Cell
Leading Edge
Essay

Heterogeneity in Cancer: Cancer Stem Cells versus Clonal Evolution

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DOI 10.1016/j.cell.2009.08.017

The identification and characterization of cancer stem cells might lead to more effective treatments for some cancers by focusing therapy on the most malignant cells. To achieve this goal it will be necessary to determine which cancers follow a cancer stem cell model and which do not, to address technical issues related to tumorigenesis assays, and to test the extent to which cancer cell heterogeneity arises from genetic versus epigenetic differences.

What Is the Cancer Stem Cell Model?

To devise more effective cancer therapies it will be critical to determine which cancer cells have the potential to contribute to disease progression. If most cancer cells can proliferate extensively and metastasize, then virtually all cells must be eliminated to cure the disease. Consistent with this view, traditional cancer therapies have sought to eliminate as many cancer cells as possible. In contrast to this approach, the cancer stem cell model proposes that the growth and progression of many cancers are driven by small subpopulations of cancer stem cells (Reya et al., 2001; Dick, 2008). The cancer stem cell model does not address the question of whether cancers arise from normal stem cells. Rather, it suggests that irrespective of the cell-of-origin, many cancers may be hierarchically organized in much the same manner as normal tissues. Just as normal stem cells differentiate into phenotypically diverse progeny with limited proliferative potential, it is argued that cancer stem cells also undergo epigenetic changes analogous to the differentiation of normal cells, forming phenotypically diverse nontumorigenic cancer cells that compose the bulk of cells in a tumor. These epigenetic changes are proposed to be associated with an irreversible (or rarely reversible) loss of tumorigenic capacity such that the vast majority of cells in these cancers have little capacity to contribute to disease progression. To characterize and eliminate the malignant cells in cancers that follow this model, it is necessary to focus on the small subpopulations of tumorigenic cells.

Consistent with the cancer stem cell model, certain cancers (including some germ cell cancers and some leukemias) have been recognized for decades to include neoplastic cells that differentiate into post-mitotic derivatives. Germ cell cancers give rise to highly differentiated cells, such as those that make neural tissue (Illmensee and Mintz, 1976). Some leukemias give rise to highly differentiated hematopoietic cells (Fearon et al., 1986; Barabe et al., 2007). These cancers are obviously hierarchically organized. The more controversial question raised by recent studies is whether many other cancers exhibit a similar hierarchical organization, even when overt differentiation is not evident among the cancer cells.

Compelling data support the cancer stem cell model in various human cancers including malignant germ cell cancers (Kleinsmith and Pierce, 1964; Illmensee and Mintz, 1976), leukemias (Lapidot et al., 1994; Bonnet and Dick, 1997), breast cancers (Al-Hajj et al., 2003), brain cancers (Singh et al., 2004), and colon cancers (Dalerba et al., 2007; O'Brien et al., 2007; Ricci-Vitiani et al., 2007). In each case, only small subpopulations of cells can transfer disease upon transplantation into immunocompromised NOD/SCID mice, and markers have been identified that distinguish the leukemogenic/tumorigenic cancer cells from the bulk populations of nonleukemogenic/tumorigenic cells. The ability to predict which cells are tumorigenic based on marker expression indicates that the tumorigenic cells are intrinsically different from nontumorigenic cancer cells. Yet, no clear morphological distinction was found between tumorigenic and nontumorigenic breast cancer cells (Al-Hajj et al., 2003), implying that differentiation need not be overt for the cells to be hierarchically organized. The observation that tumorigenic cells have tended to be rare in the cancers found so far to follow a cancer stem cell model implies that epigenetic differences distinguish tumorigenic from nontumorigenic cells because it is implausible that only rare cancer cells have a genotype permissive for extensive proliferation. Tumorigenic cancer stem cells also form phenotypically diverse nontumorigenic cells, recapitulating at least some of the heterogeneity in the tumors from which they derive.

Despite strong data supporting the stem cell model in some cancers, it is important to acknowledge a number of caveats. There is no direct evidence in these cancers that tumorigenic cells differ from nontumorigenic cells as a result of epigenetic rather than genetic differences. Moreover, the conclusion that transplanted cancer stem cells

Table 1. Models to Explain Cancer Cell Heterogeneity

	Cancer Stem Cell Model	(Stochastic) Clonal Evolution Model[a]
Frequency of cancer cells with tumorigenic potential	Rare to moderate	High
Phenotype of cancer cells	Heterogeneous	Heterogeneous or homogeneous
Tumor organization	Hierarchical	Not necessarily hierarchical
Intrinsic differences between tumorigenic and nontumorigenic cells	Stable, epigenetic	Unstable, epigenetic or genetic
Rational approach to therapy	Possible to target only tumorigenic cells	Target most or all cells
Compelling clinical evidence	Germ lineage cancers	High-grade B cell lymphoblastic leukemia[b]

[a]The clonal evolution model holds that genetic and epigenetic changes occur over time in individual cancer cells, and that if such changes confer a selective advantage they will allow individual clones of cancer cells to out-compete other clones. Clonal evolution can lead to genetic heterogeneity, conferring phenotypic and functional differences among the cancer cells within a single patient. Note that the clonal evolution and cancer stem cell models are not mutually exclusive in cancers that follow a stem cell model, as cancer stem cells would be expected to evolve by clonal evolution. However, heterogeneity in cancers that do not follow a cancer stem cell model (not hierarchically organized into epigenetically distinct tumorigenic and nontumorigenic populations) could be determined entirely by clonal evolution.

[b]B cell lymphoblastic leukemias have extraordinarily high frequencies of leukemogenic cells that are not hierarchically organized in a mouse model (Williams et al., 2007) and appear homogeneous by histopathology in patients, yet heterogeneity can arise in sensitivity to therapy through clonal genetic changes.

can recapitulate the heterogeneity of the tumors from which they derive is based on limited analyses of only two or three surface markers. It has not yet been determined whether there is also genetic heterogeneity within the primary tumors that is not recapitulated after cancer stem cell transplantation. It therefore remains possible that the functional and phenotypic diversity within these cancers is underestimated and partially genetically determined.

The cancer stem cell model has been carefully tested in only a small subset of cancers. Although this model is often assumed to apply widely to other cancers, recent data demonstrate that leukemogenic/tumorigenic potential is a common attribute of cells in some cancers (Kelly et al., 2007; Williams et al., 2007; Quintana et al., 2008) making this a questionable assumption. Second, the NOD/SCID mouse transplantation assay, which has been the source of most of the compelling data supporting the cancer stem cell model, dramatically underestimates the frequency of human cancer cells with tumorigenic potential in some cancers (Quintana et al., 2008). This suggests the need to re-evaluate some of the evidence supporting the model using assays that are more permissive for the engraftment of human cancer cells. If some cancers that are currently thought to follow a cancer stem cell model actually have common tumorigenic cells and heterogeneity is generated through genetic and epigenetic mechanisms, the progression of these cancers may be more accurately described by the clonal evolution model (Nowell, 1976) (Table 1).

The Cancer Stem Cell Model and Clinical Behavior

The response of some cancers to therapy does appear to be influenced by epigenetic differences between leukemogenic/tumorigenic cells and their nonleukemogenic/tumorigenic progeny. For example, chronic myeloid leukemia appears to be sustained by leukemic stem cells that are more resistant to the drug imatinib than their differentiated progeny (O'Hare et al., 2006; Oravecz-Wilson et al., 2009). There is also evidence that cancer stem cells in gliomas (Bao et al., 2006) and breast cancers (Li et al., 2008; Diehn et al., 2009) might be intrinsically more resistant to therapy than other cells in these cancers. Conversely, the undifferentiated cells that drive testicular cancer progression are more sensitive to cisplatin therapy than the differentiated cells they form (Masters and Koberle, 2003). This indicates that although the epigenetic state of cancer stem cells can influence the response to therapy, these cells are not always more resistant than their nontumorigenic progeny.

Although the cancer stem cell model is likely to explain the clinical behavior of some cancers, the observation that many cancers re-emerge after treatment does not necessarily imply that the cells that survive therapy are intrinsically more resistant than the cells that are killed. All cancer cells within a given patient might have a similar probability of surviving and expanding after therapy, or the surviving cells might be in a protective microenvironment. In cases where there are intrinsic differences in the sensitivity of cancer cells to therapy, these differences can be genetically determined (Sikic, 2008). Therefore, therapy-resistant cancer stem cells do not necessarily exist in many cancers. Resistance to therapy in many cancers may be explained by epigenetic and genetic differences among tumorigenic cancer cells that lack hierarchical organization, just as predicted by clonal evolution (Nowell, 1976).

Epigenetic differences between cancer stem cells and their progeny are likely to be an important determinant of the clinical behavior of some cancers but not others, whereas clonal evolution is likely to be important in all cancers. The cancer stem cell model and the clonal evolution model are not mutually exclusive in cancers that are hierarchically organized into epigenetically distinct populations of tumorigenic and nontumorigenic cancer cells. In these cancers, clonal evolution still occurs in the cancer stem cells (Barabe et al., 2007). For example, the leukemic stem cells that maintain chronic myeloid leukemia despite imatinib therapy would be selected to develop imatinib resistance mutations over time by clonal evolution (Shah et al., 2007). However, in cancers in which tumorigenic

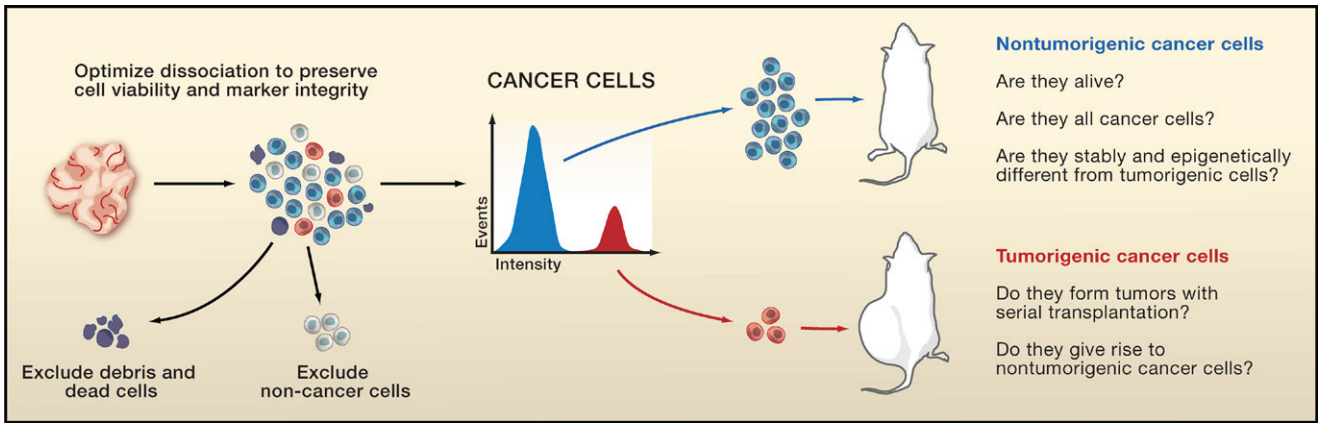


Figure 1. Testing the Cancer Stem Cell Model
During the dissociation of solid tumors (left), conditions must be optimized to maximize the preservation of cell viability and surface marker expression. During cell separation (middle), care must be taken to use viability dyes and markers to exclude dead cells, hematopoietic cells, endothelial cells, and stromal cells (if possible) by flow cytometry from the cancer cell preparation. The tumorigenicity of all cells must be tested in assays optimized for the engraftment of human cancer cells (right). For nontumorigenic cell populations, it is critical to confirm that they contain live cancer cells, rather than normal cells or debris. If markers can be identified that distinguish tumorigenic from nontumorigenic cells, an important question is whether these cancer cell populations are distinguished by epigenetic rather than genetic differences.

potential is common and there is little evidence of hierarchical organization, heterogeneity in response to therapy likely arises primarily from clonal evolution, not from epigenetic differences between cancer stem cells and their progeny.

The Cancer Stem Cell Model Addresses Potential, Not Fate

The cancer stem cell literature has addressed the *potential* of cancer cells to contribute to disease, not the actual *fate* of cells within patients. Potential describes what cells are capable of doing under permissive conditions, whereas fate describes what they actually do in a specific circumstance. The central tenet of the cancer stem cell literature has been that the vast majority of cells within at least some cancers have lost the *potential* to proliferate extensively, as revealed by their inability to transfer disease to immunocompromised mice and in some cases by their inability to proliferate in culture. It has been proposed that the nontumorigenic cancer cells can be ignored in therapy because they lack the potential to contribute to disease.

These inferences regarding tumorigenic potential are sometimes confused with the question of which cells are actually fated to contribute to disease in patients. The issue of fate is a different question that has not been addressed by the cancer stem cell field. Indeed, there are almost no data in all of cancer biology that address the question of whether many cancer cells or few cancer cells are actually fated to contribute to disease in patients because this question can only be addressed within the patient and therefore is experimentally less feasible. It is important to bear in mind that just because a cell has the potential to form a tumor does not mean that it actually does so within a patient. Cancer cells with tumorigenic potential might be held in check, transiently or permanently, by environmental or immunological mechanisms that prevent them from actually contributing to disease.

To test the cancer stem cell model, it is necessary to identify all of the cells with the potential to proliferate extensively and to contribute to disease (Figure 1). If a cancer cell has the potential to form a tumor in any assay, then it has not entered an epigenetic state in which it has lost the ability to proliferate, and it is perilous to ignore this cell when treating a patient. For this reason, tumorigenic potential is presumably the key consideration when devising therapeutic strategies because therapies must target all cells with the *potential* to contribute to disease in a patient. It is not safe to base therapeutic strategies on assumptions regarding fate because fate is context dependent. Cancer cells fated to contribute to disease in one context (such as in a primary tumor) may be quite different from cells fated to contribute to disease in other contexts (such as after metastasis or therapy). Rational approaches to therapy must therefore target all of the cells with the potential to contribute to disease.

A fundamental question is whether immunocompromised mice are reliable models for studying human cancer. The answer may depend upon the aspect of cancer biology being studied. Transplantation into highly immunocompromised mice is the best, albeit imperfect, way of assessing which human cancer cells have the potential to form tumors/leukemias. In contrast, such studies often cannot address the extent to which these cells might be positively or negatively regulated by environmental mechanisms, such as immune function, in patient tissues. For example, an interesting question is whether some cancer cells are more immunogenic than others in patients. This question should not be confused with the cancer stem cell model, which addresses the very different issue of whether intrinsic epigenetic differences among cancer cells limit their proliferative potential. The question of whether some cancer cells are more immunogenic than others in patients may not be directly testable because it requires tumorigenesis assays that replicate the syngeneic immune response that occurs in patients against their own tumors. The xenogeneic immune

response that occurs in mice against human cells is much more powerful and depends upon very different immunological mechanisms than the syngeneic response in patients does. For these reasons, it is critical to distinguish between questions of potential that can be addressed in xenograft models and questions of fate that must be addressed in human tissues or in models of mouse cancer.

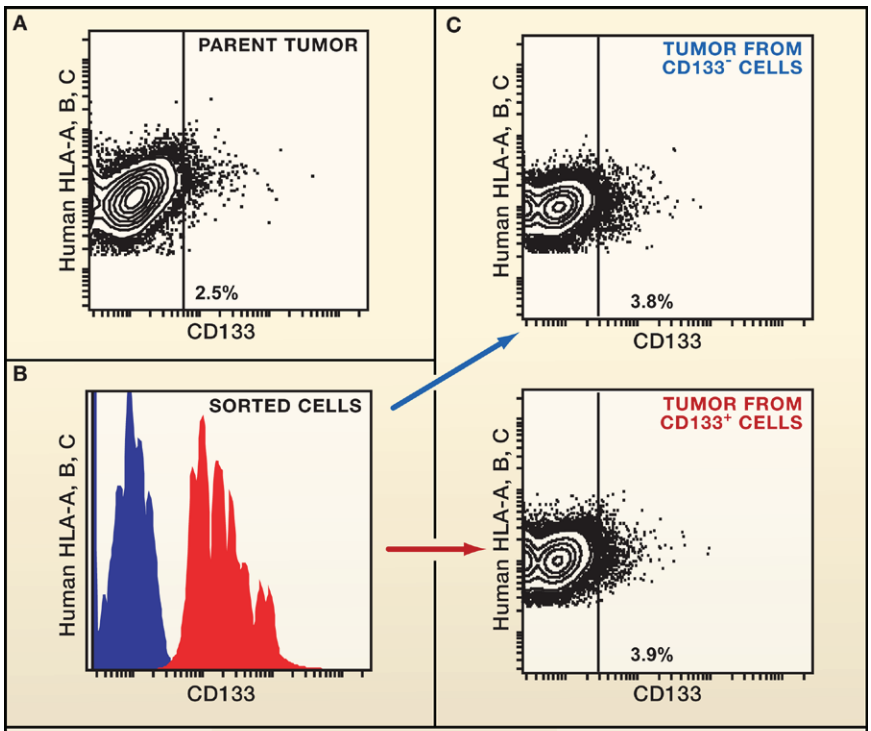
Underestimating Tumorigenic Potential

The xenogeneic immune response that mice mount against human cells is a critical variable that determines the ability of human cancer cells to engraft in mice. Even highly immunocompromised NOD/SCID mice, lacking B and T cells, retain natural killer cells that reject most transplanted human cells (McKenzie et al., 2005). As human cancer cells are transplanted into increasingly immunocompromised mice, fewer and fewer cells are required to transfer disease (Bonnet and Dick, 1997; Feuring-Buske et al., 2003; Kennedy et al., 2007; Quintana et al., 2008).

The use of NOD/SCID mice can underestimate the frequency of human cancer cells with tumorigenic potential due to the xenogeneic immune response in these mice. The percentage of melanoma cells that form tumors in NOD/SCID mice deficient in the interleukin-2 receptor γ chain (IL2Rγ^{null} mice), which lack T, B, and natural killer cells, is orders of magnitude higher than the percentage that form tumors in NOD/SCID mice. Although only ~1 in a million melanoma cells form tumors in NOD/SCID mice, 1 in 4 can form tumors in NOD/SCID IL2Rγ^{null} mice when coinjected with Matrigel (Quintana et al., 2008). Thus tumorigenic potential is a common attribute of cells in some human cancers, even though tumorigenic cells can appear to be rare in the NOD/SCID mouse transplantation assay.

Although this suggests that the frequency of leukemogenic/tumorigenic cells in other cancers should be re-evaluated in optimized assays, it does not necessarily mean that such cells will be as common in other cancers as they are in melanoma. Additional work is required to determine which cancers have common leukemogenic/tumorigenic cells and which have rare leukemogenic/tumorigenic cells (Figure 1). This is a critical issue as models and therapies must account for the full spectrum of human cancer cells with the potential to contribute to disease.

Even highly immunocompromised NOD/SCID IL2Rγ^{null} mice may underestimate the frequency of human cancer cells with tumorigenic potential due to differences between the mouse and human tissue environments. Incompatibilities between mouse ligands and human receptors for certain growth factors and adhesion molecules may impair the survival, proliferation, or migration of human cells in mice. Moreover, human cancers are often heterotopically transplanted into mice (that is, transplanted into locations that differ



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Sorted fraction	# of tumors/# of injections Cells/injections 50	10	Melanoma-initiating cell frequency (95%CI)	
Unfractionated	18/18	14/18	1/7	(1/4-1/12)
CD133⁻	17/17	12/18	1/9	(1/5-1/15)
CD133⁺	14/18	9/18	1/25	(1/15-1/40)

Figure 2. Cancers Need Not Be Hierarchically Organized to Be Heterogeneous

CD133 expression distinguishes tumorigenic from nontumorigenic cancer cells in some brain tumors and some colon cancers (Singh et al., 2004; O'Brien et al., 2007; Ricci-Vitiani et al., 2007). However, the expression of CD133 (or other stem cell markers) by small subpopulations of cells in other cancers does not necessarily mean that these cells are cancer stem cells. CD133 expression was heterogeneous in melanomas from 6 of 12 patients (Quintana et al., 2008).

(A) Representative CD133 staining in one of these melanomas (positive staining was defined using an isotype control).

(B) A reanalysis of the CD133⁻ (blue) and CD133⁺ (red) fractions after separation using magnetic beads.

(C) When these cells were transplanted into NOD/SCID IL2Rγ^{null} mice, both the CD133⁻ and CD133⁺ fractions of cells contained high frequencies of tumorigenic cells (D) (Quintana et al., 2008). The tumors that arose from CD133⁻ cells and from CD133⁺ cells contained similar proportions of CD133⁻ and CD133⁺ cells. This indicates that individual cancer cells can recapitulate the heterogeneity of the tumors from which they derive, even when there is no evidence that the cancer follows a cancer stem cell model or that tumorigenic cells are hierarchically organized.

from the locations in which the tumors normally arise in patients). Differences between the native environment of cancer cells in patients and the environment into which these cells are transplanted in mice can reduce the engraftment of cells with tumorigenic potential. These effects can be mitigated by expressing human growth factors in mice (Lapidot et al., 1994; Feuring-Buske et al., 2003) and by optimizing the site of transplantation (Kennedy et al., 2007). As tumorigenesis assays are optimized (Table S1 available online), it is likely that estimates of the frequency of cells with leukemogenic/tumorigenic potential will increase significantly in most human cancers.

The Biological Basis of Cancer Cell Heterogeneity

The cancer stem cell model posits that differences in tumorigenic potential among cancer cells from the same patient are largely epigenetically determined. Moreover, this model requires such epigenetic differences to be largely irreversible because if nontumorigenic cells could efficiently revert to the tumorigenic state it would not be possible to distinguish tumorigenic from nontumorigenic cells and such cancers would not be hierarchically organized. Consistent with the model, cancer stem cells differentiate into nontumorigenic cancer cells in germ lineage cancers (Kleinsmith and Pierce, 1964; Illmensee and Mintz, 1976), chronic myeloid leukemia (O'Hare et al., 2006), and some brain tumors (Piccirillo et al., 2006).

The extent to which cancer cells differentiate to a nontumorigenic state has not been directly tested in most other cancers. The ability to distinguish rare tumorigenic cells from nontumorigenic cells in several cancers based on marker expression implies that the tumorigenic cells are epigenetically distinct from nontumorigenic cells in these cancers (e.g., Lapidot et al., 1994; Bonnet and Dick, 1997; Al-Hajj et al., 2003; Singh et al., 2004; O'Brien et al., 2007; Ricci-Vitiani et al., 2007). However, if tumorigenic cells are much more common in some of these cancers than currently estimated, a legitimate question arises about whether they are really distinguished from nontumorigenic cells by epigenetic mechanisms. Some cancer cells would be expected to lack tumorigenic potential because they are fated to undergo cell death or senescence due to deleterious genetic changes or localization to unsupportive environments. If nontumorigenic cells represent 50% or 75% of cells in a tumor, it is conceivable that their lack of tumorigenic potential is explained entirely by genetic and environmental mechanisms. Therefore, in cancers in which tumorigenic cells are common, it will be vital to assess whether epigenetic differences distinguish tumorigenic and nontumorigenic cells to determine whether such cancers follow a cancer stem cell model. The mere observation that some cancer cells are more tumorigenic than others is entirely consistent with clonal evolution (Table 1).

Melanoma illustrates these issues. We estimate that at least 25% of human melanoma cells have tumorigenic potential, and we have not been able to identify any markers that distinguish tumorigenic from nontumorigenic melanoma cells, despite considerable effort (Quintana et al., 2008). This does not prove that melanoma does not follow a cancer stem cell model as markers that distinguish tumorigenic from nontumorigenic cells could be identified in the future. Nevertheless, the simplest interpretation of the currently available data is that many melanoma cells have a similar tumorigenic capacity, that these cells are not hierarchically organized (Figure 2), and that melanoma does not, therefore, appear to follow a cancer stem cell model. If markers are identified in future that can distinguish tumorigenic from nontumorigenic melanoma cells, it will be important to test whether these cells are distinguished by genetic differences, epigenetic differences, or localization to distinct environments.

Distinguishing Tumorigenic from Nontumorigenic Cells

Evidence that tumorigenic cells can be distinguished from nontumorigenic cells based on marker expression is a cornerstone of the cancer stem cell model. Without this evidence, it would be possible that all cancer cells have the same stochastic probability of proliferating or forming a tumor. Thus, the ability of markers to distinguish leukemogenic/tumorigenic cells from nonleukemogenic/nontumorigenic cells in leukemias (Lapidot et al., 1994; Bonnet and Dick, 1997), breast cancers (Al-Hajj et al., 2003), brain cancers (Singh et al., 2004), and colon cancers (Dalerba et al., 2007; O'Brien et al., 2007; Ricci-Vitiani et al., 2007) is strong evidence that these cancers follow a cancer stem cell model.

As markers are further evaluated in additional studies and in larger numbers of tumors, some markers will likely prove less robust than they currently appear. CD133 appeared to be a robust marker of brain tumor stem cells in initial studies (Singh et al., 2004; Bao et al., 2006), but more recent studies have found that this marker does not distinguish tumorigenic from nontumorigenic cells in many other brain tumors (Joo et al., 2008; Ogden et al., 2008; Wang et al., 2008). This raises the possibility that some brain tumors may follow a cancer stem cell model, whereas others do not. This is likely to be a general issue in a variety of cancers as many of the markers used to distinguish tumorigenic from nontumorigenic cancer cells may turn out to work in some circumstances but not in others. It is important to remember that in the absence of markers that can distinguish tumorigenic from nontumorigenic cancer cells, there is no evidence that a cancer follows a cancer stem cell model as there can be no evidence of intrinsic differences among cancer cells in tumorigenicity (without markers there is no way to rule out the possibility that all cancer cells have a similar stochastic probability of forming tumors). It will be important to determine what fraction of patients with each type of cancer actually have cancers that express informative markers and follow a cancer stem cell model. Given these uncertainties in marker robustness, markers alone should not be relied upon to assess potential biological differences between tumorigenic and nontumorigenic cells; functional assays are required to confirm differences in therapy sensitivity and other biological properties.

There are also a number of technical pitfalls that can falsely appear to distinguish tumorigenic from nontumorigenic cancer cells (Table S1). Tumors contain neoplastic cancer cells as well as non-neoplastic stromal and immune cells, but markers are rarely available to distinguish them. Many solid cancer stem cell studies have not even taken advantage of known hematopoietic and endothelial markers to exclude these cells from sorted cancer cell preparations. Thus, it is possible that the depletion of tumorigenic activity in "nontumorigenic" cell populations in some studies could be caused by the presence of non-neoplastic cells in these populations rather than by the presence of nontumorigenic cancer cells. Additionally, debris from dying cells and necrotic tissue can appear indistinguishable from live cells by flow cytometry if the debris contains little DNA and does not take up viability dyes. As a result, nontumorigenic fractions can be identified from some tumors, not because large numbers of cancer cells lack tumorigenic potential, but because the tumors yield large quantities of debris that is difficult to distinguish from live cells by flow cytometry. It is critical to exclude debris as much as possible from cancer cell preparations and to examine all sorted fractions by microscopy to ensure the presence of live cells.

Some Cancers Follow a Cancer Stem Cell Model, Others Do Not

Although studies of human cancers in immunocompromised mice can be confounded by species incompatibilities, the conclusion that some cancers follow a cancer stem cell model has been confirmed in studies of mouse cancers. Even when cells from some mouse leukemias (Deshpande et al., 2006; Krivtsov et al., 2006; Yilmaz et al., 2006), mouse breast cancers (Cho et al., 2008; Vaillant et al., 2008; Zhang et al., 2008), and mouse squamous cell carcinomas (Malanchi et al., 2008) are transplanted into fully histocompatible wild-type recipient mice, some of these cancers appear to follow a cancer stem cell model marked by the presence of small subpopulations of cancer cells that have much more leukemogenic/tumorigenic capacity than the bulk population of neoplastic cells. As a result of parallel studies of human and mouse cancers, there is strong evidence that some cancers follow a stem cell model.

Some cancers follow a stem cell model, but it is dangerous to generalize. The cancer stem cell model appears to apply to some mouse acute myeloid leukemias (Deshpande et al., 2006; Krivtsov et al., 2006; Yilmaz et al., 2006) but not others (Kelly et al., 2007). It would not be surprising if cancers with different constellations of mutations differ in the extent to which they follow a stem cell model. The same point applies to markers. Just because CD133 expression identifies cancer stem cells in some cancers does not mean this marker will be informative in other cancers (Figure 2). Markers should be validated in significant numbers of patients before they are assumed to be informative in all patients. It is necessary to rigorously test the cancer stem cell model in every circumstance in which it is hypothesized to apply, using assays that are optimized to detect the full spectrum of cells with tumorigenic potential.

The Significance of Cancer Cell Heterogeneity

Cancers that do not follow a cancer stem cell model are not necessarily homogeneous. Although we have not been able to identify any markers that distinguish tumorigenic from nontumorigenic melanoma cells, we observe considerable heterogeneity within individual melanomas in terms of cellular morphology, pigmentation, and marker expression (Quintana et al., 2008). There are likely biologically and clinically important forms of heterogeneity among cancer cells that are unrelated to distinctions between tumorigenic and nontumorigenic cells. The clonal evolution model predicts that there should be genetic heterogeneity among cancer cells that leads to heterogeneity in phenotype, function, and response to therapy. Epigenetic differences are presumably layered on top of these genetic differences to confer additional heterogeneity. Yet, the mere existence of such heterogeneity does not imply that cancers must be hierarchically organized into tumorigenic and nontumorigenic fractions, nor does the lack of hierarchical organization imply that cancers are homogeneous (Figure 2).

Although the cancer stem cell model was introduced to describe cancers in which intrinsic epigenetic differences among cancer cells cause these cells to reside in a hierarchy of tumorigenic and nontumorigenic cells (Reya et al., 2001; Dick, 2008), the term cancer stem cell is now used so promiscuously as to threaten any meaning. Some have described the normal cell that is transformed into cancer (the cell-of-origin) as the cancer stem cell, even though the cancer stem cell model does not address the cell-of-origin (for more discussion of this issue, see Dick, 2008). Others have begun to describe any clonogenic cancer cell as a cancer stem cell. The cancer stem cell model becomes meaningless and can no longer be readily distinguished from the clonal evolution model when any clonogenic cancer cell is considered a cancer stem cell. Although it is true that cancer stem cells need not be rare (Kelly et al., 2007), cancers in which most cells are similarly tumorigenic do not follow a cancer stem cell model because there can be no meaningful hierarchy within these tumors. Indeed, in cases in which most cancer cells have tumorigenic potential there is little that can be gained in terms of therapy or biology by distinguishing these cells from minority populations of nontumorigenic cells. Some cancers are just good old fashioned cancer in which nearly every cell is bad.

Whether a cancer is comprised of common tumorigenic cells that are heterogeneous as a result of clonal evolution or whether the cancer contains a hierarchy of epigenetically distinct tumorigenic and nontumorigenic cells, cancers often arise and progress due to dysregulation of self-renewal pathways borrowed from normal stem cells (Reya et al., 2001). Carcinogenic mutations can inappropriately activate normal stem cell self-renewal pathways. In some cases, carcinogenesis preserves the epigenetic programs that regulate stem cell differentiation, allowing cancer stem cells to form a hierarchy of cancer cells. In other

cases, carcinogenesis arrests differentiation, potentially allowing a plurality of cancer cells to maintain indefinite proliferative potential, avoiding hierarchical organization. The question of whether a cancer follows a cancer stem cell model is likely to be determined by the cell-of-origin, the carcinogenic mutations, and whether the mutations block the epigenetic programs that underlie differentiation. Studies of stem cell self-renewal mechanisms are likely to inform our understanding of cancer proliferation, even in cancers that are not hierarchically organized.

Conclusions

Cancers that contain a hierarchy of epigenetically distinct populations of tumorigenic and nontumorigenic cells might be more effectively studied and treated by focusing on the tumorigenic cells, particularly when those cells are rare. But this field will only achieve its promise if we carefully distinguish between cancers that follow a cancer stem cell model and those that do not. Therapies designed to eliminate only a small subpopulation of cancer cells will likely not have a clinical impact on cancers in which tumorigenic cells represent most of the cancer cells in the patient. Additional testing of the cancer stem cell model will be required in all cancers to determine what fraction of cases actually follow the model, and how often existing markers are informative. Such testing is likely to yield a complex picture involving differences between cancers, and even between patients with the same cancer, in terms of the frequency of tumorigenic cells, the degree of hierarchical organization, and the extent to which markers can distinguish tumorigenic from nontumorigenic cells.

The use of xenotransplantation models will lead to a better understanding of tumorigenesis in vivo, the developmental relationship between cancer cells, and even new therapies. However, it is critical that such models be optimized for the engraftment of human cells if we are to draw conclusions regarding the frequency of tumorigenic cells. The frequency of human cancer cells with leukemogenic/tumorigenic potential has likely been underestimated in NOD/SCID xenotransplantation assays, in some cases modestly and in other cases drastically. In cancers in which leukemogenic/tumorigenic cells are common, it will be critical to test whether these cells are distinguished from nontumorigenic cells by epigenetic or genetic differences. By optimizing xenotransplantation assays we will gain a more complete understanding of the spectrum of human cancer cells that have the potential to contribute significantly to disease. To capitalize on the promise of cancer stem cell biology, it is worthwhile to remain cognizant of the limitations of the existing data and the need to improve the precision of markers and assays.

Supplemental Data

Supplemental Data include one table and can be found with this article online at http://www.cell.com/supplemental/S0092-8674(09)01030-7.

ACKNOWLEDGMENTS

This work was supported by the Howard Hughes Medical Institute. M.S. is supported by the Human Frontiers Science Program, and E.Q. is supported by the Marie Curie Outgoing International Fellowship from the European Commission. S.J.M. is a founder of Oncomed, Inc.

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