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Cancer stem cells: mirage or reality?

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The similarities and differences between normal tissue stem cells and cancer stem cells (CSCs) have been the source of much contention, with some recent studies calling into question the very existence of CSCs. An examination of the literature indicates, however, that the CSC model rests on firm experimental foundations and that differences in the observed frequencies of CSCs within tumors reflect the various cancer types and hosts used to assay these cells. Studies of stem cells and the differentiation program termed the epithelial-mesenchymal transition (EMT) point to the possible existence of plasticity between stem cells and their more differentiated derivatives. If present, such plasticity would have major implications for the CSC model and for future therapeutic approaches.

Several recent reports have suggested that as many as 25% of the cancer cells within certain tumors have the properties of CSCs[1,2]. These findings have disputed the idea that CSCs exist only as rare subpopulations within tumors and have raised questions about the general applicability of the CSC model and even the very existence of CSCs. We believe that conclusions regarding the death of the CSC concept are premature. Rather, as we argue here, the CSC model can readily accommodate recent experimental challenges and will stand the test of time.

CSCs have been defined on the basis of their ability to seed tumors in animal hosts, to self renew and to spawn differentiated progeny (non-CSCs)[3]. Accordingly, the representation of CSCs within a population of cancer cells can be measured by the number of cells that are required, at limiting dilutions, to seed new tumors. This definition applies equally well to both primary and cultured cancer cell populations.

Pioneering work in this area originated from studies of leukemia stem cells[4] and later included demonstrations of CSCs in solid tumors, specifically human breast[5] and brain cancers[5,6]. These initial studies showed that it is possible to use cell-surface marker profiles to isolate cancer cell subpopulations that are enriched for or depleted of CSCs. Subsequent studies extended this evidence to a variety of cancer types, showing that it is possible to physically separate from a single tumor sample two distinct subpopulations of cancer cells that differ in their cell-surface protein antigen profiles and in their ability to seed new tumors *in vivo*. Importantly, these various reports showed that, after implantation *in vivo*, CSC-enriched populations generate tumors and associated cancer cell populations that are no longer enriched for CSCs. The most parsimonious explanation for this observation is that CSCs can self renew as well as give rise to non-CSC progeny. Moreover, these findings show that the cancer cells within a single tumor exist naturally in multiple states of differentiation that show distinct tumor-seeding properties.

Some of the controversy surrounding the CSC model seems to arise from confusion regarding the definition of CSCs, leading to two key objections against the use of this term. The first objection derives from the fact that, unlike the case for normal stem cells, which are usually oligo- or multipotent, it is currently unclear whether CSCs can give rise to multiple differentiated cell types. In this context, it is worth noting the most essential aspects of the stem cell model: stem cells are self renewing, capable of tissue regeneration and can give rise to non–stem cells, the latter being more differentiated and largely, if not entirely, lacking in tissue-regenerating ability. There is nothing intrinsic to the dynamics of normal stem cells that necessarily limits the use of the 'stem cell' term to define those that are oligopotent.

A second key objection to the CSC model is that it is currently unclear whether the normal cellular precursors of CSCs are, in fact, *bona fide* stem cells. It is clear, however, that the traits used to define CSCs do not rely on knowledge of their cellular origins within normal tissues. Accordingly, the CSC model must stand or fall on the basis of experimental characterizations of cancer cell populations.

The initial descriptions of CSCs reported that, in the tumors examined, these cells represented only a small fraction of the total cancer cell populations. Hence, the summary of a 2006 workshop on this topic reported "in the cancer stem cell model of tumors, there is a small subset of cancer cells, the cancer stem cells, which constitute a reservoir of self-sustaining cells with the exclusive ability to self-renew and maintain the tumor."[3] In retrospect, however, it is clear that none of the experimental findings in the initial reports on CSCs precluded the possibility that the proportion of CSCs could differ profoundly between various tumor types.

As suggested by more recent findings[1,2], CSC representation may be a function of the cell type of origin, stromal microenvironment, accumulated somatic mutations and stage of malignant progression reached by a tumor. In fact, an early report indicated that the proportion of leukemia stem cells varies 500-fold between patient samples[7]. More recent reports have suggested that individual tumors that are, at the histopathological level, relatively undifferentiated and may contain higher proportions of CSCs than their more differentiated counterparts[8–10]. In addition, CSC representation may differ substantially between cancer subtypes arising from a single tissue[11]. Hence, within some tumors, the CSCs may be as numerous as the non-CSCs with which they co-exist. In the end, the CSC model can be readily adapted to allow for these various possibilities by

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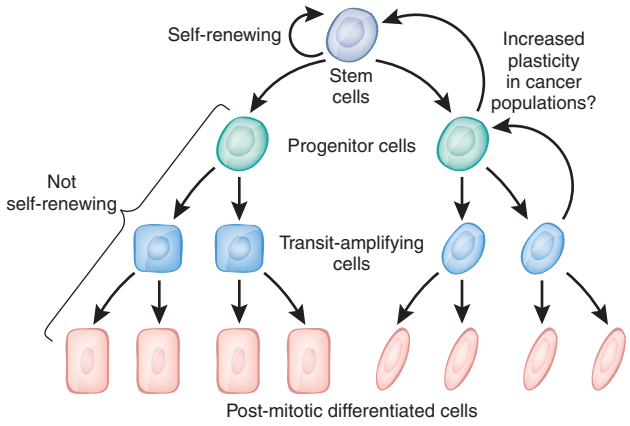

Figure 1 Stem-differentiation hierarchy. Increased plasticity may be present within cancer populations, enabling bidirectional interconvertibility between CSCs and non-CSCs.

positing only that cancer cells can exist in at least two alternative phenotypic states that show markedly different tumor-seeding potentials, without imposing any requirements on the relative proportions of the aforementioned phenotypic states.

The study of CSC biology is predicated on the ability to accurately assess CSC representation within cancer cell populations. However, measurements of CSC representation are complicated by the quality of the host tissue in which tumor-initiating ability is assessed. Thus, animal hosts that offer a hospitable environment to engrafted tumor cells will yield measures of CSCs far higher than hosts that fail to do so. Aspects of host biology that can affect cancer cell engraftment rate include vascularization at the site of implantation, extracellular matrix constitution, growth factor availability and host immunocompetence.

These considerations give rise to the thorny issue of choosing an appropriate animal model to measure CSC representation. The ideal animal model would, in principle, accurately represent tumor CSC biology as it occurs in humans. In this context, it is far from evident how recently described animal models that report reduced numbers of cells required to seed tumors compare with previously used models in terms of the accuracy of their assessment of CSC representation. In fact, the hypoxic, low-pH, low-nutrient and necrotic microenvironment within human tumors is anything but hospitable, even less so after chemotherapy. In light of these complexities, we propose an alternative solution: CSC numbers cannot presently be stated in absolute terms, but only relative to the animal model used to measure CSC representation. Even such relative evaluations may be quite useful, for example, if increased proportions of CSCs correlate with other functional attributes of interest, such as the chemosensitivity or metastatic proclivity of a tumor. In the longer term, this limitation will probably need the adoption of several animal host models, each of which will recapitulate a distinct tissue microenvironment present at a specific stage of human tumor progression.

Importantly, as has been previously noted[12,13], the studies that have shown a relatively high proportion of CSCs in tumors have also included other experimental design variables that may well have influenced measurements of CSC representation, including co-inoculation with extracellular matrix[2], use of late-stage patient samples[2] and the presence of predisposing genetic mutations that give rise to mouse tumors that fail to recapitulate the heterogeneity in human cancers[1]. It has been suggested that the proportion of CSCs in human cancer cell populations may be underestimated because of residual immunocompetence in host animals[1,2]. In fact, the presence of a small CSC subpopulation has been shown in syngeneic, immunocompetent mouse models that recapitulate the heterogeneity of human tumors, including models of breast cancer[14], leukemia[15] and glioma[16]. These findings show that CSCs are not unique to xenotransplanted human cancers.

The observations cited earlier[8–10] showing that CSC proportions differ on the basis of the stage of malignant progression reached by a tumor lead to the question of the underlying biological mechanisms that are responsible for such variations. Tumor malignancy is, in large part, gauged by the degree of dedifferentiation manifested by the cancer cells within a tumor. This suggests, in turn, that regulators of differentiation are strong determinants of CSC biology.

In fact, there is already substantial evidence to suggest that CSCs arising from mammary epithelial cells are preferentially associated with a specific state of differentiation[17,18]. Thus, EMT has been studied in morphogenesis because of its ability to convert cells from one state of differentiation to another. However, recent observations have shown that induction of EMT in transformed mammary epithelial cells creates populations of cells that are highly enriched for CSCs, as gauged by tumor-seeding ability, mammosphere formation and cell-surface marker expression[17,18]. A similar correlation has been observed between EMT induction and acquisition of certain stem-like traits in immortalized nontumorigenic mammary epithelial cells. In addition, fractionation of naturally existing normal and neoplastic mammary epithelial cells that had not been experimentally manipulated reinforced this connection: cells with surface-marker profiles that enrich for CSCs showed multiple attributes of mesenchymal transdifferentiation, including expression of mesenchymal proteins (vimentin, fibronectin) and greatly increased expression of certain EMT-inducing transcription factors[17]. Although these findings have yet to be extended to other epithelial cell types, it is plausible, if not likely, that mammary cells are not unique among epithelial cell types in showing this connection.

Although EMT has been widely studied in the context of embryonic morphogenesis, it also seems to have a key role in the acquisition of invasive and migratory traits by many types of carcinoma cells[19,20]. Carcinoma cells at the invasive edges of tumors have been observed to undergo EMT, ostensibly doing so under the influence of contextual signals that they receive from closely apposed stromal tissue[21]. This is reminiscent of embryogenesis, during which contextual signals that include transforming growth factor-β and Wnt ligands induce cells to undergo EMT[19,22]. Developmental studies have indicated that EMT induction is reversible under certain circumstances. Thus, when EMT-inducing signals are removed, cells that have been induced into EMT can sometimes revert to the epithelial state of their cellular ancestors[20]. An analogous phenomenon is observed when signals that induce EMT are transiently applied to cancer cell populations via inducible genetic factors[17].

The observed reversibility of EMT has ramifications for the perception of CSCs. In particular, whereas CSCs can differentiate into non-CSCs, the reverse process must now also be considered: non-CSCs, having received certain contextual signals, may well become reprogrammed via a process closely allied to an EMT into CSCs (**Fig. 1**). If so, this reversal places the CSC model at variance with conventional depictions of normal stem cells, which portray the differentiation of stem cells into non-stem cells but not the reverse. Hence, greater phenotypic plasticity may exist in tumor cell populations than is conventionally thought to exist in normal cell populations. Indeed, such plasticity may also eventually be found to operate in normal epithelial tissues. This phenotypic plasticity suggests that a dynamic equilibrium may exist between CSCs and non-CSCs within tumors. Moreover, this equilibrium may be shifted in one direction or another by contextual signals within the tumor

microenvironment that influence the probability of interconversion between the CSC and non-CSC compartments[23,24].

The possibility of bidirectional interconvertibility has not been expressed in existing depictions of the CSC model. The notion that CSC and non-CSC populations are interconvertible does not in itself undermine the concept of CSCs. Indeed, CSCs and non-CSCs retain their distinct identities in the sense that they can be distinguished phenotypically and functionally at any moment in a cancer cell population.

Nonetheless, the entire concept of CSCs is clearly trivialized if the two subpopulations are rapidly interconverting; that is, if the kinetic rate constants in both directions are so high that cells are continually moving in large numbers from one compartment to the other. This would be the case, for example, if a CSC-enriched subpopulation were found to represent a subpopulation of cells residing in a specific phase of the cell cycle, causing CSCs and non-CSCs to rapidly interconvert with each passage through a cell growth-and-division cycle. The existing literature, however, indicates that this is not the case; CSCs exist in a metastable state, they can perpetuate themselves indefinitely, and the flux of non-CSCs into the CSC compartment is, under most conditions, relatively low and, in some cases, possibly nonexistent[25].

We suspect that populations of non-CSCs from various types of tumors have greatly differing susceptibilities to becoming CSCs in response to contextual signals. This may explain, in part, the recent report of a high percentage of tumor-initiating cells in melanomas[2]. It is possible that CSC biology, and, more specifically, the rules that dictate the baseline levels of CSCs and the kinetic rates that govern interconvertibility, differ markedly between tumor types[24].

Such phenotypic plasticity also helps to resolve a major paradox of the CSC model. During the course of multistep tumor progression, one precursor population of premalignant cells evolves via mutation into a successor population that has a phenotypic advantage, such as an increased resistance to apoptosis or growth-inhibitory signals. The conventional depiction of the CSC model would state that the only cells within the precursor population that are qualified to evolve into a successor population are its stem cells, as only these cells are endowed with the self-renewal capability that is required to spawn unlimited numbers of progeny. If the percentage of stem cells in the precursor cell population is tiny, then the number of cells in this population that can serve as targets for genetic evolution is correspondingly small. As a consequence, the mutation rate (mutations sustained per cell generation) required to complete cancer formation must increase correspondingly, often by two orders of magnitude above the rates that have been described in recent decades for human cells. This paradox may be resolved if the non-CSCs in a precursor cell population can also serve as targets of mutation leading to clonal succession and, therefore, tumor progression.

In addition to its implications for the biology of cancer, the EMT-CSC model carries implications for cancer treatment. For example, multiple reports have shown that CSCs and cancer cells arising as the products of EMT are more resistant to a variety of conventional therapeutics than their non-CSC or nonmesenchymal counterparts[26–30]. These findings suggest that current therapeutic strategies preferentially target nonstem cancer cells, clearly underscoring the need for developing CSC-specific therapies. However, if non-CSCs can indeed give rise to CSCs, this plasticity would frustrate attempts to cure tumors by eliminating CSCs alone, as therapeutic elimination of CSCs may be followed by their regeneration from residual non-CSCs, allowing tumor regrowth and clinical relapse. We, therefore, suspect that optimal therapeutic regimens will need to incorporate agents that target both CSCs and non-CSCs if truly curative therapies are ever to be achieved.

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