Title
Invasion and metastasis—recent advances and future challenges

Permalink
https://escholarship.org/uc/item/3bg6q4ft

Authors
Chambers, AF
Werb, Z

Publication Date
2015-03-15

DOI
10.1007/s00109-015-1269-z

Peer reviewed
Introduction

Cancer cells within individual tumors often exist in distinct phenotypic states that differ in functional attributes. Within this tumor heterogeneity, cancer stem cells (CSCs) are tumor cells that have the principal properties of self-renewal, clonal tumor initiation capacity, and clonal long-term repopulation potential (Clarke et al., 2006; Nguyen et al., 2012). They also display plasticity by reversibly transitioning between stem and non-stem cell states. CSCs have the ability to evade cell death and metastasize, although they may stay dormant for long periods of time (Kreso et al., 2013). Both experimental models and clinical studies indicate that CSCs survive many commonly employed cancer therapies (Kreso and Dick, 2014). They can self-renew their own population and have long-term clonal propagation capacity so they can generate short-lived tumor heterogeneity along with genetic diversity and epigenetic modifications within tumor cells (Hanahan and Coussens, 2012). Non-CSC tumor cells are also part of the CSC niche. During the progression of tumors to a more malignant state, the CSC state in the primary tumor depends crucially on the TME and potentially on the CSC progeny with self-limited proliferative capacity (Kreso and Dick, 2014). Due to the self-renewal capacity, CSCs represent a biologically distinct subset within the total cancer cell population. According to this model, carcinogenesis occurs when a stem cell escapes regulation and gives rise to a stem-cell-like counterpart, a CSC. CSCs represent a distinct population that can be isolated from the remainder of the tumor cells. They can self-renew their own population and have long-term clone-propagating capacity so they can generate short-lived progeny with self-limited proliferative capacity (Kreso and Dick, 2014). The TME contributes to tumor heterogeneity along with genetic diversity and epigenetic modifications within tumor cells (Kreso and Dick, 2014). Two models, hierarchical and stochastic, have been used to understand tumor progression and heterogeneity. Although they differentially consider the weight that CSCs and their niches carry in driving a particular tumor, these two models are not mutually exclusive, and the concept of cellular plasticity unifies them into one model.

Models of Tumorigenesis, CSC Plasticity, and the Role of the CSC Niche

It has long been postulated that intratumoral heterogeneity contributes to disease progression, impacts therapeutic efficacy, and therefore affects patient survival (Hanahan and Weinberg, 2011). The TME contributes to tumor heterogeneity along with genetic diversity and epigenetic modifications within tumor cells (Kreso and Dick, 2014). Two models, hierarchical and stochastic, have been used to understand tumor progression and heterogeneity. Although they differentially consider the weight that CSCs and their niches carry in driving a particular tumor, these two models are not mutually exclusive, and the concept of cellular plasticity unifies them into one model.

The Hierarchical Model

The hierarchical model designates malignant tumor-propagating cells as CSCs (Figure 1). It relies on the paradigm that CSCs represent a biologically distinct subset within the total cancer cell population. According to this model, carcinogenesis occurs when a stem cell escapes regulation and gives rise to a stem-cell-like counterpart, a CSC. CSCs represent a distinct population that can be isolated from the remainder of the tumor cells.
In solid tumors, CSCs were first shown in breast cancer, as they were particularly efficient in establishing tumors upon their isolation from the tumor bulk and their transplantation into mice (Al-Hajj et al., 2003). Since then, the existence of CSCs has been shown in various cancers including various hematopoietic, head and neck, prostate, lung, brain, colon, skin, and pancreatic cancers, as well as in sarcomas (reviewed in Kreso and Dick, 2014; Oskarsson et al., 2014).

Given that cancer is characterized by proliferation and expansion yielding tissues that do not anatomically or functionally resemble the original organ, self-renewal, proliferation, and differentiation are most likely deregulated in CSCs. Indeed, the majority of evidence indicates that CSCs in most solid tumors lack true multipotency and asymmetric cell division and can only differentiate into a single type of descendant cancer cell that is unable to generate an entire array of lineages (Kreso and Dick, 2014). Consequently, some investigators have advocated the use of the term “tumor-initiating cell” (TIC), rather than CSC, to describe the subset of cells with tumorigenic potential (Hill and Perris, 2007). Although the TIC and the CSC have been used interchangeably, the TIC more appropriately denotes the cell of origin. Importantly, the hierarchical model assumes that the CSC is the cell of origin (i.e., the first abnormal cell that initiates the tumor). However, and as explained later, due to cellular plasticity, the cell of origin is not necessarily the CSC—that is, the cellular subset within the tumor that uniquely sustains primary and metastatic tumor growth. Therefore, the phenotype and characteristic gene-expression patterns of the cell of origin may differ substantially from that of the CSC (Chaffer and Weinberg, 2015).

According to the hierarchical model, the same CSC or different sets of CSCs can give rise to different cancer subtypes within a certain organ or tissue (Visvader and Lindeman, 2008), which results in the cellular heterogeneity of tumors. Those distinct subclones develop in a hierarchical fashion with their own CSCs. However, the major limitation of this model is that it conceptually precludes the interchange between differentiated and stem-like...
states within the same cell (Kreso et al., 2013). Nevertheless, it accommodates the possibility that CSCs, like their normal counterparts, may retain responsiveness to and even dependence on external cues to elicit their intrinsically determined potentialities for survival, growth, and differentiation, irrespective of how perturbed the process of differentiation may be.

**The Stochastic Model**

The stochastic model states that every cell within a tumor is equally likely to be the cell of origin and facilitate tumor initiation and progression (Figure 1). The variable activities of tumor cells are only partially determined by the environment in which the cells are found, but rather are determined by some stochastically varying intrinsic factors (Quail et al., 2012). The stochastic model relies on the premise that cancer is a disease defined by hyperproliferation and sequential acquisition of genetic mutations in cell-cycle genes that contribute to subsequent clonal expansions in an otherwise relatively quiescent normal adult somatic cell. Indeed, advanced genome sequencing has demonstrated that cancer within a single patient is a heterogeneous mixture of genetically distinct sub-clones that arise through branching evolution (Greas and Maley, 2012; Burrell et al., 2013) and seed different parts of a single tumor (Gerlinger et al., 2012). Although mutational burden is highly variable across tumor types (Lawrence et al., 2013), a typical tumor contains two to eight driver mutations that regulate three core cellular processes: cell fate, cell survival, and genome maintenance (Vogelstein et al., 2013). Whole-exome and whole-genome sequencing of thousands of tumors show that in the same tumor type there is substantial variation in driver mutations and the same driver mutations can occur in different tumor types, suggesting that the same pathways can be active in different tumors (Alexandrov et al., 2013; Kandoth et al., 2013).

Several tumor types appear to adhere to the stochastic model; good examples are some colorectal cancers (Vogelstein et al., 1988) and B cell lymphoblastic leukemias (Williams et al., 2007). However, this model focuses on genetic heterogeneity without considering that individual cells within genetically homogeneous sub-clones might still exhibit phenotypic variations due to different microenvironmental cues and therefore may not account for the heterogeneity in tumor initiation capacity.

**Cellular Plasticity Reconciles the Hierarchical and Stochastic Theories into One Model**

Phenotypic plasticity characterizes a population of cancer cells that have the capacity to interconvert between differentiated and stem-like states, through a continuum of cell fate specifications (Quail et al., 2012). Based on this characteristic, the hierarchical versus stochastic models is a false dichotomy, as hierarchically organized cell populations are more transitory between states than previously imagined and stochastic events are able to generate novel, hierarchically organized cell populations. Thus, depending on the genotype and the microenvironmental signals experienced by transit-amplifying/progenitor cells, at least in epithelial tissues, such cells may dedifferentiate and thereby enter back into the CSC pool to regain long-term tumor repopulation capacity (Chaffer and Weinberg, 2015). This dedifferentiation capacity may be either inherited (hierarchical theory) or acquired via mutations that lead to a stem-cell-like permissive epigenome (stochastic theory).

Indeed, p53 inhibition and Human telomerase reverse transcriptase (hTERT) activation (Hahn et al., 1999; Stewart et al., 2002; Hong et al., 2009) or the aberrant acquisition of stem-cell-associated factors such as Neurogenic locus notch homolog (NODAL), NOTCH, and Wingless-type MMTV integration site family (WNT) proteins facilitates such phenotypic plasticity. Moreover, the concept of cellular plasticity suggests that symmetrical cell division may not be as necessary to enlargethe CSC pool and could be secondary to asymmetrical division as progenitor cells, asymmetrically divided from CSCs, are more proliferative and can convert back to CSCs. The fact that melanoma, breast, prostate, ovarian, and lung cancer cells are all able to alter their gene expression to resemble cell types that are not part of their original lineage (Quail et al., 2012) exemplifies cancer cell plasticity that enables cancer cells to gain/lose stem cell properties (Shirakawa et al., 2002; Passalidou et al., 2002; Lim et al., 2009). Since regaining tumor-initiating capacity is potentially possible (Gupta et al., 2011), it is essential to understand how the TME and the CSC niche within it promote CSC phenotypes.

**CSC Assays Should Consider Niche Contributions**

In general, stem cell markers (Table S1) and transcriptional signatures specific to CSCs functionally correlate with aggressive behavior and are highly predictive of overall patient survival. These clinical data suggest that CSCs may be critical therapeutic targets (Suvà et al., 2009; Karnoub et al., 2007). However, it became increasingly clear that the frequency of CSCs could vary dramatically between tumor types and also between tumors of the same origin (Visvader and Lindeman, 2008). A related problem is that the variability in the frequency and identity of tumorigenic cells between patients shows that markers identified in one tumor cannot be assumed to distinguish CSCs in other tumors or in other contexts (Ricardo et al., 2011; Lopez et al., 2005; Rocco et al., 2012).

Many theoretical and experimental caveats to the CSC model have remained unexplored, largely due to technological challenges. The gold standard measure of a stem cell is maintenance of long-term clonal growth in functional repopulation assays, originally used for studies of the hematopoietic system. Until recently, most CSC studies utilized the transplantation assay to prove the existence of CSCs for a particular tumor. The markers for CSCs are primarily chosen as robust and heterogeneously expressed cell surface markers that allow the faithful flow cytometric sorting of marker-positive and -negative subsets in a certain tumor type. These subsets are transplanted into immunodeficient mice by limiting dilution, after which tumor growth is scored within several weeks or months. Different tumor initiation capacities between cell subsets are then interpreted as evidence for the presence of CSCs in the primary tumor (Clevers, 2011). Self-renewal is further demonstrated by the ability to establish or maintain the tumor clone in serial transplantation assays at clonal cell doses and give rise to daughter cells that possess limited proliferative capacity (Clarke et al., 2006). Often no clear morphological or cell-cycle distinction is obvious between the tumorigenic and non-tumorigenic cancer cells (Al-Hajj et al., 2003), and yet the tumors seem to be organized hierarchically when tested functionally.

There are several problems with the transplantation assays commonly used to identify CSC activity. The sorted and transplanted human cancer cells are challenged by various experimental manipulations and subsequently end up in a context...
that is dramatically different from the original tumor niche. The new recipient microenvironments can then differentially influence the transplanted cells based on time, species barrier, host strain, developmental stages, and even gender (LaBarge, 2010). Thus, the frequent need for the inoculation of 10⁵ cells in transplantation experiments to allow efficient tumor engraftment may not be indicative of a rare TIC but rather may represent the inability to create the proper niche. On the other hand, extremely immunodeficient models can support tumor initiation from the majority of tumor cells, even those not associated with stem cell markers, as shown for patient-derived melanoma cells (Quintana et al., 2008). It is worth noting that melanoma may represent a unique cell type that is particularly poised to enter into the CSC state, since melanocytes may be naturally inclined to stem cell states that enhance a migratory phenotype (Quintana et al., 2012). Furthermore, transplantation assays provide only a snapshot of the state of cancer cells at the time of tumor removal and basically ignore CSC plasticity (Kreso and Dick, 2014). Therefore, the host microenvironments in those assays may distort the original tumorigenic potential and frequently select for the most robust TICs that can grow due to multiple long treatments and loss of their native TME (Kreso and Dick, 2014). Conversely, some cells with tumorigenic potential do not contribute to tumor growth, because they are in a non-permissive environment or eliminated by immune effector cells, but will do so upon transplantation.

To date, most CSC markers are not selected based on a deep understanding of the underlying stem cell biology of the relevant tissue from which the cancer originates, since developmental hierarchy is still poorly characterized in most tissues that develop solid cancers. Moreover, only very few CSC markers are currently available for various solid tumors (Clevers, 2011). In some cases, the markers used to rigorously demonstrate the existence of CSCs in a particular cancer subtype were very specific for that cancer, as was shown for breast cancer cells (Clarke et al., 2006). The fact that the markers used are not widely applicable to other types of cancers does not weaken the conclusion of such studies. Nevertheless, these CSC markers only strongly enrich (even by two orders of magnitude) for CSCs within bulk populations of cancer cells, but there is no evidence that, in such enriched populations, the CSCs exist in a pure state rather than constituting a subset of the cells with a greatly heightened ability to initiate tumors. Moreover, at the time of transplantation, these cells may not necessarily possess CSC capabilities, but rather may gain them upon transplantation, which may not have happened within their native niches.

To separate between the inherent plasticity of CSCs and/or the plasticity induced or inferred by the experimental limitations discussed above, it will be crucial to continue and optimize transplantation assays potentially by development of more immune-deficient recipient mice and humanizing these with human TME and/or growth factors (Rongvaux et al., 2013), to estimate as accurately as possible the spectrum of cancer cells that retain the potential to contribute to tumor growth (Meacham and Morrison, 2013). Specifically, it was recently shown that the growth of dormant cancer sub-clones could be solely induced by microenvironmental changes caused by a sub-population of cancer cells that does not display the higher fitness commonly associated with CSCs (Marusyk et al., 2014). In addition, co-transplantation with stromal cells from myeloproliferative neoplasms enabled engraftment and expansion of neoplastic cells that was otherwise not as successful (Meyyoub et al., 2014).

Given that the major limitation of transplantation assays is that they cannot reveal the actual fate of the transplanted cell in its original tissue or tumor (Shackleton et al., 2009), it is of central importance to develop assays that can visualize and localize CSCs and their function within the primary tumor in situ. Live imaging methodologies could bring us closer to unraveling whether, in a particular niche and at a particular point in time, the cell visualized is indeed a CSC rather than a representative of a cell population that is only enriched in CSCs. It would allow us to examine whether, under a specific microenvironment, a cell is able to proliferate and produce progeny/various clones. Integration of genomic and functional properties of CSCs that have yet to be extensively utilized could further facilitate the identification of single, definitive marker genes for CSCs of a particular cancer. Based on such markers, knock-in mouse models or viral-tagging strategies may facilitate genetic lineage tracing (Kreso and Dick, 2014). Lineage tracing or fate-mapping assays are indeed a complementary measure for the long-term clonal growth of stem cells. These assess the actual fate of tumor cells in a particular context, frequently the native tumor environment rather than the potential of what these cells can do under permissive conditions. Yet, lineage-tracing experiments may also provide only limited support for the CSC model. Although intestinal adenomas were shown to be hierarchically organized by Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5)+ CSCs, both Lgr5- cells and Lgr5+ cells can act as the cell of origin via WNT-pathway activation, as exhibited by fate mapping (Schwitzela et al., 2013). This raises the question of whether adenomas that exhibit hierarchical organization lose it after they progress to malignancies. Although brain tumors may be different from carcinomas, similar concerns have been shown for markers such as CD133 in brain tumors (Meacham and Morrison, 2013). Ultimately, it will be necessary to integrate the data from both transplantation studies and fate-mapping studies of significant numbers of human and mouse tumors to understand the biological diversity. Additionally, the selective ablation of genetically defined subsets of cells (Plaks et al., 2013a) can test which tumor cells are fated to contribute to tumor growth or progression in the native tumor environment. Collectively, combining in vivo models and ex vivo systems discussed should prove useful in systematically characterizing the intricate molecular language of cell-cell communication in the CSC niche.

Cross Talk between CSCs and Their Niches
Niches are anatomically distinct microenvironments within the overall TME. Cells within the CSC niche produce factors that stimulate CSC self-renewal, induce angiogenesis, and recruit immune and other stromal cells that secrete additional factors to promote tumor cell invasion and metastasis, as reviewed in Oskarsson et al. (2014) and Ye et al., (2014) and summarized below (Figures 2 and 3).

Cancer-Associated Fibroblasts
There is evidence pointing to factors produced by CSCs and endothelial cells (ECs) in the TME that can transform normal fibroblasts into cancer-associated fibroblasts (CAFs) (reviewed
in Kalluri and Zeisberg, 2006). Compared with normal tissue fibroblasts, CAFs have increased proliferation, enhanced ECM production, and unique cytokine secretion such as CXCL12, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and hepatocyte growth factor (HGF) (Junttila and de Sauvage, 2013). CAFs (as well as other cells within the niche) stimulate stemness via activation of the WNT and NOTCH pathways. Canonical WNT is a major pathway that regulates CSCs and induces stemness in colon and other cancers (Vermeulen et al., 2010; He et al., 2004). Alternatively, epithelial non-stem cells can re-express stem cell markers upon WNT activation and can “dedifferentiate” to TICs (Schwittalla et al., 2013). NOTCH signaling has also been implicated in stem cell maintenance and cell-fate decisions (Quail et al., 2012). NOTCH prevents cells from responding to differentiation cues coming from their immediate environment (Milner and Bigas, 1999). In breast and prostate cancers, NOTCH receptors tend to be overexpressed, and their ligand expression correlates with aggressive phenotypes (Weijzen et al., 2002; Liu et al., 2006). The interplay of the WNT and NOTCH signaling with other pathways like bone morphogenic protein (BMP) (see below) and Hedgehog signaling pathways determines the differentiation state of cells (Fessler et al., 2013).

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are multipotent stromal cells that have been implicated in multiple mechanisms promoting cancer cell proliferation and metastasis, fostering angiogenesis, and generating an immunosuppressive microenvironment (Cuffo and Kamoun, 2012; Nishimura et al., 2012). They provide an advantageous TME for the restoration of CSCs, as they secrete a variety of cytokines that have both paracrine and autocrine functions in the tumor milieu. MSCs can promote cancer stemness through Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway by secreting CXCL12, Interleukin (IL) 6, and IL8 (Cibarcas et al., 2011). Moreover, MSCs can stimulate tumor progression by producing the BMP antagonist Gremlin 1 to promote the undifferentiated state (Davis et al., 2015). Furthermore, MSCs can cause elevated miR-199a expression in breast cancer cells, which leads to aberrant expression of a set of interrelated microRNAs and suppressed Forkhead box protein P2 (FOXP2) expression, providing tumor cells with CSC properties (Cuffo et al., 2014).

Inflammatory Cells

Currently, one of the areas of greatest interest is the role of the CSC niche in modulating the level of tumor immunity. The TME is characterized by chronic inflammation, which stimulates tumor cell proliferation and metastasis (Cibarcas et al., 2011). To evade immune surveillance, and thus enable tumor progression, the niche must immunosuppress the cytotoxic function and inhibition of natural killer cells (NKs) and CD8+ T cells (Kitamura et al., 2015; Casbon et al., 2015). For example, it was recently shown that a rare sub-population of anti-tumor CD103+ dendritic

Figure 2. The Molecular and Cellular Basis of the Cross Talk between CSCs and Their Niches

CSCs are metastatic cancer cells that can self-renew. Their plasticity and dormancy correlates with their therapeutic resistance. By secreting CXCL12, IL6, and IL8, MSCs promote cancer cell stemness through upregulating NF-κB while CSCs secrete IL6 to attract more MSCs. MSCs also produce the antagonist, Gremlin 1, to promote the undifferentiated state. Surrounding tumor cells produce IL4 to accumulate Treg2, which produces TNFα to upregulate the NF-κB signaling pathway and facilitates a pro-TME. In such a microenvironment, tumor cells produce M-CSF, Granulocyte macrophage colony-stimulating factor (GM-CSF), and G-CSF to induce expansion of TAMs, MDSCs, TANs, and Dc. TAM produces TNFα and TGF-β to promote NF-κB-dependent or TGF-β-dependent EMT and thus enhance CSC plasticity. TGF-β can also directly interact with NF-κB signaling pathways to further enhance cancer cell stemness. In addition, TGF-β produced by TAMs accumulates Treg cells. TAM, Trap, and the hypoxic environment inhibit immunosurveillance by inhibiting CD8+ T cell and NK cell cytotoxicity as well as macrophage phagocytosis. A subset of anti-tumor stimulatory DCs necessary for T-cell-mediated tumor rejection is kept away from the niche. Furthermore, hypoxia increases ROS, which promotes cell survival and induces EMT through the TGF-β signaling pathway. Both hypoxia and ROS induce CSCs to express HIF-1α, directly promoting EMT. Moreover, hypoxia also inhibits cell proliferation by downregulating c-Myc expression, and enhancing stemness. Hypoxia further promotes cancer cell stemness by promoting an undifferentiated state through TGF-β; the WNT signaling pathway. CSCs and CAFs produce CXCL12 to promote angiogenesis, and hypoxia causes both CSCs and ECs to produce VEGF, which further induces angiogenesis. ECs promote self-renewal of CSCs by direct cell–cell contact or by nitric oxide (NO) production via the NOTCH signaling pathway, CAFs produce TNC and HGF to enhance WNT and NOTCH signaling for CSC maintenance. CAFs also produce MMP2, 3, and 9. Along with the MMP10 produced by CSCs, these MMPs promote ECM degradation and remodeling, which enhances EMT and the CSC state. Of note, this figure does not provide spatial information as to the exact localization of CSCs in respect to niche cells.
cells (DCs), which can efficiently stimulate CD8+ T cells, is masked from tumor antigens by other tolerizing antigen-presenting myeloid cell populations (Broz et al., 2014). Numerous cell types recruited by chemokines and cytokines that are secreted by cancer cells contribute to this immunosuppression, which include tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), and a population functionally identified as myeloid-derived suppressor cells (MDSCs). TAMs secrete Transforming growth factor beta (TGF-β), which recruits T regulatory cells (Tregs) that also participate in immunosuppression (Chanmee et al., 2014). MDSCs are a heterogeneous population of cells from monocytic and granulocytic origins, which secrete IL6, TGF-β, and other cytokines and, among other functions, also recruit T helper 17 cells to promote their immunosuppressive function (Kitamura et al., 2015).

TAMs and TANs are derived from polarized macrophages and neutrophils respectively, which results in their pro-tumor phenotypes that facilitate tumor growth and stimulate angiogenesis (Lohela et al., 2014; Casbon et al., 2015). TAMs produce exosomes, enabling ingress of mRNAs and micro-RNAs (miRNAs) into various cell types (Ratajczak et al., 2006; Jing et al., 2012) for cancer cell growth and metastasis (Fabbri,
Exosomes also facilitate multidrug resistance (MDR) in tumor cells via the transfer of efflux transporters (Jaiswal et al., 2013). Transformed epithelial cells often undergo epithelial-to-mesenchymal transition (EMT)-like alterations during which they lose their cellular polarity and cell-cell adhesion and become mesenchymal like or stem cell like, gaining migratory and invasive properties (Kalunzi and Weinberg, 2009; Karreth and Tuveson, 2004). In the inflammatory TME, TAMs and CD4+ T cells secrete Tumor necrosis factor alpha (TNFα), which upregulates NF-κB signaling pathways to induce Snail homolog 2 (Slug), Snail homolog 1 (Snail), and Twist family basic helix-loop-helix transcription factor (Twist) and increase the crosstalk with the TGF-β signaling pathway which stimulates self-renewal (Smith et al., 2012; Cabarcas et al., 2011); thus, they can induce EMT and ultimately promote migration and invasion of CSCs. The correlation between stemness and EMT implies that non-CSCs can convert into CSCs through EMT-induced plasticity.

**Hypoxia and Angiogenesis**

Perturbed accessibility to vasculature results in hypoxia within various tumors. This advances stemness through activation of stem genes and dedifferentiation (Bennewith and Durand, 2004; Brurberg et al., 2006). Hypoxic CSCs impede CDB+ T cell proliferation and activation and inhibit immunosurveillance (Wei et al., 2011). Hypoxia also protects CSCs from chemotherapy and radiotherapy. Hypoxia further promotes CSC survival and EMT through reactive oxygen species (ROS)-activated stress response pathways (Liu et al., 2008) and through ROS-induced TGF-β and TNF-α signaling pathways (Pavlidis et al., 2010). Activation of TGF-β as well as WNT signaling pathways by hypoxia induces stemness by promoting an undifferentiated state in tumor cells (Anido et al., 2010; Scheel et al., 2011). In various solid cancers, ECs promote self-renewal of CSCs by direct cell-cell contact or by nitric oxide (NO) production via the NOTCH signaling pathway (Charles et al., 2010). Hypoxia-inducible factor 1 alpha (HIF-1α) also can directly increase NOTCH signaling (Quaill et al., 2012). HIF-1α antagonizes Myelocytomatosis viral oncogene homolog (c-Myc) activation, thus slowing down cell-cycle progression to protect CSCs from DNA damage and enhance stemness (Koshiji et al., 2004).

Hypoxia induces CSCs to express hypoxia-inducible factors (HIFs), which are regulated and stabilized by TGF-β (Cabarcas et al., 2011). The HIF genes are the primary factors for driving angiogenesis via induction of VEGF. Under hypoxia, both ECs and CSCs produce VEGF to stimulate tumor angiogenesis. In the hypoxic regions of the tumor, VEGF-A can recruit monocytes and macrophages (Kitamura et al., 2015). A positive correlation between TAM infiltration and angiogenesis was found in many human cancers. TAMs become pro-angiogenic through their response to Macrophage colony stimulating factor (M-CSF) (Lohe et al., 2014), secreted by tumor cells, which induces VEGF-A production and suppresses anti-angiogenic factor expression.

**ECM-Cell Interactions and Cell-Cell Contact**

The ECM is an essential noncellular component of the adult stem cell niche. In solid tumors, increased ECM stiffness can be a physical barrier blocking therapeutics and thus protect CSCs from chemotherapeutic agents (Wong and Rustgi, 2013; Ye et al., 2014). Matrix metalloproteinases (MMPs) that degrade components of ECM in tumors, release cytokines, growth factors, and other molecules from the ECM and cell surface (Noël et al., 2012) and facilitate angiogenesis, tumor cell invasion, and metastasis (Siefert and Sarkar, 2012; Kessenbrock et al., 2010). MMPs produce MMP2, 3, and 9 for ECM remodeling, which promotes EMT, enhances CSC-related marker expression, and exacerbates therapeutic resistance (Cabarcas et al., 2011). Interestingly, MMPs can increase WNT signaling and stemness (Kessenbrock et al., 2013). Increased MMP3 expression facilitates genomic instability, EMT, and tumor formation, as shown in a mouse model of breast cancer.

In normal stem cell niches, anchoring stem cells to the niche through cell-cell contacts is critical to keep them far from differentiation stimuli and physically adjacent to niche factors that specify self-renewal (Sneddon and Web, 2007; Borovski et al., 2011). CSCs also utilize cell-cell contact to preserve their phenotype and exert their functions. For example, direct cell contact is necessary for MSCs to exert their maximal effect on CSCs (Roorda et al., 2010). Hedgehog and NOTCH signaling pathways (Gilbertson and Rich, 2007) require cell-cell contact. Notch ligands are mostly transmembrane proteins, particularly Jagged and Delta (Gilbertson and Rich, 2007). Glial cells in the brain may act as a cell-cell adhesion unit to tether glioma cells (Lin et al., 2002; Riquelme et al., 2008). In addition, to protect themselves from shear forces and NK-cell-mediated lysis, and to improve their adhesion to endothelium, disseminated cancer cells surround themselves with platelets, forming a physical shield (Fessler et al., 2013). Lastly, although there is yet little evidence to support this, the development of cancer might suggest an enlargement or growth in the size of the niche to accommodate numerous CSCs (Shiozawa et al., 2011).

**CSCs and Non-CSCs**

As inferred above, also CSCs secrete a variety of factors that help recruit, activate and even create specific cell types to control the regulation of their differentiation states. Breast CSCs can produce IL6, which attracts and activates MSCs to produce the CSC-supportive cytokine CXCL7 (Liu et al., 2011). CSCs play an important role in TAM recruitment by secreting macrophage chemoattractants (Yi et al., 2013). CSCs promote angiogenesis through HIF-1α and the release of VEGF-A and CXCL12 (Ricci-Vitiani et al., 2010; Borovski et al., 2011). They help prevent ECs from undergoing hypoxia- or irradiation-induced apoptosis, resulting in resistance to vascular disrupting agents. CSCs can produce factors, such as TGF-β, to help transform fibroblasts to CAFs (Kalunzi and Zeisberg, 2006). MMP10 is highly expressed in CSCs, correlating with metastasis in many human tumor types (Jaiswal et al., 2013). Its repression leads to a loss of stem-cell-related gene expression. Tumor cells, which may not have CSC characteristics, also take part in the niche and secrete cytokines and exosomes (Fessler et al., 2013; Ye et al., 2014).

A bidirectional conversion between CSCs and non-CSCs can be triggered by an inflammatory stroma, which is characterized by elevated NF-κB signaling, enhancing Wnt activation, and inducing dedifferentiation of non-CSCs that acquire tumor-initiating capacity (Schwattala et al., 2013). Interestingly, it has been shown that tumors can be driven by a sub-population of non-CSCs. These cells that do not have higher fitness, but
CSCs and Metastasis: The Primary TME and the Metastatic Niche

As summarized below, interactions of CSCs with their niches are also critical throughout metastatic progression.

**CSCs and Metastatic CSCs**

Although CSCs may not be the only cells instigating or maintaining metastasis, the CSC-generated hierarchy of stem-like and differentiated tumor cells is able to initiate metastatic growth and is also seen in late-stage cancers and at metastatic sites (Da-larba et al., 2011; Merlos-Suárez et al., 2011; Vermeulen et al., 2008). Large-scale genome sequencing studies suggest that primary tumors accumulate most of the mutations vital to metastasis, showing a predominance of similarity between metastatic stem cells (MetSCs) and primary CSCs (Yachida et al., 2010). Gene expression signatures have identified mediators of metastatic mutations in primary tumors (as stem cell markers) that correlate with poor prognosis and relapse (Oskarsson et al., 2014). Cancer cells expressing stem cell markers have been detected in the blood of breast cancer patients; when inoculated into immunodeficient mice, these cells can generate bone, liver, and lung metastases (Baccelli et al., 2013). In addition, analysis of human colorectal cancer samples using clonal lentiviral marking demonstrates that metastases arise from primary tumor cells that display long-term self-renewal capacity and are quiescent and resistant to chemotherapy (Dieter et al., 2011; Kreso et al., 2013). Even cancers, such as melanoma, that do not appear to rely on a hierarchical organization still contain MetSCs (Meacham and Morrison, 2013). Although there is some evidence suggesting that primary tumors and metastases may arise from different cells (LaBarge, 2010), it could be that MetSCs simply develop from the original CSCs that evolved throughout tumor progression due to tumor cell plasticity or generation of MetSCs. MetSCs may be generated de novo as a result of de novo niche formation due to competition between cancer and normal stem cells for niche occupancy (Shiozawa et al., 2011). If MetSCs or disseminated tumor cells (DTCs) are primary CSCs, many of the CSC niche considerations will also apply to MetSCs.

**The TME Supports Cancer Cell Dissemination**

Beyond the passive role of circulation patterns, cancer cell dissemination is actively influenced by cancer cell autonomous functions such as invadopodia formation, paracrine factors as VEGF and Epidermal growth factor (EGF) family members, proteases as MMPs and cathepsins, and recruitment of stromal components and immunosuppressive cells as TAMs (Oskarsson et al., 2014). The tumor invasive front is a likely site for selection of metastatic traits (Cheung et al., 2013). This site is rich with blood vessels as well as niche cells and factors that support the survival and fitness of CSCs (Joyce and Pollard, 2009; Takebe et al., 2011) (Figure 2). Primary tumor stroma also select for organ-specific seeding traits by releasing exosomes that alter niche content. In the circulation, transient contact between platelets and DTCs induces EMT and a CSC-like state (Fessler et al., 2013). Endothelial tyrosine kinase positive (TIE2*, also known as CD202B*) macrophages lining the vasculature direct cancer cell migration along collagen fibers toward higher concentrations of metastasized cells. Clusters of tumor cells in blood vessels secrete EGF family members, further directing cancer cell seeding and macrophages to sites of metastasis (Noy and Pollard, 2014) (Figure 3).

**The Metastatic Niche Supports Seeding and Growth of Metastasis**

Circulating tumor cells need the right “soil” in which to seed and survive, since most metastatic sites are less hospitable than the origin (Figure 3). The survival and fitness of metastasis-initiating DTCs depends on specific components of the host environment that play the part of a niche for these cells, as inferred by massive CSC loss/apoptosis in colorectal and breast CSCs (Oskarsson et al., 2014). Although no foreign tissue may be welcoming to metastatic seeds, certain tissues may be less hostile than others. Similar to the CSC niche, the metastatic niche designates the specific locations, stromal cell types, diffusible signals, and ECM proteins that bear consequences for the metastasis of DTCs (Oskarsson et al., 2014). So beyond cell-autonomous failures, the inability to metastasize results from scarcity of survival signals in the host parenchyma, lack of a supportive stroma, and overexposure to innate immunity (Chambers et al., 2002; Fidler, 2003; Nguyen et al., 2009; Schreiber et al., 2011).

Interestingly, the traits required for metastatic dissemination are distinct from those that mediate overt metastatic colonization months or years later. Dormancy is a critical issue for tumor recurrence and metastatic spread after long lag periods in many cancers, including breast, melanoma, and leukemia (Pece et al., 2010; Roesch et al., 2010; Saïto et al., 2010). Since dormant cells are proliferatively quiescent, they survive chemotherapy and contribute to tumor regrowth, irrespective of genetic differences. Therefore, understanding the role of the microenvironment in regulating exit from dormancy is of crucial importance. The mechanisms of tumor dormancy and the ability of CSCs to remain quiescent are intertwined with angiogenic dormancy (Cabarcas et al., 2011). Restricted supplies of nutrients and oxygen due to poor vascularization cause an arrest in growth (Almog, 2010), which can also potentially result from the absence of necessary factors required by CSCs to reinitiate tumor formation or metastasis. Although angiogenic stimulators such as c-Myc, VEGF, and Fibroblast growth factor 2 (FGF-2) (Shachaf et al., 2004; Naumov et al., 2006) may play a role in mediating tumor exit from dormancy.

Although DTCs in bone marrow appear dormant, the overall DTC population is not static (Müller et al., 2005; Pantel et al., 1993). DTCs may constantly transition between dormant and active states during metastatic latency, being further selected for colonization functionality. Circulating metastatic cells co-express EMT and stem markers (Plaks et al., 2013b). Although EMT enables migration, it interferes with proliferation and metastatic growth (Ocaña et al., 2012; Stankic et al., 2013). Thus, MetSCs that have undergone EMT may need to reacquire an epithelial phenotype to seed and resume growth at the metastatic site. This reverse process is called mesenchymal-to-epithelial transition (MET) (Tsai et al., 2012; Ocaña et al., 2012; Gupta et al.,
2007). TGF-β causes EMT before extravasation, but MET after extravasation, by a yet-unknown mechanism. Despite the clinical importance of metastatic latency, mouse models lack a prolonged dormancy of MetSCs and xenograft assays may either restrict CSC detection to only the most robustly proliferating cells (Quintana et al., 2008), since they are read within months after transplantation or activate dormant cells by serial transplantation. Therefore, little is known about entering and exiting dormancy, forms of dormancy, and signaling during dormancy, and it remains an overarching challenge for successfully combating many cancers, so better models are needed (Kreso et al., 2013).

Metastatic Seeding Occurs in a Variety of Niches

DTCs may occupy normal stem cell niches in the host tissues (Figure 3). MSCs produce TGF-β family molecules, CXCL12, and Hedgehog signals in the bone marrow for hematopoietic stem cell maintenance while metastatic cancer cells from other sites occupy this niche to benefit from cues that enhance stem cell properties and deter differentiation (Shiozawa et al., 2011). The cognate chemokine receptor CXCR4 is frequently overexpressed in bone metastatic cells and provides CSCs with chemotaxis and Phosphoinositide 3-kinase (PI3K)-mediated survival signals that mediate oncogenic transformation (Müller et al., 2001; Zlotnik et al., 2011).

DTCs initiate metastatic outgrowth around blood capillaries, in perivascular niches (Figure 3). These may support MetSCs by supplying attachment, oxygen, nutrients, and paracrine factors from the activated endothelium (Butler et al., 2010; Fessler et al., 2013). The perivascular niche is a preferred residence for glioma CSCs that supplies them with Hedgehog-, NOTCH-, and PI3K-activating signals. Breast cancer, lung cancer, and melanoma cells that infiltrate the brain surround capillaries and some stretch themselves over the perivascular basal lamina (Charles and Holland, 2010; Hambardzumyan et al., 2008).

DTCs seed metastasis in distant tissue niches (Figure 3). In mouse models, breast, lung, and gastrointestinal tumors establish premetastatic niches by secreting systemic factors such as VEGF-A, TGF-β, Granulocyte colony-stimulating factor (G-CSF), TNF, and lysyl oxidase (LOX) that induce expression of chemotactic proteins (S100 calcium binding protein A8, A9 [S100A8, S100A9], and serum amyloid A3 [SAA3]), ECM-remodeling enzymes, and exosomes into the circulation and directs various cells to induce pro-metastatic changes in the lung parenchyma microenvironment before DTCs arrive (Oskarsson et al., 2014; Kaplan et al., 2005; Hiratsu et al., 2006; Casbon et al., 2015). Primary tumors induce recruitment and mobilization of VEGFR1+ bone-marrow-derived hematopoietic progenitor cells (HPCs) before the arrival of tumor cells (Kaplan et al., 2005). Pre-existing fibroblasts increase fibronectin deposition in these sites, which binds and clusters HPCs, and fibroblasts induce remodeling of stroma (Olaso et al., 1997). Macrophages, activated neutrophils, and Tregs are also recruited to the niche to promote future metastasis. Neutrophils could also potentially enhance MetSC settlement by producing neutrophil extracellular traps (NETs) (Casbon et al., 2015; Cools-Lartigue et al., 2013; Kitamura et al., 2015). The metastatic niches are populated by Gr1+ CD11b+ myeloid cells recruited by LOX and S100A proteins (Effer et al., 2009; Psaila and Lyden, 2009; Yan et al., 2010). However, direct evidence showing a pro-metastatic role for these myeloid cells through immunosuppression is lacking, even though CD11b+ Gr1+ and CD11b+ Ly6G+ cells promote metastasis processes (Yang et al., 2010; Casbon et al., 2015). The ECM component tenascin C (TNC) is found in stem cell niches, frequently supplied by CAFs and associated with increased risk of metastasis (Oskarsson et al., 2011). TNC regulates Musashi and other factors to enhance NOTCH and WNT signaling to support CSCs.

Once metastatic cells arrive, they continue to remodel their microenvironment. Breast CSCs induce the expression of the ECM molecule periostin in lung fibroblasts that binds WNT ligands to help maintain stemness of arriving CSCs. As metastatic lesions grow, the cancer cells recruit TAMs, myeloid precursors, and mesenchymal cells that establish paracrine loops feeding back to the cancer cells with various survival and self-renewal factors (Kitamura et al., 2015). In osteolytic bone metastasis of breast cancer, osteoclasts resorb bone matrix to make room for the metastatic growth and release TGF-β and other growth factors. These factors stimulate cancer cells in a feed-forward cycle of tissue destruction and metastatic expansion (Ell and Kang, 2012; Weilbaecher et al., 2011). The metastatic cells also trigger angiogenesis, and the newly forming blood vessels attract more MetSCs by expressing fibronectin and Vascular cell adhesion molecule (VCAM) (Fessler et al., 2013). These MetSCs produce CCL2 and attract CCR2+ inflammatory monocytes that become metastatic-associated macrophages and support metastatic growth (Kitamura et al., 2015).

Interestingly, in models of brain metastasis from breast and lung cancers, brain stroma takes an active role in killing the infiltrating cancer cells (Valiente et al., 2014). However, little is known about what kills the majority of DTCs. More information on how the reactive stroma repels DTCs could yield clues for how to leverage these mechanisms for therapeutic benefit.

The Stem Cell Niche as a Target for Cancer Therapy

Generally, CSCs appear to be resistant to conventional cancer therapies such as ionizing radiation and conventional anti-proliferative chemotherapy due to their quiescence (Bao et al., 2006; Li et al., 2008). On the other hand, CSCs can be more sensitive to some therapies as compared to non-tumorigenic cells. Rapamycin treatment in a mouse model of leukemia induced by conditional Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (Pten) deletion in hematopoietic cells causes the depletion of leukemia-initiating cells and restores normal hematopoietic stem cell function. Although the histological evidence of leukemia persisted, the mice were overtly healthy (Yilmaz et al., 2006). Radiation or cisplatin therapy may preferably target the undifferentiated cells that drive testicular germ cell tumors (Clevers, 2011). Differentiation therapies that specifically target CSCs by exploiting their capacity to differentiate can be effective in some cases (Meacham and Morrison, 2013). This strategy is successful in inducing cell-cycle progression in acute myeloid leukemia stem cells by supplying G-CSF to promote sensitivity to chemotherapy (Saito et al., 2010). Similarly, mouse glioblastoma stem cells can be induced to differentiate into glia by treatment with the protein BMP4, resulting in reduced proliferation, tumor growth, and tumor-initiation capability of CSCs upon transplantation (Lombardo et al., 2011; Piccirillo et al., 2006).
Tumor cell plasticity presents a huge challenge to the development of targeted cancer therapies, as tumor cell populations are continually evolving and therapeutic eradication of existing CSC populations might be followed by their regeneration from non-CSCs within the tumor under treatment (Chaffer and Weinberg, 2015). In addition, most stem cell markers used to date are not good targets for antibody therapy. Moreover, many of these markers, especially in solid tumors, fail to distinguish normal stem cells from CSCs. High-throughput screening could be an unbiased approach to uncover known or new compounds that specifically target CSCs (Clevers, 2011).

An Alternative Strategy: Targeting the Unique Aberrant Microenvironment of CSCs

Since the TME has the potential to support and initiate stem cell-like programs in cancer cells, targeting CSC niche factors that regulate plasticity may prove to be a more powerful modality for the treatment and prevention of tumor cell plasticity and progression than targeting the CSCs directly. However, it should equally be taken into account that in a particular cancer type/stage, CSCs may evolve to escape niche constraints and become independent of niches. Therefore, targeting the niche may be a critical aspect of effective cancer therapy in systems where the aberrant activation of the pathway that is about to be targeted is regulating CSCs at the cell surface level rather than a cell-autonomous mutation, which provides independence from growth factors or abolishes an apoptotic response to drive clonal expansions (Clevers, 2011). In cases where tumor progression is limited by microenvironmental constraints that cannot be overcome by a cell-autonomous increase in proliferation rates, it is possible that these secreted factors not only preferentially benefit the CSCs, enabling their clonal dominance, but also actually mediate inter-clonal interactions that could also be drivers of the tumor (Marusyk et al., 2012). Overall, it seems that the niche has a differential importance depending on the cancer type and even on the specific stage of that particular cancer. Experimental analysis and clinical diagnostics still need to take place in order to elucidate such mechanisms in various cancers.

Some attempts to target the niche has already shown promise. Antibodies that abrogate the activation of c-Met by HGF significantly inhibit xenograft growth of colon tumors (Hoey et al., 2009). Fibronectin and hyaluronic acid facilitate a quiescent state from growth factors or abolishes an apoptotic response to drive clonal expansions (Clevers, 2011). In cases where tumor progression is limited by microenvironmental constraints that cannot be overcome by a cell-autonomous increase in proliferation rates, it is possible that these secreted factors not only preferentially benefit the CSCs, enabling their clonal dominance, but also actually mediate inter-clonal interactions that could also be drivers of the tumor (Marusyk et al., 2012). Overall, it seems that the niche has a differential importance depending on the cancer type and even on the specific stage of that particular cancer. Experimental analysis and clinical diagnostics still need to take place in order to elucidate such mechanisms in various cancers.

A successful approach in combating tumors is targeting immune checkpoints by either blocking immunosuppressive mechanisms to restore T cell function (such as Programmed cell death 1 [PD1] and its ligand PDL1) or enhancing immune function by engaging co-stimulatory receptors such as Tumor necrosis factor receptor superfamily member 4 (OX40) with agonist antibodies. Most successful is the use of a monoclonal antibody targeting the negative immune checkpoint protein Cytotoxic T-lymphocyte-associated protein (CTLA-4) (Junttila and de Sauvage, 2013). Other technologies that are currently in clinical development attempt to directly engage T-cell-mediated killing. Adoptive cell-transfer therapy, which involves the ex vivo expansion and reinfusion of tumor-reactive T cells, is emerging as a potential curative treatment for patients with advanced-stage cancer (Klebanoff et al., 2012). Overall, immunotherapy is an emerging field, and the exact mechanism by which these therapies may abrogate the ability of CSCs to reinitiate tumors is still under investigation.

Combinatorial treatment with conventional cancer therapies may be an effective strategy. Interferon gamma (IFN-γ) shows synergistic effects with the conventional anticancer drug oxaliplatin to eliminate both CSCs and differentiated cancer cells in colorectal cancer (Ni and Huang, 2013). Depletion of TAMs or IMs by inhibiting either CCR2 or M-CSF receptor resulted in decreased CSCs in pancreatic tumors, improved chemotherapeutic efficacy, inhibited metastasis, and increased antitumor T cell responses (Mitchem et al., 2013). Targeting components of the innate immune system along with conventional therapy is also under clinical evaluation. For example, the anti-CD40 agonist antibody and gemcitabine combination therapy has shown early clinical promise in treating pancreatic cancer (Junttila and de Sauvage, 2013). Targeting the bulk of the tumor with standard cancer therapy could help remodel the CSCs niche, exposing crucial niche component(s) and making it more receptive to niche-targeted therapeutics. For example, using conventional cancer therapeutics to expose anti-tumor DCs to antigens that are otherwise inaccessible to them (Broz et al., 2014) with a combination of immunotherapy using engineered DCs with enhance ability to stimulate T-cell-mediated tumor rejection could potentially be a successful strategy to eradicate CSCs.

Concluding Remarks

It is now accepted that most cancers originate from cells that gained tumor-initiating capacity and that these cells are plastic in nature. The tumor-initiating capacity or cancer stemness of these cells could therefore be influenced by extrinsic factors. It is also postulated that in many cancers the TME and especially the closely related niches have detrimental effects on the ability of these cells to initiate a tumor and/or metastasize. Due to their plasticity and given that CSCs need to be eradicated to prevent malignancy and metastasis, targeting specific niche components relevant to that particular cancer type in addition to standard cancer therapy that tackles the bulk of the tumor bears therapeutic promise. A better understanding of CSC biology and niche factors of each cancer subtype as well as their modulation using various therapeutic designs is paramount for this paradigm to be fully applicable in the clinic.
SUPPLEMENTAL INFORMATION

Supplemental Information includes one tables and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2015.02.015.

AUTHOR CONTRIBUTIONS

V.P. conceived the ideas and figures and wrote the manuscript. N.K. summarized the relevant literature and designed and produced figures. Z.W. guided the overall approach and trajectory of this piece and edited the manuscript.

ACKNOWLEDGMENTS

We would like to thank Renske van Den Biggaart for help with designing the figures. This study was supported by funds from the National Cancer Institute to Z.W. (CA057821 and CA180039) and by a Department of Defense postdoctoral fellowship to V.P. (W81XWH-11-01-0139). Given the length constraints, we were not able to cite all the papers that are relevant to this topic. We apologize to the scientists whose papers were left out.

REFERENCES


stem cells are regulated by mesenchymal stem cells through cytokine networks. Cancer Res. 71, 614–624.


Perspective

Cell Stem Cell


