HHS Public Access

Author manuscript

Clin Chem. Author manuscript; available in PMC 2018 November 07.

Published in final edited form as:

Clin Chem. 2013 January ; 59(1): 168–179. doi:10.1373/clinchem.2012.184655.

Cell Plasticity and Heterogeneity in Cancer

Nemanja D. Marjanovic[1], **Robert A. Weinberg**[1,2], and **Christine L. Chaffer**[1,*]

[1]Whitehead Institute for Biomedical Research, Cambridge, MA

[2]Ludwig MIT Center for Molecular Oncology, Cambridge, MA.

Abstract

BACKGROUND: Heterogeneity within a given cancer arises from diverse cell types recruited to the tumor and from genetic and/or epigenetic differences amongst the cancer cells themselves. These factors conspire to create a disease with various phenotypes. There are 2 established models of cancer development and progression to metastatic disease. These are the clonal evolution and cancer stem cell models.

CONTENT: The clonal evolution theory suggests that successive mutations accumulating in a given cell generate clonal outgrowths that thrive in response to microenvironmental selection pressures, dictating the phenotype of the tumor. The alternative cancer stem cell (CSC) model suggests that cancer cells with similar genetic backgrounds can be hierarchically organized according to their tumorigenic potential. Accordingly, CSCs reside at the apex of the hierarchy and are thought to possess the majority of a cancer's tumor-initiating and metastatic ability. A defining feature of this model is its apparent unidirectional nature, whereby CSCs undergo symmetric division to replenish the CSC pool and irreversible asymmetric division to generate daughter cells (non-CSCs) with low tumor-igenic potential. However, evolving evidence supports a new model of tumorigenicity, in which considerable plasticity exists between the non-CSC and CSC compartments, such that non-CSCs can reacquire a CSC phenotype. These findings suggest that some tumors may adhere to a plastic CSC model, in which bidirectional conversions are common and essential components of tumorigenicity.

SUMMARY: Accumulating evidence surrounding the plasticity of cancer cells, in particular, suggests that aggressive CSCs can be created de novo within a tumor. Given the current focus on therapeutic targeting of CSCs, we discuss the implications of non-CSC-to-CSC conversions on the development of future therapies.

[*]Address correspondence to this author at: Whitehead Institute for Biomedical Research, Nine Cambridge Center Cambridge, MA 02142. chaffer@wi.mit.edu.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors' Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Employment or Leadership: None declared.

Consultant or Advisory Role: R.A. Weinberg, Verastem Inc.

Stock Ownership: R.A. Weinberg, Verastem Inc.

Honoraria: None declared.

Research Funding: R.A. Weinberg, NIH.

Expert Testimony: None declared.

Arguably the most challenging facets of neoplastic disease are its last phases, when cancer cells from primary tumors spread to distant sites in the body. This process of cell dissemination, termed metastasis, accounts for 90% of cancer-related deaths. The lethality of meta-static cancer is due in large part to its resistance to the currently available therapeutics. This explains why early detection and removal of primary tumors is still the most effective way to prevent metastasis and thereby improve patient survival.

Cancer is not a single disease but instead is manifested in numerous subtypes, each with its own distinct histopathological and biological features. One aspect common to all cancers is abnormal cell proliferation, which offers a target for possible therapeutic approaches to the disease. Thus, chemotherapies remain among the most useful anticancer therapies because of their ability to exert cytotoxic effects on rapidly dividing cells. Nonetheless, cancers often become refractory to these treatments and patients are left with few or no treatment alternatives. The development of more effective therapies requires a better understanding of the specific driving forces behind different subtypes of cancer. As we are learning, carcinomas, which represent the great majority of clinical cases and are the focus of this review, can employ diverse and even sophisticated strategies to establish and maintain their proliferative and metastatic ability. In this review, we focus our attention on these strategies, specifically those used by cancer cells to create heterogeneous cell populations with different functional properties. Our discussion therefore encompasses recent results in the emerging field of cellular plasticity—an area of cancer research that is rapidly attracting considerable attention.

Carcinomas Are Driven by Cell Intrinsic and Extrinsic Factors

To begin to understand the intratumoral diversity driving cancer development and metastasis, we categorize known facets of the disease into cell-intrinsic and cell-extrinsic components. Intrinsic cell features, sometimes termed cell-autonomous properties, are the inherent properties of a cell that contribute to its oncogenic phenotype, whereas extrinsic features are the components of its surrounding microenvironment that act on this cell to influence its phenotype and thus perturb the course of neoplastic disease (Fig. 1).

CELL-INTRINSIC VARIABILITY

Arguably the most well-known and widely studied aspects of cancer biology are the genetic mutations underlying primary tumor formation. Indeed, the permutations of mutations accrued by progressing cancer cells are a major source of cell-intrinsic variability among cancers, in which the dominant driver mutation can have a profound impact on cells and thus disease phenotype (1–3). When driver mutations or pathways can be identified, targeted treatments may be beneficial to patients. For example, patients with breast cancer driven by human epidermal growth factor receptor 2 (HER2)[3] benefit from therapies inhibiting HER2 signaling. Similarly, understanding key muta tions can provide useful information on disease course and progression, as is the case with patients harboring *BRCA1* (breast cancer 1, early onset)[4] or *BRCA2* (breast cancer 2, early onset) germline mutations. Whole-genome

[3]Nonstandard abbreviations: HER2, human epidermal growth factor receptor 2; CSC, cancer stem cell; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition.

sequencing analyses that characterize somatic mutations in tumor cell genomes demonstrate that in a field of multiple somatic mutations within a single cancer cell genome, only a relatively small subset, serving as its driver mutations, are responsible for determining the disease phenotype. The remainder—the so-called passenger mutations—are acquired as incidental by-products of the cancer cell's heightened mu tability and, by definition, play no role in determining its biology. Utilizing appropriate analytical tools to enumerate the driver mutations within a tumor or a subtype of cancer should provide the oncologist with a powerful means to determine appropriate, highly specific therapies (4, 5).

Recently it has become evident that genetic mutations are not the sole determinants of either tumori-genesis or cancer heterogeneity. Each population of cells forming a given tumor derives from a normal cell-of-origin that expressed a particular differentiation program before the onset of tumorigenesis. Such differentiation programs are the products of ordered alterations in the epigenome occurring during normal development. Of relevance here, major components of these epigenetic programs resist disruption during multistep tumorigenesis and therefore continue to strongly influence the phenotype of all but the most aggressive tumor cells.

This epigenetic program undergoes modification from 2 sources during tumorigenesis. To begin, the driver mutations acquired during tumor development perturb a wide spectrum of transcriptional programs and thus components of the epigenome. Of equal and possibly greater importance are the stochastic changes in the epigenome that occur during tumor progression, many of which clearly benefit the evolving populations of preneoplastic cells. Thus, alterations of a cell's epigenome perform major roles in determining cancer cell phenotype, as indicated by many observations that disruption of DNA methylation, histone modification, and chromatin compartments are common accompaniments of human cancer development (6). The extent of epigenetic deregulation in cancer may be diverse and may include dramatic global changes such as loss of 5-methylcytosine genomic content, concomitant dense hypermethylation in discrete genomic regions, and site-specific CpG-island hypermethylation in the promoters of genes encoding cell cycle regulators [pRB (retinoblastoma protein), $p16^{INK4a}$ (cyclin-dependent kinase inhibitor 2A)] or mediators of DNA repair [*BRCA1*, mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli) (*MLH1*)] (6–8). Mutations in histone modifiers have been identified as a major feature of small cell lung carcinoma (6, 9). Similarly, it has been demonstrated that breast cancer–associated single-nucleotide polymorphisms are enriched for the H3K4me1 histone modification in a cancer-specific manner (10). It is becoming increasingly apparent that epigenomes play a major role in cell-type–specific transcriptional programs and disease development.

Alongside a central role in cancer initiation and/or progression, epigenetic modifications may also play a more subtle role in cancer by determining a cell's ability to respond to an evolving and even taxing microen vironment. In normal development, the multipotent nature

[4]Genes: *BRCA1*, breast cancer 1, early onset; *BRCA2*, breast cancer 2, early onset; *MLH1*, mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli); *Pax5*, paired box gene 5; BMI1, BMI1 polycomb ring finger oncogene; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *CDH1*, cadherin 1, type 1, E-cadherin (epithelial).

of stem and progenitor cells is maintained by a fine balance between maintenance of pluripotency genes and inhibition of lineage-specific genes. Importantly, the multipotent state is metastable, because in order for differentiation to occur, cells must be able to shut down stemlike genes and concomitantly activate lineage-specification genes. These broad cellular changes are almost entirely driven by microenviron-mental cues. Important in this discussion is the notion that the ability of cancer cells to respond to microenvironmental cues by modulating their epigenetic status may determine their ability to survive and even thrive under difficult and continuously changing conditions.

Gene expression analyses of highly aggressive tumors show a compelling overlap of their gene expression profiles with those of normal stem cells (11, 12). These findings imply that oncogenic transformation of stem or progenitor cells yields more aggressive tumors compared to oncogenic transformation of lineage-committed cells. Alternatively, it is possible that transformation of more differentiated cells leads to more differentiated tumors that may thereafter evolve, via dedifferentiation processes, to stemlike phenotypic states. In either case, stem-cell–specific features, such as self-renewal, anchorage-independent growth and long-term proliferative capacity, are advantageous traits for successful completion of the invasion-metastatic cascade, and indeed they have been shown to be associated with a more aggressive cancer cell phenotype (13).

CELL-EXTRINSIC VARIABILITY

The cell-intrinsic features described above must be considered in a context-dependent manner. Thus, as is the case in normal tissues, cell behavior is strongly influenced by factors in the microenvironment acting to promote or inhibit the aggressive cancer phenotype (14). Indeed, once certain normal microenvironmental controls have been disrupted, disease can progress with rapidity (15).

Multiple factors in the microenvironment contribute to tumor cell behavior and thus to intratumoral heterogeneity (Fig. 1). These factors can directly impact the behavior of individual cancer cells or can exert global influences on a tumor and thereby become strong determinants of the course of cancer progression.

The most obvious example here is the development of a tumor’s blood supply. Seminal work by Judah Folkman and colleagues demonstrated that the recruitment of blood vessels is critical for the onset of tumorigenesis, the tumor’s escape from dormancy, and the persistence of unrestricted tumor growth. Accordingly, inhibition of angiogenesis can impair the deregulated proliferation associated with neoplasia (16, 17). The prevailing functions of the vasculature in tumorigenesis were once limited to supplying nutrients and trophic and growth factors to the tumor site, evacuating metabolic wastes, and providing routes through which the cells in tumors could disseminate to distant organs (18). More recently, it has been established that the vasculature can also provide a specialized niche for cancer stem cells (CSCs) (19, 20), the cancer cells thought to be responsible for the maintenance of tumor growth (see discussion below).

Together with the recruitment of endothelial cells to generate an adequate blood supply for the developing tumor, an assortment of other stromal cells is conscripted to the site of

tumorigenesis. These cells include inflammatory cells, fibroblasts, and pluripotent mesenchymal stem cells. Once present at the tumor site, these diverse stromal cells become activated, altering their normal secretion patterns of cytokines, growth factors, and extracellular matrix (ECM) components to create a “reactive stroma.” These stromal cells play prominent roles in promoting cancer progression through cell proliferation and degrading basement membranes to enhance invasive ability.

In addition to direct cell–cell interactions that occur between cancer cells and their stromal neighbors, stromal cells, in particular fibroblasts, also play an important role in determining the structure, organization, and function of the ECM in the tumor microenvironment (21). The ECM is highly dynamic and undergoes rapid remodeling in response to stimuli, including wound healing, angiogenesis, and cancer. Under normal circumstances, the ECM plays an important instructive role in tissue function, including the regulation of cell differentiation, stem cell proliferation, migration, and the formation of growth factor gradients. In the cancer setting, abnormal processing of the ECM can lead to aberrant cell proliferation, invasion, and loss of neoplastic cell differentiation. ECM components can also act as chemoattractants for stromal cells including endothelial and inflammatory cells that further influence the tumor microenvironment and disease progression (22, 23).

The importance of the ECM in the development of metastases was recently highlighted by studies of the mouse mammary tumor virus–PyMT cell line murine breast cancer model. Periostin is a component of the ECM secreted by fibroblasts in the stromata of the normal mouse mammary gland. It is also found in the stromata of mammary tumors growing in mice. Of note, colonization of metastases in the lung was dependent on tumor cells inducing stromal cells at the metastatic site to secrete periostin (24). Together these studies demonstrated that the ECM exerts influential effects on the development and progression of primary tumors and their metastatic derivatives.

As we continue to unravel the complexity of cancer, it is evident that cell-autonomous and -nonautonomous processes cooperate to enhance the aggressive cancer cell phenotype. Conversely, these processes may collaborate to keep cancer cells in check. Indeed, it is now certain that cell-extrinsic and -intrinsic properties conspire to shape the course of cancer development and progression (25). In that respect, identifying essential cell–cell signaling pathways driving a particular cancer is important for the development of effective therapies (4).

Tumor Heterogeneity

The above discussion highlights the variety of factors that can conspire in specific contexts to generate cancer cells with diverse phenotypes, ranging from relatively benign to highly aggressive, metastatic cell states. A further level of complexity to the understanding of cancer biology comes from the knowledge that the neoplastic cell populations within tumors comprise heterogeneous cell populations, in which cells having different tumorigenic potential coinhabit single tumors.

Two models have been proposed to explain how tumor heterogeneity arises and contributes to disease progression. The first model is the clonal evolution theory, which suggests that cancer cells evolve progressively during multistep tumorigenesis and that tumor cell heterogeneity, which is created by heritable genetic and epigenetic changes, creates the raw material for the selection and clonal outgrowth of novel cell populations. A second model is the CSC theory, which proposes that residence in different states of stemness and differentiation generates tumor cell heterogeneity (Fig. 2). It is likely that both the clonal evolution and CSC theories apply to human cancers and that, in some cases, tumors exhibit traits that are generated by both models (26).

In considering the clonal evolution model of cancer development, it is thought that an initial oncogenic insult is acquired that generates a benign neoplasia. Transformation to the malignant phenotype then requires successive mutations occurring in a cell lineage that progressively alters the phenotype of a lineage of cells. Moreover, heritable changes in the epigenome can also confer advantageous traits on variant sub-populations, favoring their clonal expansion. This description—essentially modeled on the neo-Darwinian model of organismic evolution—must also take into account the selection pressures imposed by the tumor microenvironment on the selective outgrowth of clones with more malignant phenotypes (27). Since the generation of variants, especially in late stages of tumor progression, can occur more rapidly than the elimination of less-fit clones, this may be responsible for the considerable interclonal heterogeneity observed within individual human tumors.

As cited above, an alternative and possibly complementary source of tumor heterogeneity is explained by the CSC hypothesis (28, 29). In contrast to the clonal evolution theory, this model focuses on the internal heterogeneity within individual clonal subpopulations of a tumor. Thus, in this narrative, all the cells in a heterogeneous population are genetically identical to one another. According to the CSC model, the most tumorigenic cells within such a clonal population reside at the apex of a cellular hierarchy and are capable of undergoing self-renewal to generate daughters that once again exhibit the CSC phenotype. Alternatively, they can undergo asymmetric division to create daughters (non-CSCs) with limited tumorigenic and meta-static potential that have initiated differentiation programs. In this case, the divergent cell phenotypes are regulated by instructive stimuli arising in the microen vironment. These stimuli can activate specific growth factor pathways that, in turn, affect subtle epigenetic changes in the CSCs and their non-CSC progeny.

A central concept of the CSC model is that a small subpopulation of cells within a tumor drives the growth and progression of the tumor as a whole. The CSC concept has fuelled the idea that the non-CSC component of a tumor plays a comparatively minor role in tumorigenesis, i.e., in the initiation of tumor. However, in established tumors, the non-CSCs, being in the great majority, are responsible for expressing many of the phenotypic traits that determine the traits of the tumor as a whole. Importantly, diverse observations have indicated that CSCs are resistant to many of the current therapeutic regimes (30–33).

Evidence for the applicability of the CSC model to human cancers was first demonstrated in a model of acute myeloid leukemia, in which the leukemia-initiating cells were shown to

possess the differentiation, proliferative, and self-renewal capacities expected of a normal stem cells (28, 29). The CSC hierarchical model has subsequently been extended to many solid human tumors, including carcinomas and glioblastomas (34). Many of these initial studies were done by isolating both CSCs and non-CSC populations by flow cytometry from human tumors and assessing tumorigenicity by transplantation into immunocompromised mice. Recently, several in vivo models have demonstrated that the CSC model is indeed applicable to autochthonous tumors, i.e., those growing in the tissue in which tumor initiation began (35, 36).

The question still remains as to the general applicability of the CSC theory to both experimental and clinical models of cancer. For example, analysis of clinical disease has revealed that metastases often recapitulate the heterogeneity and morphology of their respective primary tumors. According to the CSC model of tumorigenicity, in which a CSC-like cell initially seeds metastatic deposits, CSCs in those deposits must subsequently undergo both symmetric (to increase in number) and asymmetric (to generate more differentiated progeny) divisions. The CSCs behaving in this way can thereby recreate the hierarchical organization and histopathological appearance that existed previously in the corresponding primary tumor.

In some cases, however, metastases retain an un-differentiated phenotype, perhaps suggesting that the differentiation of disseminated CSCs is not applicable to all metastases. There are several ways in which un-differentiated metastases may arise while still retaining some resemblance to a CSC-like model (Fig. 3).

First, it is possible that CSCs are actually heterogeneous cell populations with functionally distinct biological properties. Accordingly, tumors may comprise classical CSCs, unconventional CSCs, or a mixture of both. The classical CSC would be expected to undergo symmetric and asymmetric divisions and give rise to metastases that recapitulate the heterogeneity observed in its corresponding primary tumor, whereas an unconventional CSC may exhibit some, but not all, features of the classical CSC. For example, it is possible that unconventional CSCs arise that undergo only symmetric divisions. CSCs of this sort may be expected to give rise to aggressive metastases composed almost entirely of CSCs (Fig. 3). The key here is that metastatic outgrowth can be achieved by both classical and unconventional CSCs, and the histopathology of resulting outgrowths may vary dramatically.

Second, it is possible that a classical CSC could give rise to undifferentiated metastases when the microenvironment plays a dominant role in determining symmetric vs asymmetric division (Fig. 3). In this case, the same CSC may display context-dependent behavior. For example, in the case in which a secondary microenvironment enables the classical CSC to undergo both symmetric and asymmetric division, the resulting out growth would recapitulate the histopathology of the primary tumor. Alternatively, if the secondary microenvironment favors symmetric division, the classical CSC may behave like an unconventional CSC and give rise to a CSC-rich undifferentiated metastasis.

Third, undifferentiated metastases may arise if classical CSCs acquire additional mutations or display some degree of genomic instability at either the primary or metastatic site (Fig. 3). In this situation, the biology that we now attribute to CSCs may no longer apply. One causal mechanism may involve a mutation occurring that prevents a CSC from undergoing asymmetric divisions. In this case, retention of a CSC phenotype may be advantageous for cells in certain sites of dissemination, resulting in the selective outgrowth of cells that have lost the ability to exit from the CSC state. See a recent review by Thomas Brabletz for further discussion on the consequences of differing CSC biologies and their impact on metastatic outgrowth (37).

Although heterogeneity may exist within the pool of cells deemed CSCs, accounting for biological deviations from the classical CSC model, it is also possible that the cancer cells-of-origin, combined with specific types and frequencies of mutations, give rise to cancers for which the CSC model does not apply. See the following references for further discussion on the applicability and limitations of the CSC theory (38–40).

Cell Plasticity and the Generation of CSCs

The phenotypic switch from the CSC to non-CSC state has been largely portrayed as a unidirectional process (Fig. 2). Thus, similar to models of normal tissue stem cells, once a CSC has generated a daughter that has exited the CSC state, the latter should become committed to a non-CSC, poorly tumorigenic state and, quite possibly, to spawn more differentiated, possibly post-mitotic descendants. According to this thinking, non-CSCs are unable to move back up the hierarchy and acquire CSC-like activity.

The alternative is a plastic process by which committed, nonstem cells and non-CSCs, can undergo a dedifferentiation process and reenter the stem cell/CSC state. In the normal tissue, stem cell behavior is tightly regulated by various cells and signals that constitute the stem cell niche. Despite the tight regulatory mechanisms in place for controlling stem cell function in normal tissues, aberrant changes in gene expression may occur that lead to dedifferentiation, enabling a differentiated cell to acquire stemlike activity. In the hematopoietic system, recent work has shown that differentiation of lymphoid progenitor cells to a committed mature B-cell identity is dependent on the induction of the transcription factor PAX5 (41). Interestingly, conditional deletion of PAX5 allowed mature B cells to dedifferentiate back into uncommitted progenitors, which, in turn, were able to rescue T lymphopoiesis in the thymus of T-cell–deficient mice. Moreover, PAX5 is also deregulated in some human B-cell malignancies, and mice lacking *Pax5* (paired box gene 5) in mature B cells also developed aggressive lymphomas exhibiting a progenitor cell phenotype (42). These results demonstrate a surprising level of plasticity in mature differentiated cells in both normal and neoplastic settings and illustrate how shutting down just one key lineage-commitment factor can allow cells to move back up a cellular hierarchy. Important to this discussion is the demonstration here that non-CSCs, in this case transformed mature B cells, can give rise to aggressive cancer cells with a CSC-like phenotype.

An analogous dedifferentiation route to the CSC state may arise when differentiated cells stochastically acquire genetic or epigenetic mutations in genes governing the CSC state. One

such example arises in the case of tumor cells expressing the BMI1 polycomb ring finger oncogene (BMI1), an important regulator of normal stem cell self-renewal. BMI1 is frequently over-expressed in human cancers acting to promote tumor-igenesis by downregulating the expression of tumor-suppressor genes, such as the proteins $p16^{INK4a}$ and $p14^{ARF}$ encoded by cyclin-dependent kinase inhibitor 2A (*CDKN2A*), and to prevent cellular senescence (43). BMI1 overexpression can also induce telomerase activity, which can lead in turn to cell immortalization (44). Of note here, when BMI1 is overexpressed in transformed epithelial cells, it can promote their conversion into a CSC-like state, thereby increasing tumorigenicity and metastatic ability (45). Similar observations have been made in models of leukemia, in which oncogene fusion proteins such as MOZ-TIF2 and MLL-ENL confer CSC-like properties on otherwise short-lived progenitor cells (46, 47). In contrast, overexpression of the BCR-ABL oncogene fusion protein in committed progenitors does not confer leukemia-initiating properties in vivo (46). These findings demonstrate that non-CSCs can be forced in an oncogene-dependent manner into a CSC-like state, thereby endowing otherwise poorly tumorigenic cells with tumor-initiating and, potentially meta-static ability.

Molecular and Cellular Mechanisms of Reentering the Stem Cell State

Recently, another set of findings was published illustrating a method whereby epithelial cells can coopt an embryonic developmental program, the epithelial-tomesenchymal transition (EMT), which also led to a type of cell dedifferentiation (48, 49). The EMT is a well-characterized developmental process required for early embryonic events, such as gastrulation and neural crest formation, as well as in adult tissues for tissue homeostasis and repair (50). In the cancer setting, the EMT had been invoked as a mechanism by which immotile cancer cells might acquire a more invasive and motile phenotype that could enhance a cancer's invasive and metastatic potential. In support of this idea, the EMT has been documented at the invasive fronts of several cancer types (50–52).

In the cited work, the authors overexpressed EMT-inducing transcription factors, notably Twist1, and Snail1, in differentiated epithelial cancer cells, which caused these cells to undergo EMT as anticipated. Surprisingly, this work demonstrated that the network of EMT signaling extends far more broadly than its known role in increasing migration and invasion in cancer. In addition to the loss of epithelial markers, gain of mesenchymal markers, and the associated increase in migratory and invasive potential, transformed mammary epithelial cells forced to overexpress EMT-inducers acquired the $CD44^{hi}CD24^{lo}$ cell-surface marker profile characteristic of breast CSCs, acquired self-renewal properties, and most importantly, acquired enhanced tumor-initiating ability (48, 49). These studies reinforced the possibility that the CSC phenotype in carcinoma can be an acquired state, rather than arising exclusively via oncogenic transformation of normal tissue stem cells. In this case, the authors illustrated that the reawakening of an embryonic pathway is an alternative way by which cells can undergo a dedifferentiation-like process. Together these studies demonstrate that under conditions of genetic manipulation, non-CSCs can indeed be forced to revert to a CSC phenotype, hinting at a bidirectional CSC model.

The question remains as to whether the forced transition of non-CSCs into a CSC-like state via experimental genetic manipulation resembles a process used by carcinomas under physiologic conditions. Addressing that question in part, another mechanism was recently described whereby populations of human mam-mary epithelial cells could spontaneously switch to a stemlike state (53). Moreover, oncogenic derivatives of those cells displayed increased rates of transition to the CSC state in vitro relative to their nontransformed counterparts. Importantly, and in contrast to the previous demonstrations of cell plasticity mentioned above, purifiedpopulationsofnon-CSCCD44^{lo}CD24^{+}cells created their own CD44^{hi}CD24$^{–}$CSC population in vivo without genetic manipulation (53). The results of this study thereby demonstrated that select cell populations are predisposed to undergo a non-CSC–to-CSC conversion, highlighting a novel mechanism by which cells can move back up a cell hierarchy and achieve a CSC-like state (Fig. 2). Mathematical models have been de rived to quantify the rates of cell switching between different states (54). Importantly, the generation of CSCs by this spontaneous mechanism pertains to a switch in cell phenotype that is not driven by additional genetic mutations (53, 54).

In further examples of cell plasticity, other work has demonstrated that leukemia-initiating cells that recreated the phenotypic diversity of acute myeloid leukemia could be found in fractions other than the originally described leukemia-initiating CD34^{+}CD38$^{–}$fraction (28, 55), although the tumor-initiating frequency was markedly lower in these alternatively derived populations. This work hinted at the possibility that more mature types of leukemic cells can revert to a CSC state and thereby acquire the ability to initiate leukemia. Similarly, phenotypically diverse melanoma cells can also recapitulate the heterogeneity of their primary tumors with high frequency, demonstrating a high degree of functional plasticity and reversibility in the expression of cell-surface markers. Together, these diverse findings suggest that the extent of plasticity between non-CSC and CSC populations may vary greatly across tumor types, among which melanoma in particular represents tumors at the far end of the spectrum displaying high cell plasticity. This idea is consistent with the high tumor-initiating frequency of melanoma cells (56).

Other examples of tumor cell plasticity include the demonstration that melanoma cells expressing the hi-stone demethylase JARID1B are required for continuous tumor growth, and that such JARID1B-positive cells can spontaneously arise from JARID1B-negative cells (57). Another important example of tumor cell plasticity pertains to the reversibility of a drug-tolerant state. Work by Sharma et al. (58) demonstrated that a small subpopulation of non–small cell lung carcinoma cells marked by JARID1A existed in a drug-tolerant state. Importantly, cells could exit that state and yet later reenter it. The idea that cell plasticity can lead to drug tolerance, either connected with or independent of residence in the CSC state, has broad implications for achieving effective therapeutics.

As mentioned earlier, under normal physiologic conditions the microenvironment is an important determinant of cell behavior. It follows that in circumstances in which the microenvironment becomes disrupted, cell fate decisions may go awry, resulting in aberrant differentiation programs. For example, in the hematopoietic system, the common lymphoid progenitor (a bone marrow-resident cell that gives rise exclusively to lymphocytes), can be redirected to the myeloid lineage by stimulation through exogenously expressed

interleukin-2 and GM-CSF (granulocyte-macrophage colony-stimulating factor) receptors (59). Other examples utilizing growth factor and cyto kine signaling to force epithelial cancer cells to alter cell states have been achieved, particularly in enabling non-CSCs to switch to a CSC-like state (48, 60, 61).

Together these studies highlight the fact that the potential for cells to undergo phenotypic switching is likely a combination of cell-intrinsic properties, i.e., the natural epigenetic status of a cell, together with instructive stimuli from the microenvironment, may act to promote or inhibit phenotypic conversions. Given the right combination of cell-intrinsic and cell-extrinsic events, it is possible that interconversions between the non-CSC and CSC states may be common events during tumorigenesis. Moreover, in cases in which tumors display a well-differentiated and poorly metastatic phenotype, the ability of those cancer cells to spontaneously create their own pool of highly aggressive and metastatic cells may even be a rate-limiting step for a tumor to acquire an aggressive phenotype and progress to metastatic disease. Altogether, the evidence currently accumulating suggests that the presiding CSC model of tumorigenicity is more dynamic than previously described. In particular, the data demonstrate that the CSC hierarchy should encompass bidirectional conversions between non-CSC and CSC states.

These discussions also bear on the earlier dichotomy in which we cited 2 alternative routes to intratu-moral heterogeneity: One involved genetic and epigenetic changes that create distinct clonal subpopulations that coexist within individual tumors. The alternative relates to the distinct phenotypic states in which cells exist at different levels of the stem-cell hierarchy. We consider it plausible that both situations operate in actual tumors. More specifically, it seems that all clonal populations—including normal, preneoplastic, and frankly neoplastic cells—contain their own complements of stem cells and nonstem cells. Hence, there are 2 dimensions of intratumoral heterogeneity that define the organization of most naturally arising tumors.

Implications of Cell Plasticity for the CSC Model and Targeted Therapeutics

The implications of non-CSC–to-CSC bidirectional interconversions are far reaching. To begin, where the CSC phenotype was first thought to arise via oncogenic transformation of a normal tissue stem cell, the pool of cells capable of undergoing oncogenic transformation is consequently relatively small. In contrast, the idea that CSCs can arise from more differentiated cell types suggests that the pool of cancer-initiating cells is far larger. In an extension to this idea, the fact that non-CSCs can spontaneously switch to a CSC state suggests that CSCs can be created *de novo* at different stages of tumorigenesis, and that the pool of CSCs within a tumor may be continually renewing and/or expanding in some tumor types, as implied above. From a therapeutic standpoint there are several aspects of this dynamic bidirectional CSC model that should be considered.

First, CSCs may be derived in a number of fashions, whether by oncogenic transformation of a normal tissue stem cell, by non-CSC–to-CSC plasticity driven by EMT, by a spontaneous conversion process, or by mutations in key regulators of differentiation or the stem cell state. These alternative paths to acquiring a CSC-like state may have important

implications for our understanding of CSCs and their behavior: CSCs may comprise a group of heterogeneous and functionally distinct subpopulations. As such, the factors driving CSC self-renewal and asymmetric division likely differ to some extent on the basis of the cellular origin of the CSC. Therefore, therapies aimed at disrupting CSC proliferation, self-renewal, or survival may need to target vastly different biological pathways between cancer types, and possibly even between cancer subtypes.

Furthermore, the alternative routes to deriving CSCs may result in CSCs with very distinct functional properties. For example, the CSC model suggests that therapies aimed to push CSCs toward a comparatively benign non-CSC state may curb cancer progression and metastatic outgrowth. In cases in which self-renewal and differentiation pathways remain intact, as may occur in oncogenic transformation of normal adult tissue stem cells, this type of differentiation therapy may be possible. However, as discussed earlier, CSCs may be generated from non-CSCs due to mutations affecting self-renewal pathways. In this case, one might expect that the acquired CSC-like phenotype becomes a permanent cell state that is continuously maintained and reinforced by oncogenic activation. In such a setting, therapies promoting CSC differentiation toward a non-CSC state may not be possible due to the dominant overriding power of genetic mutations that drive self-renewal and symmetric division, doing so in an irreversible fashion. Under these circumstances, therapies focused on inhibiting CSC proliferation may be far more successful in preventing cancer progression and metastasis than therapies aimed at promoting CSC differentiation. An example comes here from carcinomas in which the E-cadherin gene [cadherin 1, type 1, E-cadherin (epithelial) (*CDH1*)] is inactivated by mutation, resulting in activation of an EMT program and thus tumor-wide entrance into the mesenchymal and CSC state that is essentially irreversible.

Another important consideration is the notion that therapies aimed at eradicating CSCs may ultimately fail if non-CSC populations can recreate the CSC pool. In instances in which non-CSCs escape the confines of the primary tumor and either lodge in or migrate to a secondary site, they may initially reside there as dormant cells. However, if at some stage those non-CSCs reenter the cell cycle, it is likely that they may also succeed in spawning a new pool of CSCs, initiating metastatic outgrowth or disease relapse. Here, the microenvironment may play a prominent inductive role in promoting non-CSC–to-CSC conversions. In these cases, patients may benefit from adjuvant therapies that inhibit non-CSC–to-CSC transitions. Indeed, identifying hallmarks of cancers with the potential to undergo phenotype switching will be of fundamental importance.

Concluding Remarks

As we continue to unravel the intricacies underlying cancer initiation and metastasis, it is becoming increasingly evident that the success of future therapeutics lies in understanding cancer cell heterogeneity at both the genetic and epigenetic levels. Although the evidence supports the role of highly aggressive subpopulations of CSC-like cells as drivers of metastatic disease, the discussion here points to the diversity that can exist under the umbrella of the CSC phenotype. Moreover, a new and important consideration for cancer biology is the dynamic phenotypic switching that has recently been documented between

non-CSC and CSC cell populations, in particular, the fact that non-CSCs can give rise to CSC-like populations with various degrees of efficiency. These data suggest that the cells responsible for driving aggressive and metastatic disease may be moving targets. From a clinical standpoint, combining therapies targeting CSC proliferation, forcing CSCs to exit the CSC-state, and also preventing non-CSCs from switching to a CSC-state may eventually provide the most effective therapeutic regimes and ultimately improve patient survival.

Acknowledgments

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References

1. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000;406: 747–52. [PubMed: 10963602]
2. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869–74. [PubMed: 11553815]
3. Guedj M, Marisa L, de Reynies A, Orsetti B, Schiappa R, Bibeau F, et al. A refined molecular taxonomy of breast cancer. Oncogene 2012;31: 1196–206. [PubMed: 21785460]
4. Boehm JS, Hahn WC. Towards systematic functional characterization of cancer genomes. Nat Rev Genet 2011;12:487–98. [PubMed: 21681210]
5. Salk JJ, Fox EJ, Loeb LA. Mutational heterogeneity in human cancers: origin and consequences. Annu Rev Pathol 2010;5:51–75. [PubMed: 19743960]
6. Berdasco M, Esteller M. Aberrant epigenetic landscape in cancer: how cellular identity goes awry. Dev Cell 2010;19:698–711. [PubMed: 21074720]
7. Esteller M Epigenetics provides a new generation of oncogenes and tumour-suppressor genes. Br J Cancer 2006;94:179–83. [PubMed: 16404435]
8. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128:683–92. [PubMed: 17320506]
9. Peifer M, Fernandez-Cuesta L, Sos ML, George J, Seidel D, Kasper LH, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet 2012;44: 1104–10. [PubMed: 22941188]
10. Cowper-Sal Lari R, Zhang X, Wright JB, Bailey SD, Cole MD, Eeckhoute J, et al. Breast cancer risk-associated SNPs modulate the affinity of chromatin for FOXA1 and alter gene expression. Nat Genet 2012;44:1191–8. [PubMed: 23001124]
11. Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A, Weinberg RA. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat Genet 2008;40:499–507. [PubMed: 18443585]
12. Eppert K, Takenaka K, Lechman ER, Waldron L, Nilsson B, van Galen P, et al. Stem cell gene expression programs influence clinical outcome in human leukemia. Nat Med 2011;17:1086–93. [PubMed: 21873988]
13. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science 2011;331:1559–64. [PubMed: 21436443]
14. Mintz B, Illmensee K. Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc Natl Acad Sci U S A 1975;72:3585–9. [PubMed: 1059147]
15. Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med 2011; 17:320–9. [PubMed: 21383745]
16. Ferrara N, Alitalo K. Clinical applications of angiogenic growth factors and their inhibitors. Nat Med 1999;5:1359–64. [PubMed: 10581076]
17. Folkman J, Watson K, Ingber D, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 1989;339: 58–61. [PubMed: 2469964]

18. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011;473:298–307. [PubMed: 21593862]
19. Beck B, Driessens G, Goossens S, Youssef KK, Kuchnio A, Caauwe A, et al. A vascular niche and a VEGF-Nrp1 loop regulate the initiation and stemness of skin tumours. Nature 2011;478: 399–403. [PubMed: 22012397]
20. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, et al. A perivascular niche for brain tumor stem cells. Cancer Cell 2007;11: 69–82. [PubMed: 17222791]
21. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. Nature 2004;432:332–7. [PubMed: 15549095]
22. Hynes RO. The extracellular matrix: not just pretty fibrils. Science 2009;326:1216–9. [PubMed: 19965464]
23. Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol 2011;3 pii: a005058. [PubMed: 21917992]
24. Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Delaloye JF, Huelsken J. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature 2012;481: 85–9.
25. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74. [PubMed: 21376230]
26. Shackleton M, Quintana E, Fearon ER, Morrison SJ. Heterogeneity in cancer: cancer stem cells versus clonal evolution. Cell 2009;138:822–9. [PubMed: 19737509]
27. Nowell PC. The clonal evolution of tumor cell populations. Science 1976;194:23–8. [PubMed: 959840]
28. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3:730–7. [PubMed: 9212098]
29. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;367:645–8. [PubMed: 7509044]
30. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006;444:756–60. [PubMed: 17051156]
31. Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, et al. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst 2008;100:672–9. [PubMed: 18445819]
32. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 2009;458:780–3. [PubMed: 19194462]
33. Ishikawa F, Yoshida S, Saito Y, Hijikata A, Kitamura H, Tanaka S, et al. Chemotherapy-resistant human AMl stem cells home to and engraft within the bone-marrow endosteal region. Nat Biotechnol 2007;25:1315–21. [PubMed: 17952057]
34. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008;8:755–68. [PubMed: 18784658]
35. Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, Parada LF. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 2012;488:522–6. [PubMed: 22854781]
36. Driessens G, Beck B, Caauwe A, Simons BD, Blanpain C. Defining the mode of tumour growth by clonal analysis. Nature 2012;488:527–30. [PubMed: 22854777]
37. Brabletz T To differentiate or not: routes towards metastasis. Nat Rev Cancer 2012;12:425–36. [PubMed: 22576165]
38. Gupta PB, Chaffer CL, Weinberg RA. Cancer stem cells: mirage or reality? Nat Med 2009;15: 1010–2. [PubMed: 19734877]
39. Visvader JE, Lindeman GJ. Cancer stem cells: current status and evolving complexities. Cell Stem Cell 2012;10:717–28. [PubMed: 22704512]

40. Valent P, Bonnet D, De Maria R, Lapidot T, Cop-land M, Melo JV, et al. Cancer stem cell definitions and terminology: the devil is in the details. Nat Rev Cancer 2012;12:767–75. [PubMed: 23051844]
41. Cobaleda C, Schebesta A, Delogu A, BusslingerM. Pax5: the guardian of B cell identity and function. Nat Immunol 2007;8:463–70. [PubMed: 17440452]
42. Cobaleda C, Jochum W, Busslinger M. Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. Nature 2007;449: 473–7. [PubMed: 17851532]
43. Valk-Lingbeek ME, Bruggeman SW, van LohuizenM. Stem cells and cancer; the polycomb connection. Cell 2004;118:409–18. [PubMed: 15315754]
44. Dimri GP, Martinez JL, Jacobs JJ, Keblusek P, Itahana K, Van Lohuizen M, et al. The Bmi-1 oncogene induces telomerase activity and immortalizes human mammary epithelial cells. Cancer Res 2002;62:4736–45. [PubMed: 12183433]
45. Yu CC, Lo WL, Chen YW, Huang PI, Hsu HS, Tseng LM, et al. Bmi-1 regulates snail expression and promotes metastasis ability in head and neck squamous cancer-derived ALDH1 positive cells. J Oncol 2011;2011 pii: 609259. [PubMed: 20936121]
46. Huntly BJ, Shigematsu H, Deguchi K, Lee BH, Mizuno S, Duclos N, et al. MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. Cancer Cell 2004;6:587–96. [PubMed: 15607963]
47. Cozzio A, Passegue E, Ayton PM, Karsunky H, Cleary ML, Weissman IL. Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. Genes Dev 2003;17:3029–35. [PubMed: 14701873]
48. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008;133:704–15. [PubMed: 18485877]
49. Morel AP, Lievre M, Thomas C, Hinkal G, Ansieau S, Puisieux A. Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS One 2008;3:e2888. [PubMed: 18682804]
50. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009;139:871–90. [PubMed: 19945376]
51. Spaderna S, Schmalhofer O, Hlubek F, Berx G, Eger A, Merkel S, et al. A transient, EMT-linked loss of basement membranes indicates metastasis and poor survival in colorectal cancer. Gastroenterology 2006;131:830–40. [PubMed: 16952552]
52. Prall F Tumour budding in colorectal carcinoma. Histopathology 2007;50:151–62. [PubMed: 17204028]
53. Chaffer CL, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, et al. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. Proc Natl Acad Sci U S A 2011;108:7950–5. [PubMed: 21498687]
54. Gupta PB, Fillmore CM, Jiang G, Shapira SD, Tao K, Kuperwasser C, Lander ES. Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell 2011;146: 633–44. [PubMed: 21854987]
55. Sarry JE, Murphy K, Perry R, Sanchez PV, Secreto A, Keefer C, et al. Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rgammac-deficient mice. J Clin Invest 2011;121:384–95. [PubMed: 21157036]
56. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. Nature 2008;456:593–8. [PubMed: 19052619]
57. Roesch A, Fukunaga-Kalabis M, Schmidt EC, Za bierowski SE, Brafford PA, Vultur A, et al. A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. Cell 2010;141:583–94. [PubMed: 20478252]
58. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, et al. A chromatin-mediated reversible drug tolerant state in cancer cell sub-populations. Cell 2010;141:69–80. [PubMed: 20371346]
59. Kondo M, Scherer DC, Miyamoto T, King AG, Akashi K, Sugamura K, Weissman IL. Cell-fate conversion of lymphoid-committed progenitors by instructive actions of cytokines. Nature 2000; 407:383–6. [PubMed: 11014194]

60. Scheel C, Eaton EN, Li SH, Chaffer CL, Reinhardt F, Kah KJ, et al. Paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast. Cell 2011;145:926–40. [PubMed: 21663795]

61. Vermeulen L, De Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski T, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol 2010;12:468–76. [PubMed: 20418870]

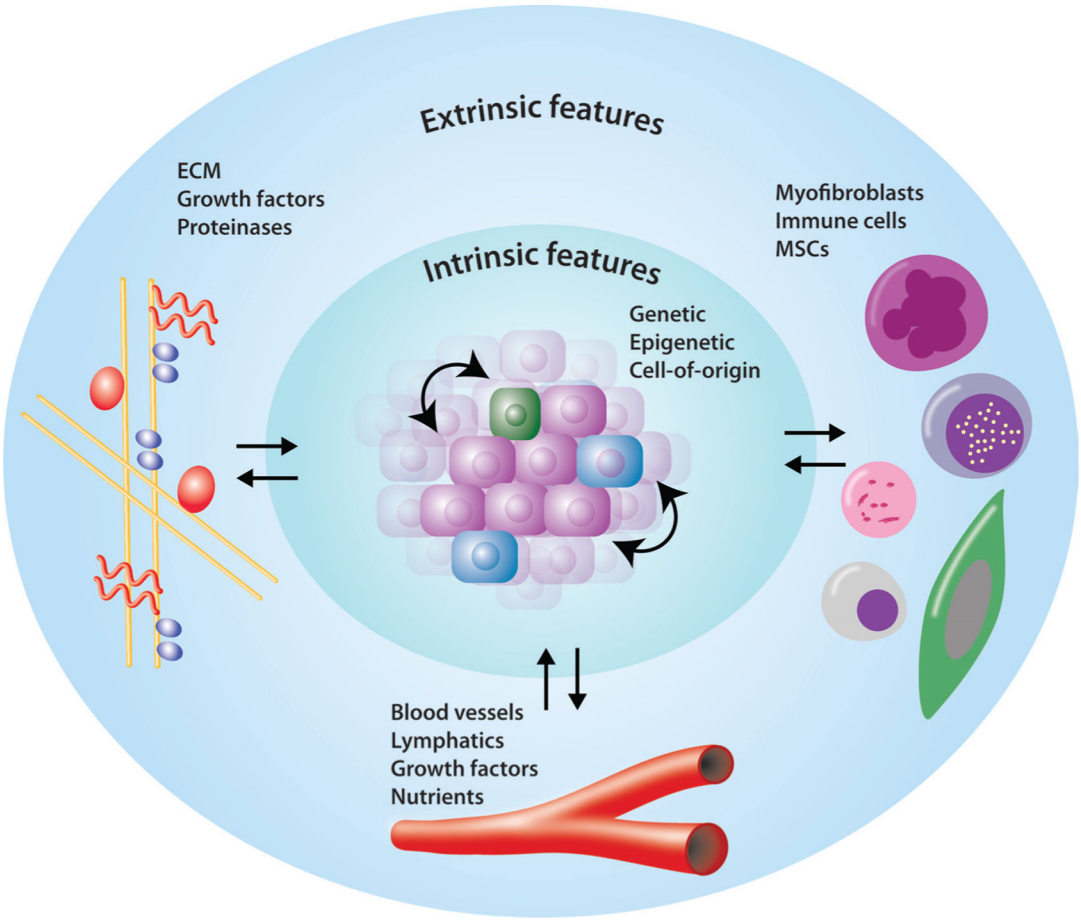

Fig. 1. Cell-intrinsic and cell-extrinsic features contribute to cancer cell heterogeneity.

Carcinomas comprise heterogeneous cell populations. A variety of factors contribute to the diverse biological phenotypes of cancer cells existing both within a given tumor and between tumor subtypes. This diversity arises from (*a*) cell-intrinsic properties, including variability in the genetics, epigenetics, and biology of a tumor's cell-of-origin, and (*b*) cell-extrinsic properties arising from factors in the microenvironment that include the composition of the extracellular matrix and factors sequestered to its constituents, a tumor's ability to recruit an adequate blood supply, and to recruit stromal cell types that aid tumor growth.

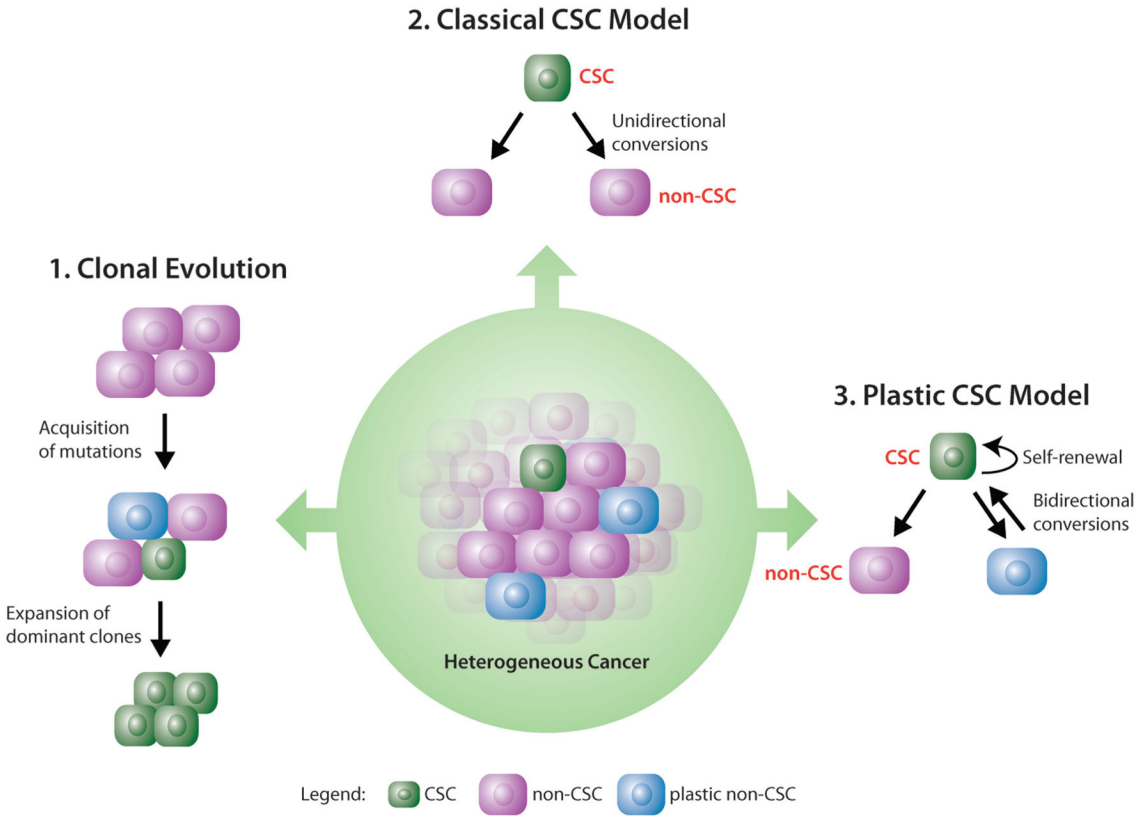

Fig. 2. Models of tumor heterogeneity.

The clonal evolution theory was the first model to describe a way in which cancer cells with diverse phenotypes could arise within a tumor. In this model, distinct cancer cell populations evolve progressively during multistep tumorigenesis due to heritable genetic and epigenetic changes. These stochastic events create the raw material for the selection and clonal outgrowth of novel cell populations arising from the acquisition of accumulating mutations. A second model is described by the classical CSC theory, which proposes that tumor heterogeneity arises when cancer cells within a given tumor reside in different states of stemness or differentiation. Critical to this model is the notion that CSC-to–non-CSC conversion is a unidirectional process. The plastic cancer stem cell theory describes a third and evolving model in which bidirectional conversions exist between non-CSCs and CSCs. This model implies that non-CSCs can continually create CSC populations throughout tumorigenesis.

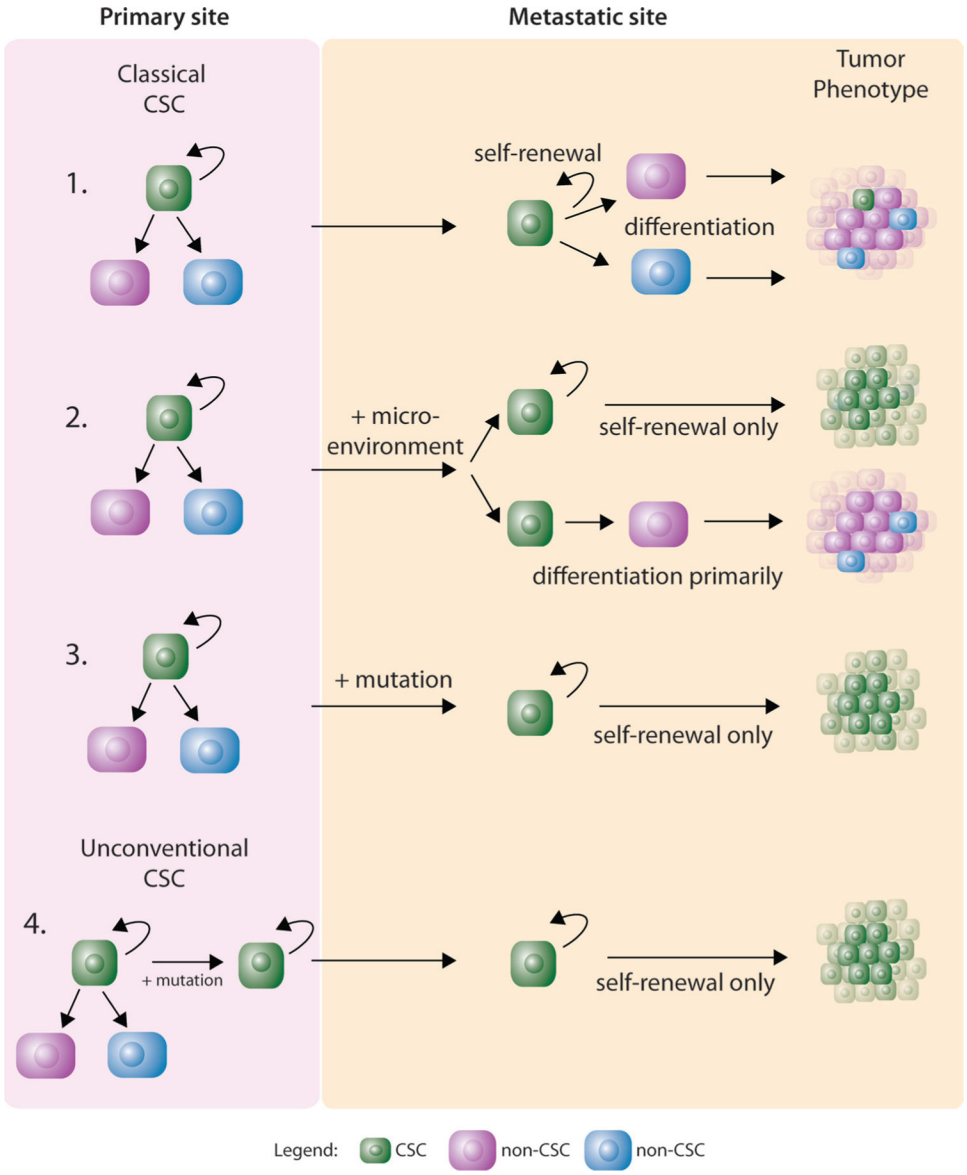

Fig. 3. Variations of the CSC model.

CSCs may display context-dependent behavior. In this way, a CSC may give rise to a metastasis with a phenotype different from that of its corresponding primary tumor. There are several ways in which this can occur: 1. The classical CSC undergoes symmetric and asymmetric division at equal frequencies at both the primary and metastatic site. The outcome is a heterogeneous metastasis comprising CSCs in small numbers and more differentiated progeny with high frequency. In this case it is expected that the phenotype of the primary tumor and its metastasis are similar. 2. The classical CSC may be responsive to microenvironmental cues present at the metastatic site. The nature of those cues may enforce the CSC state by promoting symmetric division or inhibiting asymmetric division, giving rise to a metastasis comprising almost entirely CSCs. Alternatively, contextual signals at the secondary site may inhibit symmetric division and favor asymmetric division. The resultant metastasis would be expected to comprise almost entirely non-CSCs. In either case, the

metastases display a markedly different phenotype to their corresponding primary tumors. 3. A classical CSC may acquire additional mutations at the metastatic site that lock it in the CSC state. In this scenario a CSC-rich metastasis would ensue. 4. An unconventional CSC may preexist in the primary tumor, for example, due to acquisition of an additional mutation in a self-renewal pathway. In this situation only symmetric division becomes possible and metastases arising from this type of CSC comprise almost entirely CSCs.