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CANCER STEM CELLS (BO WILLIAMS, SECTION EDITOR)

Dynamic Interactions Between Cancer Stem Cells and Their Stromal Partners

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Abstract The cancer stem cell (CSC) paradigm presumes the existence of self-renewing cancer cells capable of regenerating all tumor compartments and exhibiting stem cell-associated phenotypes. Recent interpretations of the CSC hypothesis envision stemness as a dynamic trait of tumor-initiating cells rather than a defined and unique cell type. Bidirectional crosstalk between the tumor microenvironment and the cancer bulk is well-described in the literature, and the tumor-associated stroma, vasculature, and immune infiltrate have all been implicated as direct contributors to tumor development. These non-neoplastic cell types have also been shown to organize specific niches within the tumor bulk, where they can control the intratumor CSC content and alter the fate of CSCs and tumor progenitors during tumorigenesis to acquire phenotypic features for invasion, metastasis, and dormancy. Despite the complexity of the tumor–stroma interactome, novel therapeutic approaches envision combining tumor-ablative treatment with manipulation of the tumor microenvironment. We will review the currently available literature that provides clues about the complex cellular network that regulates the CSC phenotype and its niches during tumor progression.

Keywords Cancer stem cells · Tumor-initiating cells · Tumor microenvironment · Mesenchymal stem/stromal cells · Tumor progression

Abbreviations

AML	Acute myeloid leukemia
BM-MSC	Bone marrow-derived mesenchymal stem cell
CML	Chronic myeloid leukemia
CSC	Cancer stem cell
ECM	Extracellular matrix
EMT	Epithelial-to-mesenchymal transition
ESC	Embryonic stem cell
Hh	Hedgehog
HIF	Hypoxia-inducible factor
HGF	Hepatocyte growth factor
HSC	Hematopoietic stem cell
iPSC	Induced pluripotent stem cell
MSC	Mesenchymal stem/stromal cell
PDGF	Platelet-derived growth factor
PDGFR	Platelet derived growth factor-receptor
TAF	Tumor-associated fibroblast
TAM	Tumor-associated macrophage
VEGF	Vascular endothelial growth factor

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VEGFR2 Vascular endothelial growth fact receptor 2
VM Vascular mimicry

Introduction

Most cancers are characterized by marked phenotypic and functional heterogeneity within the tumor bulk that can result from the accumulation of intrinsic (genetic and epigenetic) insults and extrinsic signals from the microenvironment [1]. Despite the absence of comprehensive organization among all cancer types, several mechanisms have been postulated to model the acquisition of intratumor cellular heterogeneity, including the clonal evolution theory [2] and the cancer stem cell (CSC) hypothesis [3]. The latter has become increasingly popular after the identification of defined tumor subsets endowed with tumorigenic activity and exhibiting phenotypic features of normal stem cells [4]. Although the existence of tumor cells displaying CSC features has been well-described in the literature for a number of cancers, no single CSC phenotype can be generalized to all cancers, and several distinct populations within a unique tumor may display CSC features [5•]. In tumors that incorporate cells having a CSC phenotype, the CSC compartment concentrates most of the tumor-initiating activity and has also been implicated in tumor progression, invasion, and metastasis [5•]. Due to their propensity to exhibit metabolic and transport activities usually associated with normal stem cells, CSCs represent an attractive culprit for the augmented radio- and chemotherapy resistance that plagues cancer recurrence. However, the evolution of the CSC phenotype accompanying distinct steps of tumor progression has not been clearly established. The acquisition of CSC features by non-CSC subsets has been described in a number of studies, mostly involving cancer cell lines. Dedifferentiation has been especially proposed to be a possible feature of metastasis and relapse [6]. Metastatic CSCs display distinct properties that separate them from CSCs detected in primary tumors, including long-term self-renewal [7] or heightened chemoresistance [8], and the expression of CXCR4 has also been used to differentiate pancreatic CSCs having metastatic potential [9]. The contribution of microenvironmental cues to cancer progression is well-described in the literature [10], and the identification of several niches within the tumor microenvironment revealed interactions between stromal, vascular, or immune populations and CSCs that influence the fate of the CSC compartment during tumor progression (Fig. 1). Here, we will review the recent literature pertaining to the interactions between CSCs and niche-resident stromal cells, and we will discuss their complex crosstalk, including its incidence for possible therapeutics.

Experimental Designs to study CSC–Stroma Interactions

The tumor microenvironment is heterogeneous (including stroma, vasculature, and inflammatory cells) and recruited cells often display an activated phenotype upon interactions with tumor cells to augment their pro-tumorigenic activities. Thus, the study of interactions between putative CSCs and the stromal microenvironment remains challenging, due to high heterogeneity and variability in both cellular compartments. In vivo recapitulation of interactions between human CSCs and their niche is typically attempted using immunodeficient rodent models [4]. Modulation of the microenvironment can be achieved using transgenic animals, orthotopic transplantation, or co-injection of stromal cells and engineered niches. Alteration of the medullar hematopoietic niche using transgenic immunodeficient animal strains was used to evaluate the effects of unbalanced hematopoietic cytokines on the fate of $CD34^+$ leukemic CSCs [11]. Co-injection of basement membrane matrix protein has been shown to support tumor initiation and growth of putative CSCs [12]. The site of injection has also been reported to be of critical importance and can modify the frequency of tumor-initiating cells that can be measured in these assays. Mammary fat pads were the most reliable site of injection to study the tumor-initiation potential of ovarian tumors [13], and represents an orthotopic injection site for breast CSC studies [14•]. Orthotopic transplantation assays via the co-injection of human tumor and stromal cells to a humanized microenvironment is gaining acceptance [4], but most models involve a single component, either endothelial [15, 16] or stromal [17]. Finally, current CSC sorting strategies from primary isolates often do not distinguish CSC subsets or CSCs from non-CSC tumor-initiating progenitors. Putative breast CSCs were originally identified by their $CD44^+CD24^-$ phenotype. Further refinement of the breast tumorigenic compartment can be achieved using additional markers such as CD90 [18], or by including CSC-associated phenotypes such as multidrug resistance (MDR) transporter expression and activity [19] or cellular size via light scatter properties [14•, 18]. A small, resting CSC-like phenotype was associated with a tumorigenic activity at low doses without requirement for stromal support, whereas larger progenitor-like cells either required injection of a larger number of cells or the presence of supportive stroma to retain tumor-initiating activity [14•].

Current Approach to the CSC Paradigm

The modern interpretation of the CSC hypothesis was formulated over a decade ago following studies on acute

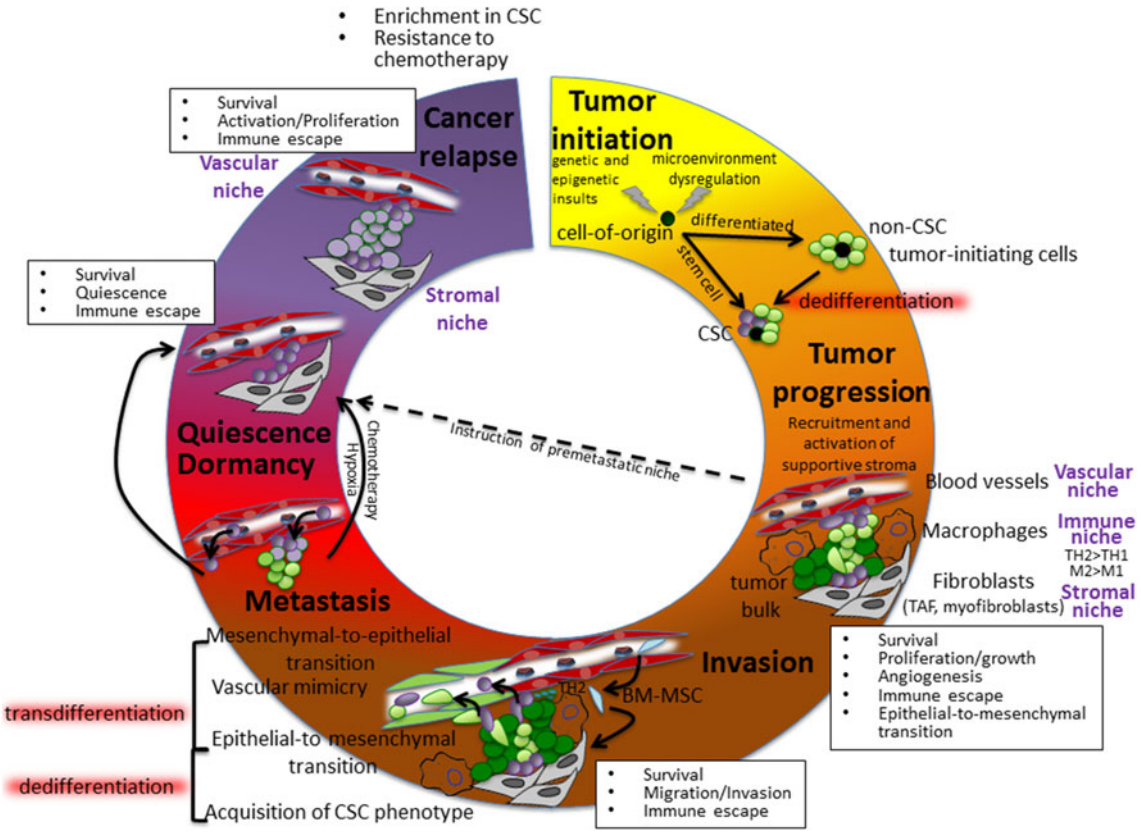

Fig. 1 Evolving cancer stem cell–niche interactions during tumor progression. The tumor cell-of origin may initially display CSC features or CSCs may appear during tumor progression. Complex interactions between all components of the microenvironment and CSCs organize distinct niches that govern tumor proliferation, immune escape, invasion, metastasis, dormancy, and cancer relapse. CSCs have been located in close relationship with two distinct niches: the stroma and the vasculature. Both niches have been shown to play a critical role in regulating CSC phenotypes and initiate invasive, metastatic, or dormant behaviors. The immune infiltrate also plays critical roles, modulating these niches or directly interacting with CSCs. Circulating BM-MSCs can be recruited at the primary tumor site, where they can contribute both directly and indirectly to the primary tumor niche or can participate in the establishment of the metastatic niche. CSC transdifferentiation has been suggested, based on CSC acquisition of endothelial (vascular mimicry) or mesenchymal (epithelial-to-mesenchymal/mesenchymal-to-epithelial transitions) traits, to support tumor growth or invasiveness. The involvement of the microenvironment in the possible dedifferentiation of non-CSCs to a CSC phenotype has also been suggested. The acquisition of CSC features is often partially reminiscent of embryonic phenotypes and a possible dedifferentiation process may involve signaling routes exploited by induced pluripotency. The metastatic process is highly inefficient, but instruction of a premetastatic niche by the primary tumor and acquisition of a CSC phenotype by invasive cells may favor survival and engraftment of circulating cancer cells in secondary niches. While the osteoblast niche seems to regulate the fate of leukemic CSCs for cancer relapse, the vascular niche has been involved in the establishment and exit of breast cancer dormancy

myeloid leukemia, AML [5•]. Other putative CSC subsets were subsequently identified in a variety of solid tumors [5•]. The identification of rare, self-renewing cancer cell subsets capable of serially generating heterogeneous tumors in xenograft models and the convergence of signaling pathways that are dysregulated during oncogenesis and that govern the self-renewal/differentiation fate of normal stem cells has led to the hypothesis that CSCs in primary tumors may arise from the transformation of normal stem cells, or alternatively via the acquisition of stem cell features (i.e., self-renewal) by more differentiated cells harboring genetic or epigenetic insults (Fig. 1). The initial concept of a clonogenic tumor-initiating CSC atop heterogeneous cancer cell progenies was extrapolated from the hierarchical differentiation model of the hematopoietic system, a unidirectional differentiation scheme in which self-renewing multipotent stem cells give rise to pools of proliferating intermediate progenitor cells and ultimately all mature cell types. Most recent iterations to define CSCs have emphasized the inherent complexity and fluctuation of the CSC compartment within a unique tumor and have embraced a definition of the CSC phenotype as a dynamic cell state rather than a distinct cell type [1, 5•, 6, 20]. Beyond the clonogenic properties of CSCs (self-renewal and differentiation/tumorigenicity), numerous analogies have been made between putative CSCs and their normal counterparts to identify them within the heterogeneous tumor bulk, including surface marker expression, cell cycle

state, migratory properties, immune escape, or metabolic and transporter activities [5•, 6]. Recent studies have exposed that CSCs may also arise from the dedifferentiation of more differentiated tumor cells [21], after receiving specific signals from the local microenvironment (Fig. 1). Data obtained in the Donnenberg Laboratory using clinical isolates seems to support such a scenario. Unlike small CSC-like, MDR+, breast cancer-initiating cells, high-light scatter MDR–$CD90^+$ breast cancer progenitors exhibit tumorigenic activity only when injected at high cell numbers [19] or when co-injected with adipose-derived stromal cells [14•]. Yet, these progenitors were shown to be able to generate tumors that recapitulated the heterogeneity of original patient tumors, including the rare, small, resting CSC population. These observations support the plasticity of tumor initiating cells and highlight the link between tumorigenicity, expression of mesenchymal associated markers, and stromal interactions.

Tumor Microenvironment Cellular Components

The tumor microenvironment consists of an extra-cellular matrix (ECM) and multiple cell types. The tumor ECM mainly results from the extravasation of plasma proteins and dense deposits of collagen delivered by the fibrotic component. The cellular components include a substantial inflammatory infiltrate (i.e., macrophages, dentritic cells, and T-cells), which is reminiscent of chronic inflammatory and fibrotic lesions and has now become an attractive target for the development of anti-cancer immunomodulating therapies [22]. Tumor-associated macrophages, TAMs, often accumulate in hypoxic areas and can support angiogenesis via the release of proangiogenic factors (e.g., vascular endothelial growth factor, VEGF) sequestered in the ECM, or can facilitate revascularization via the release of metalloproteinases [23]. TAMs have been shown to interact with CSCs in several cancers, including breast, hepatocellular, and colon carcinomas and gliomas [24–27]. Under hypoxic conditions, glioma CSCs can inhibit TAM phagocytosis, as well as T cell proliferation and activation via STAT3 signaling [28]. Although the tumor microenvironment is often considered to promote tumorigenicity by inhibition of the innate and adaptive responses [22], including dendritic cell maturation and subsequent antigen presentation [29], immune cells such as follicular dendritic cells can directly support the maintenance of the tumorigenic CSC state, as occurs in some cases of therapy-resistant follicular lymphoma [30]. The microenvironment also incorporates tumor-associated fibroblasts (TAFs), vascular cells, and local or recruited progenitors [bone marrow-derived mesenchymal stem cells (BM-MSCs), endothelial progenitors (EPC)]. TAFs display an activated phenotype that often resembles myofibroblasts and secrete a battery of growth factors and cytokines at the primary site of the tumor to support both cancer cell proliferation and survival [31]. TAFs not only directly regulate tumor growth, but can also support local angiogenesis via the recruitment of endothelial progenitors [32]. Possible crosstalk between CSCs and myofibroblasts is supported by their close localization at the invasive front of epithelial tumors [18, 33•]. Interactions between CSCs and vascular lineages are particularly prominent in highly vascularized brain tumors [34, 35], but have also been reported to govern the metastatic activities of dormant breast cancer cells [36•]. The perivascular niche of glioblastoma tumors can self-regulate a tumor's growth in a loop fashion in which CSCs stimulate local angiogenesis by releasing paracrine factors and endothelial cells control migratory and tumorigenic CSC activities [35]. The effects of BM-MSCs on tumor cells have been reviewed in [37, 38]. Large numbers of BM-MSCs can be mobilized and recruited to the local microenvironment via the release of endocrine and paracrine signals during tumor development. Both pro- and anti-tumorigenic activities of BM-MSCs have been acknowledged in the literature [37, 38]. BM-MSCs interact with all other stroma–resident populations. They can replenish intratumor TAFs via differentiation, regulate local angiogenesis, and modulate innate immunity via interactions with macrophages [37, 38]. Several studies have suggested that MSCs can contribute to the acquisition of a CSC phenotype by non-CSC tumor cells or support epithelial-to-mesenchymal transition (EMT) leading to invasion and metastasis [37, 39].

The CSC Niche

Stem cells reside in a specific microenvironment (or niche) that can regulate their self-renewal and differentiation. A similar niche concept has been extrapolated to cancer in which microenvironmental cues regulate the CSC fate during tumor development [40].

Quiescent hematopoietic stem cells (HSCs) reside in an osteoblastic niche, although HSC can also occupy a vascular niche within the sinusoidal endothelium [41•]. Assuming leukemia–CSCs occupy similar niches in the BM, several studies have investigated the effects of microenvironment modulation on the fate of cancer cells. The injection of leukemia–CSCs into transgenic animals revealed an instructive role of the microenvironment [11]. Adhesion signals involving the glycoprotein CD44 seem to play a critical role in leukemia–CSC–niche interactions, as the disruption of CD44 signaling altered the myeloproliferative and homing activities of chronic myeloid leukemia (CML)- [42] and AML- [43] CSCs. Disturbance of the

osteoblastic niche has direct repercussions on leukemia–CSCs. The activation of osteoblasts via Dicer1 deletion can induce myelodysplasia and secondary leukemia [44]. Similarly, modulation of the osteoblastic niche via parathyroid hormone signaling can alter the myeloproliferative activity of leukemia–CSCs [45•], although in the latter, bone remodeling resulted in increased transforming growth factor beta (TGF-β) release by medullar osteoblasts, which was detrimental to the myeloproliferative neoplasia and engraftment of CML cells. The same approach improved engraftment and tumorigenicity of AML–CSCs [45•]. TGF-β signaling had been previously implicated in the maintenance of a CSC phenotype in CML cells [46]. Yet, in this study, in vitro inhibition of TGF-β actually impaired the colony-forming ability of CML cells, and the combination of TGF-β, SCF, and Foxo3a inhibition depleted the CML in vivo. While the results obtained in these studies are contradictory, they confirm a pivotal role of TGF-β signaling in the regulation of the leukemia–CSC niche. The diverging effects of TGF-β signaling on CSC activities probably reflect the complexity of the interplay between CSCs and their cellular partners in the niche.

In highly vascularized brain tumors such as gliomas, CSCs are tightly regulated by the tumor endothelium [34]. Brain CSCs are chemoattracted towards endothelial cells in vitro and their tumorigenicity in animal models can be enhanced via the co-injection of endothelial cells [34]. Niche–glioma CSC interactions have been shown to be bidirectional [35, 47]. Other CSC niches can be observed at the invasive front of epithelial tumors [18, 33•] where stromal cells are suspected to control tumor invasion [6, 48]. The high density of myofibroblasts at the tumor–stroma interface in colon cancer coincides with an enrichment of tumor cells with high nuclear expression of β-catenin, which is mediated by the myofibroblast secretion of hepatocyte growth factor, HGF [33•]. Similarly, a population of $CD44^{+}CD90^{+}$ CSCs has been shown to reside in direct contact with a layer of $CD90^{+}$ stromal cells at the periphery of invasive nests and trabeculae observed throughout breast tumors [18], supporting the possible regulation of the invasive phenotype of breast CSCs by the adjacent stroma.

Tumor Initiation and CSC Pool Regulation

The acquisition of CSC features by tumor cells upon interaction with the microenvironment has been reported for a variety of cancers. Restricted leukemia progenitors can reexpress a CSC phenotype via the reactivation of self-renewal programs [49]. Hedgehog (Hh) signaling seems to be essential for the maintenance of leukemia–CSCs [50, 51], possibly involving activation of β-catenin signaling [52]. Recently, stromal cells have been shown to modulate Hh signaling and proliferation in myeloid neoplasms via expression of the Hh-interacting protein [53]. Induction of Hh signaling in epithelial cancers upon interaction with TAMs has also been reported [25].

Stroma-mediated regulation of the CSC phenotype is well-described in epithelial tumors. Cancer-associated MSCs were shown to rely on altered BMP production to regulate ovarian CSCs and their tumorigenesis [54]. Similarly, pancreatic stromal cells can enhance the CSC phenotype in pancreatic cancer cells [55] and promote their self-renewal and invasiveness [56]. Infiltrating immune cells also exert control over the CSC pool. The secretion of interleukin-6 (IL-6) by innate immune cells stimulates the proliferation of colon CSCs [57]. IL-6 was also found to enhance the conversion of breast cancer progenitors to a CSC phenotype via a positive feedback loop involving NFκb, Lin28, and Let7miRNA [58], and was identified among other TAM-secreted cytokines as an inducer of tumor-initiating capacity and chemotherapy resistance in colon and lung cancer cells [25].

TAFs were shown to promote a CSC phenotype in colorectal carcinoma cells via the production of collagen type I [59], but can also induce a CSC phenotype in non-tumorigenic cancer cells via a reactivation of the Wnt pathway and HGF signaling [33•]. Wnt signaling has also been proposed to be essential to maintaining a CSC phenotype in epidermal tumors [60]. Wnt activation by the surrounding microenvironment and HGF signaling seem to be redundant mechanisms to promote tumor activation [33•, 52, 61–63]. HGF promoted a CSC phenotype in various cell lines [64, 65] and is implicated in the acquisition of an invasive phenotype via EMT [66]. Gastric TAFs were suggested to exploit another EMT-related growth factor (i.e., TGF-β) to regulate CSC content [67].

Low physiological oxygen favors the acquisition of CSC features in various cancer cells including glioblastoma [68] and ovarian cancer cell lines [69]. Hypoxic conditions increased expression of the ABC transporter ABCG2 in ovarian CSCs [61] and acquisition of the pro-inflammatory phenotype by breast CSCs via Wnt signaling [62]. Hypoxic and acidic microenvironments potentiate the maintenance or the acquisition of CSC features via the induction of hypoxia inducible factor 2α, HIF2α [35, 47]. HIF2α expression promoted the local release of angiogenic factors [35] and acquisition of a CSC phenotype [47, 70, 71] that was marked by an upregulation of stem cell-associated networks such as the pluripotency-associated factors OCT4, NANOG, or c-MYC. A similar acquisition of human embryonic stem cell (ESC) markers in various cancer lines following hypoxia was recently reported [72]. Tumorigenicity shares many features with pluripotency and induced pluripotency, exploiting factors that are known

oncogenes (MYC) or are commonly detected in tumors (NANOG, SOX2, OCT4) [73]. Non-tumorigenic mammary cells and differentiated populations of luminal-like breast cancer cells can acquire a CSC phenotype using cellular reprogramming [74•, 75•]. Interestingly, hypoxia also enhances the efficiency of induced pluripotent stem cell (iPSC) generation from mouse embryonic fibroblast and human dermal fibroblasts [76], suggesting that it may play a critical role in dedifferentiation. The Zambidis Laboratory has derived human iPSC lines using a methodology involving both low oxygen and microenvironmental stroma-priming that dramatically enhanced cellular reprogramming of myeloid progenitors to pluripotency [77•]. BM-MSC-secreted factors that were active during the progenitor reprogramming included known MSC-released cytokines such as platelet-derived growth factor (PDGF), CCL2, and IL-6, which have already been implicated in the acquisition of CSC features. For example, CCL2 has been shown to mediate crosstalk between cancer cells and stromal fibroblasts that augments the CSC phenotype and self-renewal of breast cancer cell lines [78]. BM-MSC secretion of IL-6 has also been suggested to modulate the CSC content of breast cancer [79]. In another study, BM-MSC secretion of IL-6, CCL5, and IL-8 resulted in the activation of β-catenin/Wnt signaling in various cancer cell lines and the promotion of a CSC phenotype [80].

Vascular Regulation

Bidirectional crosstalk between CSCs and vascular cells has been demonstrated in the perivascular niche of highly vascularized tumors (i.e., glioblastoma). Local endothelial cells support retention of the stem cell phenotype and tumorigenicity by CSCs [34], while glioma CSCs closely promote local angiogenesis through the release of VEGF and stromal-derived factor 1 [34, 81–84]. Glioma CSC self-renewal has been shown to be mediated by activation of the Notch pathway following the release of nitric oxide by endothelial cells [85]. Glioma CSCs not only promote recruitment and expansion of the local vascular network by releasing VEGF [81, 86], but also protect vascular cells from hypoxia and irradiation-induced apoptosis [87, 88]. Skin carcinoma CSCs have also been shown to populate a vascular niche [89•]. Both niches seem to resolve upon an autocrine VEGF loop that regulates both CSC and niche self-renewal [89•, 90•]. Glioblastoma CSCs can also transdifferentiate into vascular cells and contribute to the microvasculature via a process termed vascular mimicry, VM [91, 92]. VM is a new pattern for tumor vascularization involving the formation by tumor cells of highly patterned vascular channels (Fig. 1) that has been observed primarily in aggressive types of cancer [93]. Although these tumor tubes are deprived of endothelial cells, they include a basement membrane and have been demonstrated to anastomose to the vasculature [93].

Invasion and Metastasis Regulation

Numerous components of the tumor microenvironment have been implicated in local and distal dissemination of tumor cells [48]. Stroma–tumor interactions have been shown to promote acquisition by CSCs of an invasive phenotype in various cancers including pancreatic [94] and bladder [95] carcinomas. A population of CSCs is observed at the invasive front of epithelial cancers [18, 33•]. The breach of the basement membrane by carcinoma cells facilitates tumor–stroma interactions and recruitment of circulating stromal components (i.e., immune cells, EPCs, MSCs). TAMs can participate in the acquisition of an invasive phenotype by CSCs. While bidirectional interactions between carcinoma CSCs and macrophages can modulate metastasis [26], TAMs and resident microglia can also regulate the invasive phenotype of glioma CSCs via TGF-β signaling [27]. Invasion is often accompanied by a transdifferentiation process (Fig. 1), termed EMT. Epithelial cancer cells that undergo EMT exhibit mesenchymal features (loss of polarized epithelial morphology and acquisition of spindle shape) that favor motility, invasiveness, and survival [96]. Carcinogenic EMT is a critical step for invasion/metastasis that is partially reminiscent to embryonic developmental programs [39]. EMT is often associated with dedifferentiation and the acquisition of CSC features (Fig. 1). Induction of EMT in normal, immortalized human mammary epithelial cells led to augmented expression of CSC markers, self-renewal capacity, and tumor-initiating activity [97].

Cancer EMT can be triggered by various factors including HGF, PDGF, and TGF-β [66]. Hypoxia can regulate the expression of EMT-associated genes [98] and promote the acquisition of an invasive phenotype via the activation of Wnt signaling in breast, colon, hepatic, and pancreatic cancer cell lines [99]. HGF secretion by myofibroblasts at the invasive front in colorectal cancer can induce a CSC phenotype in non-tumorigenic cancer cells [33•]. In lung adenocarcinoma, putative CSCs expressing cytokeratin and the EMT-associated markers CD44 and -90 are rare in primary tumors, but prevalent in metastatic pleural effusions [100].

Metastasis does not occur randomly, and recent studies suggest that primary tumors can instruct the microenvironment of distant organs to develop premetastatic niches (Fig. 1) [101]. Premetastatic plasma and BM-MSCs of advanced breast cancer patients facilitate the transendothelial migration of breast cancer cell lines and may

participate in the remodeling of the BM prior to colonization by cancer cells [102]. Other cell types, including tumor-associated T-cells, can participate in the organization of the premetastatic niche [103]. A reciprocal role of the metastatic niche in the control of CSC content and invasive phenotype at the primary site of tumor has also been suggested. Peritoneal mesothelial cells contributed to the acquisition of a CSC phenotype and invasive phenotype by ovarian cancer cells via SDF1–CXCR4 signaling [104]. Periostin, a component of tumor ECM, was found to be critical for CSC colonization of a metastatic niche [105•]. Periostin deposition by stromal fibroblasts was induced in the secondary target organ upon interactions with infiltrating CSCs [105•]. Local stroma–resident populations can also accompany metastatic cancer cells to facilitate their engraftment at distal niches. Pancreatic stellate cells were shown to intravasate/extravasate to and from blood vessels and to accompany metastatic pancreatic cells to distant metastatic nodules where they stimulate angiogenesis [106].

Metastatic CSCs specifically migrate and incorporate into a suitable niche [101] where they can potentially lay dormant until reactivation by niche signals (Fig. 1). Although cancer dormancy is not a defining hallmark of CSCs, significant phenotypic overlap between dormant cancer cells and CSCs (quiescence, radio- and chemotherapy resistance, immune escape, response to angiogenic factors) suggest at least an overlap of both phenotypes [107]. The local microenvironment has been proposed to play a critical role in the establishment and maintenance of cancer dormancy [108]. Activation of osteoblasts can disrupt the local niche and induce myelodysplasia and secondary leukemia [44]. The perivascular niche has recently been implicated in the regulation of breast tumor dormancy [36•]. Using engineered microvascular niches, the authors determined the factors critical to maintaining dormancy or promoting reactivation of disseminated tumorigenic cells. Secretion of thrombospondin-1 by the vasculature was critical to sustaining cancer cell quiescence. Inversely, active angiogenesis resulted in a sprouting neovasculature and release of TGF-β and periostin, leading to micrometastatic outgrowth. A vascularized "inhibitory niche" was also reconstructed ex vivo using stromal cell lines and umbilical cord vascular endothelial cells, and shown to support a resting state in breast cancer cell lines [109].

Conclusions: Therapeutic Perspectives

Many of the aspects of tumor progression and resistance to treatment result from the interplay between the neoplastic cells and the surrounding non-malignant stroma [110]. The tumor–stroma has become an attractive target for anti-cancer therapies due to its global contribution to tumorigenesis and direct interactions with therapy-refractory CSCs. Tumors in which interactions between CSC and vascular cells are closely regulated are possible targets for anti-angiogenesis strategies. For example, anti-angiogenic treatment was shown to decrease the glioblastoma CSC content, resulting in reduced overall tumor growth and higher sensitivity to cytotoxic agents [81, 111]. In another study, the use of inhibitors of VEGF receptor 2 (VEGFR2) and PDGF-receptor (PDGFR)-β targeted both endothelium and pericytes, resulting in diminished vascular supply of glioma tumors [31]. Disruption of Notch signaling in glioblastoma CSCs resulted in higher sensitivity to radiotherapy by disrupting the attachment of CSCs to their vascular niche [112]. Yet, inhibition of local angiogenesis in other models elicited increased invasiveness and metastasis [113] and created a local hypoxic niche, which could also result in the expansion of a radioresistant CSC phenotype [70]. In colon cancer, CSCs have been shown to display resistance to antiangiogenic therapy [114]. Other therapies envision augmenting the local vasculature to facilitate delivery of chemotherapeutic agents. Coadministration of gemcitabine and IPI-926, a drug that depletes tumor-associated stromal cells via inhibition of Hh signaling, produced a transient increase in intratumor vascular density and the intratumor concentration of gemcitabine, leading to temporary stabilization of the disease [115].

Targeted strategies against non-endothelial contributors to the tumor microenvironment have also been investigated. TAF depletion via T-cell-mediated killing was shown to lead to a significant reduction of tumor growth and metastasis in colon cancer, as well as improved chemotherapy efficacy [116]. Anti-HGF signaling treatments (anti-MET antibodies) prevented colon cancer tumor growth in vivo [117]. A number of strategies have been targeted to microenvironmental support to invasion and metastasis. Disruption of the CXCR4–CXCL12 axis has proven to affect the migratory properties of leukemia [118–120], follicular lymphoma [30], and colon [121] CSCs. Combined blockade of CXCR4 and dacarbazine treatment efficiently inhibited tumor growth and metastasis in a chemoresistant melanoma CSC model by modifying the lymphatic microenvironment [122]. MT1-MMP and MMP9 targeting has been proposed to reduce CSC content and the invasive phenotype in medulloblastoma [123]. CCL2 targeting disrupted CSC–TAF interactions and reduced tumorigenesis [78]. Metformin has been shown to specifically target CSCs in several cancers including glioblastoma [124] and ovarian carcinoma [125]. Combination therapy including metformin and a stroma-targeting, smoothened inhibitor was able to reduce pancreatic tumor CSC content and affect their proinvasion phenotype [126]. Finally, some approaches rely on microenvironment

manipulation to disrupt dormancy and target chemoresistant CSCs. The induction of oxidative stress disrupted the quiescence of leukemia–CSCs, leading to their entry into cycling and significant sensitivity to cytosine arabinoside and apoptosis [127]. Overall, an improved understanding of the interactions between CSCs and their specific niches during tumor progression has the potential to reveal new ways in which to target radio- and chemoresistant CSC populations that are often selected during cancer recurrence.

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Compliance with Ethics Guidelines

Conflict of Interest Tea Soon Park, Vera S. Donnenberg, Albert D. Donnenberg, Elias T. Zambidis, and Ludovic Zimmerlin declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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