

Is Tumor Growth Sustained by Rare Cancer Stem Cells or Dominant Clones?

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Abstract

A key issue for cancer biology and therapy is whether the relentless growth of a tumor is driven by a substantial proportion of its cells or exclusively by a rare subpopulation, commonly termed “cancer stem cells.” Support for the cancer stem cell model has been stimulated by experiments in which human tumor cells were transplanted into immunodeficient mice. Most notably, in human acute myeloid leukemia, only a minute proportion of the cells, displaying a defined phenotype, could seed leukemia in mice. Xenotransplantation, however, may fail to reveal many tumor growth-sustaining cells because the foreign microenvironment precludes essential interactions with support cells. In studies that instead have transplanted mouse leukemias and lymphomas into syngeneic animals, most of the tumors seem to be maintained by the dominant cell population, and only a few types of mouse leukemia seem to be sustained by a minor tumor growth-sustaining subpopulation. The collective evidence suggests that various tumors may span the spectrum between the extremes represented by the two models. If tumor growth can indeed be sustained either by rare cancer stem cells or dominant clones or both, as current evidence suggests, curative therapy for many types of tumors will most likely require targeting *all* the tumor cell populations. [Cancer Res 2008;68(11):4018–21]

Two Models for Tumor Propagation

How the inexorable growth of a tumor is sustained is hotly debated. In the cancer stem cell model (Fig. 1A), tumor growth, like the normal development of a tissue, relies exclusively on rare cells within it (1, 2). This view, inspired by the unique capacity of the rare normal hematopoietic stem cells to restore long-term hematopoiesis to myeloablated mice, posits that the vast majority of the cells of the tumor, although derived by differentiation from the cancer stem cell, lack self-renewal potential and, hence, do not contribute significantly to tumor perpetuation. In this model, heterogeneity within the tumor is ascribed entirely to somewhat aberrant differentiation from the cancer stem cell. In an alternative view (Fig. 1B), which can be termed the “stochastic” (1) or “clonal evolution model” (3), most of the tumor cells are capable of self-renewal and can contribute substantially to tumor maintenance. Tumor heterogeneity in this model is ascribed not only to differentiation but also to intraclonal genetic and epigenetic variation plus microenvironmental influences. In this view,

differences in phenotype within a tumor may reflect subclones at different stages of neoplastic transformation, each having a growth and survival advantage over normal cells, albeit to varying extents. Thus, whereas the cancer stem cell model is highly hierarchical with a unique self-renewing cell type at the apex, the clonal evolution model attributes much of the intratumor variation to subclonal differences in mutational profile, and all except the terminally differentiated cells may well have some self-renewal capacity.

Although the term cancer stem cells has sometimes also been used to designate the normal cell in which tumorigenesis first began (the “cell of origin”), we will follow here the current consensus that its use be restricted to the cell that maintains an established tumor (2). However, we will often use more operational terms such as “tumor growth-sustaining” or “tumor-propagating” cell.

The Complexity of Xenotransplantation

The cancer stem cell model has arisen primarily from studies in which human tumor cells are transplanted into immunodeficient mice. Support for it was first galvanized by the intriguing reports that only 1 in 10^4 to 10^7 of the cells in many samples of human AML, which can be heterogenous, could elicit leukemia in sublethally irradiated nonobese diabetic-severe combined immunodeficient (NOD-SCID) mice (1, 4). More recently, putative cancer stem cell populations have been identified in similar experiments with several types of human solid tumors, including for example, ones from breast, colon, and brain (5–7). Xenotransplantation is problematic, however, because the growth of tumor cells requires an intricate network of interactions with diverse support cells (including fibroblasts, endothelial cells, macrophages, mast cells, and mesenchymal stem cells; ref. 8), and many of the cytokines and receptors required for these two-way interactions are incompatible between mice and humans (9). Furthermore, whether many human tumor cells can home efficiently to an appropriate niche in the mouse is unclear. The complexity of xenotransplantation is underlined by the demonstration that 50% of human acute myeloid leukemia (AML) samples did not engraft irradiated NOD-SCID mice even when 10^7 or 10^8 cells were introduced (10).

Advocates of the cancer stem cell hypothesis argue that the model is proven for human AML by evidence that cell populations prospectively isolated from the leukemia samples by surface markers (e.g., $CD34^+CD38^-$) seed leukemia in mice, whereas the majority cell population lacking that phenotype does not (1, 2, 4). This argument, however, relies on the unproven premise that the observed differences in engraftment must reflect differences in self-renewal ability within the patients. Rather than lacking self-renewal activity, the nontransplantable human AML cell population might instead simply lack a critical feature for obtaining stromal support in the foreign microenvironment, such as a cytokine receptor responsive to mouse factors or a chemokine receptor that attracts

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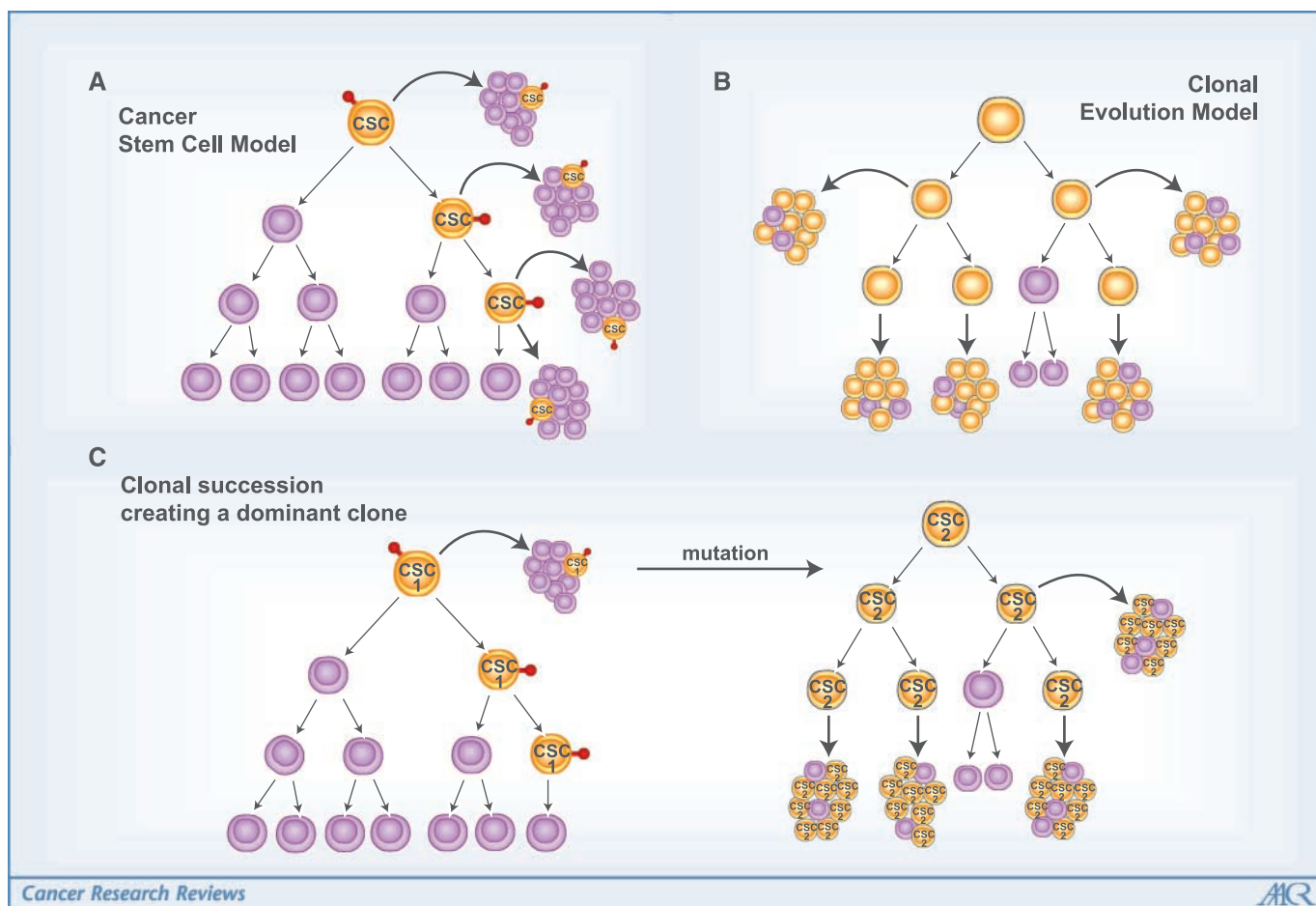


Figure 1. Models for the nature of sustained tumor growth. *A*, in the cancer stem cell (CSC) model, only the CSC (gold), which can be isolated prospectively by surface markers (red), possesses self-renewal activity and, hence, represents the only relevant target for therapy. *B*, in the clonal evolution model, a substantial proportion of the tumor cells (gold) can sustain its growth, and hence, therapy must attempt to eliminate all the cells. *C*, in a mixed model, whereas a tumor is originally driven primarily by rare cells of one phenotype (CSC1), a mutation enhancing self-renewal in a differentiated derivative creates a dominant subclone driven by cells of a different phenotype (CSC2). In some human tumors (e.g., AML), CSC1 but not CSC2 might be able to engraft mice.

the cells to a nurturing niche. Conversely, rather than being uniquely responsible for AML growth, the human transplantable population may simply have inadvertently acquired (perhaps by epigenetic changes) features that endow those cells with the ability to survive in the foreign milieu. A more interesting possibility is that these rare cells could be the founders of the original human disease, but subsequent mutations within the clone have created a dominant, more aggressive and mature, derivative that drives the AML but cannot engraft mice (Fig. 1C). If so, the transplantable population might represent only a minor component among the cancer stem cells sustaining the human leukemia.

The development of solid tumors is considerably more complex than that of hematopoietic ones, requiring a more complex microenvironment, greater reliance on angiogenesis, escape from tissue barriers and, eventually, the morphologic and other changes required for metastasis. Furthermore, in most solid tissues, the differentiation pathways are not nearly as well-understood as in the hematopoietic system. Not surprisingly, it is generally accepted that the evidence for cancer stem cells in solid tumors is less advanced than that for AML (2). No stem cells from solid tumors have as yet been highly purified (5-7), and because some studies place them within subpopulations (eg, CD133⁺) that can contain up to 20% of the total cells, they need not be rare. In some cases, the apparent

rarity of the human transplantable cells might reflect the need to cotransfer an essential support cell that happens to display similar cell surface markers. Thus, cotransfer of support and tumor cells that are both CD133⁺ might explain the paradox that the colon cancer CD133⁺ population was estimated to contain 20 times more tumor-propagating cells than the unfractionated population (11). For example, CD133⁺ endothelial cell precursors can enhance growth of transplanted human cancer cells (12). Pertinently, engraftment of mouse solid tumors requires far more cells than hematopoietic ones, but the number required is markedly reduced if irradiated tumor cells are coinjected, suggesting that the solid tumors rely greatly on stromal support (13). Such studies with the human tumors might reveal additional populations of tumor-propagating cells and far higher frequencies.

Thus, for these and other reasons (3, 14, 15), although the findings with human malignancies seem consistent with the cancer stem cell model, the case is far from proven.

Mouse Tumors with Abundant Transplantable Cells

To test the cancer stem cell hypothesis without the complexity of xenotransplantation, several recent studies have evaluated syngeneic transfers of mouse leukemias or lymphomas. Although a few

of the results are compatible with that hypothesis (see below), a number of others challenge the notion that tumor growth need be driven by a minor subpopulation of tumor stem cells. For example, in three types of well-characterized primary monoclonal mouse tumors (B lymphomas of $E\mu$ -*myc* transgenic mice, T lymphomas of $E\mu$ -*Nras* transgenic mice, and the AML that develops in animals lacking PU.1), >10% of cells from each tumor readily seeded tumor growth in nonirradiated recipients, and 3 of the 8 single-cell transfers attempted with a B lymphoma succeeded (16). Particularly germane to human AML, transplantation of colonies of primitive mouse hematopoietic cells transformed by the MLL-AF9 oncogene revealed that a quarter of *all* myeloid cells could initiate leukemogenesis in mice, although (apparently due to poor homing ability) by limit dilution, only 1 in 100 leukemia cells could seed tumors in recipients (17). Notably, the leukemia-propagating cells had a predominantly mature (Mac-1⁺ Gr-1⁺) phenotype rather than features of the hematopoietic stem cell. Similarly, in another MLL-AF9 model, up to 50% of granulocyte-macrophage progenitors could initiate leukemia (18). Finally, as few as 20 BCR-ABL-transduced Arf-null pre-B cells, and virtually all colonies generated by them, could rapidly induce acute lymphocytic leukemia (19). Thus, in all these studies, the leukemia-propagating cells were abundant and displayed relatively mature phenotypes rather than resembling a stem cell (16–19). Hence, certain oncogenes clearly can activate extensive self-renewal in more differentiated cells, perhaps by enhancing expression of genes that can impose stem cell character, such as those of the Wnt- β -catenin pathway (20) or *bmi-1* and certain *hox* genes (21).

A high frequency of tumor-propagating cells is not confined to genetically engineered models. In several classic studies of spontaneous mouse leukemias and lymphomas, the frequency of transplantable tumor cells ranged from >1% to the majority of cells, and, in several striking examples, tumors could be induced by transplantation of a single tumor cell (13, 22). Thus, a variety of monoclonal mouse hematopoietic malignancies arising with stochastic onset, including ones that closely model human counterparts and involve equivalent genetic changes, have been found to be sustained by a substantial proportion of their cells (at least 10%), and no functionally distinct subpopulation has been evident (16–19). These results favor a model in which growth of the tumor is sustained by a large proportion of its cells (Fig. 1B), perhaps by most of the cells that can form colonies *in vitro* under optimal conditions, rather than exclusively by a minor subpopulation, as expected on the cancer stem cell model (Fig. 1A). The disparity with the human AML results may mean that xenotransplantation greatly underestimates the proportion of cells, and range of cell types, within the human leukemias that drive neoplastic growth.

On the other hand, three types of mouse leukemia (23–25) and one type of murine breast cancer (26) have been reported to display some features expected from the cancer stem cell model. In a blast crisis model of chronic myeloid leukemia (CML), the leukemogenic subpopulation did have the phenotype of a primitive cell (24), although in the human blast crisis disease, the relatively mature granulocyte-monocyte progenitors have acquired extensive self-renewal (20). In mouse AML generated by the *MOZ-TIF2* gene, irrespective of whether stem cells or late progenitor cells had been targeted, the leukemic cells had a relatively mature phenotype (CD34⁺Mac1⁺) but only 10⁻⁴ of them could seed leukemia on transplantation (23). Finally, a leukemogenic subpopulation was identified in AML induced by the *CALM/AF10* fusion gene,

although curiously, its phenotype and genotype resembled that of a pro-B lymphocyte (B220⁺ and D-J rearrangement; ref. 25). Finally, six of seven cancers arising in MMTV-*Wnt-1* transgenic mice seemed to be propagated by a subpopulation corresponding to 1 in 200 cells; presumably, the seventh tumor did not fit this model (26). Moreover, because Wnt-1 but not Neu seems to augment mammary stem cell numbers (27), probably by inducing self-renewal (20), whether other breast cancer models will behave similarly remains to be determined.

Implications for Tumor Propagation and Therapy

As we have seen, many of the results with mouse leukemias and lymphomas challenge the generality of the cancer stem cell hypothesis because a substantial proportion of their cells can seed tumors in syngeneic animals and no functionally distinct subpopulations are evident (16–19). The high transplantability of many mouse leukemias suggests that xenotransplantation may seriously underestimate the frequency of cells, and range of cell types, within a human tumor that sustain its growth. More compelling tests of the cancer stem cell hypothesis might be provided by analysis of more mouse tumor models; by studies with human tumors that include cotransfer of appropriate human stromal cells or irradiated tumor tissue, or that exploit mice in which the relevant human support cells have been installed; and by purification of the stem cells using more specific surface markers (28). Expression profiling and genomic sequence analysis of multiple subclones from the same tumor would help to determine whether heterogeneity and differences in transplantability are simply due to differentiation, as postulated by the cancer stem cell model, or instead often reflect a varied complement of mutations, as expected from the clonal evolution model.

In any case, a few types of mouse tumors have yielded results broadly consistent with the cancer stem cell model (23–26), and the collective evidence suggests that each of the models in Fig. 1 probably holds for different types of tumors. For example, the cancer stem cell model (Fig. 1A) is almost certainly valid for the chronic phase of CML because the characteristic BCR-ABL chromosome translocation can be detected in multiple hematopoietic lineages from the same patient. At the other extreme, we suggest that few if any lymphoid tumors or lymphoid leukemias will follow that model because virtually all the cells in those malignancies exhibit a clonotypic rearrangement of their antigen receptor genes, and that distinctive hallmark of relatively mature differentiation invariably remains in transplants of such tumors. Hence, all lymphoid tumors, with the possible exception of those lacking such gene rearrangements, are likely to follow the clonal evolution model (Fig. 1B). Why might tumors be propagated in two disparate ways? Most of the studies with mouse tumors cited here favors the view that the nature of the key oncogenic mutations each has suffered determines the model followed. Another possibility is that a tumor tends to follow the cancer stem cell model if the key mutation occurred in a normal stem or primitive progenitor cell, as originally suggested (4), whereas the clonal evolution model predominates among tumors that originate in more mature cells. Naturally, both the nature of the mutations and the cell of origin may well contribute. It is also conceivable that many tumors that initially follow the cancer stem cell paradigm progress on acquiring additional mutations to resemble the clonal evolution model (Fig. 1C). Such tumors might exhibit features of both models, such as a relatively high frequency of tumor-propagating cells as well as

a substantial proportion unable to sustain tumor growth. For example, metastatic neuroblastoma seems to be highly enriched for tumorigenic cells because as few as 10 cells could engraft mice and no hierarchical organization was evident (29).

Much of the excitement about the cancer stem cell model has been engendered by the prospect that it might provide a new approach to therapy (1, 2). For any tumors in which all self-renewal resided in the cancer stem cells (Fig. 1A), those cells would be the critical therapeutic targets, whereas elimination of the bulk of the tumor cells might have negligible effect on long-term patient survival. In particular, if that subpopulation, which is thought to be largely quiescent, were more refractory than other tumor cell populations to most conventional therapeutic agents, these cells might be primarily responsible for the relapses that eventuate with so many tumor types. On that model, more durable or even curative therapies might result from treatments targeted to the cancer stem cells, particularly if normal stem cells can be spared. Thus far, the evidence that therapy will improve is limited, but cell populations containing the putative cancer stem cells are reportedly more refractory to irradiation and chemotherapy (30, 31), and administration to mice of an antibody to CD44, an antigen expressed on human AML-initiating cells, markedly reduced leukemic repopulation (32).

Therapy targeted to cancer stem cells may well have great promise for tumors that behave exclusively as predicted by the cancer stem cell model, if those tumors can be identified. It is noteworthy, however, that imatinib has revolutionized the management of CML, although the stem cells that drive this leukemia may be refractory, preventing true cures (33). Thus, even with this paradigm disease of cancer stem cells, a drug that attacks the vast bulk of the tumor cells can be highly effective. Furthermore, on available evidence, we surmise that many tumors more nearly resemble the clonal evolution model (Fig. 1B), or some mixture of the two models (Fig. 1C). Hence, it seems likely that curative therapy of most tumors will require targeting *all* the tumor populations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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