Cell Stem Cell
Perspective

Cancer Stem Cells: Current Status and Evolving Complexities

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The cancer stem cell (CSC) model has been established as a cellular mechanism that contributes to phenotypic and functional heterogeneity in diverse cancer types. Recent observations, however, have highlighted many complexities and challenges: the CSC phenotype can vary substantially between patients, tumors may harbor multiple phenotypically or genetically distinct CSCs, metastatic CSCs can evolve from primary CSCs, and tumor cells may undergo reversible phenotypic changes. Although the CSC concept will have clinical relevance in specific cases, accumulating evidence suggests that it will be imperative to target all CSC subsets within the tumor to prevent relapse.

Introduction

Phenotypic and functional heterogeneity is a defining feature of many leukemias and solid tumors. Several factors contribute to this heterogeneity, including genetic mutations, epigenetic changes, interactions with the microenvironment, and the presence or absence of a cellular hierarchy. Different cellular mechanisms have been postulated to account for intratumoral heterogeneity. The acquisition of genetic (or epigenetic) alterations underpins the clonal evolution theory (Nowell, 1976) in which cells in the dominant clonal population(s) possess similar tumorigenic potential. Conversely, the cancer stem cell (CSC) model postulates a hierarchical organization of cells such that only a small subset is responsible for sustaining tumorigenesis and establishing the cellular heterogeneity inherent in the primary tumor. Although CSCs exhibit the stem cell properties of self-renewal and differentiation, they do not necessarily originate from the transformation of normal tissue stem cells (Figure 1). This model has received wide attention because it provides an explanation for resistance to both radiation and chemotherapy and eventual tumor relapse. In addition, quiescent or slow cycling CSCs may survive therapeutic intervention and result in recurrence. The first prospective identification of a CSC was made by Dick and colleagues for AML (Bonnet and Dick, 1997; Lapidot et al., 1994). CSCs were subsequently demonstrated to occur in diverse solid tumors (reviewed in Visvader and Lindeman, 2008). Although the existence of CSCs has been well established for specific cancers, it is clear that the CSC model does not account for functional heterogeneity in all tumors.

Over the last few years, emphasis in the CSC field has shifted more toward the use of freshly isolated tumor specimens and early-passage xenografts for transplantation studies rather than the use of cultured tumor cells. Furthermore, there is increased awareness that the nature of the xenotransplantation assay is critical for evaluating the existence of CSCs (Quintana et al., 2008). Here we review recent developments in this rapidly moving field, including the variable phenotype of CSCs, the presence of multiple CSC pools within individual tumors, the ability of CSCs to undergo genetic evolution, and the potential of non-CSCs to switch to CSC-like cells (Figure 2). These observations highlight the dynamic nature of CSCs and further indicate that the clonal evolution and CSC models can act in concert. They also somewhat dampen the original therapeutic promise of the CSC model, as it seems that all CSC subsets within the tumor will need to be defined and targeted in order to influence clinical outcome. In the case of solid tumors, which exhibit extraordinary genomic instability, it will probably be necessary to target both CSCs and non-CSCs to achieve durable remission. Despite these complexities, the recent derivation of a stem cell-like or "self-renewal" gene expression signature that is predictive of patient outcome in human leukemia lends credence to the CSC hypothesis and its clinical relevance (Eppert et al., 2011; Gentles et al., 2010).

CSC Markers Are Not Universal for Any Cancer Type

CSCs must be defined functionally by well-validated assays such as in vivo transplantation rather than on the basis of immunophenotype alone. Nonetheless, a number of markers have proven useful for the isolation of subsets enriched for CSCs in multiple types of solid tumors, including CD133, CD44, EpCAM, and ALDH activity. In the case of human leukemia, a combination of CD34, CD38, and IL3Rα has enabled the prospective isolation of leukemia stem cells. It should be noted that none of these markers are exclusively expressed by CSCs. With the passage of time, it has become increasingly evident that the CSC phenotype varies between individual patient tumors of the same subtype, raising the question of whether the markedly different clinical outcomes reflect differences in their CSC populations. CD133 (prominin) is one example of a cell surface protein that has been widely explored as a CSC marker. CD133 was initially described as a CSC marker for glioblastoma multiforme (Singh et al., 2004). Moreover, direct imaging of matched CSCs and non-CSCs in the same in vivo microenvironment of primary glioblastoma tumors demonstrated that only the CD133$^+$ subset had the ability to maintain tumorigenesis and generate heterogeneity (Lathia et al., 2011a). However, CD133 does not always mark

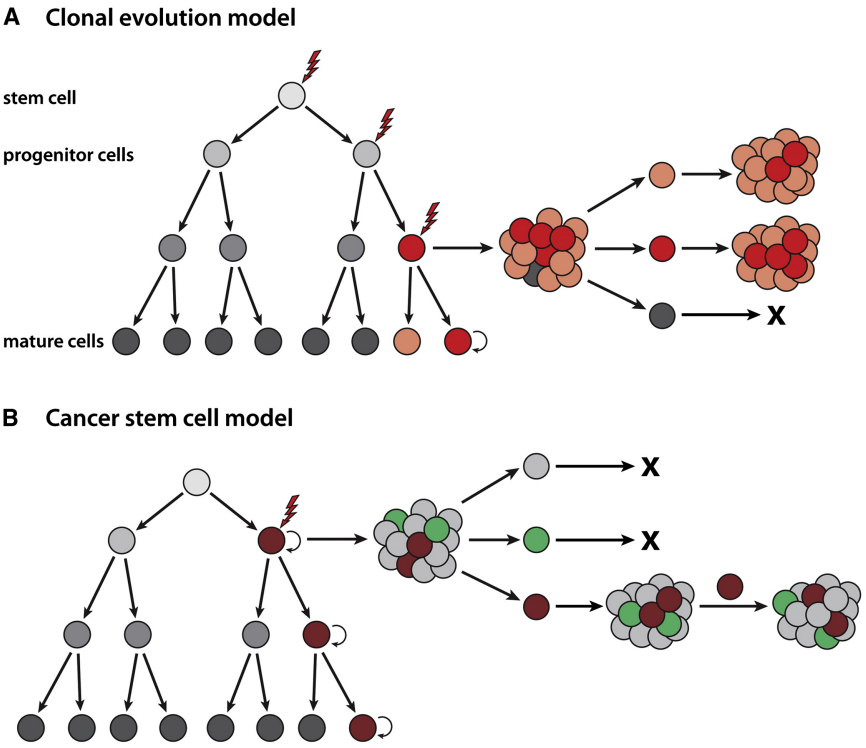

Figure 1. Schemata of the Clonal Evolution and Cancer Stem Cell Models

(A) The clonal evolution model is a nonhierarchical model where mutations arising in tumor cells confer a selective growth advantage. Depicted here is a cell (red) that has acquired a series of mutations and produced a dominant clone. Tumor cells (red and orange) arising from this clone have similar tumorigenic capacity. Other derivatives (grey) may lack tumorigenicity due to stochastic events. Tumor heterogeneity results from the diversity of cells present within the tumor.

(B) The cancer stem cell model is predicated on a hierarchical organization of cells, where a small subset of cells has the ability to sustain tumorigenesis and generate heterogeneity through differentiation. In the example shown, a mutation(s) in a progenitor cell (depicted as the brown cell) has endowed the tumor cell with stem cell-like properties. These cells have self-renewing capability and give rise to a range of tumor cells (depicted as gray and green cells), thereby accounting for tumor heterogeneity.

CSCs and appears to be modulated by extrinsic factors. The search for more robust markers of CSCs in glioblastoma and other brain tumors has revealed SSEA-1/CD15/Lewis X and α6-integrin. SSEA-1 (stage-specific embryonic antigen) was identified as a CSC marker in both human glioblastoma and syngeneic mouse models of medulloblastoma (Read et al., 2009; Son et al., 2009; Ward et al., 2009). Despite a high proportion of specimens lacking $CD133^+$ cells, SSEA-1 enriched for CSCs by 100-fold in almost every human glioblastoma tumor evaluated (Son et al., 2009). In another approach, Rich and colleagues examined the perivascular microenvironment in which brain CSCs reside and identified α6-integrin as a CSC marker that was required for maintenance of CSCs in vivo (Lathia et al., 2010). Coexpression of CD133 and α6-integrin was observed in some but not all tumors.

The limited overlap evident between the phenotypes of CSCs isolated from the same tumor type may reflect the presence of multiple CSC pools or technical variation arising from differing enzymatic digestion conditions, the use of cultured versus freshly sorted cells, or extensively passaged versus early xenograft tumors. Another confounding factor is that stringent assays to prove self-renewing activity have not always been applied. The genetic mutation profile may also influence the nature and phenotype of CSCs, as suggested by studies on different genetic mouse models of lung adenocarcinoma (Curtis et al., 2010), whereas epigenetic changes in regulatory genes could impact marker expression itself. In breast cancer, although CD44 and CD24 have been extensively used to isolate CSCs, they should not be viewed as universal markers. CD44 and CD24 did not selectively enrich for CSCs in ER-negative and triple-negative breast tumors as shown by the fact that CSCs were found in both the $CD44^+CD24^-$ and $CD44^+CD24^+$ fractions (Meyer et al., 2010). Furthermore, the $ALDH^{hi}$ and $CD44^{hi}CD24^{lo}$ CSC-enriched subsets in breast cancer bear little overlap within the same tumor (Ginestier et al., 2007). A similar story holds true for colorectal cancer in which the $EpCAM^{hi}CD44^+$ CSC subpopulation shared minor overlap with CD133 (Dalerba et al., 2007), and for pancreatic cancer, where overlap between the $CD133^+$ and $CD44^+CD24^+$ populations varied considerably between specimens (Hermann et al., 2007). In ovarian cancers, strikingly little concordance was found between CD133 and reported ovarian CSC markers including CD117, CD44, and ALDH1 activity (Curley et al., 2009; Stewart et al., 2011), most probably explained by many groups relying on cultured cells as opposed to freshly sorted tumors. Finally, in patients with non-small cell lung cancer, although CD133, CD44, and EpCAM proved ineffective for the isolation of CSCs, CD166 emerged as a robust marker in more than 50% of cases (Zhang et al., 2012). Nevertheless, a combination of markers can refine the CSC phenotype. For example, CD44 expression combined with high levels of the tyrosine kinase receptor c-MET provided robust selection of pancreatic CSCs (Li et al., 2011), and high ALDH activity together with CD133 expression resulted in significant enrichment such that 1 in 11 ovarian tumor cells exhibited CSC properties (Silva et al., 2011).

Highly Variable Frequency of CSCs between Tumors

The true frequency of CSCs in most human tumors has probably been underestimated because of barriers imposed by xenotransplantation, species-specific differences in growth factors/receptors, and the level of immune recognition. However, CSCs and tumor-initiating cells in many solid tumors tend to be relatively infrequent, even when measured under more permissive conditions (Ishizawa et al., 2010; Stewart et al., 2011). The term "tumor-initiating" is generally used by the field as an operational term to define cells that initiate tumors upon transplantation but it is not necessarily synonymous with a CSC.

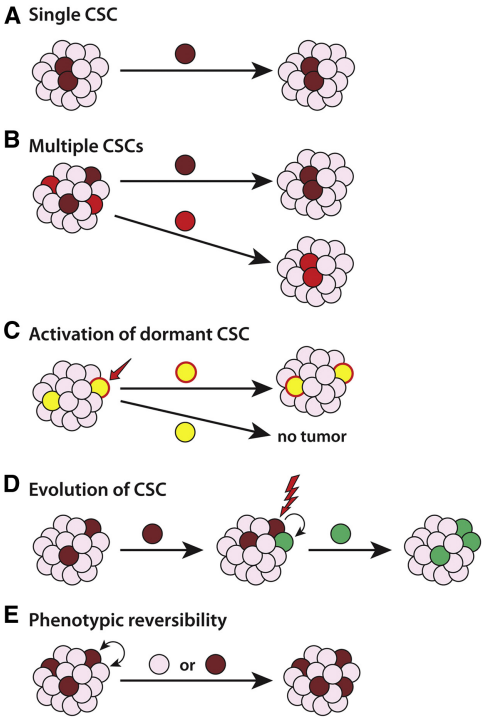

Figure 2. Schematic Models of Tumor Propagation by CSCs Depicting Variations that Can Contribute to Tumor Heterogeneity
(A) One CSC subset may be present within the tumor. As described in Figure 1, non-CSCs are incapable of generating a tumor.
(B) Multiple distinct CSC pools, each independently capable of tumor propagation, may exist with an individual tumor.
(C) Long-lived dormant CSCs may produce local and/or distant tumor recurrence after activation (depicted here as a yellow CSC with a red rim) many years after anticancer therapy.
(D) As tumor progression occurs, a second distinct CSC may arise as a result of clonal evolution. This may result from the acquisition of an additional mutation or by epigenetic modification. The more aggressive CSC will become dominant and drive tumor formation.
(E) The CSC phenotype may be unstable, resulting in phenotypic reversion of cell surface markers and switching of the CSC phenotype. This may occur in response to cell-intrinsic or microenvironmental cues.

Tumors that do not follow a CSC model also contain tumor-initiating cells but these do not exhibit stem cell-like properties. In head and neck, pancreatic, and non-small cell lung cancers, the frequency of tumor-initiating cells varied dramatically but always comprised a very small population (<0.02%). Interestingly, the frequency was not dependent on the immune status of the recipient (Ishizawa et al., 2010). CSCs in serous ovarian cancers were also found to be infrequent (<0.04%) and again varied substantially among patients (Stewart et al., 2011). Analogous to these observations, evaluation of leukemic stem cells (LSCs) under improved xenotransplantation conditions revealed highly variable LSC frequencies in the range of 1 in 10^3–10^6 cells (Eppert et al., 2011).

CSCs may not necessarily constitute a minor component of the tumor. A relatively high proportion of leukemia-propagating cells has been observed in specific syngeneic mouse models of lymphomas and leukemias, whereas lower frequencies of CSCs generally occur in murine models of epithelial and other solid tumors (reviewed in Visvader and Lindeman, 2008). Interestingly, initiating "driver" mutations were found to affect the frequencies of LSCs in mouse models of AML (Heuser et al., 2009; Somervaille et al., 2009). In high-grade tumors from patients, an increased pool of CSCs may underlie their aggressive behavior (Boiko et al., 2010; Pece et al., 2010; Zhang et al., 2012). Indeed, the CSC model of heterogeneity may apply more readily to early-stage than to advanced tumors, in which dominant clones probably drive tumor progression.

Instability of the CSC Phenotype

Substantial differences in the immunophenotype of tumor-propagating cells between primary cancer specimens and their corresponding xenografts have been reported. Whereas most primary serous ovarian cancers contained $CD133^+$ CSCs, the majority of xenografted tumors contained significant numbers of $CD133^-$ tumor-initiating cells that could not be attributed to contamination (Stewart et al., 2011). The marked changes in copy number variation between these primary and xenografted tumors suggest that genetic change is driving tumor progression, although the pre-existence of subclonal diversity cannot be excluded. These data not only reflect heterogeneity within the tumor-propagating compartment but also indicate that the CSC phenotype may not be stable upon xenograft passaging. Although the frequency of CSCs for some ovarian (Stewart et al., 2011) and breast cancer (Meyer et al., 2010) xenografts remained constant, the frequency of CSCs has been observed to increase during serial transplantation, thus emphasizing the need to study early-passage tumors (Boiko et al., 2010; Ishizawa et al., 2010).

Existence of Multiple CSC Pools within Individual Tumors

Cancers can harbor heterogeneous and biologically distinct populations of CSCs. Recent studies have identified molecularly distinct leukemic stem cell populations defined by CD34, CD38, and/or IL3Rα expression. In the majority of AML patients, two hierarchically organized LSCs were shown to coexist. These populations are more closely related to normal progenitor subtypes than hematopoietic stem cells (HSCs) (Goardon et al., 2011), implying that the progenitors have aberrantly acquired stem cell properties. Complementary findings were made with a large number of AML patient samples, in which both progenitor and more primitive HSC-like fractions contained LSCs and generated the same phenotypic diversity found in the primary samples (Eppert et al., 2011; Sarry et al., 2011). Notably, another study revealed that $CD38^+$ AML cells may have previously escaped detection because of their unexpected clearance by the antibody (Taussig et al., 2008). In a HoxA9-Meis1-driven mouse model of AML, multiple phenotypically distinct LSCs were identified, and each was capable of recapitulating the original disease histopathology (Gibbs et al., 2012). Collectively, these findings demonstrate heterogeneity within the LSC compartment of individual patient specimens and also indicate that AML often appears as a progenitor disease. In at least some of these AMLs, a hierarchical relationship appears to exist among the different LSC subsets. The observation that different pools can clonally recapitulate the immunophenotype of the primary specimen suggests that LSCs may dedifferentiate or exhibit phenotypic interconversion.

Heterogeneous CSC compartments have recently been unmasked in solid tumors. In ovarian, breast, and squamous cell carcinomas, distinct CSC populations that regenerate the phenotypic and functional heterogeneity of the parental tumor have been described (Meyer et al., 2010; Schober and Fuchs, 2011; Stewart et al., 2011). In the case of primary colorectal cancers, three different types of tumor-initiating cells were resolved on the basis of clonal sphere cultures from individual patient tumors: a rare subset of CSCs that maintained tumor growth on serial transplantation, a tumor-initiating cell with limited self-renewal capacity (therefore not defined as a CSC), and a more latent CSC that apparently was activated in secondary or tertiary transplantation assays (Dieter et al., 2011). Because spheres generated from single cells comprised three cell types defined by differences in self-renewal, epigenetic rather than genetic mechanisms may account for the functional differences. Clonal heterogeneity among tumor-initiating cells was also observed in PTEN-deficient glioblastoma, in which a series of phenotypically distinct self-renewing cells was observed in both the $CD133^+$ and $CD133^-$ fractions (Chen et al., 2010). These cells were arranged in a linear hierarchy and generated tumors with different growth kinetics in serial transplantation experiments. However, both of these studies relied on sphere cultures of cells maintained under specific conditions, and therefore need to be validated with fresh primary tumor samples. Although tumor-propagating ability can reflect sphere-forming capacity, it is important to note that they do not always equate because the selection of specific cells may occur in vitro (Read et al., 2009).

Adding a further layer of complexity, distinct CSC subsets within a tumor have the potential to interconvert. In skin squamous cell carcinomas, two CSC subsets located along the tumor-stroma interface displayed different tumor growth kinetics and could interchange phenotype (Schober and Fuchs, 2011). These may not represent distinct CSC pools but rather stochastic variation within a single CSC population in response to microenvironmental signals. Phenotypic conversion also occurs among nonhierarchically organized tumor cells in melanoma (Quintana et al., 2010).

Metastatic CSCs May Be the Same or Distinct from the Primary CSC

There is growing evidence for the existence of functionally distinct subsets of tumor cells that impart metastatic activity. CSC subsets within primary tumors may harbor CSC subsets with tumor-propagating and/or metastatic capacity (Figure 3). In breast cancer, noninvasive imaging indicated that primary tumor CSCs characterized by CD44 expression are directly involved in metastasis (Liu et al., 2010). Similarly, in colorectal cancer, metastasis was almost exclusively a property of the CSCs that exhibited long-term self-renewing capacity (Dieter et al., 2011). Multiple disseminating CSCs homed to the bone marrow and generated liver metastases but only single clones were detected in peripheral blood, suggesting that metastatic CSCs enter the circulation transiently. In a related study on colorectal cancer, a subset of $CD26^+$ cells resident within primary and metastatic tumors demonstrated tumor propagation, chemoresistance, and liver metastatic potential after implantation at the orthotopic site (the cecal wall). Significantly, the presence

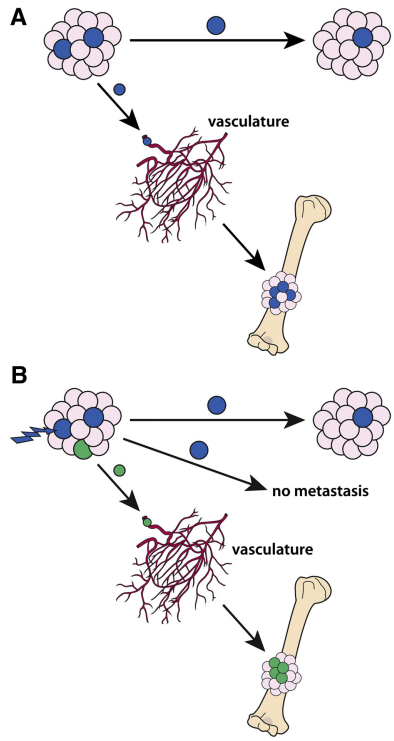

Figure 3. CSC Subsets within Primary Tumors May Harbor Metastatic and Tumor-Propagating Capacity

(A) A CSC may be responsible for both local and disseminated tumor propagation. In the example shown, a CSC (blue) enters the vasculature and metastasizes to a distant organ, where it seeds a heterogeneous tumor deposit exhibiting the hallmark features of the primary tumor.

(B) Alternatively, genetic and/or epigenetic mechanisms acting in the primary CSC could lead to the emergence of a self-renewing metastatic CSC (green) expressing distinct markers from the original CSC. This metastatic CSC, through a series of invasive processes, seeds secondary tumors in distant organs.

of $CD26^+$ cells in primary tumors also predicted metastasis in patients (Pang et al., 2010). In these epithelial malignancies, the epithelial mesenchymal transition (EMT) may underlie the metastatic process and give rise to precursors of metastatic CSCs at the invading edge of the tumor (Mani et al., 2008).

In other tumors, a unique subset of metastatic CSCs may drive metastasis. In pancreatic cancer, only $CD133^+CXCR4^+$ cells (not $CD133^+CXCR4^-$ cells) demonstrated metastatic activity, despite both subsets having tumor-propagating capacity (Hermann et al., 2007). Moreover, inhibition of CXCR4 signaling profoundly reduced the metastatic potential of pancreatic tumors without altering their tumorigenic potential. This metastatic CSC may have evolved from the primary tumor CSC or, alternatively, from a non-CSC within the tumor. The delineation of functionally distinct pools of CSCs will ultimately require cell tracing studies in vivo, via either mouse models relevant to human disease or minimally manipulated human cells for transplantation. Tracking of tumor cells in the circulation should also provide insight into metastatic CSCs.

Not All Cancers Harbor CSCs

Not all cancers will be sustained by CSCs. In melanoma, the high proportion of tumorigenic cells (as many as 50%) assayed

under more permissive conditions and with a wide spectrum of markers (e.g., CD271), argues against a CSC model of heterogeneity (Quintana et al., 2010). On the other hand, there are data from two groups indicating that melanoma lesions contain a CSC subset characterized by CD271 expression with nude recipient mice (Boiko et al., 2010; Civenni et al., 2011). However, in more immunocompromised strains such as NOD-SCID-IL2R$\gamma^{-/-}$ mice, both subsets were found to be tumorigenic, although the CD271^{-} fraction did not phenocopy the original tumor histology (Civenni et al., 2011). One factor contributing to these disparities (besides recipient strain) will include the use of trypsin during tissue dissociation (Quintana et al., 2010), given that the CD271 antigen was shown to be sensitive to this enzyme (Civenni et al., 2011). Thus, the inclusion of trypsin in the dissociation procedure will result in contamination of the negative fraction with cells that actually express the antigen. Nevertheless, a large number of markers evaluated by Morrison and colleagues (Quintana et al., 2010) yielded cell populations that were tumorigenic irrespective of marker expression. It seems plausible that other parameters such as implantation conditions and tumor grade also contribute to the discrepant findings. Another potential issue is that the frequency of CSCs can vary widely from 2.5% to 41% (Boiko et al., 2010), suggesting that the CSC model is not applicable to those tumors containing a high proportion of tumor-forming cells. Melanoma may also use distinct cellular mechanisms from most other solid malignancies, given the highly migratory nature of neural crest cells and their ability to respond to immune-based therapies.

Role of the CSC Niche

Cancers comprise malignant cells together with inflammatory cells, hematopoietic cells, associated stroma, and vasculature. Although some CSCs conceivably do not require a dedicated niche, others will be dependent on a specific set of extrinsic interactions with their microenvironment. The niche effect on tumor cells may be inductive or selective but will inevitably differ for every tumor subtype. The perivascular niche of CSCs in brain cancers is the best characterized to date (reviewed in Gilbertson and Rich, 2007). In at least some glioblastomas, the relationship between the CSC and local environment appears to be bidirectional: the niche can alter the cellular fate of cancer cells and, conversely, CSCs can modify their microenvironment (Heddleston et al., 2009; Hjelmeland et al., 2011; Ricci-Vitiani et al., 2010; Wang et al., 2010b). Indeed, CSCs in glioblastoma have been demonstrated to secrete VEGF that directly supports the development of the local vasculature (Gilbertson and Rich, 2007). In the reverse direction, endothelial cells secrete nitric oxide that induces Notch signaling in glioma cells (Charles et al., 2010). It is relevant that CSCs but not non-CSCs in gliomas were shown to be dependent on nitric oxide synthase-2 (Eyler et al., 2011). Intriguingly, CSCs in glioblastomas can directly contribute to the microvasculature through their transdifferentiation into vascular cells (Ricci-Vitiani et al., 2010; Wang et al., 2010b), underscoring the close relationship between brain CSCs and their niche. The perivascular niche also serves a crucial role in the case of cutaneous squamous cell carcinomas. CSCs in this vascular niche establish an autocrine loop in which VEGF promotes CSC activity by governing both the microenvironment and intrinsic self-renewal pathways in CSCs (Beck et al., 2011). Even in nonsolid tumors, microenvironmental cues from cytokines, growth factors, or the immune-deficient strain play an instructive role in determining the lineage fate of LSCs in a human model of leukemia (Wei et al., 2008). Hence, there has been considerable interest in targeting the putative CSC niche.

Cells within the tumor-associated stroma, such as myofibroblasts, are likely to have a prominent role in controlling CSC homeostasis in many tumor types. In colorectal cancer, myofibroblasts secrete HGF that maintains CSC function by activating the Wnt pathway. Interestingly, tumor cells with an active Wnt canonical pathway were preferentially located adjacent to stromal myofibroblasts (Vermeulen et al., 2010). Moreover, HGF-mediated activation of the Wnt pathway could induce CSC features and tumorigenic capacity in differentiated cancer cells that otherwise had limited tumorigenic capacity. Although these studies used spheroid cultures of primary colorectal cancers rather than fresh patient specimens, they suggest that the microenvironment can govern tumor cell "stemness." Selective targeting of myofibroblasts or the HGF/c-MET pathway would be predicted to interfere with the maintenance of CSCs and to potentially prevent the generation of CSCs from the non-CSC compartment. Notably, HGF is a potent inducer of the EMT, which plays a role in mediating invasion and metastasis. Using a mouse model of mammary tumorigenesis, another stromal factor, periostin, was shown to be essential for metastatic colonization by governing interactions between CSCs and their metastatic niche (Malanchi et al., 2012). Finally, the perturbation of other stromal mesenchymal cells such as osteoprogenitors can disrupt homeostasis, resulting in myelodysplasia and secondary leukemia (Raaijmakers et al., 2010). These findings support the notion of niche-induced transformation and suggest that selective targeting of the tumor microenvironment may represent an alternative or adjunct to targeting the CSC.

Even though it will be extraordinarily difficult to delineate the niche for human tumor CSCs and recapitulate the immune system of cancer patients, it is crucial to use an orthotopic transplantation assay to mimic the tumor environment as closely as possible. The coinoculation of human stromal cells to create a more appropriate environment for tumor development is also a relevant parameter to consider. The site of injection has been shown to directly influence the frequency of tumor-initiating cells, underscoring the relevance of context. For example, the frequency of tumor-initiating cells in ovarian tumors was highest and most reliably read-out by the mammary fat pad assay rather than the ovarian bursa (Stewart et al., 2011). However, the questions arise as to why these microenvironments differentially influence cell tumorigenicity and whether the tumor-initiating cells measured in the fat pad are in fact different from those assayed in the ovarian bursa.

Pathways Regulating CSC Function

Elucidation of the pathways that regulate the maintenance and survival of CSCs is important for the development of novel therapies. Not surprisingly, many CSC subsets and normal tissue stem cells seem to share core regulatory genes and developmental pathways such as c-*myc*, Bmi-1, and the Hedgehog (Hh), Notch, and Wnt pathways. Indeed, there is substantial evidence that restricted progenitors can generate LSCs by the reactivation of distinct self-renewal programs (Krivtsov et al.,

2006; Somervaille and Cleary, 2006; Somervaille et al., 2009). In chronic myeloid leukemia (CML), Hh signaling is essential for the maintenance and function of LSCs, and loss of Hh activity via disruption of Smoothened led to depletion of LSCs in vivo and prolonged animal survival (Dierks et al., 2008; Zhao et al., 2009). In the transition to the blast cell crisis phase, the LSC appears to originate from the granulocyte-macrophage progenitor cell through the acquired activation of the Wnt pathway (Jamieson et al., 2004). The Wnt pathway also plays a prominent role in the generation and self-renewal of LSCs in AML (Wang et al., 2010c). Significantly, β-catenin activation endowed progenitor cells with self-renewing capability but was not essential for the renewal of normal adult HSCs. Parallel findings were made for cutaneous CSCs versus normal skin stem cells in mouse models of skin cancer (Malanchi et al., 2008). Hence, the genetic programs governing self-renewal may be differentially active in normal and malignant stem cells, thereby opening therapeutic avenues.

Cell polarity and metabolic pathways have recently been implicated in governing the function of CSCs. TAZ, a transcriptional effector in the Hippo pathway, was found to be frequently overexpressed in high-grade breast cancers and to maintain the self-renewing capacity of tumorigenic cells isolated from established cell lines (Cordenonsi et al., 2011). A key link was established between the Hippo pathway and the cell polarity gene Scribbled, suggesting that cell polarity pathways may impact CSC function. It will be important to extend these studies to fresh tumor specimens because cancer cell lines do not reflect in vivo tumor cell behavior. In a metabolic context, glycine decarboxylase was demonstrated to regulate the activity of tumor-propagating cells in non-small cell lung cancer (Zhang et al., 2012). Aberrant expression of glycine decarboxylase occurs in multiple cancer types and leads to changes in glycine/serine metabolism. The observation that CSC activity was dependent on glycine decarboxylase function provides a direct link between glycine metabolism and tumorigenesis. Although it is presumed that metabolic processes play a crucial role in all tumor cells, it is intriguing that they can selectively influence CSC function.

CSCs and Stemness Signatures

In spite of the heterogeneity exhibited by CSCs, recent gene expression profiling studies have provided important insights into the prognostic significance of CSCs. The molecular analyses of functionally defined LSC populations from AML patients led to the generation of a LSC signature that largely reflects a self-renewal or stemness signature (Eppert et al., 2011). This signature was found to be a strong predictor of poor prognosis, with the implication that it may be possible to identify patients at highest risk and to inform both the type and duration of their therapy. In a murine model of AML, a conserved signal transduction network was unveiled among different LSCs (Gibbs et al., 2012). Other recently derived "stem cell" signatures also exhibit prognostic value. In colorectal cancer, a gene signature derived for adult intestinal stem cells predicted relapse in patients and identified EphB2-positive CSCs in tumors (Merlos-Suárez et al., 2011). Moreover, an embryonic stem cell (ESC)- and metastatic cell-based stem cell signature was found to increase with tumor grade and mortality in multiple tumor types (Shats et al., 2011), while an ESC-like transcriptional program evident in diverse epithelial cancers predicted poor prognosis (Wong et al., 2008). Preliminary data suggest that activation of this transcriptional program in adult cells may lead to the generation of CSCs. Another important player in ESCs, STAT3, was implicated in maintaining the stemness of glioma CSCs (Guryanova et al., 2011). Overall, these findings suggest that targeting self-renewal pathways may represent one of the most effective strategies for eradicating CSCs (see below).

Therapeutic Strategies to Target CSCs

From a clinical perspective, it is important to decipher mechanisms of chemo- and radioresistance that operate in CSCs. Quiescent CSCs are thought to be more resistant to therapies while most CSCs seem to evade cytotoxic or radiotherapy through active mechanisms. There is clinical evidence for a subpopulation of chemotherapy-resistant "CSCs" in a number of solid tumors including breast cancer (Li et al., 2008; Yu et al., 2007). Furthermore, the analysis of breast tissue taken from patients pre- and postendocrine therapy or chemotherapy for gene expression changes revealed that residual breast cancers may be enriched for tumor cells with CSC-like and mesenchymal characteristics (Creighton et al., 2009). In patients with del(5q) myelodysplastic syndrome, rare stem cells were found to be refractory to therapeutic targeting in individuals in remission, which probably accounts for relapse (Tehranchi et al., 2010).

Different aspects of CSCs have been explored in recent targeting strategies including quiescence, self-renewal pathways, radioresistance, and CSC-specific cell surface molecules. Several reports, predominantly for hematopoietic malignancies, indicate that CSCs can be selectively targeted without ablating normal stem cell function. Stem cell maintenance pathways are emerging as prime targets to eradicate CSCs. This approach, however, will be applicable only if the genetic programs controlling self-renewal are differentially active in malignant versus normal stem cells. It will be imperative to carefully evaluate the toxicity of anti-CSC agents on normal stem cell function in preclinical models. There are little data on the use of differentiation therapy in the context of CSCs but BMPs may be effective in inducing glial differentiation in glioblastomas and attenuating tumor growth (Piccirillo et al., 2006). In all likelihood, given the large number of mutations incurred by solid tumors such as breast (Wood et al., 2007), it will be essential to target multiple pathways that have been activated in CSCs in a given tumor.

Quiescence or dormancy is a property of at least some CSCs such as those in leukemia. This feature has recently been exploited to provide a window for therapeutic intervention. Cytokines such as G-CSF efficiently induced quiescent LSCs in AML to enter the cell cycle, thus sensitizing them to different chemotherapeutic agents (Saito et al., 2010). Indeed, combined G-CSF with chemotherapy elicited profound apoptosis and eradication of human AML stem cells in vivo. Inhibition of DNA repair mechanisms may also be harnessed for eradication of slow cycling LSCs (Viale et al., 2009). Although LSCs may reside in a more quiescent state, emerging evidence suggests that solid tumor CSCs follow a different pattern. In glioblastoma, CSCs are actively self-renewing and cellular diversity is most probably generated through symmetric cell division (Lathia et al., 2011b). Despite the observation that mouse mammary CSCs appear to

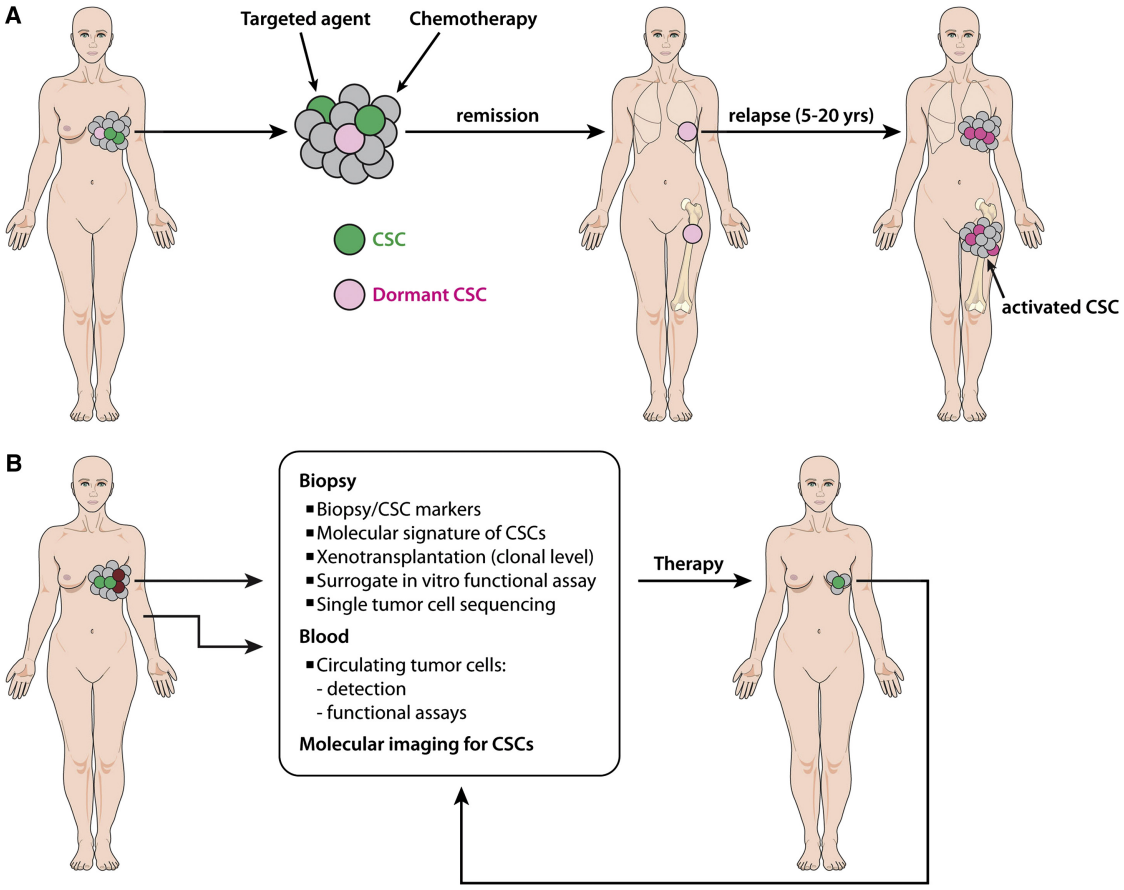

Figure 4. Possible Mechanisms of Metastatic Relapse after Anticancer Therapy and Evaluation of Anti-CSC Treatments

(A) Late relapse can be accounted for by CSC dormancy. Here a dormant CSC (pink) that is resistant to both chemotherapy and targeted therapy has seeded to distant organs. After a considerable latency period, reactivation of a CSC will result in tumor growth and clinical emergence of metastases. Intriguingly, the clinical appearance of metastases is often synchronous in breast cancer.

(B) One of the challenges facing the field is the clinical translation of anti-CSC therapies. Strategies will need to be deployed in clinical trials that enable reproducible assays of CSC activity. In the scenario depicted here, biopsy material from a newly diagnosed breast cancer is subjected to a variety of assays to measure CSCs, including functional assays and gene expression profiling, in parallel with the collection of blood samples and tumor imaging. The latter could include nanoparticle-labeling of anti-CSC markers coupled with in vivo imaging. These assays can be repeated after neoadjuvant therapy to determine whether the therapy has elicited a response against putative CSCs.

undergo more frequent symmetrical division than normal mammary stem cells in mammosphere cultures (Cicalese et al., 2009), it is tempting to speculate that the long period between primary tumor detection and relapse in patients with ER-positive breast cancer (up to 20 years) may reflect a dormant stem cell subset (Figure 4).

Of the key developmental pathways frequently deregulated in CSCs, considerable progress has been made in the case of targeted therapies against Notch and Hh, but the development of Wnt inhibitors has proven difficult. Significantly, pharmacologic inhibition of the Hh pathway in human and mouse leukemias inhibited the expansion of imatinib-resistant CML (Dierks et al., 2008; Zhao et al., 2009). These findings have profound implications because they suggest that treatment of imatinib-resistant recurrence in CML patients may be achievable via targeting the Hh pathway. However, because Hh pathway activity is required for maintenance of normal HSCs, it will be crucial to determine the effects of these anticancer agents on all aspects of normal HSC function. Pharmacologic or siRNA-mediated inhibition of Hh signaling in CSCs in glioblastoma, medulloblastoma, breast, pancreatic adenocarcinoma, and multiple myeloma has resulted in markedly reduced tumorigenic potential and, in some cases, ameliorated metastasis (reviewed in Merchant and Matsui, 2010). Hh ligands may play a dual role in the maintenance of CSCs and their niche, given the high stromal expression of these ligands. In terms of Notch signaling, CSCs in brain cancer were rendered more sensitive to radiation by blockade of this pathway (Wang et al., 2010a). Notably, Notch pathway inhibition via a neutralizing antibody against the DLL4 ligand was effective in reducing CSC numbers in diverse solid tumor xenografts (Hoey et al., 2009), whereas inhibition of Notch-4 expressed within the CSC subset largely ablated breast tumor growth (Harrison et al., 2010). A combination of Notch and Hh signaling may drive the self-renewal of CSCs in certain tumors such as undifferentiated pleomorphic sarcomas (Wang et al., 2012). Other self-renewal programs such as those regulated by Nodal and Activin, factors important for ESC maintenance, are also candidate targets. Pharmacologic inhibition of

the Nodal/Activin pathway sensitized CSCs to gemcitabine in a human xenograft model and significantly prolonged survival when combined with a stroma-targeting Hh inhibitor to improve drug delivery (Lonardo et al., 2011).

There has been considerable interest in the development of monoclonal antibodies to target CSCs. Markers differentially expressed between normal stem cells and LSCs have been utilized to specifically target LSCs in human AML, including CD44 (Jin et al., 2006), IL3R (Jin et al., 2009), and the immunoglobulin mucin TIM-3 (Kikushige et al., 2010). In each case, treatment with antibodies against these cell surface molecules dramatically decreased leukemogenicity and eradicated LSCs as assessed by AML reconstitution in mice. Furthermore, the targeting of CD44 provides a paradigm for targeting CSC-niche interactions. Blocking antibodies against CD47, which serves as a "don't eat me" signal to tumor macrophages, may also be effective in eliminating LSCs in ALL that express higher levels of this antigen than their normal counterpart (Chao et al., 2011).

Several studies have highlighted the radioresistance of CSCs in solid tumors, particularly in brain cancer. CSCs in fresh glioblastoma specimens or glioma xenografts are more resistant to ionizing irradiation (IR) in vivo than non-CSCs because of enhanced DNA repair pathways operating in CSCs (reviewed in Gilbertson and Rich, 2007). In medulloblastoma, targeting of cells in the perivascular region with Akt inhibitors enhanced responsiveness to radiation (Hambardzumyan et al., 2008), indicating that the CSC niche itself may serve as a therapeutic target. Interestingly, the DNA damage checkpoint response and radioresistance of CSCs in glioma is regulated in part by the adhesion molecule L1CAM through the activation of the ATM kinase pathway (Cheng et al., 2011). Similarly, radioresistance has been implicated in breast CSC-like populations that are thought to repair DNA damage more efficiently. Inhibition of the Akt pathway led to the selective targeting of CSCs by blocking canonical WNT signaling and repair of DNA damage in these cells, thus sensitizing them to ionizing radiation (Zhang et al., 2010). In some breast tumors, lower ROS levels were found in certain CSC subsets compared with their nontumorigenic counterparts, perhaps conferring resistance to ionizing radiation (Diehn et al., 2009).

Other therapeutic targets currently being pursued in the context of CSCs include growth factor receptor signaling networks. In pancreatic cancer, inhibition of the c-MET tyrosine kinase receptor diminished the CSC population and prevented metastasis, either alone or in combination with gemcitabine (Li et al., 2011). These inhibitors may also prove efficacious in colorectal cancer (Vermeulen et al., 2010). Recent studies on squamous carcinoma revealed that selective inhibition of VEGF signaling reduced CSC activity and led to tumor regression (Beck et al., 2011), implying that it may be necessary to target both CSCs and the stroma in which they reside. Cytokine pathways such as IL-8/CXCR1 are also emerging as important modulators of CSC activity. Repertaxin-mediated inhibition of this pathway reduced breast tumorigenesis and metastasis but only in combination with chemotherapy (Ginestier et al., 2010).

In the search for novel drug discovery platforms, high throughput screens using small molecule, miRNA, or siRNA libraries have become an area of increasing focus. The application of a high throughput screen to target breast CSCs revealed a class of compounds that had previously not been implicated as cancer drugs: salinomycin-reduced tumor growth and lung metastases, possibly via direct targeting of breast CSCs (Gupta et al., 2009). An analogous screen to identify small molecule inhibitors of LSCs in AML led to the recent discovery of kinetic riboside (McDermott et al., 2012). MicroRNA-based therapies are emerging as novel modes of therapeutic intervention. Notably, systemic delivery of miR-34a, which is expressed at low levels in prostate CSCs, inhibited metastasis of prostate cancer cells and prolonged survival of mice (Liu et al., 2011). It is relevant that miR-34a targets CD44, a cell surface marker used to enrich prostate CSCs. Further large-scale screens should be enabled by the development of improved surrogate in vitro culture assays that maintain the integrity of primary tumor-derived CSCs, rather than the use of cell lines. Ultimately, the testing of all putative anti-CSC agents requires preclinical mouse models containing early passage xenografts (or leukemic cells) to obviate any changes that occur upon prolonged passage.

Evolution of CSCs and Tumor Cell "Plasticity"

It is important to note that the CSC and clonal evolution concepts are not mutually exclusive. Two recent papers have highlighted a high degree of convergence between these models in leukemia. LSCs in acute lymphoblastic leukemia harboring the ETV6-Runx1 translocation were shown to be genetically diverse, exhibiting different degrees of self-renewing and leukemogenic activity in vivo (Anderson et al., 2011). This study provides evidence that CSCs within individual cancer patients can be genetically heterogeneous, presumably accounting for their variable biological properties such as self-renewal potential. Moreover, in BCR-ABL acute lymphoblastic leukemia patients, the leukemia-initiating population displayed profound genetic diversity, with multiple genetically distinct tumor-initiating subclones at diagnosis (Notta et al., 2011). Although it has not yet been determined whether these tumor-initiating cells follow the CSC model, it seems likely. In parallel with the other study, a nonlinear, branching model of tumor evolution was identified. Taken together, these studies illustrate the importance of complementing functional assays of cellular heterogeneity with genetic fingerprinting of the different subsets.

In addition to genetic variegation, tumor cell plasticity may contribute to phenotypic and functional heterogeneity. Many cell surface markers on melanoma cells are reversibly expressed, such that phenotypically diverse melanoma cells can recapitulate tumor heterogeneity of the parent tumor, irrespective of whether they arose from marker-positive or marker-negative cells (Quintana et al., 2010). Regulatory genes may also be transiently or stochastically expressed. JARID1B-mediated histone demethylation was demonstrated to be reversibly expressed in melanoma cell lines and to be essential for the maintenance of tumorigenic activity (Roesch et al., 2010). Slow cycling JARID1B-expressing cells could arise from a negative population even when initiated from a single cell, suggesting that a nontumorigenic cell may reacquire stem cell-like properties. In addition, cells within breast cancer cell lines were found to transition stochastically between states to establish a stable phenotypic equilibrium (Gupta et al., 2011), and CSC-like cells could arise de novo from transformed breast epithelial cells

(Chaffer et al., 2011). These data suggest that cellular interconversion might occur in a stochastic manner.

CSC function may be induced by specific microenvironmental cues from growth factors or in stress-related contexts. For example, HGF has been implicated in the reprogramming of non-CSCs toward a CSC-like phenotype in colon cancer (Vermeulen et al., 2010). Furthermore, induction of an EMT in immortalized human breast epithelial cells can endow them with stem cell-like properties and potentially promote the generation of CSCs from tumor cells (Mani et al., 2008). *HIF2α*, induced as a cellular response to a hypoxic microenvironment, has been implicated in the maintenance of CSCs in glioblastoma and may promote the interconversion of nonstem ($CD133^{-}$) to CSC-like cells (Heddleston et al., 2009; Li et al., 2009). The reversible state, in its various guises discussed above, has profound implications for the treatment and management of patients. Moreover, therapeutic resistance itself may potentially reflect a reversible state (Sharma et al., 2010).

Finally, it should be noted that many of the studies described in this section used cell lines or cultured primary cells, and that at present there is no evidence for dedifferentiation occurring in primary tumors in vivo. However, it is noteworthy that dedifferentiation can occur at a low frequency in normal tissues such as the testis (Barroca et al., 2009). Because definitive cell surface markers are lacking for most stem cells and their descendants, at least in solid organs, it is difficult to study cellular plasticity. Further elucidation of differentiation hierarchies may eventually enable "plasticity" and interconversion of cells between nontumorigenic and tumorigenic states to be formally tested by clonal cell tracking analysis in vivo.

Conclusions

An emerging consensus in the field is that "cellular state" rather than phenotype is important when defining a CSC. The uniformity between LSC signatures that has emerged across diverse samples, despite interpatient variation in CSC markers, confirms that current phenotypic markers are not a reliable measure of CSCs. One corollary of high interpatient variation is that an extensive range of markers will need to be validated in a large number of patient samples, perhaps even necessitating functional assessment for each patient. Indeed, the purification of CSCs using a robust set of markers, even from a given tumor subtype, remains a major challenge for the field. If CSCs exist in a dynamic state in certain tumors, then this will inevitably confound their prospective isolation. The existence of multiple CSC pools or evolving intratumoral clones in individual tumors demands the monitoring of these populations in pre- and posttreatment samples by multicolor flow cytometry or high-resolution molecular imaging to identify residual cells that might drive relapse (Figure 4). The derivation of robust signatures that distinguish CSCs from normal stem cells may also facilitate the evaluation of clinically relevant residual cells.

The clinical applicability of the CSC concept to predicting patient response remains a fundamental question. Most putative anti-CSC therapies to date have attenuated rather than eradicated solid tumors in preclinical models, and efficacious response often required concomitant chemotherapy. In order to improve the evaluation of efficacy of anti-CSC agents in clinical trials, there is a pressing need to optimize assays for CSC targeting and measurement of tumor response. In standard clinical trials, tumor response criteria depend on measurements of tumor size, which largely reflects tumor response in the non-CSC tumor bulk. Specific response criteria that provide a readout of response to anti-CSC agents in clinical trials remain elusive (Figure 4). Tumor sphere-forming assays and measurement of CSC marker expression are unlikely to provide robust surrogate markers in a clinical setting. The incorporation of other measures such as self-renewal activity into therapeutic strategies will almost certainly be required. We speculate that for most tumor types it will still prove necessary to test novel anti-CSC therapies in combination with tumor debulking (non-CSC) therapy, such as conventional chemotherapy.

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REFERENCES

Anderson, K., Lutz, C., van Delft, F.W., Bateman, C.M., Guo, Y., Colman, S.M., Kempski, H., Moorman, A.V., Titley, I., Swansbury, J., et al. (2011). Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature *469*, 356–361.

Barroca, V., Lassalle, B., Coureuil, M., Louis, J.P., Le Page, F., Testart, J., Allemand, I., Riou, L., and Fouchet, P. (2009). Mouse differentiating spermatogonia can generate germinal stem cells in vivo. Nat. Cell Biol. *11*, 190–196.

Beck, B., Driessens, G., Goossens, S., Youssef, K.K., Kuchnio, A., Caauwe, A., Sotiropoulou, P.A., Loges, S., Lapouge, G., Candi, A., et al. (2011). A vascular niche and a VEGF-Nrp1 loop regulate the initiation and stemness of skin tumours. Nature *478*, 399–403.

Boiko, A.D., Razorenova, O.V., van de Rijn, M., Swetter, S.M., Johnson, D.L., Ly, D.P., Butler, P.D., Yang, G.P., Joshua, B., Kaplan, M.J., et al. (2010). Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. Nature *466*, 133–137.

Bonnet, D., and Dick, J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. *3*, 730–737.

Chaffer, C.L., Brueckmann, I., Scheel, C., Kaestli, A.J., Wiggins, P.A., Rodrigues, L.O., Brooks, M., Reinhardt, F., Su, Y., Polyak, K., et al. (2011). Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. Proc. Natl. Acad. Sci. USA *108*, 7950–7955.

Chao, M.P., Alizadeh, A.A., Tang, C., Jan, M., Weissman-Tsukamoto, R., Zhao, F., Park, C.Y., Weissman, I.L., and Majeti, R. (2011). Therapeutic antibody targeting of CD47 eliminates human acute lymphoblastic leukemia. Cancer Res. *71*, 1374–1384.

Charles, N., Ozawa, T., Squatrito, M., Bleau, A.M., Brennan, C.W., Hambardzumyan, D., and Holland, E.C. (2010). Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. Cell Stem Cell *6*, 141–152.

Chen, R., Nishimura, M.C., Bumbaca, S.M., Kharbanda, S., Forrest, W.F., Kasman, I.M., Greve, J.M., Soriano, R.H., Gilmour, L.L., Rivers, C.S., et al. (2010). A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. Cancer Cell *17*, 362–375.

Cheng, L., Wu, Q., Huang, Z., Guryanova, O.A., Huang, Q., Shou, W., Rich, J.N., and Bao, S. (2011). L1CAM regulates DNA damage checkpoint response of glioblastoma stem cells through NBS1. EMBO J. *30*, 800–813.

Cicalese, A., Bonizzi, G., Pasi, C.E., Faretta, M., Ronzoni, S., Giulini, B., Brisken, C., Minucci, S., Di Fiore, P.P., and Pelicci, P.G. (2009). The tumor

suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. Cell *138*, 1083–1095.

Civenni, G., Walter, A., Kobert, N., Mihic-Probst, D., Zipser, M., Belloni, B., Seifert, B., Moch, H., Dummer, R., van den Broek, M., and Sommer, L. (2011). Human CD271-positive melanoma stem cells associated with metastasis establish tumor heterogeneity and long-term growth. Cancer Res. *71*, 3098–3109.

Cordenonsi, M., Zanconato, F., Azzolin, L., Forcato, M., Rosato, A., Frasson, C., Inui, M., Montagner, M., Parenti, A.R., Poletti, A., et al. (2011). The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. Cell *147*, 759–772.

Creighton, C.J., Li, X., Landis, M., Dixon, J.M., Neumeister, V.M., Sjolund, A., Rimm, D.L., Wong, H., Rodriguez, A., Herschkowitz, J.I., et al. (2009). Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc. Natl. Acad. Sci. USA *106*, 13820–13825.

Curley, M.D., Therrien, V.A., Cummings, C.L., Sergent, P.A., Koulouris, C.R., Friel, A.M., Roberts, D.J., Seiden, M.V., Scadden, D.T., Rueda, B.R., and Foster, R. (2009). CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. Stem Cells *27*, 2875–2883.

Curtis, S.J., Sinkevicius, K.W., Li, D., Lau, A.N., Roach, R.R., Zamponi, R., Woolfenden, A.E., Kirsch, D.G., Wong, K.K., and Kim, C.F. (2010). Primary tumor genotype is an important determinant in identification of lung cancer propagating cells. Cell Stem Cell *7*, 127–133.

Dalerba, P., Dylla, S.J., Park, I.K., Liu, R., Wang, X., Cho, R.W., Hoey, T., Gurney, A., Huang, E.H., Simeone, D.M., et al. (2007). Phenotypic characterization of human colorectal cancer stem cells. Proc. Natl. Acad. Sci. USA *104*, 10158–10163.

Diehn, M., Cho, R.W., Lobo, N.A., Kalisky, T., Dorie, M.J., Kulp, A.N., Qian, D., Lam, J.S., Ailles, L.E., Wong, M., et al. (2009). Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature *458*, 780–783.

Dierks, C., Beigi, R., Guo, G.R., Zirlik, K., Stegert, M.R., Manley, P., Trussell, C., Schmitt-Graeff, A., Landwerlin, K., Veelken, H., and Warmuth, M. (2008). Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. Cancer Cell *14*, 238–249.

Dieter, S.M., Ball, C.R., Hoffmann, C.M., Nowrouzi, A., Herbst, F., Zavidij, O., Abel, U., Arens, A., Weichert, W., Brand, K., et al. (2011). Distinct types of tumor-initiating cells form human colon cancer tumors and metastases. Cell Stem Cell *9*, 357–365.

Eppert, K., Takenaka, K., Lechman, E.R., Waldron, L., Nilsson, B., van Galen, P., Metzeler, K.H., Poeppl, A., Ling, V., Beyene, J., et al. (2011). Stem cell gene expression programs influence clinical outcome in human leukemia. Nat. Med. *17*, 1086–1093.

Eyler, C.E., Wu, Q., Yan, K., MacSwords, J.M., Chandler-Militello, D., Misuraca, K.L., Lathia, J.D., Forrester, M.T., Lee, J., Stamler, J.S., et al. (2011). Glioma stem cell proliferation and tumor growth are promoted by nitric oxide synthase-2. Cell *146*, 53–66.

Gentles, A.J., Plevritis, S.K., Majeti, R., and Alizadeh, A.A. (2010). Association of a leukemic stem cell gene expression signature with clinical outcomes in acute myeloid leukemia. JAMA *304*, 2706–2715.

Gibbs, K.D., Jr., Jager, A., Crespo, O., Goltsev, Y., Trejo, A., Richard, C.E., and Nolan, G.P. (2012). Decoupling of tumor-initiating activity from stable immunophenotype in HoxA9-Meis1-driven AML. Cell Stem Cell *10*, 210–217.

Gilbertson, R.J., and Rich, J.N. (2007). Making a tumour's bed: glioblastoma stem cells and the vascular niche. Nat. Rev. Cancer *7*, 733–736.

Ginestier, C., Hur, M.H., Charafe-Jauffret, E., Monville, F., Dutcher, J., Brown, M., Jacquemier, J., Viens, P., Kleer, C.G., Liu, S., et al. (2007). ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell *1*, 555–567.

Ginestier, C., Liu, S., Diebel, M.E., Korkaya, H., Luo, M., Brown, M., Wicinski, J., Cabaud, O., Charafe-Jauffret, E., Birnbaum, D., et al. (2010). CXCR1 blockade selectively targets human breast cancer stem cells in vitro and in xenografts. J. Clin. Invest. *120*, 485–497.

Goardon, N., Marchi, E., Atzberger, A., Quek, L., Schuh, A., Soneji, S., Woll, P., Mead, A., Alford, K.A., Rout, R., et al. (2011). Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer Cell *19*, 138–152.

Gupta, P.B., Onder, T.T., Jiang, G., Tao, K., Kuperwasser, C., Weinberg, R.A., and Lander, E.S. (2009). Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell *138*, 645–659.

Gupta, P.B., Fillmore, C.M., Jiang, G., Shapira, S.D., Tao, K., Kuperwasser, C., and Lander, E.S. (2011). Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell *146*, 633–644.

Guryanova, O.A., Wu, Q., Cheng, L., Lathia, J.D., Huang, Z., Yang, J., MacSwords, J., Eyler, C.E., McLendon, R.E., Heddleston, J.M., et al. (2011). Nonreceptor tyrosine kinase BMX maintains self-renewal and tumorigenic potential of glioblastoma stem cells by activating STAT3. Cancer Cell *19*, 498–511.

Hambardzumyan, D., Becher, O.J., Rosenblum, M.K., Pandolfi, P.P., Manova-Todorova, K., and Holland, E.C. (2008). PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. Genes Dev. *22*, 436–448.

Harrison, H., Farnie, G., Howell, S.J., Rock, R.E., Stylianou, S., Brennan, K.R., Bundred, N.J., and Clarke, R.B. (2010). Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. Cancer Res. *70*, 709–718.

Heddleston, J.M., Li, Z., McLendon, R.E., Hjelmeland, A.B., and Rich, J.N. (2009). The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. Cell Cycle *8*, 3274–3284.

Hermann, P.C., Huber, S.L., Herrler, T., Aicher, A., Ellwart, J.W., Guba, M., Bruns, C.J., and Heeschen, C. (2007). Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell *1*, 313–323.

Heuser, M., Sly, L.M., Argiropoulos, B., Kuchenbauer, F., Lai, C., Weng, A., Leung, M., Lin, G., Brookes, C., Fung, S., et al. (2009). Modeling the functional heterogeneity of leukemia stem cells: role of STAT5 in leukemia stem cell self-renewal. Blood *114*, 3983–3993.

Hjelmeland, A.B., Wu, Q., Heddleston, J.M., Choudhary, G.S., MacSwords, J., Lathia, J.D., McLendon, R., Lindner, D., Sloan, A., and Rich, J.N. (2011). Acidic stress promotes a glioma stem cell phenotype. Cell Death Differ. *18*, 829–840.

Hoey, T., Yen, W.C., Axelrod, F., Basi, J., Donigian, L., Dylla, S., Fitch-Bruhns, M., Lazetic, S., Park, I.K., Sato, A., et al. (2009). DLL4 blockade inhibits tumor growth and reduces tumor-initiating cell frequency. Cell Stem Cell *5*, 168–177.

Ishizawa, K., Rasheed, Z.A., Karisch, R., Wang, Q., Kowalski, J., Susky, E., Pereira, K., Karamboulas, C., Moghal, N., Rajeshkumar, N.V., et al. (2010). Tumor-initiating cells are rare in many human tumors. Cell Stem Cell *7*, 279–282.

Jamieson, C.H., Ailles, L.E., Dylla, S.J., Muijtjens, M., Jones, C., Zehnder, J.L., Gotlib, J., Li, K., Manz, M.G., Keating, A., et al. (2004). Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N. Engl. J. Med. *351*, 657–667.

Jin, L., Hope, K.J., Zhai, Q., Smadja-Joffe, F., and Dick, J.E. (2006). Targeting of CD44 eradicates human acute myeloid leukemic stem cells. Nat. Med. *12*, 1167–1174.

Jin, L., Lee, E.M., Ramshaw, H.S., Busfield, S.J., Peoppl, A.G., Wilkinson, L., Guthridge, M.A., Thomas, D., Barry, E.F., Boyd, A., et al. (2009). Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells. Cell Stem Cell *5*, 31–42.

Kikushige, Y., Shima, T., Takayanagi, S., Urata, S., Miyamoto, T., Iwasaki, H., Takenaka, K., Teshima, T., Tanaka, T., Inagaki, Y., and Akashi, K. (2010). TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. Cell Stem Cell *7*, 708–717.

Krivtsov, A.V., Twomey, D., Feng, Z., Stubbs, M.C., Wang, Y., Faber, J., Levine, J.E., Wang, J., Hahn, W.C., Gilliland, D.G., et al. (2006). Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. Nature *442*, 818–822.

Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., Paterson, B., Caligiuri, M.A., and Dick, J.E. (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature *367*, 645–648.

Lathia, J.D., Gallagher, J., Heddleston, J.M., Wang, J., Eyler, C.E., Macswords, J., Wu, Q., Vasanji, A., McLendon, R.E., Hjelmeland, A.B., and

Rich, J.N. (2010). Integrin alpha 6 regulates glioblastoma stem cells. Cell Stem Cell *6*, 421–432.

Lathia, J.D., Gallagher, J., Myers, J.T., Li, M., Vasanji, A., McLendon, R.E., Hjelmeland, A.B., Huang, A.Y., and Rich, J.N. (2011a). Direct in vivo evidence for tumor propagation by glioblastoma cancer stem cells. PLoS ONE *6*, e24807.

Lathia, J.D., Hitomi, M., Gallagher, J., Gadani, S.P., Adkins, J., Vasanji, A., Liu, L., Eyler, C.E., Heddleston, J.M., Wu, Q., et al. (2011b). Distribution of CD133 reveals glioma stem cells self-renew through symmetric and asymmetric cell divisions. Cell Death Dis *2*, e200.

Li, X., Lewis, M.T., Huang, J., Gutierrez, C., Osborne, C.K., Wu, M.F., Hilsenbeck, S.G., Pavlick, A., Zhang, X., Chamness, G.C., et al. (2008). Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J. Natl. Cancer Inst. *100*, 672–679.

Li, Z., Bao, S., Wu, Q., Wang, H., Eyler, C., Sathornsumetee, S., Shi, Q., Cao, Y., Lathia, J., McLendon, R.E., et al. (2009). Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. Cancer Cell *15*, 501–513.

Li, C., Wu, J.J., Hynes, M., Dosch, J., Sarkar, B., Welling, T.H., Pasca di Magliano, M., and Simeone, D.M. (2011). c-Met is a marker of pancreatic cancer stem cells and therapeutic target. Gastroenterology *141*, 2218–2227, e5.

Liu, H., Patel, M.R., Prescher, J.A., Patsialou, A., Qian, D., Lin, J., Wen, S., Chang, Y.F., Bachmann, M.H., Shimono, Y., et al. (2010). Cancer stem cells from human breast tumors are involved in spontaneous metastases in orthotopic mouse models. Proc. Natl. Acad. Sci. USA *107*, 18115–18120.

Liu, C., Kelnar, K., Liu, B., Chen, X., Calhoun-Davis, T., Li, H., Patrawala, L., Yan, H., Jeter, C., Honorio, S., et al. (2011). The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Nat. Med. *17*, 211–215.

Lonardo, E., Hermann, P.C., Mueller, M.T., Huber, S., Balic, A., Miranda-Lorenzo, I., Zagorac, S., Alcala, S., Rodriguez-Arabaolaza, I., Ramirez, J.C., et al. (2011). Nodal/Activin signaling drives self-renewal and tumorigenicity of pancreatic cancer stem cells and provides a target for combined drug therapy. Cell Stem Cell *9*, 433–446.

Malanchi, I., Peinado, H., Kassen, D., Hussenet, T., Metzger, D., Chambon, P., Huber, M., Hohl, D., Cano, A., Birchmeier, W., and Huelsken, J. (2008). Cutaneous cancer stem cell maintenance is dependent on beta-catenin signalling. Nature *452*, 650–653.

Malanchi, I., Santamaria-Martínez, A., Susanto, E., Peng, H., Lehr, H.A., Delaloye, J.F., and Huelsken, J. (2012). Interactions between cancer stem cells and their niche govern metastatic colonization. Nature *481*, 85–89.

Mani, S.A., Guo, W., Liao, M.J., Eaton, E.N., Ayyanan, A., Zhou, A.Y., Brooks, M., Reinhard, F., Zhang, C.C., Shipitsin, M., et al. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell *133*, 704–715.

McDermott, S.P., Eppert, K., Notta, F., Isaac, M., Datti, A., Al-Awar, R., Wrana, J., Minden, M.D., and Dick, J.E. (2012). A small molecule screening strategy with validation on human leukemia stem cells uncovers the therapeutic efficacy of kinetin riboside. Blood *119*, 1200–1207.

Merchant, A.A., and Matsui, W. (2010). Targeting Hedgehog—a cancer stem cell pathway. Clin. Cancer Res. *16*, 3130–3140.

Merlos-Suárez, A., Barriga, F.M., Jung, P., Iglesias, M., Céspedes, M.V., Rossell, D., Sevillano, M., Hernando-Momblona, X., da Silva-Diz, V., Muñoz, P., et al. (2011). The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse. Cell Stem Cell *8*, 511–524.

Meyer, M.J., Fleming, J.M., Lin, A.F., Hussnain, S.A., Ginsburg, E., and Vonderhaar, B.K. (2010). CD44posCD49fhiCD133/2hi defines xenograft-initiating cells in estrogen receptor-negative breast cancer. Cancer Res. *70*, 4624–4633.

Notta, F., Mullighan, C.G., Wang, J.C., Poeppl, A., Doulatov, S., Phillips, L.A., Ma, J., Minden, M.D., Downing, J.R., and Dick, J.E. (2011). Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. Nature *469*, 362–367.

Nowell, P.C. (1976). The clonal evolution of tumor cell populations. Science *194*, 23–28.

Pang, R., Law, W.L., Chu, A.C., Poon, J.T., Lam, C.S., Chow, A.K., Ng, L., Cheung, L.W., Lan, X.R., Lan, H.Y., et al. (2010). A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell *6*, 603–615.

Pece, S., Tosoni, D., Confalonieri, S., Mazzarol, G., Vecchi, M., Ronzoni, S., Bernard, L., Viale, G., Pelicci, P.G., and Di Fiore, P.P. (2010). Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell *140*, 62–73.

Piccirillo, S.G., Reynolds, B.A., Zanetti, N., Lamorte, G., Binda, E., Broggi, G., Brem, H., Olivi, A., Dimeco, F., and Vescovi, A.L. (2006). Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature *444*, 761–765.

Quintana, E., Shackleton, M., Sabel, M.S., Fullen, D.R., Johnson, T.M., and Morrison, S.J. (2008). Efficient tumour formation by single human melanoma cells. Nature *456*, 593–598.

Quintana, E., Shackleton, M., Foster, H.R., Fullen, D.R., Sabel, M.S., Johnson, T.M., and Morrison, S.J. (2010). Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. Cancer Cell *18*, 510–523.

Raaijmakers, M.H., Mukherjee, S., Guo, S., Zhang, S., Kobayashi, T., Schoonmaker, J.A., Ebert, B.L., Al-Shahrour, F., Hasserjian, R.P., Scadden, E.O., et al. (2010). Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature *464*, 852–857.

Read, T.A., Fogarty, M.P., Markant, S.L., McLendon, R.E., Wei, Z., Ellison, D.W., Febbo, P.G., and Wechsler-Reya, R.J. (2009). Identification of CD15 as a marker for tumor-propagating cells in a mouse model of medulloblastoma. Cancer Cell *15*, 135–147.

Ricci-Vitiani, L., Pallini, R., Biffoni, M., Todaro, M., Invernici, G., Cenci, T., Maira, G., Parati, E.A., Stassi, G., Larocca, L.M., and De Maria, R. (2010). Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. Nature *468*, 824–828.

Roesch, A., Fukunaga-Kalabis, M., Schmidt, E.C., Zabierowski, S.E., Brafford, P.A., Vultur, A., Basu, D., Gimotty, P., Vogt, T., and Herlyn, M. (2010). A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. Cell *141*, 583–594.

Saito, Y., Uchida, N., Tanaka, S., Suzuki, N., Tomizawa-Murasawa, M., Sone, A., Najima, Y., Takagi, S., Aoki, Y., Wake, A., et al. (2010). Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. Nat. Biotechnol. *28*, 275–280.

Sarry, J.E., Murphy, K., Perry, R., Sanchez, P.V., Secreto, A., Keefer, C., Swider, C.R., Strzelecki, A.C., Cavelier, C., Récher, C., et al. (2011). Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rγc-deficient mice. J. Clin. Invest. *121*, 384–395.

Schober, M., and Fuchs, E. (2011). Tumor-initiating stem cells of squamous cell carcinomas and their control by TGF-β and integrin/focal adhesion kinase (FAK) signaling. Proc. Natl. Acad. Sci. USA *108*, 10544–10549.

Sharma, S.V., Lee, D.Y., Li, B., Quinlan, M.P., Takahashi, F., Maheswaran, S., McDermott, U., Azizian, N., Zou, L., Fischbach, M.A., et al. (2010). A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell *141*, 69–80.

Shats, I., Gatza, M.L., Chang, J.T., Mori, S., Wang, J., Rich, J., and Nevins, J.R. (2011). Using a stem cell-based signature to guide therapeutic selection in cancer. Cancer Res. *71*, 1772–1780.

Silva, I.A., Bai, S., McLean, K., Yang, K., Griffith, K., Thomas, D., Ginestier, C., Johnston, C., Kueck, A., Reynolds, R.K., et al. (2011). Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. Cancer Res. *71*, 3991–4001.

Singh, S.K., Hawkins, C., Clarke, I.D., Squire, J.A., Bayani, J., Hide, T., Henkelman, R.M., Cusimano, M.D., and Dirks, P.B. (2004). Identification of human brain tumour initiating cells. Nature *432*, 396–401.

Somervaille, T.C., and Cleary, M.L. (2006). Identification and characterization of leukemia stem cells in murine MLL-AF9 acute myeloid leukemia. Cancer Cell *10*, 257–268.

Somervaille, T.C., Matheny, C.J., Spencer, G.J., Iwasaki, M., Rinn, J.L., Witten, D.M., Chang, H.Y., Shurtleff, S.A., Downing, J.R., and Cleary, M.L. (2009). Hierarchical maintenance of MLL myeloid leukemia stem cells employs a transcriptional program shared with embryonic rather than adult stem cells. Cell Stem Cell *4*, 129–140.

Son, M.J., Woolard, K., Nam, D.H., Lee, J., and Fine, H.A. (2009). SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell Stem Cell *4*, 440–452.

Stewart, J.M., Shaw, P.A., Gedye, C., Bernardini, M.Q., Neel, B.G., and Ailles, L.E. (2011). Phenotypic heterogeneity and instability of human ovarian tumor-initiating cells. Proc. Natl. Acad. Sci. USA *108*, 6468–6473.

Taussig, D.C., Miraki-Moud, F., Anjos-Afonso, F., Pearce, D.J., Allen, K., Ridler, C., Lillington, D., Oakervee, H., Cavenagh, J., Agrawal, S.G., et al. (2008). Anti-CD38 antibody-mediated clearance of human repopulating cells masks the heterogeneity of leukemia-initiating cells. Blood *112*, 568–575.

Tehranchi, R., Woll, P.S., Anderson, K., Buza-Vidas, N., Mizukami, T., Mead, A.J., Astrand-Grundström, I., Strömbeck, B., Horvat, A., Ferry, H., et al. (2010). Persistent malignant stem cells in del(5q) myelodysplasia in remission. N. Engl. J. Med. *363*, 1025–1037.

Vermeulen, L., De Sousa E Melo, F., van der Heijden, M., Cameron, K., de Jong, J.H., Borovski, T., Tuynman, J.B., Todaro, M., Merz, C., Rodermond, H., et al. (2010). Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat. Cell Biol. *12*, 468–476.

Viale, A., De Franco, F., Orleth, A., Cambiaghi, V., Giuliani, V., Bossi, D., Ronchini, C., Ronzoni, S., Muradore, I., Monestiroli, S., et al. (2009). Cell-cycle restriction limits DNA damage and maintains self-renewal of leukaemia stem cells. Nature *457*, 51–56.

Visvader, J.E., and Lindeman, G.J. (2008). Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat. Rev. Cancer *8*, 755–768.

Wang, J., Wakeman, T.P., Lathia, J.D., Hjelmeland, A.B., Wang, X.F., White, R.R., Rich, J.N., and Sullenger, B.A. (2010a). Notch promotes radioresistance of glioma stem cells. Stem Cells *28*, 17–28.

Wang, R., Chadalavada, K., Wilshire, J., Kowalik, U., Hovinga, K.E., Geber, A., Fligelman, B., Leversha, M., Brennan, C., and Tabar, V. (2010b). Glioblastoma stem-like cells give rise to tumour endothelium. Nature *468*, 829–833.

Wang, Y., Krivtsov, A.V., Sinha, A.U., North, T.E., Goessling, W., Feng, Z., Zon, L.I., and Armstrong, S.A. (2010c). The Wnt/beta-catenin pathway is required for the development of leukemia stem cells in AML. Science *327*, 1650–1653.

Wang, C.Y., Wei, Q., Han, I., Sato, S., Ghanbari-Azarnier, R., Whetstone, H., Poon, R., Hu, J., Zheng, F., Zhang, P., et al. (2012). Hedgehog and Notch signaling regulate self-renewal of undifferentiated pleomorphic sarcomas. Cancer Res. *72*, 1013–1022.

Ward, R.J., Lee, L., Graham, K., Satkunendran, T., Yoshikawa, K., Ling, E., Harper, L., Austin, R., Nieuwenhuis, E., Clarke, I.D., et al. (2009). Multipotent CD15+ cancer stem cells in patched-1-deficient mouse medulloblastoma. Cancer Res. *69*, 4682–4690.

Wei, J., Wunderlich, M., Fox, C., Alvarez, S., Cigudosa, J.C., Wilhelm, J.S., Zheng, Y., Cancelas, J.A., Gu, Y., Jansen, M., et al. (2008). Microenvironment determines lineage fate in a human model of MLL-AF9 leukemia. Cancer Cell *13*, 483–495.

Wong, D.J., Liu, H., Ridky, T.W., Cassarino, D., Segal, E., and Chang, H.Y. (2008). Module map of stem cell genes guides creation of epithelial cancer stem cells. Cell Stem Cell *2*, 333–344.

Wood, L.D., Parsons, D.W., Jones, S., Lin, J., Sjöblom, T., Leary, R.J., Shen, D., Boca, S.M., Barber, T., Ptak, J., et al. (2007). The genomic landscapes of human breast and colorectal cancers. Science *318*, 1108–1113.

Yu, F., Yao, H., Zhu, P., Zhang, X., Pan, Q., Gong, C., Huang, Y., Hu, X., Su, F., Lieberman, J., and Song, E. (2007). let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell *131*, 1109–1123.

Zhang, M., Atkinson, R.L., and Rosen, J.M. (2010). Selective targeting of radiation-resistant tumor-initiating cells. Proc. Natl. Acad. Sci. USA *107*, 3522–3527.

Zhang, W.C., Shyh-Chang, N., Yang, H., Rai, A., Umashankar, S., Ma, S., Soh, B.S., Sun, L.L., Tai, B.C., Nga, M.E., et al. (2012). Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell *148*, 259–272.

Zhao, C., Chen, A., Jamieson, C.H., Fereshteh, M., Abrahamsson, A., Blum, J., Kwon, H.Y., Kim, J., Chute, J.P., Rizzieri, D., et al. (2009). Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature *458*, 776–779.