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Cancer stem cells: impact, heterogeneity, and uncertainty

Jeffrey A. Magee, **Elena Piskounova**, and **Sean J. Morrison**[1]
Howard Hughes Medical Institute, Children's Research Institute, and Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX, 75390, USA

Abstract

The differentiation of tumorigenic cancer stem cells into non-tumorigenic cancer cells confers heterogeneity to some cancers beyond that explained by clonal evolution or environmental differences. In such cancers, functional differences between tumorigenic and non-tumorigenic cells influence response to therapy and prognosis. However, it remains uncertain whether the model applies to many, or few, cancers due to questions about the robustness of cancer stem cell markers and the extent to which existing assays underestimate the frequency of tumorigenic cells. In cancers with rapid genetic change, reversible changes in cell states, or biological variability among patients the stem cell model may not be readily testable.

Sources of heterogeneity within cancer

Many tumors contain phenotypically and functionally heterogeneous cancer cells (Fidler and Hart, 1982; Fidler and Kripke, 1977; Heppner, 1984; Nowell, 1986). This heterogeneity among cancer cells in the same patient can arise in multiple ways. The most well established mechanism involves intrinsic differences among cancer cells caused by stochastic genetic (Nowell, 1976) or epigenetic (Baylin and Jones, 2011) changes (clonal evolution; Figure 1A). Differences can also arise among cancer cells through extrinsic mechanisms in which different microenvironments within a tumor confer phenotypic and functional differences upon cancer cells in different locations (Figure 1B) (Polyak et al., 2009; Bissell and Hines, 2011). Finally, some cancers follow a stem cell model in which tumorigenic cancer stem cells "differentiate" into non-tumorigenic cancer cells, creating a hierarchical organization (Figure 1C; Table 1) (Dick, 2008; Reya et al., 2001; Shackleton et al., 2009). The differentiation of cancer stem cells provides a mechanism for generating phenotypic and functional heterogeneity beyond the heterogeneity that can be attributed to clonal evolution or environmental differences (Figure 1D). However, the fact that heterogeneity can arise through multiple mechanisms means that heterogeneity alone does not imply the existence of a cancer stem cell hierarchy.

The cancer stem cell model

The cancer stem cell model is not a new idea (Hamburger and Salmon, 1977). It has been clear for decades that some cancers, including some germ lineage cancers (Kleinsmith and

[1]Author for correspondence: Children's Research Institute, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Texas, 75390-8502; phone 214-633-1791 fax 214-648-5517; Sean.Morrison@UTSouthwestern.edu.

Pierce, 1964), some neuroblastomas (Shimada et al., 1984), and some myeloid leukemias (Fearon et al., 1986; Ogawa et al., 1970), can differentiate into progeny that have limited proliferative potential despite retaining the oncogenic mutations of their malignant progenitors.

Some germ lineage cancers contain rapidly dividing cells that differentiate into postmitotic derivatives (mature teratoma elements) in a process that resembles aberrant embryogenesis (Chaganti and Houdsworth, 2000). The presence of only mature differentiated cells in residual tumor masses after chemotherapy is a favorable prognostic factor, while the presence of residual undifferentiated cells predicts disease recurrence (Stenning et al., 1998). These and other data suggest that undifferentiated cells are primarily responsible for tumor growth and disease progression, consistent with the cancer stem cell model.

Neuroblastomas also exhibit variable degrees of differentiation (Ambros et al., 2002; Shimada et al., 1999a; Shimada et al., 1999b; Shimada et al., 1984). Neuroblastomas with widespread differentiation have a better prognosis than those with limited differentiation (Shimada et al., 1999b). Highly differentiated neuroblastic tumors are typically focal and can often be cured with surgery (Nitschke et al., 1988). Conversely, poorly differentiated neuroblastomas are often widely disseminated and are usually fatal despite aggressive treatment (Matthay et al., 2009; Matthay et al., 1999; Shimada et al., 1999b). Therapies that promote differentiation significantly improve survival (Matthay et al., 2009; Matthay et al., 1999). In some infants disseminated tumors undergo spontaneous differentiation, leading to a favorable outcome even without therapy (Baker et al., 2010). While staging of neuroblastoma is complex and involves a number of variables other than differentiation status, these clinical observations are consistent with the cancer stem cell model in suggesting that undifferentiated neuroblastoma cells sometimes drive disease progression.

While the overt differentiation in some germ lineage cancers and some neuroblastomas provided clinical evidence consistent with the cancer stem cell model, these rare and unusual malignancies are of uncertain relevance to more prevalent adult cancers. Thus, the cancer stem cell model gained increased attention when evidence emerged supporting the model in leukemia and breast cancer. The advent of flow cytometry made it possible to separate phenotypically distinct subpopulations of live cancer cells to compare their tumorigenic potential. Using this approach, some human acute myeloid leukemias (AMLs) (Bonnet and Dick, 1997; Lapidot et al., 1994) and breast cancers (Al-Hajj et al., 2003) were found to follow the cancer stem cell model, suggesting that a broad spectrum of cancers might be hierarchically organized into tumorigenic and non-tumorigenic components. In each of these studies, cells capable of forming leukemias/tumors were rare when transplanted into immunocompromised mice but could be enriched by selecting cells that expressed specific combinations of surface markers: leukemia-initiating cells were $CD34^{+}CD38^{-}$ (Bonnet and Dick, 1997; Lapidot et al., 1994) while breast cancer-initiating cells were $CD44^{+}CD24^{-/low}$ (Al-Hajj et al., 2003). This suggested that in some cancers only a small minority of cells can proliferate extensively and that some therapies that shrink tumors might not be curative because they fail to eliminate cancer stem cells.

Since these studies were published, other studies have taken similar approaches to provide evidence that other human cancers also follow the cancer stem cell model including colon cancer (Dalerba et al., 2007; O'Brien et al., 2007; Ricci-Vitiani et al., 2007), pancreatic cancer (Li et al., 2007), brain tumors (Bao et al., 2006; Piccirillo et al., 2006; Singh et al., 2004) and ovarian cancer (Alvero et al., 2009; Curley et al., 2009; Stewart et al., 2011; Zhang et al., 2008b). In each case, the capacity to propagate the malignancy appeared to be restricted to a small, phenotypically-distinct subpopulation of cancer cells. In tumorigenesis assays, many unfractionated cells had to be transplanted in order to transfer disease, suggesting that tumorigenic cells were rare. These studies suggested that many cancers follow the stem cell model and might be more effectively treated by targeting cancer stem cells.

Sources of heterogeneity among cancers

Despite these studies the generalizability of the model remained uncertain. Does the model apply to all cancers, or only some? Do all AMLs and breast cancers follow the cancer stem cell model, or only in certain patients? Differences among cancers in driver mutations and in the cell-of-origin create great diversity in cancer biology. Sometimes these differences are reflected in the histopathology of the cancer but in other cases they may influence the underlying biology without recognized effects on histopathology. Nonetheless, these sources of heterogeneity complicate the testing of the cancer stem cell model and mean that observations in a cancer from one patient may be true of cancers in certain other patients but not all patients.

Some cancers, including hierarchically organized cancers that follow the stem cell model, can arise from normal stem cells through mutations that over-activate self-renewal mechanisms (Barker et al., 2009; Merlos-Suarez et al., 2011; Yang et al., 2008). Other cancers, including hierarchically organized cancers, can arise from restricted progenitors or differentiated cells as a result of mutations that ectopically activate self-renewal mechanisms in these cells (Figure 2A) (Cozzio et al., 2003; Huntly et al., 2004; Krivtsov et al., 2006; Schuller et al., 2008; Yang et al., 2008; Zhao et al., 2010). Thus, hierarchical organization in a cancer does not imply that it originated from normal stem cells and the cancer stem cell model does not address the cell-of-origin (Wang and Dick, 2005). However, the cell-of-origin can influence the hierarchical organization of cancers as it influences the mutations that are competent to transform (Wang et al., 2010). The interaction of driver mutations with cellular context influences the frequency of leukemogenic cells and perhaps the degree of hierarchical organization (Heuser et al., 2009; Somervaille et al., 2009). Nonetheless, these inferences are all based upon experimentally induced cancers. The cell-of-origin for most cancers that spontaneously arise in patients has not been identified, at least not with precision, making it difficult to assess which biological differences among human cancers reflect differences in cell-of-origin.

Spatial differences in the cell-of-origin can also influence cancer properties. In some tissues, regional differences in cellular properties influence the driver mutations that are competent to transform. Medulloblastomas can arise either from Sonic Hedgehog pathway activation in granule neuron precursors of the cerebellum or from Wnt pathway activation in dorsal

brainstem progenitors (Gibson et al., 2010; Schuller et al., 2008; Yang et al., 2008) (Figure 2B). Medulloblastomas are hierarchically organized, consistent with the cancer stem cell model (Read et al., 2009; Singh et al., 2004; Ward et al., 2009), so it is likely that the regional identity of the cell-of-origin influences cancer stem cell properties though this has not yet been tested. Ependymomas that arise from different regions of the central nervous system have different mutations, different patterns of gene expression, and different prognoses (Johnson et al., 2010; Taylor et al., 2005). Since ependymomas appear to arise from radial glia and are hierarchically organized into tumorigenic and non-tumorigenic components (Taylor et al., 2005), these results suggest that regional differences in radial glia lead to regional differences in driver mutations and cancer stem cell properties.

Temporal differences in the cell-of-origin also affect the properties of leukemogenic cells. Stem cell properties and self-renewal mechanisms change with age (He et al., 2009; Levi and Morrison, 2009). This likely alters the types of mutations that are competent to initiate cancers. Consistent with this, the mutation spectrum changes with age in human leukemias. Some driver mutations are found primarily in older patients (e.g. in FLT3, Nucleophosmin1, and Dnmt3a), whereas other mutations occur throughout life (e.g. in Ras) or more commonly in young patients (e.g. translocations involving AML1, MLL, and NUP98) (Figure 2C) (Armstrong and Look, 2005; Berman et al., 2011; Brown et al., 2007; Downing and Shannon, 2002; Falini et al., 2005; Ho et al., 2011; Kiyoi et al., 1999; Kottaridis et al., 2001; Ley et al., 2010; Meshinchi et al., 2001; Stirewalt et al., 2001; Thiede et al., 2006; Vogelstein et al., 1990; Zwaan et al., 2003). Since many AMLs are hierarchically organized (Lapidot et al., 1994; Yilmaz et al., 2006), and driver mutations influence the frequency of leukemogenic cells (Heuser et al., 2009; Somervaille et al., 2009), temporal changes in the cell-of-origin likely influence cancer stem cell properties.

Heterogeneity among patients in cancer stem cell phenotype

Differences in driver mutations and cell-of-origin among patients raise the question of whether similar hierarchies of tumorigenic and non-tumorigenic cells, with similar markers, are conserved among patients with similar cancers. Initial studies suggested that AMLs in many patients adopted a similar hierarchical organization in which rare leukemogenic cells were distinguished from non-leukemogenic progeny by having a $CD34^+CD38^-$ surface marker phenotype, largely irrespective of AML subtype or blast cell maturation state (Bonnet and Dick, 1997; Lapidot et al., 1994). Many studies from other laboratories went on to characterize “leukemic stem cell” properties, such as gene expression signatures, by isolating $CD34^+CD38^-$cells without verifying these markers distinguished leukemogenic from non-leukemogenic cells in the patients they studied.

It was subsequently determined that there are leukemia-initiating cells among $CD34^-$ and $CD38^+$ cells in some AMLs (Sarry et al., 2011; Taussig et al., 2008; Taussig et al., 2010). Dick and colleagues systematically addressed this issue by comparing the leukemogenic capacity of $CD34^+CD38^-$, $CD34^+CD38^+$, $CD34^-CD38^+$, and $CD34^-CD38^-$ AML cells from 16 patients (Eppert et al., 2011). In the 13 AMLs that engrafted, there was leukemogenic activity in the $CD34^+CD38^-$fraction; however, leukemogenic cells were also detected in at least one other fraction in most patients. Most leukemogenic cells were

contained in the $CD34^+CD38^-$ fraction in half of the patients and in the $CD34^+CD38^+$ fraction in the other half of patients. In one case there were similar frequencies of leukemogenic cells in all fractions, and a second case had leukemogenic cells in 3 of 4 fractions. Leukemogenic activity is therefore not usually restricted to the $CD34^+CD38^-$ fraction and there is heterogeneity among patients in leukemogenic cell phenotype (Figure 3). Some AMLs might not follow the cancer stem cell model at all.

The frequency and phenotype of leukemogenic cells is also highly variable in mouse AMLs. Deletion of *Pten* from adult mouse hematopoietic cells leads to the development of AML upon transplantation into wild-type mice (Yilmaz et al., 2006). In these AMLs, leukemogenic activity is most highly enriched among rare cells with a surface marker phenotype similar to normal HSCs but cells that express mature myeloid markers possess lower levels of leukemogenic activity. Mouse AMLs induced by MLL-AF9 expression appear to have much higher frequencies of leukemogenic cells than observed after *Pten* deletion (Krivtsov et al., 2006; Somervaille and Cleary, 2006; Somervaille et al., 2009). In these leukemias, cells bearing GMP-like surface markers have the highest frequency of leukemogenic cells (Krivtsov et al., 2006); however, leukemogenic activity is also found in other cell fractions (Somervaille and Cleary, 2006; Somervaille et al., 2009). Differences in oncogenic mutations can thus have profound effects on the frequency and phenotype of leukemia-initiating cells.

The same is true in solid cancers. Tumorigenic cells are enriched within the $CD44^+CD24^{-/low}$ population of some breast cancers (Al-Hajj et al., 2003). However, other breast cancers studied by Al-Hajj et al. had more phenotypically diverse breast cancer-initiating cells, demonstrating that the $CD44^+CD24^{-/low}$ surface marker phenotype does not universally distinguish tumorigenic from non-tumorigenic breast cancer cells (Al-Hajj et al., 2003). In mouse models of mammary cancer different driver mutations give rise to cancers that differ in the extent to which they follow the stem cell model (Cho et al., 2008; Vaillant et al., 2008; Zhang et al., 2008a). For example, CD61 enriched for cancer stem cell activity in mice with Wnt1 driven breast cancers but not in mice with Neu/ErbB2 driver breast cancers (Vaillant et al., 2008). Mouse models of lung cancer with different transforming mutations have tumorigenic cells with different surface marker phenotypes (Curtis et al., 2010). Thus, different oncogenic mutations give rise to cancers that differ in the extent to which they follow the cancer stem cell model and in tumorigenic cell phenotype.

These results demonstrate the importance of testing cancer stem cell markers in significant numbers of patients to appreciate the heterogeneity among patients. Yet studies of cancer stem cell characteristics often assume that markers identified in one study can be applied without validation to other patients, to cell lines, or to cells transformed in culture. The conclusions in such studies may be undermined by heterogeneity among patients or by context or oncogene-dependent effects on tumorigenic cell phenotype.

The implications of the cancer stem cell model for therapy

In cancers that follow the stem cell model, the functional differences between tumorigenic and non-tumorigenic cells can have important implications for therapy. For example, the

BCR-ABL inhibitor imatinib has been incredibly effective at restoring the health and prolonging the lives of patients with chronic myeloid leukemia (CML) (Druker et al., 2006); however, these patients must remain on imatinib indefinitely since many patients fail to completely eliminate BCR-ABL expressing cells from their bone marrow (Bhatia et al., 2003; Chu et al., 2011) and even patients that achieve a complete molecular remission often relapse upon withdrawal of therapy (Rousselot et al., 2007).

These clinical observations appear to be explained by the persistence of rare imatinib-resistant CML stem cells (Chu et al., 2011; Graham et al., 2002; Holtz et al., 2002). A mouse model of CML-like disease driven by HIP1-PDGFR and AML1-ETO fusion proteins had leukemogenic and non-leukemogenic subpopulations of cancer cells distinguished by differences in surface marker expression (Oravecz-Wilson et al., 2009). The leukemogenic cells were rare and much more resistant to imatinib than their non-leukemogenic progeny. Imatinib treatment dramatically enriched leukemogenic cells despite reducing overall leukemia burden. Evidence has also been presented for radiation resistance in brain tumor-initiating cells (Bao et al., 2006) and breast cancer-initiating cells (Diehn et al., 2009).

While it has become fashionable to consider therapy-resistance a defining feature of cancer stem cells, the sensitivity of tumorigenic and non-tumorigenic cells to therapy depends upon the cancer and the therapy. Some therapies actually exploit the capacity of tumorigenic cells to differentiate into non-tumorigenic cells by inducing differentiation. Acute promyelocytic leukaemia is treated with arsenic trioxide and trans-retinoic acid, which induce rapid terminal differentiation, growth arrest, and apoptosis of the cancer cells (de The and Chen, 2010). Differentiation therapy has also been exploited experimentally in glioblastoma by treating with bone morphogenetic protein 4 (BMP4) to induce glial differentiation, reducing proliferation, tumor growth, and tumorigenic cell frequency (Piccirillo et al., 2006). BMP4 also promotes glial differentiation by normal CNS stem cells (Gross et al., 1996) suggesting tumorigenic cancer cells sometimes inherit differentiation pathways from normal stem cells in the same tissue. Cis-retinoic acid also induces glial differentiation and improves survival in high-risk neuroblastoma patients (Matthay et al., 1999; Thiele et al., 1985). Thus, tumorigenic cells are specifically targeted by some therapies.

Therapy resistance can also arise through genetic mechanisms that are not necessarily related to cancer stem cells. While the intrinsic resistance of CML-initiating cells to imatinib allows these cells to persist in treated patients who are in remission, the ongoing administration of imatinib generally prevents relapse until the CML-initiating cells acquire an amplification of BCR-ABL or point mutations that confer imatinib resistance (Gorre et al., 2001; Roumiantsev et al., 2002). Melanomas carrying V600E BRAF mutations become resistant to the BRAF inhibitor vemurafenib through a variety of genetic mechanisms (Johannessen et al., 2010; Nazarian et al., 2010; Poulikakos et al., 2011; Poulikakos and Rosen, 2011; Villanueva et al., 2010) and we are unable to find any evidence that melanoma follows a cancer stem cell model (Quintana et al., 2010; Quintana et al., 2008). Therefore, cancer progression and therapy resistance may be influenced by the properties of cancers stem cells in cancers that follow the model, but therapy resistance and disease progression can also arise through genetic changes unrelated to the question of whether a cancer follows the stem cell model.

Tumorigenesis assays

The xenotransplantation of human cancer cells into mice differs in a number of important respects from the growth of human cancer cells in patients. Mouse tissues differ from human tissues in terms of architecture and stromal cells (Kuperwasser et al., 2004). Mouse growth factors and adhesion molecules sometimes do not bind human receptors (Manz, 2007). Autologous immune cells are an important element of the tumor microenvironment as they can either promote or impair tumor growth (de Visser et al., 2006). Yet there are profound differences in immune regulation between the autologous and xenogeneic settings. Human cells transplanted into mice are subject to powerful xenogeneic immune responses that kill most human cells before they have an opportunity to proliferate (Auchincloss and Sachs, 1989). That is why human cancer cells must be transplanted into highly immunocompromised mice to assay tumorigenic capacity. Even NOD/SCID mice retain an attenuated xenogeneic barrier. Transplantation into more highly immunocompromised mice (such as NOD/SCID IL2Rγ^{null}) can significantly increase the frequency of tumorigenic cells that is detected in some, but not all, human cancers (Ishizawa et al., 2010; Kennedy et al., 2007; Quintana et al., 2008).

Some have speculated that less immunocompromised mice may represent “better models” for studying human cancers because the preservation of some immune activity makes these mice more similar to patients. However, the mechanisms by which mouse immune cells respond to transplanted human cells bear little resemblance to the mechanisms by which human immune cells sometimes respond to autologous cancer cells (Auchincloss and Sachs, 1989). No xenotransplantation assay can model the immune responses that sometimes occur in patients against their own tumors. Normal human hematopoietic stem cells (HSCs) and cancer-initiating cells are therefore more likely to be detected in more highly immunocompromised mice: NOD/SCID mice treated with anti-CD122 antibody to deplete NK cells or NOD/SCID IL2Rγ^{null} mice (Eppert et al., 2011; McDermott et al., 2010; Quintana et al., 2008).

It is also critical to recognize that transplantation assays, particularly xenotransplantation assays, test the potential of cells to form tumors, not their actual fate in the tumor in which they are born (Figure 4). There are many environmental variables in the tumor environment, such as hypoxia and immune responses, which can prevent cells that have the potential to form a tumor from actually doing so in their normal environment. Consequently, nobody knows whether many, or few, cells with tumorigenic potential actually contribute to disease progression in patients. The question of which cells are actually fated to contribute to disease progression is highly context dependent and is probably not testable in patients because of the experimental manipulations that would be required; however, this is testable by fate mapping of cells in mouse tumors. It will be interesting to compare the results of side-by-side fate-mapping experiments and transplantation assays to assess whether the cells that have the potential to form tumors upon transplantation are the same cells that drive disease progression *in situ*.

Tumorigenic cells are abundant in some cancers

Not all hematopoietic malignancies contain rare cancer-initiating cells. Tumorgenic/leukemogenic cells are common in certain mouse models of B-cell lymphoma, T-cell lymphoma, and AML in which it is possible to transfer disease to wild-type recipients by transplanting only 10 cells (Kelly et al., 2007). In a mouse model of B-cell acute lymphoblastic leukemia (ALL) at least 50% of cancer cells are capable of transferring disease into wild-type recipient mice (Williams et al., 2007). In these malignancies the abundance of tumorgenic/leukemogenic cells suggests that if there is any hierarchy it must be much more shallow than observed in many human AMLs, which so far have consistently appeared to have rare leukemogenic cells (Bonnet and Dick, 1997; Lapidot et al., 1994).

Tumorigenic cells are also common in some human cancers. When human melanomas were first transplanted into NOD/SCID mice it was estimated that only 1 in a million melanoma cells were capable of forming tumors and melanoma was proposed to follow a cancer stem cell model (Schatton et al., 2008). However, simple changes in tumorigenesis assay conditions (including the use of NOD/SCID IL2Rγ^{null} mice) increased the detected frequency of tumorigenic cells to 1 in 4 (Quintana et al., 2008). We now routinely transplant single cells directly from patients into NOD/SCID IL2Rγ^{null} mice and on average 30% of single cells form tumors (Quintana et al., 2010). We have quantified the frequency of tumorigenic cells from more than 30 patients with diverse stages and sites of disease and in every case the frequency of tumorigenic cells has been high, even in primary cutaneous melanomas obtained directly from patients (Quintana et al., 2010). Similar results have been published with mouse models of melanoma (Held et al., 2010). Thus xenotransplantation assays sometimes dramatically underestimate the frequency of human cancer cells with tumorigenic potential.

Is melanoma unique among solid cancers in having common tumorigenic cells? We addressed this question in mouse models of malignant peripheral nerve sheath tumors (MPNSTs) (Buchstaller et al., 2011). We found that approximately 20% of mouse MPNST cells have the potential to form tumors, even when transplanted into fully immunocompetent mice. This suggests that tumorigenic cells may be common in a number of solid cancers, though it is important to note that we have not tested whether MPNSTs are hierarchically organized and therefore it is unknown whether this cancer has a shallow hierarchical organization.

An important question is whether we have systematically underestimated the frequency of tumorigenic cells by using transplantation assays that have not been optimized to detect the full range of cells with the potential to form tumors. Work on human HSCs and AML has identified numerous assay improvements that increased estimates of stem cell frequency by orders-of-magnitude (Eppert et al., 2011; Kennedy et al., 2007; McDermott et al., 2010; McKenzie et al., 2005; Notta et al., 2010). But assays for tumorigenic cells from solid cancers generally have not been studied to the same extent. Enzymatic dissociation conditions, sorting conditions, transplantation site, the extracellular matrix environment, and the recipient mouse (sex and strain) all affect the ability to detect tumorigenic cells. Other undiscovered assay parameters may also be important. Estimates of tumorigenic cell

frequencies may continue to increase in many cancers as assays improve, though tumorigenic cells will likely remain rare in some cancers despite such improvements (Eppert et al., 2011; Ishizawa et al., 2010).

Do tumorigenesis assays test the ability to recapitulate tumor heterogeneity?

A fundamental element of the stem cell model is that cancer stem cells give rise to phenotypically diverse progeny that recapitulate the heterogeneity of the tumor from which they derive (Figure 1C). This is presumed to occur through epigenetic mechanisms akin to the differentiation of normal stem cells; however, nobody has experimentally confirmed that epigenetic differences distinguish tumorigenic from non-tumorigenic cells. The differentiation of cancer stem cells into non-tumorigenic progeny is commonly assumed to be the major driver of heterogeneity in cancers that follow the stem cell model; however, the degree of genetic heterogeneity in such cancers is unknown. Therefore, some of the phenotypic and functional differences among cancer cells that have been attributed to the differentiation of cancer stem cells might derive from genetic differences that arise through clonal evolution (Figure 5A).

Since genetic changes are irreversible and stochastic, no tumorigenic cell can recapitulate the genetic heterogeneity of the tumor from which it derives. If the degree of genetic heterogeneity within a tumor is low, then cancer stem cells may be able to largely recapitulate the heterogeneity of the tumors from which they derive by differentiating into non-tumorigenic cells (Figure 5B). However, if there is extensive genetic heterogeneity then every tumorigenic cell may form a genetically distinct tumor and no tumorigenic cell will recapitulate the heterogeneity of the primary tumor (Figure 5C). Increasing evidence suggests that there is more genetic heterogeneity within tumors than previously thought (Navin et al., 2011; Yachida et al., 2010). However, driver mutations are rare among the genetic changes observed in cancer. So the degree of genetic heterogeneity that influences cancer cell function may be more modest. Nonetheless, if there is widespread genetic heterogeneity, functional and phenotypic differences among cancer cells cannot be assumed to be driven by epigenetic hierarchies rather than genetic differences (Campbell et al., 2010; Ding et al., 2010).

In cancers that follow the stem cell model, cancer stem cells would be expected to undergo genetic change over time. Consistent with this, leukemia-initiating cells in B-ALLs undergo clonal evolution (Anderson et al., 2011; Notta et al., 2011) though these studies did not test whether the B-ALLs were hierarchically organized according to the cancer stem cell model. In cancers that do not follow the cancer stem cell model, genetic change would also occur over time and would be predicted to introduce phenotypic and functional heterogeneity. Thus, the cancer stem cell and clonal evolution models can be interacting, or independent, sources of heterogeneity depending on the cancer.

Another issue concerns the extent to which existing assays reliably test phenotypic heterogeneity. Some have suggested that melanomas have intrinsically different populations of tumorigenic and non-tumorigenic cells that can be distinguished based on ABCB5

(Schatton et al., 2008) or CD271 (Civenni et al., 2011; Boiko et al., 2010) expression. However, neither of these markers correlate with the frequency of tumorigenic cells and in our hands small numbers of cells that are positive or negative for ABCB5 or CD271 have a similar capacity to form tumors and to recapitulate the phenotypic heterogeneity of the tumors from which they derive (Quintana et al., 2010). We also identified 20 other markers that are heterogeneously expressed by human melanoma cells and transplanted just 10 cells that were either positive or negative for each of these markers into NOD/SCID IL2Rγ^{null} mice (Quintana et al., 2010). All subpopulations exhibited a similar capacity to form phenotypically heterogeneous tumors. Thus we have subdivided melanomas from many patients into almost 50 subpopulations of cells based on differences in marker expression and have not found any subpopulation that lacks the ability to form a phenotypically heterogeneous tumor.

The simplest interpretation of our data (Quintana et al., 2010) and other data (Pinner et al., 2009; Roesch et al., 2010), is that melanoma cells are phenotypically plastic, reversibly turning on and off markers. In contrast, Civenni et al. reported that both CD271^{+} and CD271^{-}melanoma cells formed tumors in NOD/SCID IL2Rγ^{null} mice but that the tumors formed by CD271^{-} cells were not heterogeneous for CD271 expression and that only CD271^{+} cells formed tumors in NOD/SCID mice (Civenni et al., 2011). They speculated that the difference relative to our results reflected different dissociation conditions. In our hands, both dissociation methods yield similar results with CD271^{+} and CD271^{-} melanoma cells each forming heterogeneous tumors that can be serially passaged, irrespective of whether they are transplanted in to NOD/SCID or NOD/SCID IL2Rγ^{null} mice (E. Quintana, U. Eskiocak, and S.J. Morrison, unpublished data); however, if differences in enzymatic dissociation methods in solid cancers can sometimes generate reproducible differences in the phenotype of tumorigenic cells this would illustrate the complexity of reproducing “cancer stem cell” markers.

If there are certain cells that reproducibly form tumors in NOD/SCID IL2Rγ^{null} mice and reproducibly fail to form tumors in NOD/SCID mice what would this mean? Are some human cells more able to evade rejection by mouse immune cells? If so, does this have any physiological relevance for these cells in humans? If some tumorigenic cells are more able to generate phenotypically heterogeneous progeny than others what does this mean? Could this reflect genetic differences among the cells (Campbell et al., 2010; Ding et al., 2010) rather than hierarchical epigenetic differences? Ultimately, a cell that has the potential to form a tumor in any assay has the potential to contribute to disease progression in a patient and cannot be ignored during therapy.

Uncertainty in cancer stem cell markers

In some cases it has proven difficult to confirm markers that originally appeared to robustly distinguish tumorigenic from non-tumorigenic cells. For example, CD133 was reported as a marker of tumorigenic brain cancer cells and even large numbers of CD133 negative brain tumor cells were reported to lack the ability to form tumors (Bao et al., 2006; Singh et al., 2004). On this basis, gliomas and medulloblastomas were concluded to follow a cancer stem cell model. Yet subsequent studies found tumorigenic activity among both CD133^{+} and

CD133^{-}brain tumor cells (Beier et al., 2007; Chen et al., 2010; Joo et al., 2008; Wang et al., 2008). These discrepancies could reflect differences among patients, methodological differences among laboratories, or ascertainment/tumor selection bias that led early studies to overestimate the robustness of markers. Work with other markers continues to support the conclusion that gliomas and medulloblastomas follow a cancer stem cell model (Read et al., 2009; Son et al., 2009; Ward et al., 2009); however, to confirm the existence and identity of cancer stem cells it is necessary to identify markers that reproducibly distinguish tumorigenic and non-tumorigenic cells, at least in specific subsets of patients.

Tumorigenic ovarian cancer cells have been reported to be enriched in the CD44^{+} (Alvero et al., 2009), CD44^{+}CD117^{+} (Zhang et al., 2008b), and CD133^{+} (Curley et al., 2009) subpopulations of ovarian cancer cells. However, when Stewart et al. evaluated a large cohort of ovarian cancers, they were unable to find CD44^{+}CD117^{+} cells in most ovarian cancers and the CD44^{+}CD117^{+} cells they did find were depleted for tumorigenic activity (Stewart et al., 2011). CD133 enriched tumorigenic ovarian cancer cells in some cases and not in other cases. CD133 expression changed on some tumorigenic cells during passaging (Stewart et al., 2011). This suggests that CD133 only marks ovarian cancer stem cells under defined conditions in some patients and that the hierarchical organization of some ovarian cancers is not stable. It remains uncertain what fraction of ovarian cancers follow the stem cell model.

Cancer stem cell markers have proven difficult to broadly confirm in a number of solid cancers, raising questions about whether we have over-estimated the number of cancers that follow this model and making it difficult to study the biology of these cells. Alternatively, it is possible that many cancers are hierarchically organized but that there is considerable diversity among patients in terms of the markers that distinguish tumorigenic from non-tumorigenic cells. This is a key issue that must be resolved and which may require approaches other than the traditional dependence upon cell surface markers. For example, the separation of live cancer cells based on functional measures, such as signaling pathway activation (Vermeulen et al., 2010), might reduce phenotypic variability among tumorigenic cells. Unfortunately, the genetic approaches currently required to do this are only possible in mice where the use of inbred genetic backgrounds and targeted mutations already reduce variability in cancer models relative to what is observed in patients.

Do non-tumorigenic cells sometimes form tumorigenic cells?

Recent studies have suggested that the differentiation of cancer stem cells into non-tumorigenic cells may be reversible (Chaffer et al., 2011; Gupta et al., 2011). In culture, immortalized human mammary epithelial cells (HMECs) undergo an epithelial to mesenchymal transition (EMT) following sustained expression of the transcription factors Snail or Twist, silencing of E-cadherin, or exposure to TGF-p (Mani et al., 2008; Morel et al., 2008; Scheel et al., 2011). This EMT was interpreted as conferring stem cell properties upon normal or transformed epithelial cells in culture, partly because the cells acquired a CD44^{+}CD24^{-}phenotype, similar to breast cancer stem cells. The idea that cancer cells might reversibly transition between epigenetically-defined tumorigenic and non-tumorigenic states is appealing, partly because mechanisms that generate reversible heterogeneity can confer

therapy-resistance (Roesch et al., 2010; Sharma et al., 2010). However, it is not clear whether CD44 and CD24 consistently distinguish tumorigenic from non-tumorigenic cells in cultured cell lines or whether non-tumorigenic cells acquire tumorigenic potential by EMT in breast cancers in vivo.

The idea that non-tumorigenic cancer cells could sometimes revert to having tumorigenic capacity is plausible given that restricted progenitors de-differentiate into stem cells in certain normal tissues. In the Drosophila testis and ovary, spermatogonia and cytocytes de-differentiate into germline stem cells under certain circumstances (Brawley and Matunis, 2004; Kai and Spradling, 2004). In mouse testis, spermatogonial progenitors can also de-differentiate into spermatogonial stem cells (Barroca et al., 2009). So far this de-differentiation has only been observed in normal tissues at low frequencies or under restricted circumstances. Nonetheless, if non-tumorigenic cancer cells revert to a tumorigenic state at an appreciable rate in certain cancers then this would undermine the ability to distinguish tumorigenic from non-tumorigenic cells as even the “non-tumorigenic” cells would be expected to form tumors at some level.

It is not clear whether the cancer stem cell model would effectively describe cancers in which there are tumorigenic and non-tumorigenic states that can reversibly interconvert. If cells in the non-tumorigenic state only convert to the tumorigenic state under restricted circumstances or with low efficiency, then it may still be possible to identify markers that distinguish populations that are enriched or depleted for tumorigenic capacity (Figure 5D). However, if cells in the non-tumorigenic state convert to the tumorigenic state with high efficiency, it should not be possible to distinguish tumorigenic from non-tumorigenic cells, potentially rendering the cancer stem cell model untestable in such cancers (Figure 5E). New models may be required to describe the heterogeneity in such cancers

Cancer stem cells and metastasis

Metastasis requires cells from a primary tumor to detach, invade the vascular or lymphatic system, migrate to distant sites, extravasate, then proliferate extensively and recruit new vasculature (Nguyen et al., 2009). There is much discussion regarding the metastasis of cancer stem cells, though nobody has actually tested whether the cells that are enriched for tumorigenic activity in transplantation assays are also enriched for the capacity to metastasize under physiological conditions. A fundamental question is whether metastatic potential is confined to a single population of tumorigenic cells or whether it arises stochastically through genetic or epigenetic changes that occur in many cancer cells without regard to their competence to form tumors prior to the stochastic change.

Some studies have suggested that subpopulations of tumorigenic pancreatic cancer (Hermann et al., 2007) and colon cancer (Pang et al., 2010) cells are enriched for the capacity to metastasize. However, the relationship between tumorigenic cells and metastasis has not yet been addressed systematically by examining all of the cancer cells that can be found in circulation and their tumorigenic capacity. Therefore, it remains uncertain whether non-tumorigenic cancer cells have a similar capacity to disseminate as tumorigenic cells, or

whether non-tumorigenic cells from a primary tumor can sometimes acquire tumorigenic capacity after migrating to a new environment.

It has been proposed that cancer cells acquire metastatic potential by undergoing an EMT (Kalluri and Weinberg, 2009). As cancer cells lose their epithelial characteristics, they lose intracellular adhesions and polarity while acquiring more mesenchymal features such as the ability to migrate, invade and resist apoptosis (Thiery et al., 2009). EMT has been proposed as a requisite step for breast cancer metastasis (Yang et al. 2004) and induction of an EMT also confers upon cultured cells a surface marker phenotype ($CD44^{+}CD24^{-}$) similar to tumorigenic breast cancer cells (Mani et al., 2008; Scheel et al., 2011). If cancer cells that have no tumorigenic capacity in transplantation assays can acquire this potential by undergoing an EMT then any cancer cell could acquire metastatic potential.

Although the cancer stem cell model and work on EMTs focus on epigenetic differences between tumorigenic and non-tumorigenic cells, there is good evidence that irreversible genetic mutations also confer metastatic potential (Vogelstein et al., 1988). In pancreatic cancer and medulloblastoma genetically distinct sub-clones initiate metastatic disease (Campbell et al., 2010; Wu et al., 2012; Yachida et al., 2010). This raises the question of whether only cancer stem cells are competent to acquire genetic changes that confer metastatic potential or whether non-tumorigenic cells can acquire genetic changes that confer both tumorigenic and metastatic potential. These fundamental questions remain to be studied in primary tumors in vivo so the implications of the cancer stem cell model for metastasis are unresolved.

Conclusions and future directions

- Some cancers are hierarchically organized into undifferentiated cells that can drive disease progression and differentiated cells with less capacity to drive disease progression, consistent with the cancer stem cell model.
- Cancers that exhibit this kind of hierarchical organization have functional differences among undifferentiated and differentiated cancer cells that affect response to therapy and prognosis.
- There remain a number of uncertainties that make it difficult to assess whether many, or few, cancers follow the stem cell model. Some “cancer stem cell” markers have proven difficult to confirm and tumorigenic cell frequencies can sometimes increase dramatically as a result of changes in assay conditions.
- Many studies have assumed that markers discovered in earlier studies were universally able to distinguish tumorigenic from non-tumorigenic cells, even in independent patient cohorts or in cultured cell lines. Given the heterogeneity that is evident among patients and the context-dependence of some markers, “cancer stem cell” markers should be confirmed in functional assays in each patient or under each experimental circumstance in which they are used.
- It remains unclear to what extent we have systematically underestimated tumorigenic cell frequencies by using assays that are not optimized for the

engraftment of transplanted cells. In some cases, the underestimates may be modest and may not affect conclusions. In other cases, cancers thought to have only rare tumorigenic cells may actually have common tumorigenic cells.

- It will be necessary to systematically assess the degree to which changes in assay conditions affect the spectrum of cancer cells that can form tumors. If a cancer cell has the potential to proliferate extensively in any assay then it has the potential to contribute to disease progression and it is perilous to ignore that cell during therapy.
- It will be important to fate map cells within tumors thought to follow the cancer stem cell model to test whether only small populations of phenotypically distinct cells promote actually tumor growth and disease progression.
- Few studies have assessed the degree of genetic heterogeneity among cancer cells within the same tumor or the extent to which this causes phenotypic and functional differences. In some cancers, genetic heterogeneity may confound the assumption that heterogeneity arises from hierarchical epigenetic differences, rendering the cancer stem cell model difficult to test.
- It will be important to test whether primary tumors in vivo contain cells that reversibly transition between tumorigenic and non-tumorigenic states. The cancer stem cell model may not effectively describe cancers in which the efficiency of interconversion is high.
- It will be necessary to distinguish cancers that follow the stem cell model from those that do not to avoid testing agents that target specific subpopulations of cancer cells in patients who have no chance of benefitting from them.

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REFERENCES

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA. 2003; 100:3983–3988. [PubMed: 12629218]

Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, Silasi DA, Steffensen KD, Waldstrom M, Visintin I, et al. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle. 2009; 8:158–166. [PubMed: 19158483]

Ambros IM, Hata J, Joshi VV, Roald B, Dehner LP, Tuchler H, Potschger U, Shimada H. Morphologic features of neuroblastoma (Schwannian stroma-poor tumors) in clinically favorable and unfavorable groups. Cancer. 2002; 94:1574–1583. [PubMed: 11920516]

Anderson K, Lutz C, van Delft FW, Bateman CM, Guo Y, Colman SM, Kempski H, Moorman AV, Titley I, Swansbury J, et al. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature. 2011; 469:356–361. [PubMed: 21160474]

Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. J Clin Oncol. 2005; 23:6306–6315. [PubMed: 16155013]

Auchincloss, H.; Sachs, DH. Transplantation and Graft Rejection. In: Paul, WE., editor. Fundamental Immunology. New York: Raven Press; 1989. p. 889-922.

Baker DL, Schmidt ML, Cohn SL, Maris JM, London WB, Buxton A, Stram D, Castleberry RP, Shimada H, Sandler A, et al. Outcome after reduced chemotherapy for intermediate-risk neuroblastoma. The New England Journal of Medicine. 2010; 363:1313–1323. [PubMed: 20879880]

Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006; 444:756–760. [PubMed: 17051156]

Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ, Clevers H. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature. 2009; 457:608–611. [PubMed: 19092804]

Barroca V, Lassalle B, Coureuil M, Louis JP, Le Page F, Testart J, Allemand I, Riou L, Fouchet P. Mouse differentiating spermatogonia can generate germinal stem cells in vivo. Nat Cell Biology. 2009; 11:190–196. [PubMed: 19098901]

Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nature Reviews Cancer. 2011; 11:726–734. [PubMed: 21941284]

Beier D, Hau P, Proescholdt M, Lohmeier A, Wischhusen J, Oefner PJ, Aigner L, Brawanski A, Bogdahn U, Beier CP. CD133(+) and CD133(−) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Research. 2007; 67:4010–4015. [PubMed: 17483311]

Berman JN, Gerbing RB, Alonzo TA, Ho PA, Miller K, Hurwitz C, Heerema NA, Hirsch B, Raimondi SC, Lange B, et al. Prevalence and clinical implications of NRAS mutations in childhood AML: a report from the Children's Oncology Group. Leukemia. 2011; 25:1039–1042. [PubMed: 21358716]

Bhatia R, Holtz M, Niu N, Gray R, Snyder DS, Sawyers CL, Arber DA, Slovak ML, Forman SJ. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. Blood. 2003; 101:4701–4707. [PubMed: 12576334]

Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nature Medicine. 2011; 17:320–329.

Boiko AD, Razorenova OV, van de Rijn M, Swetter SM, Johnson DL, Ly DP, Butler PD, Yang GP, Joshua B, Kaplan MJ, Longaker MT, Weissman IL. Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. Nature. 2010; 466:133–137. [PubMed: 20596026]

Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nature Medicine. 1997; 3:730–737.

Brawley C, Matunis E. Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. Science. 2004; 304:1331–1334. [PubMed: 15143218]

Brown P, McIntyre E, Rau R, Meshinchi S, Lacayo N, Dahl G, Alonzo TA, Chang M, Arceci RJ, Small D. The incidence and clinical significance of nucleophosmin mutations in childhood AML. Blood. 2007; 110:979–985. [PubMed: 17440048]

Buchstaller J, McKeever PE, Morrison SJ. Tumorigenic cells are common in mouse MPNSTs but their frequency depends upon tumor genotype and assay conditions. Cancer Cell. 2011 In Press.

Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, Stebbings LA, Morsberger LA, Latimer C, McLaren S, Lin ML, et al. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature. 2010; 467:1109–1113. [PubMed: 20981101]

Chaffer CL, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, Brooks M, Reinhardt F, Su Y, Polyak K, et al. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. Proc Natl Acad Sci USA. 2011; 108:7950–7955. [PubMed: 21498687]

Chaganti RS, Houldsworth J. Genetics and biology of adult human male germ cell tumors. Cancer Research. 2000; 60:1475–1482. [PubMed: 10749107]

Charles N, Ozawa T, Squatrito M, Bleau AM, Brennan CW, Hambardzumyan D, Holland EC. Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. Cell Stem Cell. 2010; 6:141–152. [PubMed: 20144787]

Chen R, Nishimura MC, Bumbaca SM, Kharbanda S, Forrest WF, Kasman IM, Greve JM, Soriano RH, Gilmour LL, Rivers CS, et al. A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. Cancer Cell. 2010; 17:362–375. [PubMed: 20385361]

Cho RW, Wang X, Diehn M, Shedden K, Chen GY, Sherlock G, Gurney A, Lewicki J, Clarke MF. Isolation and molecular characterization of cancer stem cells in MMTV-Wnt-1 murine breast tumors. Stem Cells. 2008; 26:364–371. [PubMed: 17975224]

Chu S, McDonald T, Lin A, Chakraborty S, Huang Q, Snyder DS, Bhatia R. Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment. Blood. 2011

Civenni G, Walter A, Kobert N, Mihic-Probst D, Zipser M, Belloni B, Seifert B, Moch H, Dummer R, van den Broek M, et al. Human CD271-positive melanoma stem cells associated with metastasis establish tumor heterogeneity and long-term growth. Cancer Research. 2011; 71:3098–3109. [PubMed: 21393506]

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; 17:3029–3035. [PubMed: 14701873]

Curley MD, Therrien VA, Cummings CL, Sergent PA, Koulouris CR, Friel AM, Roberts DJ, Seiden MV, Scadden DT, Rueda BR, et al. CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. Stem Cells. 2009; 27:2875–2883. [PubMed: 19816957]

Curtis SJ, Sinkevicius KW, Li D, Lau AN, Roach RR, Zamponi R, Woolfenden AE, Kirsch DG, Wong KK, Kim CF. Primary tumor genotype is an important determinant in identification of lung cancer propagating cells. Cell Stem cell. 2010; 7:127–133. [PubMed: 20621056]

Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, et al. Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci USA. 2007; 104:10158–10163. [PubMed: 17548814]

de The H, Chen Z. Acute promyelocytic leukaemia: novel insights into the mechanisms of cure. Nature Reviews Cancer. 2010; 10:775–783. [PubMed: 20966922]

de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nature Reviews Cancer. 2006; 6:24–37. [PubMed: 16397525]

Dick JE. Stem cell concepts renew cancer research. Blood. 2008; 112:4793–4807. [PubMed: 19064739]

Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Qian D, Lam JS, Ailles LE, Wong M, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature. 2009

Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, Harris CC, McLellan MD, Fulton RS, Fulton LL, et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature. 2010; 464:999–1005. [PubMed: 20393555]

Downing JR, Shannon KM. Acute leukemia: a pediatric perspective. Cancer Cell. 2002; 2:437–445. [PubMed: 12498712]

Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, Deininger MW, Silver RT, Goldman JM, Stone RM, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. The New England Journal of Medicine. 2006; 355:2408–2417. [PubMed: 17151364]

Eppert K, Takenaka K, Lechman ER, Waldron L, Nilsson B, van Galen P, Metzeler KH, Poeppl A, Ling V, Beyene J, et al. Stem cell gene expression programs influence clinical outcome in human leukemia. Nature Medicine. 2011; 17:1086–1093.

Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, La Starza R, Diverio D, Colombo E, Santucci A, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. The New England Journal of Medicine. 2005; 352:254–266. [PubMed: 15659725]

Fearon ER, Burke PJ, Schiffer CA, Zehnbauer BA, Vogelstein B. Differentiation of leukemia cells to polymorphonuclear leukocytes in patients with acute nonlymphocytic leukemia. The New England Journal of Medicine. 1986; 315:15–24. [PubMed: 3086723]

Fidler IJ, Hart IR. Biological diversity in metastatic neoplasms: origins and implications. Science. 1982; 217:998–1003. [PubMed: 7112116]

Fidler IJ, Kripke ML. Metastasis results from preexisting variant cells within a malignant tumor. Science. 1977; 197:893–895. [PubMed: 887927]

Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, Eden C, Kranenburg TA, Hogg T, Poppleton H, Martin J, et al. Subtypes of medulloblastoma have distinct developmental origins. Nature. 2010; 468:1095–1099. [PubMed: 21150899]

Goardon N, Marchi E, Atzberger A, Quek L, Schuh A, Soneji S, Woll P, Mead A, Alford KA, Rout R, et al. Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer Cell. 2011; 19:138–152. [PubMed: 21251617]

Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science. 2001; 293:876–880. [PubMed: 11423618]

Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, Holyoake TL. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood. 2002; 99:319–325. [PubMed: 11756187]

Gross RE, Mehler MF, Mabie PC, Zang Z, Santschi L, Kessler JA. Bone Morphogenetic Proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells. Neuron. 1996; 17:595–606. [PubMed: 8893018]

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; 146:633–644. [PubMed: 21854987]

Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. Science. 1977; 197:461–463. [PubMed: 560061]

He S, Nakada D, Morrison SJ. Mechanisms of stem cell self-renewal. Annual Review of Cell and Developmental Biology. 2009; 25:377–406.

Held MA, Curley DP, Dankort D, McMahon M, Muthusamy V, Bosenberg MW. Characterization of melanoma cells capable of propagating tumors from a single cell. Cancer Research. 2010; 70:388–397. [PubMed: 20048081]

Heppner GH. Tumor heterogeneity. Cancer Research. 1984; 44:2259–2265. [PubMed: 6372991]

Hermann PC, Huber SL, Herrier T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell. 2007; 1:313–323. [PubMed: 18371365]

Heuser M, Sly LM, Argiropoulos B, Kuchenbauer F, Lai C, Weng A, Leung M, Lin G, Brookes C, Fung S, et al. Modeling the functional heterogeneity of leukemia stem cells: role of STAT5 in leukemia stem cell self-renewal. Blood. 2009; 114:3983–3993. [PubMed: 19667399]

Ho PA, Kutny MA, Alonzo TA, Gerbing RB, Joaquin J, Raimondi SC, Gamis AS, Meshinchi S. Leukemic mutations in the methylation-associated genes DNMT3A and IDH2 are rare events in pediatric AML: a report from the Children's Oncology Group. Pediatr Blood Cancer. 2011; 57:204–209. [PubMed: 21504050]

Holtz MS, Slovak ML, Zhang F, Sawyers CL, Forman SJ, Bhatia R. Imatinib mesylate (STI571) inhibits growth of primitive malignant progenitors in chronic myelogenous leukemia through reversal of abnormally increased proliferation. Blood. 2002; 99:3792–3800. [PubMed: 11986238]

Huntly BJ, Shigematsu H, Deguchi K, Lee BH, Mizuno S, Duclos N, Rowan R, Amaral S, Curley D, Williams IR, et al. MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. Cancer Cell. 2004; 6:587–596. [PubMed: 15607963]

Ishizawa K, Rasheed ZA, Karisch R, Wang Q, Kowalski J, Susky E, Pereira K, Karamboulas C, Moghal N, Rajeshkumar NV, et al. Tumor-initiating cells are rare in many human tumors. Cell Stem Cell. 2010; 7:279–282. [PubMed: 20804964]

Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, Johnson LA, Emery CM, Stransky N, Cogdill AP, Barretina J, et al. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature. 2010; 468:968–972. [PubMed: 21107320]

Johnson RA, Wright KD, Poppleton H, Mohankumar KM, Finkelstein D, Pounds SB, Rand V, Leary SE, White E, Eden C, et al. Cross-species genomics matches driver mutations and cell compartments to model ependymoma. Nature. 2010; 466:632–636. [PubMed: 20639864]

Joo KM, Kim SY, Jin X, Song SY, Kong DS, Lee JI, Jeon JW, Kim MH, Kang BG, Jung Y, et al. Clinical and biological implications of CD133-positive and CD133-negative cells in glioblastomas. Lab Investigation. 2008; 88:808–815.

Kai T, Spradling A. Differentiating germ cells can revert into functional stem cells in Drosophila melanogaster ovaries. Nature. 2004; 428:564–569. [PubMed: 15024390]

Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. Journal of Clinical Investigation. 2009; 119:1420–1428. [PubMed: 19487818]

Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. Science. 2007; 317:337. [PubMed: 17641192]

Kennedy JA, Barabe F, Poeppl AG, Wang JC, Dick JE. Comment on “Tumor growth need not be driven by rare cancer stem cells”. Science. 2007; 318:1722. author reply 1722. [PubMed: 18079385]

Kiyoi H, Naoe T, Nakano Y, Yokota S, Minami S, Miyawaki S, Asou N, Kuriyama K, Jinnai I, Shimazaki C, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood. 1999; 93:3074–3080. [PubMed: 10216104]

Kleinsmith LJ, Pierce GB. Multipotentiality of single embryonal carcinoma cells. Cancer Research. 1964; 24:1544–1551. [PubMed: 14234000]

Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, Walker H, Wheatley K, Bowen DT, Burnett AK, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood. 2001; 98:1752–1759. [PubMed: 11535508]

Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, Levine JE, Wang J, Hahn WC, Gilliland DG, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. Nature. 2006; 442:818–822. [PubMed: 16862118]

Kuperwasser C, Chavarria T, Wu M, Magrane G, Gray JW, Carey L, Richardson A, Weinberg RA. Reconstruction of functionally normal and malignant human breast tissues in mice. Proc Natl Acad Sci USA. 2004; 101:4966–4971. [PubMed: 15051869]

Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukemia after transplantation into SCID mice. Nature. 1994; 17:645–648. [PubMed: 7509044]

le Viseur C, Hotfilder M, Bomken S, Wilson K, Rottgers S, Schrauder A, Rosemann A, Irving J, Stam RW, Shultz LD, et al. In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. Cancer Cell. 2008; 14:47–58. [PubMed: 18598943]

Levi BP, Morrison SJ. Stem Cells Use Distinct Self-renewal Programs at Different Ages. Cold Spring Harb Symp Quant Biol. 2009; 73:539–553. [PubMed: 19150957]

Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, Kandoth C, Payton JE, Baty J, Welch J, et al. DNMT3A mutations in acute myeloid leukemia. New England Journal of Medicine. 2010; 363:2424–2433. [PubMed: 21067377]

Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. Cancer Research. 2007; 67:1030–1037. [PubMed: 17283135]

Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008; 133:704–715. [PubMed: 18485877]

Manz MG. Human-hemato-lymphoid-system mice: opportunities and challenges. Immunity. 2007; 26:537–541. [PubMed: 17521579]

Matthay KK, Reynolds CP, Seeger RC, Shimada H, Adkins ES, Haas-Kogan D, Gerbing RB, London WB, Villablanca JG. Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a children's oncology group study. J Clin Oncol. 2009; 27:1007–1013. [PubMed: 19171716]

Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, Swift P, Shimada H, Black CT, Brodeur GM, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. The New England Journal of Medicine. 1999; 341:1165–1173. [PubMed: 10519894]

McDermott SP, Eppert K, Lechman ER, Doedens M, Dick JE. Comparison of human cord blood engraftment between immunocompromised mouse strains. Blood. 2010; 116:193–200. [PubMed: 20404133]

McKenzie JL, Gan OI, Doedens M, Dick JE. Human short-term repopulating stem cells are efficiently detected following intrafemoral transplantation into NOD/SCID recipients depleted of CD122+ cells. Blood. 2005; 106:1259–1261. [PubMed: 15878972]

Merlos-Suarez A, Barriga FM, Jung P, Iglesias M, Cespedes MV, Rossell D, Sevillano M, Hernando-Momblona X, da Silva-Diz V, Munoz P, et al. The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse. Cell Stem Cell. 2011; 8:511–524. [PubMed: 21419747]

Meshinchi S, Woods WG, Stirewalt DL, Sweetser DA, Buckley JD, Tjoa TK, Bernstein ID, Radich JP. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. Blood. 2001; 97:89–94. [PubMed: 11133746]

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]

Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, Cook K, Stepansky A, Levy D, Esposito D, et al. Tumour evolution inferred by single-cell sequencing. Nature. 2011; 472:90–94. [PubMed: 21399628]

Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, Chen Z, Lee MK, Attar N, Sazegar H, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature. 2010; 468:973–977. [PubMed: 21107323]

Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. Nature Reviews Cancer. 2009; 9:274–284. [PubMed: 19308067]

Nitschke R, Smith EI, Shochat S, Altshuler G, Travers H, Shuster JJ, Hayes FA, Patterson R, McWilliams N. Localized neuroblastoma treated by surgery: a Pediatric Oncology Group Study. J Clin Oncol. 1988; 6:1271–1279. [PubMed: 3411339]

Notta F, Doulatov S, Dick JE. Engraftment of human hematopoietic stem cells is more efficient in female NOD/SCID/IL-2Rgc-null recipients. Blood. 2010; 115:3704–3707. [PubMed: 20207983]

Notta F, Mullighan CG, Wang JC, Poeppl A, Doulatov S, Phillips LA, Ma J, Minden MD, Downing JR, Dick JE. Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. Nature. 2011; 469:362–367. [PubMed: 21248843]

Nowell PC. The clonal evolution of tumor cell populations. Science. 1976; 194:23–28. [PubMed: 959840]

Nowell PC. Mechanisms of tumor progression. Cancer Research. 1986; 46:2203–2207. [PubMed: 3516380]

O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature. 2007; 445:106–110. [PubMed: 17122772]

Ogawa M, Fried J, Sakai Y, Strife A, Clarkson BD. Studies of cellular proliferation in human leukemia. VI. The proliferative activity, generation time, and emergence time of neutrophilic granulocytes in chronic granulocytic leukemia. Cancer. 1970; 25:1031–1049. [PubMed: 5266628]

Oravecz-Wilson KI, Philips ST, Yilmaz OH, Ames HM, Li L, Crawford BD, Gauvin AM, Lucas PC, Sitwala K, Downing JR, et al. Persistence of leukemia-initiating cells in a conditional knockin model of an imatinib-responsive myeloproliferative disorder. Cancer Cell. 2009; 16:137–148. [PubMed: 19647224]

Pang R, Law WL, Chu AC, Poon JT, Lam CS, Chow AK, Ng L, Cheung LW, Lan XR, Lan HY, et al. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell. 2010; 6:603–615. [PubMed: 20569697]

Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, Brem H, Olivi A, Dimeco F, Vescovi AL. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature. 2006; 444:761–765. [PubMed: 17151667]

Pinner S, Jordan P, Sharrock K, Bazley L, Collinson L, Marais R, Bonvin E, Goding C, Sahai E. Intravital imaging reveals transient changes in pigment production and Brn2 expression during metastatic melanoma dissemination. Cancer Research. 2009; 69:7969–7977. [PubMed: 19826052]

Polyak K, Haviv I, Campbell IG. Co-evolution of tumor cells and their microenvironment. Trends Genet. 2009; 25:30–38. [PubMed: 19054589]

Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, Shi H, Atefi M, Titz B, Gabay MT, et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). Nature. 2011; 480:387–390. [PubMed: 22113612]

Poulikakos PI, Rosen N. Mutant BRAF melanomas--dependence and resistance. Cancer Cell. 2011; 19:11–15. [PubMed: 21251612]

Quintana E, Shackleton M, Foster HR, Fullen DR, Sabel MS, Johnson TM, Morrison SJ. Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. Cancer Cell. 2010; 18:510–523. [PubMed: 21075313]

Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. Nature. 2008; 456:593–598. [PubMed: 19052619]

Read TA, Fogarty MP, Markant SL, McLendon RE, Wei Z, Ellison DW, Febbo PG, Wechsler-Reya RJ. Identification of CD15 as a marker for tumor-propagating cells in a mouse model of medulloblastoma. Cancer Cell. 2009; 15:135–147. [PubMed: 19185848]

Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001; 414:105–111. [PubMed: 11689955]

Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. Nature. 2007; 445:111–115. [PubMed: 17122771]

Roesch A, Fukunaga-Kalabis M, Schmidt EC, Zabierowski SE, Brafford PA, Vultur A, Basu D, Gimotty P, Vogt T, Herlyn M. A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. Cell. 2010; 141:583–594. [PubMed: 20478252]

Roumiantsev S, Shah NP, Gorre ME, Nicoll J, Brasher BB, Sawyers CL, Van Etten RA. Clinical resistance to the kinase inhibitor STI-571 in chronic myeloid leukemia by mutation of Tyr-253 in the Abl kinase domain P-loop. Proc Natl Acad Sci USA. 2002; 99:10700–10705. [PubMed: 12149456]

Rousselot P, Huguet F, Rea D, Legros L, Cayuela JM, Maarek O, Blanchet O, Marit G, Gluckman E, Reiffers J, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. Blood. 2007; 109:58–60. [PubMed: 16973963]

Sarry JE, Murphy K, Perry R, Sanchez PV, Secreto A, Keefer C, Swider CR, Strzelecki AC, Cavelier C, Recher C, et al. Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rgammac-deficient mice. Journal of Clinical Investigation. 2011; 121:384–395. [PubMed: 21157036]

Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C, et al. Identification of cells initiating human melanomas. Nature. 2008; 451:345–349. [PubMed: 18202660]

Scheel C, Eaton EN, Li SH, Chaffer CL, Reinhardt F, Kah KJ, Bell G, Guo W, Rubin J, Richardson AL, et al. Paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast. Cell. 2011; 145:926–940. [PubMed: 21663795]

Schuller U, Heine VM, Mao J, Kho AT, Dillon AK, Han YG, Huillard E, Sun T, Ligon AH, Qian Y, et al. Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell

competence to form Shh-induced medulloblastoma. Cancer Cell. 2008; 14:123–134. [PubMed: 18691547]

Shackleton M, Quintana E, Fearon ER, Morrison SJ. Sources of heterogeneity in cancer: cancer stem cells versus clonal evolution. Cell. 2009; 138:822–829. [PubMed: 19737509]

Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, McDermott U, Azizian N, Zou L, Fischbach MA, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell. 2010; 141:69–80. [PubMed: 20371346]

Shimada H, Ambros IM, Dehner LP, Hata J, Joshi VV, Roald B. Terminology and morphologic criteria of neuroblastic tumors: recommendations by the International Neuroblastoma Pathology Committee. Cancer. 1999a; 86:349–363. [PubMed: 10421272]

Shimada H, Ambros IM, Dehner LP, Hata J, Joshi VV, Roald B, Stram DO, Gerbing RB, Lukens JN, Matthay KK, et al. The International Neuroblastoma Pathology Classification (the Shimada system). Cancer. 1999b; 86:364–372. [PubMed: 10421273]

Shimada H, Chatten J, Newton WA Jr, Sachs N, Hamoudi AB, Chiba T, Marsden HB, Misugi K. Histopathologic prognostic factors in neuroblastic tumors: definition of subtypes of ganglioneuroblastoma and an age-linked classification of neuroblastomas. Journal of the National Cancer Institute. 1984; 73:405–416. [PubMed: 6589432]

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature. 2004; 432:396–401. [PubMed: 15549107]

Somervaille TC, Cleary ML. Identification and characterization of leukemia stem cells in murine MLL-AF9 acute myeloid leukemia. Cancer Cell. 2006; 10:257–268. [PubMed: 17045204]

Somervaille TC, Matheny CJ, Spencer GJ, Iwasaki M, Rinn JL, Witten DM, Chang HY, Shurtleff SA, Downing JR, Cleary ML. Hierarchical maintenance of MLL myeloid leukemia stem cells employs a transcriptional program shared with embryonic rather than adult stem cells. Cell Stem Cell. 2009; 4:129–140. [PubMed: 19200802]

Son MJ, Woolard K, Nam DH, Lee J, Fine HA. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell Stem Cell. 2009; 4:440–452. [PubMed: 19427293]

Stenning SP, Parkinson MC, Fisher C, Mead GM, Cook PA, Fossa SD, Horwich A, Jones WG, Newlands ES, Oliver RT, et al. Postchemotherapy residual masses in germ cell tumor patients: content, clinical features, and prognosis. Medical Research Council Testicular Tumour Working Party. Cancer. 1998; 83:1409–1419. [PubMed: 9762943]

Stewart JM, Shaw PA, Gedye C, Bernardini MQ, Neel BG, Ailles LE. Phenotypic heterogeneity and instability of human ovarian tumor-initiating cells. Proc Nat Acad Sci USA. 2011; 108:6468–6473. [PubMed: 21451132]

Stirewalt DL, Kopecky KJ, Meshinchi S, Appelbaum FR, Slovak ML, Willman CL, Radich JP. FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. Blood. 2001; 97:3589–3595. [PubMed: 11369655]

Taussig DC, Miraki-Moud F, Anjos-Afonso F, Pearce DJ, Allen K, Ridler C, Lillington D, Oakervee H, Cavenagh J, Agrawal SG, et al. Anti-CD38 antibody-mediated clearance of human repopulating cells masks the heterogeneity of leukemia-initiating cells. Blood. 2008; 112:568–575. [PubMed: 18523148]

Taussig DC, Vargaftig J, Miraki-Moud F, Griessinger E, Sharrock K, Luke T, Lillington D, Oakervee H, Cavenagh J, Agrawal SG, et al. Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34(−) fraction. Blood. 2010; 115:1976–1984. [PubMed: 20053758]

Taylor MD, Poppleton H, Fuller C, Su X, Liu Y, Jensen P, Magdaleno S, Dalton J, Calabrese C, Board J, et al. Radial glia cells are candidate stem cells of ependymoma. Cancer Cell. 2005; 8:323–335. [PubMed: 16226707]

Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, Ehninger G. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood. 2006; 107:4011–4020. [PubMed: 16455956]

Thiele CJ, Reynolds CP, Israel MA. Decreased expression of N-myc precedes retinoic acid-induced morphological differentiation of human neuroblastoma. Nature. 1985; 313:404–406. [PubMed: 3855502]

Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009; 139:871–890. [PubMed: 19945376]

Vaillant F, Asselin-Labat ML, Shackleton M, Forrest NC, Lindeman GJ, Visvader JE. The mammary progenitor marker CD61/beta3 integrin identifies cancer stem cells in mouse models of mammary tumorigenesis. Cancer Research. 2008; 68:7711–7717. [PubMed: 18829523]

Vermeulen L, De Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski T, Tuynman JB, Todaro M, Merz C, Rodermond H, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nature Cell Biology. 2010; 12:468–476. [PubMed: 20418870]

Villanueva J, Vultur A, Lee JT, Somasundaram R, Fukunaga-Kalabis M, Cipolla AK, Wubbenhorst B, Xu X, Gimotty PA, Kee D, et al. Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. Cancer Cell. 2010; 18:683–695. [PubMed: 21156289]

Vogelstein B, Civin CI, Preisinger AC, Krischer JP, Steuber P, Ravindranath Y, Weinstein H, Elfferich P, Bos J. RAS gene mutations in childhood acute myeloid leukemia: a Pediatric Oncology Group study. Genes Chromosomes Cancer. 1990; 2:159–162. [PubMed: 2278970]

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. New England Journal of Medicine. 1988; 319:525–532. [PubMed: 2841597]

Wang J, Sakariassen PO, Tsinkalovsky O, Immervoll H, Boe SO, Svendsen A, Prestegarden L, Rosland G, Thorsen F, Stuhr L, et al. CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. Int J Cancer. 2008; 122:761–768. [PubMed: 17955491]

Wang JC, Dick JE. Cancer stem cells: lessons from leukemia. Trends Cell Biol. 2005; 15:494–501. [PubMed: 16084092]

Wang Y, Krivtsov AV, Sinha AU, North TE, Goessling W, Feng Z, Zon LI, Armstrong SA. The Wnt/beta-catenin pathway is required for the development of leukemia stem cells in AML. Science. 2010; 327:1650–1653. [PubMed: 20339075]

Ward RJ, Lee L, Graham K, Satkunendran T, Yoshikawa K, Ling E, Harper L, Austin R, Nieuwenhuis E, Clarke ID, et al. Multipotent CD15+ cancer stem cells in patched-1-deficient mouse medulloblastoma. Cancer Research. 2009; 69:4682–4690. [PubMed: 19487286]

Williams RT, den Besten W, Sherr CJ. Cytokine-dependent imatinib resistance in mouse BCR-ABL+, Arf-null lymphoblastic leukemia. Genes Dev. 2007; 21:2283–2287. [PubMed: 17761812]

Wu X, Northcott PA, Dubuc A, Dupuy AJ, Shih DJ, Witt H, Croul S, Bouffet E, Fults DW, Eberhart CG, et al. Clonal selection drives genetic divergence of metastatic medulloblastoma. Nature. 2012; 482:529–533. [PubMed: 22343890]

Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JR, Nowak MA, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature. 2010; 467:1114–1117. [PubMed: 20981102]

Yang ZJ, Ellis T, Markant SL, Read TA, Kessler JD, Bourboulas M, Schuller U, Machold R, Fishell G, Rowitch DH, et al. Medulloblastoma can be initiated by deletion of Patched in lineage-restricted progenitors or stem cells. Cancer Cell. 2008; 14:135–145. [PubMed: 18691548]

Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell. 2004; 117:927–939. [PubMed: 15210113]

Yilmaz OH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, Morrison SJ. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature. 2006; 441:475–482. [PubMed: 16598206]

Zhang M, Behbod F, Atkinson RL, Landis MD, Kittrell F, Edwards D, Medina D, Tsimelzon A, Hilsenbeck S, Green JE, et al. Identification of tumor-initiating cells in a p53-null mouse model of breast cancer. Cancer Research. 2008a; 68:4674–4682. [PubMed: 18559513]

Zhang S, Balch C, Chan MW, Lai HC, Matei D, Schilder JM, Yan PS, Huang TH, Nephew KP. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Research. 2008b; 68:4311–4320. [PubMed: 18519691]

Zhao Z, Zuber J, Diaz-Flores E, Lintault L, Kogan SC, Shannon K, Lowe SW. p53 loss promotes acute myeloid leukemia by enabling aberrant self-renewal. Genes Dev. 2010; 24:1389–1402. [PubMed: 20595231]

Zwaan CM, Meshinchi S, Radich JP, Veerman AJ, Huismans DR, Munske L, Podleschny M, Hahlen K, Pieters R, Zimmermann M, et al. FLT3 internal tandem duplication in 234 children with acute myeloid leukemia: prognostic significance and relation to cellular drug resistance. Blood. 2003; 102:2387–2394. [PubMed: 12816873]

Article highlights

- Cancer stem cells give rise to a hierarchy of tumorigenic and non-tumorigenic cells
- It is uncertain whether many, or few, cancers follow the cancer stem cell model
- The frequency of tumorigenic cells remains uncertain in many cancers
- The model may not be testable in genetically or epigenetically unstable cancers

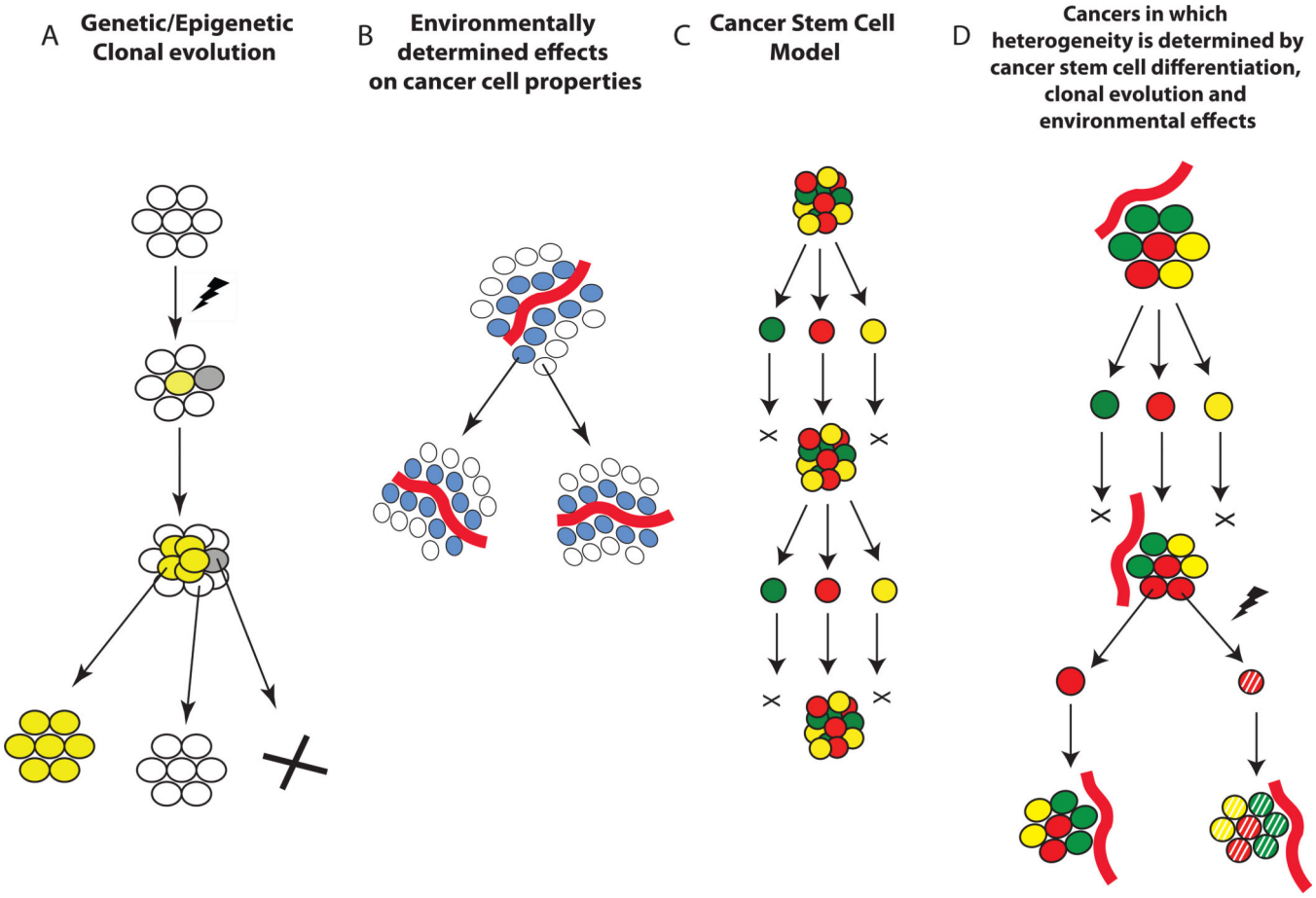

Figure 1. Sources of heterogeneity within cancer

A) Heterogeneity can arise within tumors through stochastic genetic (Nowell, 1976) and epigenetic (Baylin and Jones, 2011) changes that confer heritable phenotypic and functional differences upon cancer cells. This process is known as clonal evolution because the genetic/epigenetic changes are subject to selection within tumors. This process tends to lead to more aggressive cancers over time; however, some cancer cells (grey) would be predicted to lose their tumorigenic capacity as a consequence of disadvantageous genetic changes. B) Heterogeneity can arise in response to extrinsic environmental differences within tumors: cancer cells (blue) adjacent to blood vessels (red) are different from cancer cells further from blood vessels (white) (Charles et al., 2010). The differences are shown as being reversible, though environmental differences could also cause irreversible changes in cancer cell properties. C) Cancers that follow the stem cell model contain intrinsically different subpopulations of tumorigenic (red) and non-tumorigenic cells (yellow and green) organized in a hierarchy in which a minority population of tumorigenic cells gives rise to phenotypically diverse non-tumorigenic cells. Non-tumorigenic cells are thought to compose the bulk of tumors but have little capacity to contribute to cancer progression (Dick, 2008; Reya et al., 2001; Shackleton et al., 2009). Tumorigenic cells can be serially transplanted, re-establishing phenotypic heterogeneity with each passage. D) Cancers that follow the stem cell model are also subject to clonal evolution as well as heterogeneity from environmental

differences within tumors. Thus, these sources of heterogeneity are not mutually exclusive and may each apply to variable extents depending on the cancer.

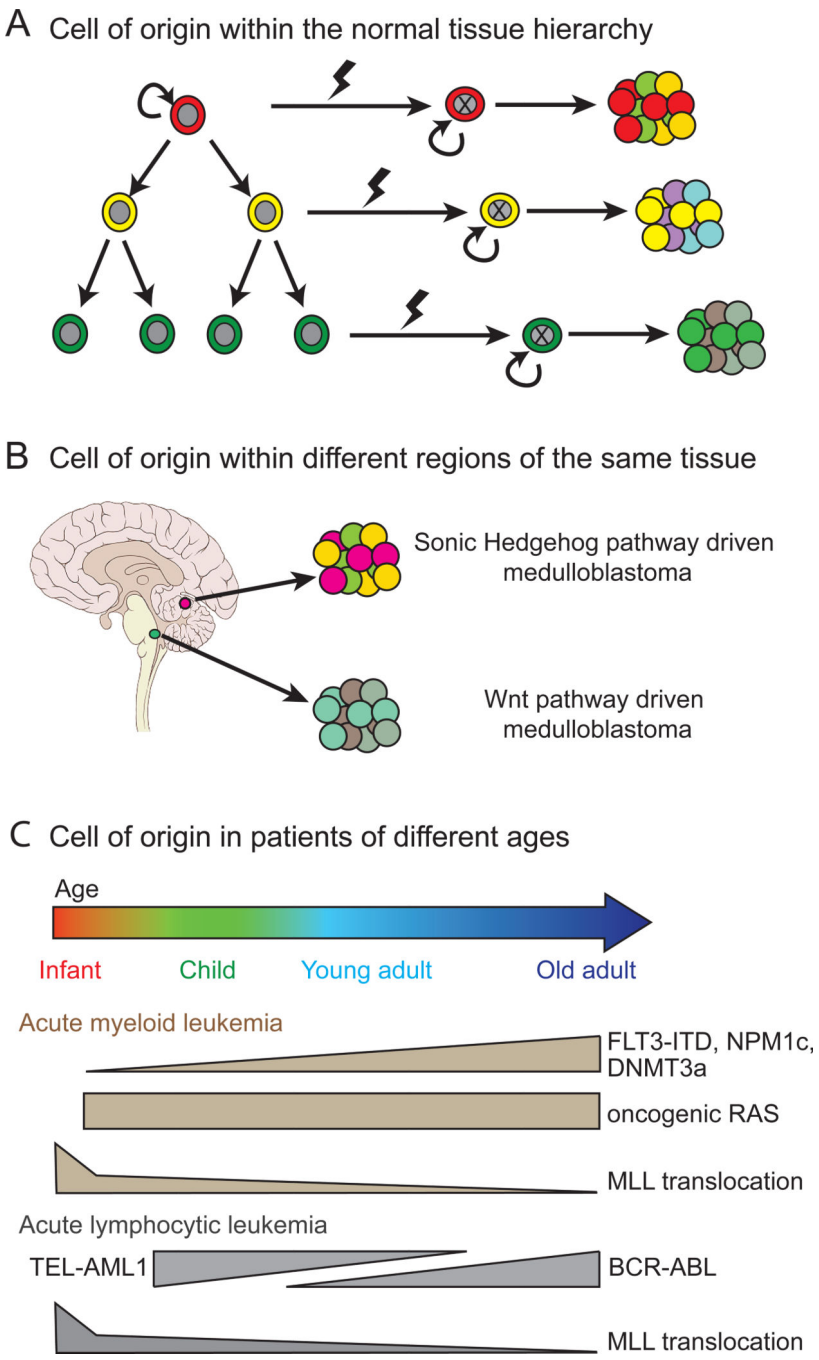

Figure 2. Sources of heterogeneity among cancers

Differences in the cell-of-origin can directly and indirectly influence the phenotype of tumorigenic cells and, perhaps, whether or not the cancer is hierarchically organized. A) Different cell types in a stem/progenitor cell hierarchy within a normal tissue may be transformed into cancer cells. The properties of the cell-of-origin influence the types of mutations that are competent to transform and the properties of the resulting cancer (Huntly et al., 2004; Wang et al., 2010). (B) Spatial differences in the identity of the cell-of-origin within tissues influence the types of mutations that are competent to transform and the

properties of the resulting cancer (Gibson et al., 2010; Johnson et al., 2010). (C) Temporal differences in the cell-of-origin also influence the types of mutations that are competent to transform and the properties of the resulting cancer (Magee and Morrison, unpublished data), consistent with the observation that the driver mutation spectrum changes with age in patients (Downing and Shannon, 2002)(see text for references regarding age-related changes in the incidence of specific mutations).

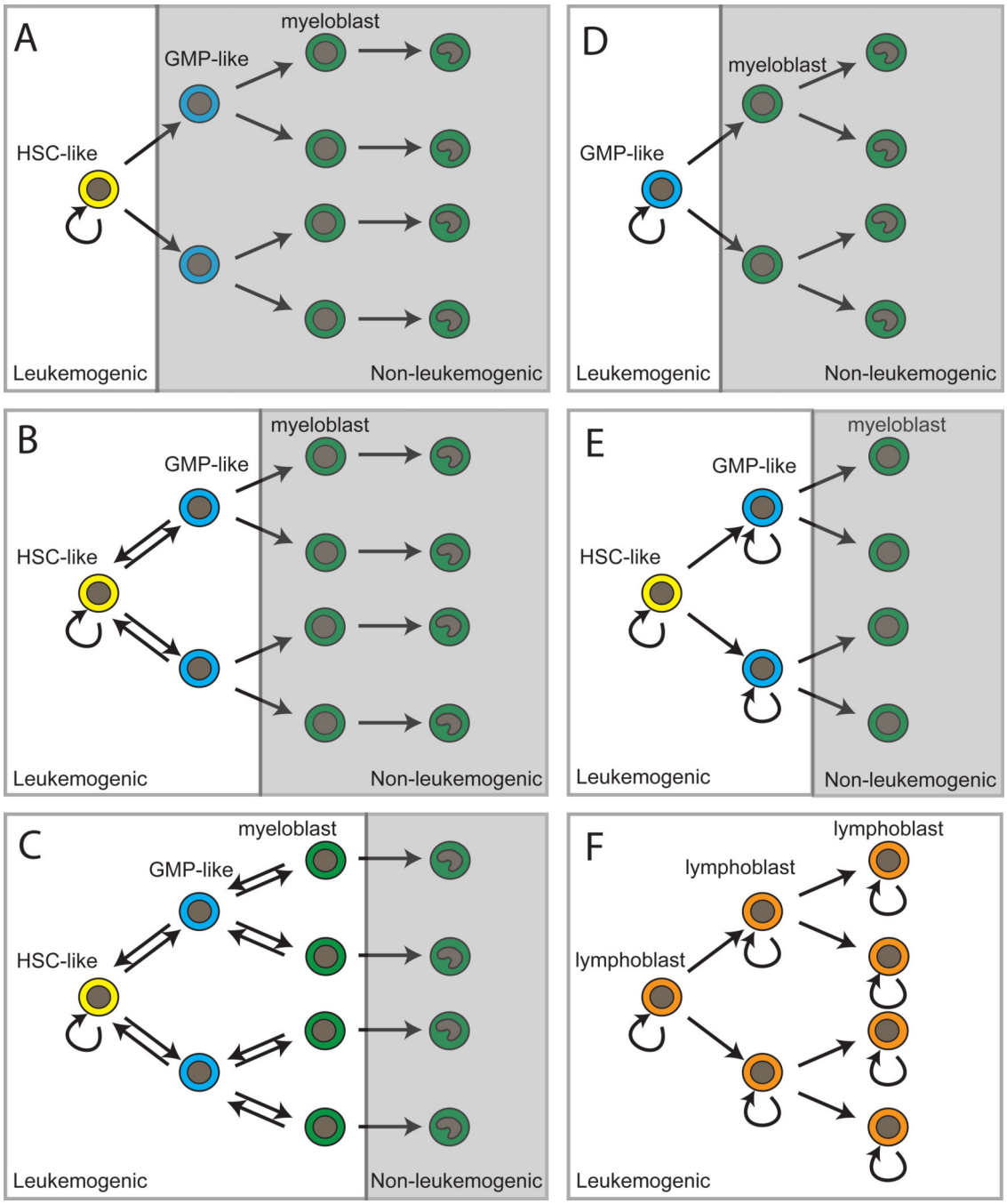

Figure 3. Variation among leukemias in the degree and nature of hierarchical organization

Although most AMLs follow a cancer stem cell model, the surface marker phenotypes of the leukemogenic cells vary from patient to patient. (A–C) Different oncogenic mutations can transform cells at different levels within the hematopoiesis hierarchy (Wang et al., 2010), potentially influencing the frequency, spectrum, and phenotype of cells with leukemogenic potential. Interconversion between leukemogenic cell populations would allow any population to recapitulate the heterogeneity of the leukemogenic cell pool (Eppert et al., 2011; Sarry et al., 2011). (D) Some mutations, such as MLL-AF9 translocations, can confer

leukemogenic activity upon restricted progenitors (Cozzio et al., 2003; Huntly et al., 2004; Krivtsov et al., 2006; Zhao et al., 2010). (E) If multiple populations have leukemogenic capacity but do not interconvert, then only the most immature cells can recapitulate the full heterogeneity of the parent leukemia but multiple levels of the hierarchy may be able to drive disease progression (Goardon et al., 2011). (F) In some ALLs many cells have leukemogenic activity despite heterogeneity in marker expression (le Viseur et al., 2008; Williams et al., 2007).

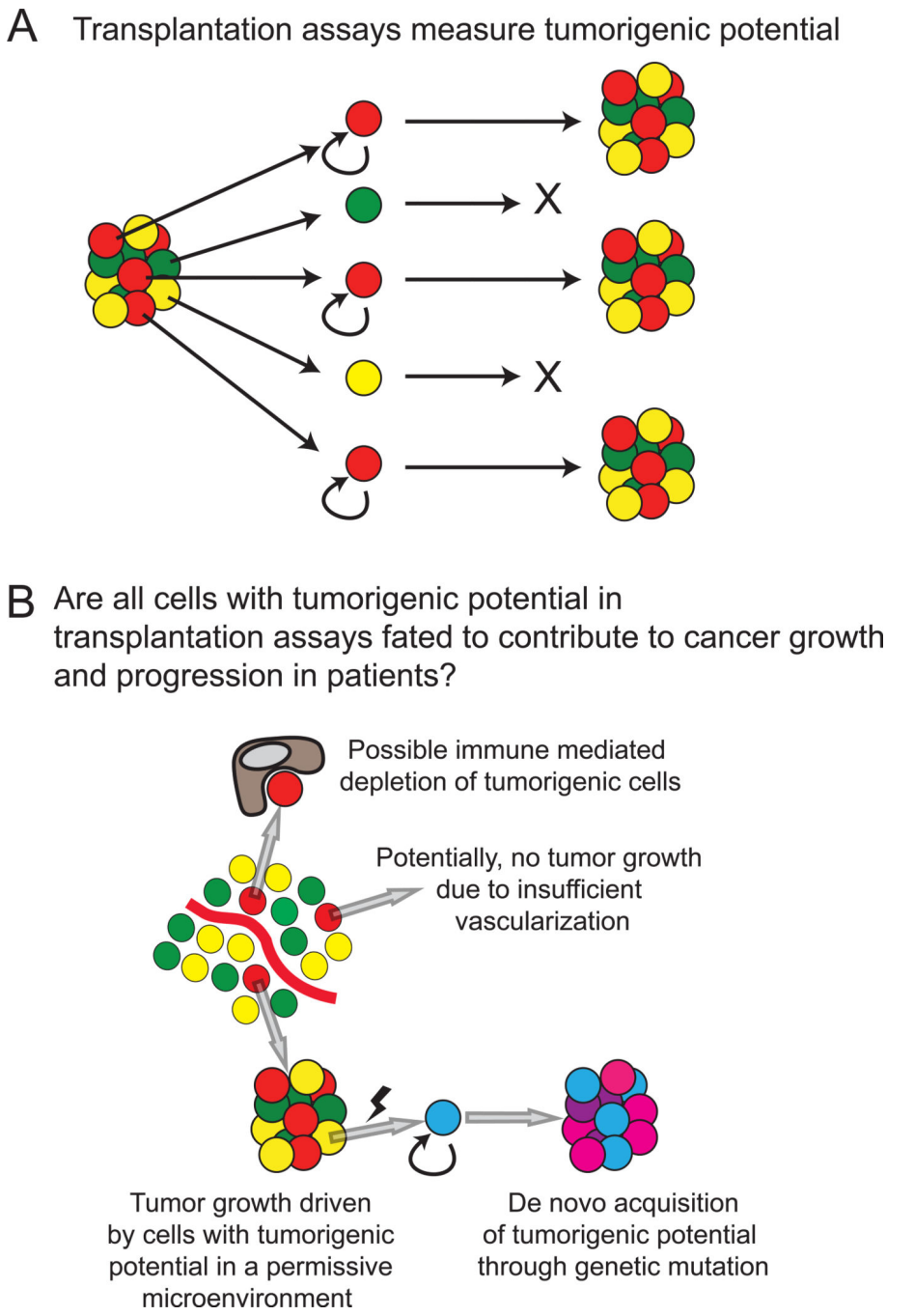

Figure 4. Fate versus potential in cancer

A) Potential describes what cells can do in a permissive environment. The cancer stem cell model, and the transplantation assays (black arrows) on which it is largely based, address the potential of cancer cells to form tumors. B) Fate reflects what cells actually do in a specific environment. In the context of cancer, the question is which cells are fated to contribute to tumor growth and disease progression in their actual environment in the patient. Many of the cells that have the potential to form tumors upon transplantation may not be fated to contribute to disease progression in a particular patient because they are not in a permissive

environment. For example, some cancer cells undergo cell death due to hypoxia or immune effector activity. Some of these cells might have the potential to form a tumor if transplanted into another environment, but are fated to undergo cell death in the tumor environment in which they actually reside in the patient. There may also be cells that lack the ability to form a tumor upon transplantation but that are nonetheless fated to contribute to disease progression in the patient, such as if they acquire a new mutation that increases their proliferation. Transplantation assays only assess potential, not fate in a patient, and therefore should attempt to detect the full range of cells with the potential to form tumors. No transplantation assay mimics the environment within patient tissues and such assays are neither designed nor capable of assessing cell fate in patients. Consequently, very little is known about the spectrum of cells fated to contribute to tumor growth and disease progression in patients, or the extent to which it overlaps with the spectrum of cells that can form a tumor upon transplantation.

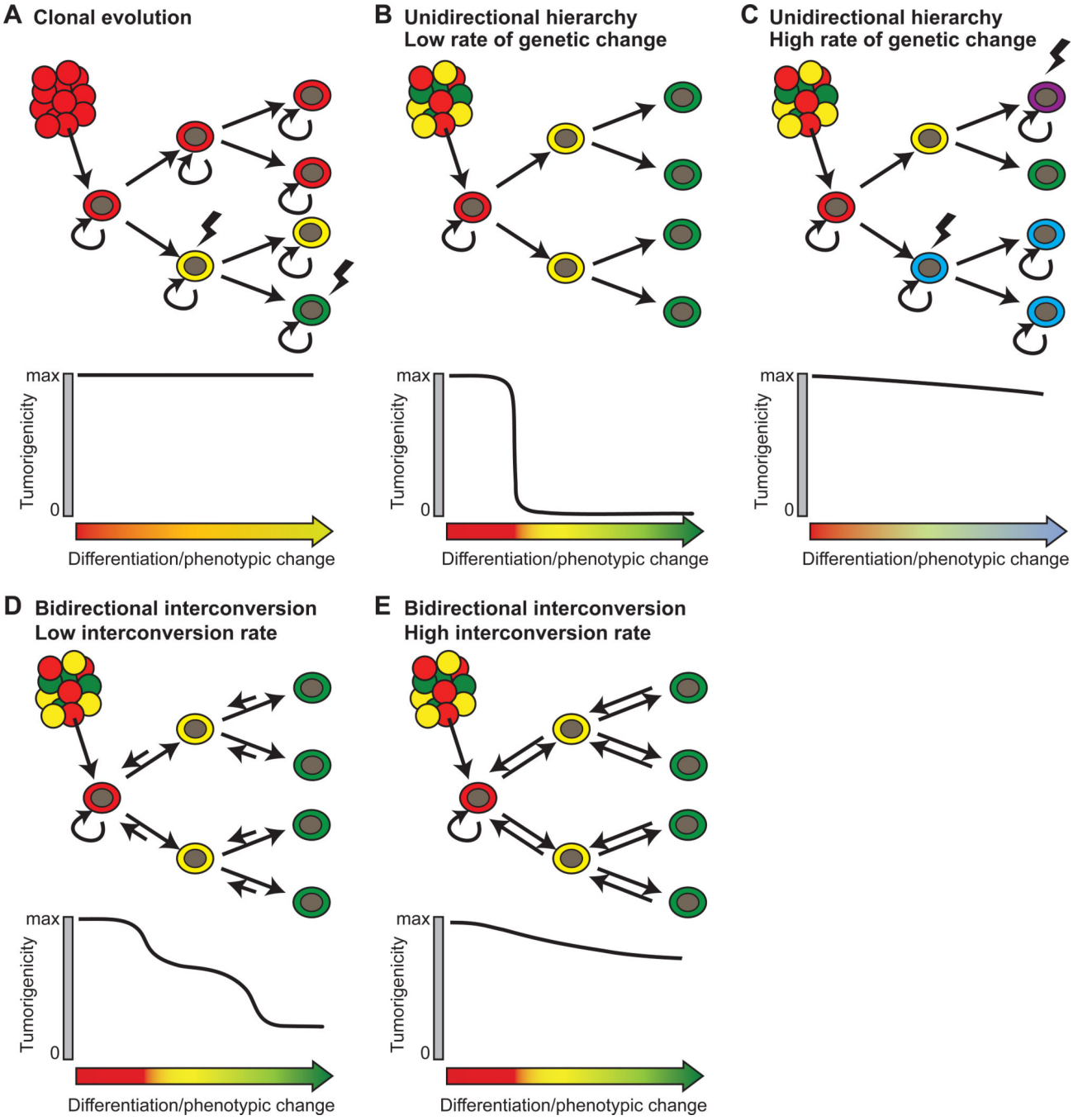

Figure 5. The influences of genetic change and reversible transitions in cell states on hierarchical organization in cancer

A) According to the clonal evolution model many cancer cells have tumorigenic potential (circular self-renewal arrows) and heterogeneity arises through stochastic genetic/epigenetic changes (lightning bolt). Changes in cell phenotype are not necessarily associated with changes in tumorigenic potential. B) For cancers that follow a stem cell model in which only the cells at the top of the hierarchy retain tumorigenic capacity, the differentiation of these cells into non-tumorigenic progeny creates tumor heterogeneity. Differentiation is associated with a loss of tumorigenic potential. C) For cancers with a high rate of genetic change,

clones of cells within the hierarchy depicted in panel B may acquire tumorigenic potential as a consequence of new mutations. Phenotypic changes are sometimes associated with changes in tumorigenic potential and sometimes not. Note that it would be difficult to experimentally distinguish this model from the model in panel A. D) A cancer that is hierarchically organized according to the cancer stem cell model but in which non-tumorigenic cells can inefficiently revert to higher levels of the hierarchy. In this case, tumorigenic cells could be enriched or depleted using markers but “non-tumorigenic” cells from the bottom of the hierarchy would always retain some tumorigenic capacity due to their ability to revert to tumorigenic states. E) A hierarchically organized cancer in which cells readily and reversibly interconvert between tumorigenic (red) and non-tumorigenic (yellow and green) states. Note that it would be difficult to experimentally distinguish case (E) from case (C). In cancers that are genetically unstable or subject to efficient reversible cell transitions, the cancer stem cell model may be untestable as it may be difficult to experimentally distinguish from cancers in which there is no hierarchy but where heterogeneity arises through clonal evolution.

Table 1

Testing the cancer stem cell model.

Property of cancers that follow the stem cell model	**Experimental evidence**
Phenotypic and functional heterogeneity	Flow cytometry distinguishes phenotypically distinct subpopulations of cancer cells that are transplanted to test whether some are tumorigenic while others are non-tumorigenic
Hierarchical organization	Cancer stem cells are tumorigenic cells that give rise to a hierarchy of tumorigenic and non-tumorigenic progeny. Upon transplantation, tumorigenic cells should give rise to more tumorigenic cells as well as phenotypically distinct non-tumorigenic cells
Properties sometimes ascribed to cancer stem cells but not required by the model	
Therapy resistance	Are tumorigenic cells more likely to survive therapy than non-tumorigenic cells? Are tumorigenic cells enriched by therapy?
Rarity	Tumorigenic cells have been rare in many studies that supported the cancer stem cell model but in principle such cells do not have to be rare in a hierarchically organized cancer
Quiescence	While cancer stem cells are sometimes claimed to be quiescent little data support this assertion and the cell cycle distribution of most cancer stem cells is unknown
Asymmetric division	Cancer stem cells are sometimes claimed to divide asymmetrically but this has never been demonstrated in vivo and cannot be an obligate property because it would prevent the numerical expansion of cancer stem cells
Derive from normal stem cells	Experimentally, cancer stem cells can arise from either normal stem cells or from restricted progenitors/differentiated cells. In practice, the cell of origin of most cancers is unknown.