–mediated killing significantly reduced tumor growth and metastasis in, among others, colon cancer (34). In addition, efficacy of chemotherapy was improved, implying that TAFs are also involved in tumor chemoresistance. Recently, we reported that high Wnt activity levels mark the colon CSC population and are orchestrated by myofibroblasts residing in the tumor microenvironment (35). More precisely, we found that hepatocyte growth factor (HGF) produced by myofibroblasts is capable of enhancing Wnt signaling activity in colon CSCs, suggesting a strong link between the microenvironment and CSC features in colorectal cancer as well.

**Cancer Stem Cells, Dedifferentiation, and the Epithelial-Mesenchymal Transition**

Intriguingly, the effects of the microenvironment on CSCs are beyond just preservation and protection of this compartment. We observed that HGF-producing myofibroblasts were able to dedifferentiate nontumorigenic tumor cells into more immature cells by reactivating the Wnt pathway. These dedifferentiated cancer cells displayed all characteristics of CSCs, including expression of stem cell–associated genes, such as LGR5, and high tumorigenic potential (35). Our experiments imply that the tumor microenvironment, more specifically the CSC niche, is capable of inducing a CSC phenotype in differentiated tumor cells.

What would be the consequences of an ongoing process of dedifferentiation in malignancies? Cancer cells possess a certain level of plasticity that allows them to change their phenotype and acquire different functions and properties under the influence of the environment. Processes that reflect their plasticity are the EMT and its reverse, the mesenchymal-epithelial transition, highly conserved programs that are involved in embryonic development but also in carcinogenesis (36). EMT is one of the crucial, early steps in the invasion–metastasis cascade and has been associated with poor clinical outcome of patients in many types of tumors. Epithelial tumor cells that undergo EMT lose cell–cell adhesion properties and polarity and acquire a more mesenchymal-like phenotype, including motility, invasiveness, and increased resistance to apoptosis. Importantly, tumor cells undergoing EMT acquire CSC-like features as can be concluded from experiments in which induction of EMT in immortalized human mammary epithelial cells led to the expression of CSC markers, increased self-renewal capacity, and enhanced tumor formation (37). EMT, thus, provides disseminated tumor cells with self-renewing properties and increased proliferative capacity, enhancing their chances to seed at a distant site and grow metastases. Considering the fact that the EMT promotes the generation of a CSC phenotype, it is crucial to know how this process is controlled and regulated. A variety of signals that can induce the EMT, such as hypoxia, are being received from the tumor microenvironment. Hypoxia was shown to regulate the plasticity of GBM cells as well. Heddleston and colleagues found hypoxia to promote the self-renewal capability and stem cell phenotype in the non–stem cell population, increasing their neurosphere-forming capacity and upregulating important stem cell factors, such as OCT4, NANOG, and c-MYC (38). Furthermore, expression of HIF2α in the non–stem cell population enhanced their tumorigenic potential. It should be noted, though, that, in addition to regulating cell plasticity, hypoxia, and other EMT-inducing factors from the microenvironment, such as TGF-β, are also known to stimulate proliferation and expansion of the preexisting CSC pool, thereby further increasing the chances for metastatic spread (37, 39, 40). One of the mechanisms of hypoxia-induced invasiveness is the activation of the Wnt signaling pathway via inhibition of glycogen synthase kinase β, which leads to the induction of the key EMT-inducing transcription factor Snail (41). Expression of SNAIL protein was detected at the tumor–stroma interface in diverse human cancers including colon cancer (42). Correspondingly, it was mentioned previously that colorectal cancer cells with nuclear β-catenin, a marker of colon CSCs on one hand and an inducer of EMT on the other, mostly reside at the host–tumor interface (30). Intriguingly, HGF, which we have described as being able to induce CSC properties in differentiated colon cancer cells, was used to induce cell scattering of MDCK cells in the initial studies on EMT (43). The ability of reacquiring stem cell features in the more differentiated cells by HGF-producing myofibroblasts puts these cells at the crucial position in the EMT-CSC framework.

**The Premetastatic Niche**

We have described how TAFs are involved in maintenance of CSCs and the induction of CSC features in more differentiated tumor cells, potentially via mechanisms related to EMT. Moreover, we have put forward how this model provides an elegant explanation for the intimate connection between CSC features, the EMT, and tumor cell invasion. However, the microenvironment might also be implicated in the final steps of the metastatic cascade. It is known that metastases selectively occur in certain organs such as lungs, liver, brain, and bones. This observation led to the so-called seed and soil hypothesis. According to this hypothesis, the local microenvironment of these organs seems to be more receptive to disseminated tumor cells from particular malignancies than other organs. Thus, occurrence of metastasis does not happen randomly, but disseminated tumor cells need to meet a hospitable microenvironment in order to initiate a secondary tumor. Furthermore, in recent years, evidence suggested that the primary tumor itself is actively involved in adapting these so-called premetastatic niches for tumor cells to come, by secreting systemic factors and directing bone marrow–derived cells and macrophages to certain tissues, thereby priming certain tissues for tumor cell engraftment (44, 45). Accordingly, VEGFR1-positive bone marrow–derived hematopoietic progenitor cells (HPC) were shown to localize to premetastatic sites and form clusters before the arrival of tumor cells (44). Eradication of these cells from the bone marrow prevents the formation of premetastatic clusters and, subsequently, tumor metastasis. In addition to homing of HPCs, preexisting fibroblasts are noted to increase fibronectin deposition on these sites, which most likely binds to VLA4, a
fibronectin receptor expressed on HPCs, and facilitates accumulation of these cells. Furthermore, activated fibroblasts were shown to induce remodeling of stroma required for liver metastasis in a murine melanoma model (46). Thus, in addition to their contribution to the CSC niche at the primary tumor site, fibroblasts are suggested to have a critical role in premetastasis niche formation as well.

Conclusions

In conclusion, the reviewed data point out a central role of the CSC niche in virtually every step of the tumorigenic cascade (Fig. 1). In primary tumors, the CSC niche is an important regulator of stemness. The importance of this interaction is supported by the fact that the loss of a niche environment mainly leads to the loss of CSCs. The reliance of CSCs on niche signals seems to be a general phenomenon and has been shown in a whole variety of different tumors. In addition to maintaining the CSC pool and supporting the growth of primary tumors, the niche plays a role in reverting nontumorigenic cells into CSCs by processes related to the EMT, leading to tumor invasion and dissemination. Finally, the putative premetastatic niche supposedly assures successful homing of cancer cells to distant organs and the development of metastasis. Whether premetastatic niches are also capable of installing a CSC phenotype in more differentiated cells or whether these particular environments are only capable of maintaining the function of metastasized CSCs is still a matter of speculation. Either way, the supporting role of the microenvironment in tumor growth and progression, including metastasis formation, clearly puts the CSC niche and, especially, the mediators of this interaction in the spotlight as future therapeutic targets. Emerging therapies are already targeting this strategy. For example, we previously highlighted the importance of HGF production by the microenvironment in colorectal cancer. HGF acts via the tyrosine kinase receptor MET on cancer cells and triggers its downstream targets. Recently, it has been shown that anti-MET antibodies prevent HGF binding to MET and, subsequently, inhibit colon cancer tumor growth (47). This example is only one of the extensive studies that explore the modulation of the interaction between cancer cells and niche cells as a therapeutic strategy that could lead to major advances in cancer treatment in the years to come.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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