Stem cells, cancer, and cancer stem cells

Tannishtha Reya*§||, Sean J. Morrison†||, Michael F. Clarke‡ & Irving L. Weissman*

**Departments of Pathology and Developmental Biology, Stanford University School of Medicine, Palo Alto, California 94305, USA (e-mail: irv@stanford.edu)*
†Howard Hughes Medical Institute, and Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109-0934, USA
‡Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109-0936, USA
§Present address: Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, USA
||These authors contributed equally to this work

Stem cell biology has come of age. Unequivocal proof that stem cells exist in the haematopoietic system has given way to the prospective isolation of several tissue-specific stem and progenitor cells, the initial delineation of their properties and expressed genetic programmes, and the beginnings of their utility in regenerative medicine. Perhaps the most important and useful property of stem cells is that of self-renewal. Through this property, striking parallels can be found between stem cells and cancer cells: tumours may often originate from the transformation of normal stem cells, similar signalling pathways may regulate self-renewal in stem cells and cancer cells, and cancer cells may include 'cancer stem cells' — rare cells with indefinite potential for self-renewal that drive tumorigenesis.

Stem cells are defined as cells that have the ability to perpetuate themselves through self-renewal and to generate mature cells of a particular tissue through differentiation. In most tissues, stem cells are rare. As a result, stem cells must be identified prospectively and purified carefully in order to study their properties. Although it seems reasonable to propose that each tissue arises from a tissue-specific stem cell, the rigorous identification and isolation of these somatic stem cells has been accomplished only in a few instances. For example, haematopoietic stem cells (HSCs) have been isolated from mice and humans[1–4], and have been shown to be responsible for the generation and regeneration of the blood-forming and immune (haematolymphoid) systems (Fig. 1). Stem cells from a variety of organs might have the potential to be used for therapy in the future, but HSCs — the vital elements in bone-marrow transplantation — have already been used extensively in therapeutic settings (reviewed in ref. 5).

The recent discovery that bone marrow[6–8], as well as purified HSCs[9,10], can give rise to non-haematopoietic tissues suggests that these cells may have greater differentiation potential than was assumed previously. Definitive experiments are needed to determine whether the cells from the bone marrow that are capable of giving rise to different non-haematopoietic lineages are indeed HSCs or another population. If further studies support the idea of HSC plasticity, this will undoubtedly open new frontiers for understanding the developmental potential of HSCs, as well as expand their therapeutic application.

As the characteristics of HSCs, their differentiation potential and clinical applications have been covered in earlier reviews, here we discuss emerging evidence that stem cell biology could provide new insights into cancer biology. In particular, we focus on three aspects of the relationship between stem cells and tumour cells: first, the similarities in the mechanisms that regulate self-renewal of normal stem cells and cancer cells; second, the possibility that tumour cells might arise from normal stem cells; and third, the notion that tumours might contain 'cancer stem cells' — rare cells with indefinite proliferative potential that drive the formation and growth of tumours. Through much of this review we focus on the haematopoietic system because both normal stem cells and cancer cells from this tissue are well characterized. Moreover, cancers of the haematopoietic system (that is, leukaemias) provide the best evidence that normal stem cells are the targets of transforming mutations, and that cancer cell proliferation is driven by cancer stem cells.

Self-renewal of haematopoietic stem cells

One of the most important issues in stem cell biology is understanding the mechanisms that regulate self-renewal. Self-renewal is crucial to stem cell function, because it is required by many types of stem cells to persist for the lifetime of the animal. Moreover, whereas stem cells from different organs may vary in their developmental potential, all stem cells must self-renew and regulate the relative balance between self-renewal and differentiation. Understanding the regulation of normal stem cell self-renewal is also fundamental to understanding the regulation of cancer cell proliferation, because cancer can be considered to be a disease of unregulated self-renewal.

In the haematopoietic system, stem cells are heterogeneous with respect to their ability to self-renew. Multipotent progenitors constitute 0.05% of mouse bone-marrow cells, and can be divided into three different populations: long-term self-renewing HSCs, short-term self-renewing HSCs, and multipotent progenitors without detectable self-renewal potential[2,11]. These populations form a lineage in which the long-term HSCs give rise to short-term HSCs, which in turn give rise to multipotent progenitors[11]. As HSCs mature from the long-term self-renewing pool to multipotent progenitors, they progressively lose their potential to self-renew but become more mitotically active. Whereas long-term HSCs give rise to mature haematopoietic cells for the lifetime of the mouse, short-term HSCs and multipotent progenitors reconstitute lethally irradiated mice for less than eight weeks.

Although the phenotypic and functional properties of HSCs have been extensively characterized (reviewed in

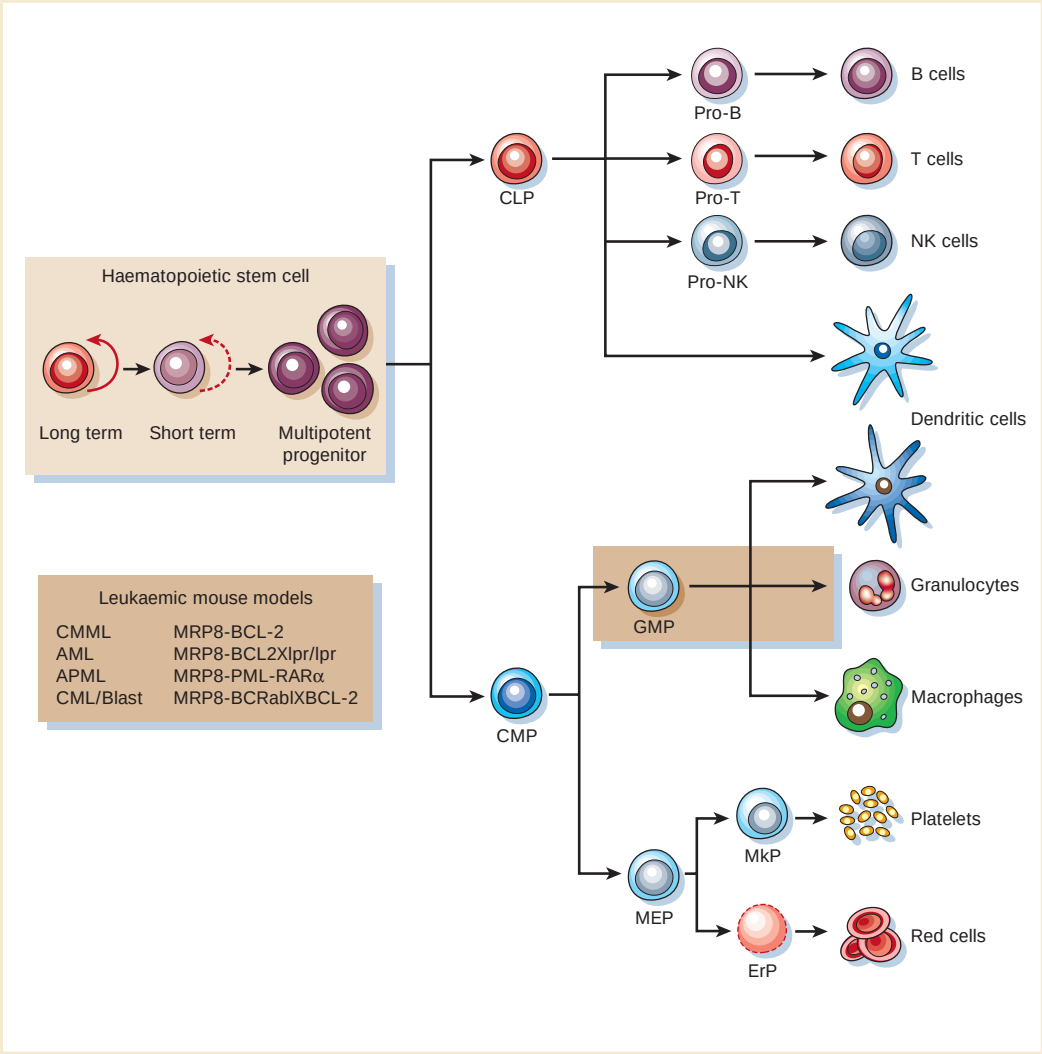

Figure 1 Development of haematopoietic stem cells. HSCs can be subdivided into long-term self-renewing HSCs, short-term self-renewing HSCs and multipotent progenitors (red arrows indicate self-renewal). They give rise to common lymphoid progenitors (CLPs; the precursors of all lymphoid cells) and common myeloid progenitors (CMPs; the precursors of all myeloid cells). Both CMPs/GMPs (granulocyte macrophage precursors) and CLPs can give rise to all known mouse dendritic cells. The isolation of precursors in the haematopoietic system has allowed the generation of a series of mouse models for myeloid leukaemia (see box, lower left). The expression of the oncogenes BCL-2, BCR–Abl and PML–RARα under the control of the *hMRP8* promoter, individually or together, and in combination with Fas deficiency, results in diseases that resemble several human leukaemias, including chronic myelomonocytic leukaemia (CMML), acute myeloid leukaemia (AML), acute promyelocytic leukaemia (APML)[77], and chronic myeloid leukaemia (CML)/Blast (S. Jaswal, K. Akashi and I.L.W., submitted). ErP, erythrocyte precursor; MEP, megakaryocyte erythrocyte precursor; MkP, megakaryocyte precursor; NK, natural killer.

ref. 12), the fundamental question of how self-renewal is regulated remains unanswered. In most cases, combinations of growth factors that can induce potent proliferation cannot prevent the differentiation of HSCs in long-term cultures. Although progress has been made in identifying culture conditions that maintain HSC activity in culture (for example, see ref. 13), it has proved exceedingly difficult to identify combinations of defined growth factors that cause a significant expansion in culture in the number of progenitors with transplantable HSC activity.

Pathways regulating stem cell self-renewal and oncogenesis

Because normal stem cells and cancer cells share the ability to self-renew, it seems reasonable to propose that newly arising cancer cells appropriate the machinery for self-renewing cell division that is normally expressed in stem cells. Evidence shows that many pathways that are classically associated with cancer may also regulate normal stem cell development (Fig. 2). For example, the prevention of apoptosis by enforced expression of the oncogene *bcl-2* results in increased numbers of HSCs *in vivo*, suggesting that cell death has a role in regulating the homeostasis of HSCs[14,15].

Other signalling pathways associated with oncogenesis, such as the Notch, Sonic hedgehog (Shh) and Wnt signalling pathways, may also regulate stem cell self-renewal (reviewed in ref. 16). Notch activation in HSCs in culture using the ligand Jagged-1 have consistently increased the amount of primitive progenitor activity that can be observed *in vitro* and *in vivo*, suggesting that Notch activation promotes HSC self-renewal, or at least the maintenance of multipotentiality[17,18]. Shh signalling has also been implicated in the regulation of self-renewal by the finding that populations highly enriched for human HSCs ($CD34^+Lin^-CD38^-$) exhibit increased self-renewal in response to Shh stimulation *in vitro*, albeit in combination with other growth factors[19]. The involvement of Notch and Shh in the self-renewal of HSCs is especially interesting in light of studies that implicate these pathways in the regulation of self-renewal of stem cells from other tissues as well (Fig. 2, and see review in this issue by Spradling and colleagues, pages 98–104).

One particularly interesting pathway that has also been shown to regulate both self-renewal and oncogenesis in different organs is the Wnt signalling pathway (Fig. 2). Wnt proteins are intercellular signalling molecules[20] that regulate development in several organisms[21] and contribute to cancer when dysregulated. The expression of Wnt proteins in the bone marrow[22] suggests that they may influence HSCs as well. Using highly purified mouse bone-marrow HSCs, we have shown that overexpression of activated β-catenin (a downstream activator of the Wnt signalling pathway) in long-term cultures of HSCs expands the pool of transplantable HSCs determined by both phenotype ($Thy1.1^{lo}Lin^{-/lo}Sca1^+c\text{-}kit^+$) and function (ability to reconstitute the haematopoietic system *in vivo*). Moreover, ectopic expression of Axin, an inhibitor of Wnt signalling, leads to inhibition of HSC proliferation, increased death of HSCs *in vitro*, and reduced reconstitution *in vivo* (T.R. *et al.*, submitted). In separate studies, soluble Wnt proteins from conditioned supernatants have also been shown to influence the proliferation of haematopoietic progenitors from mouse fetal liver and human bone marrow[23,24].

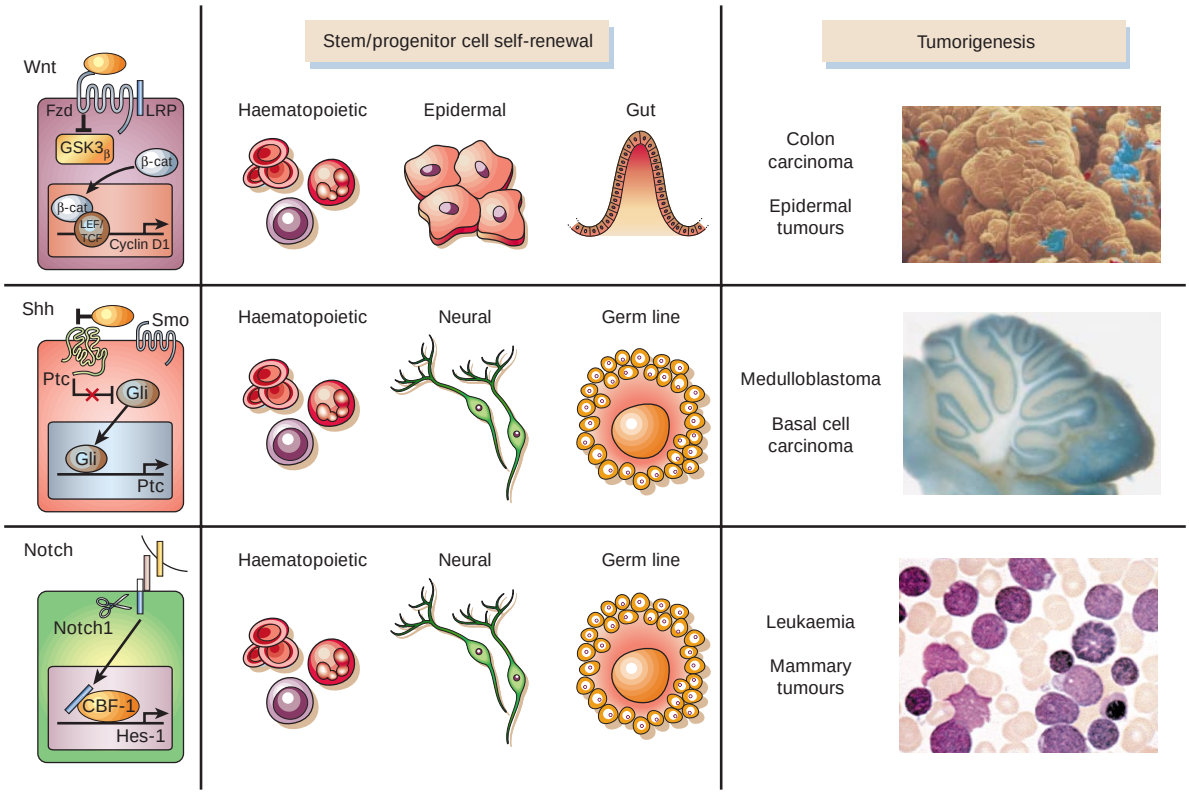

Figure 2 Signalling pathways that regulate self-renewal mechanisms during normal stem cell development and during transformation. Wnt (refs 25, 27; and T.R. *et al.*, submitted), Shh[19,78,79] and Notch pathways[17,80,81] have been shown to contribute to the self-renewal of stem cells and/or progenitors in a variety of organs, including the haematopoietic and nervous systems. When dysregulated, these pathways can contribute to oncogenesis. Mutations of these pathways have been associated with a number of human tumours, including colon carcinoma[37] and epidermal tumours[82] (Wnt), medulloblastoma[83] and basal cell carcinoma[84] (Shh), and T-cell leukaemias[85] (Notch). (Images courtesy of Eye of Science/SPL and R. Wechsler-Reya/M. Scott/Annual Reviews.)

Studies of epidermal and gut progenitors suggest that the Wnt signalling pathway may contribute to the regulation of stem cell/progenitor cell self-renewal in other tissues. Cultured human keratinocytes with higher proliferative potential have increased levels of β-catenin compared with keratinocytes with lower proliferative capacity. Moreover, retroviral transduction of activated β-catenin results in increased epidermal stem cell self-renewal and decreased differentiation[25]. *In vivo* data from transgenic mice suggest that activation of the Wnt signalling pathway in epidermal stem cells leads to epithelial cancers[26]. Furthermore, mice lacking TCF-4, one of the transcriptional mediators of the Wnt signalling pathway, quickly exhaust the undifferentiated progenitors in the crypts of the gut epithelium during fetal development[27], suggesting that this pathway is required for the maintenance or self-renewal of gut epithelial stem cells.

Cumulatively, the above findings suggest that Wnt signalling may promote stem cell self-renewal in a variety of different epithelia in addition to HSCs. The molecular mechanisms by which Wnt signalling influences stem cells remain to be elucidated. It will also be important to determine whether the Wnt, Notch and Shh pathways interact to regulate stem and progenitor cell self-renewal.

Self-renewal and leukaemogenesis

If the signalling pathways that normally regulate stem cell self-renewal lead to tumorigenesis when dysregulated, then are stem cells themselves the target of transformation in certain types of cancer[28,29]? There are two reasons to think that this may be the case. First, because stem cells have the machinery for self-renewal already activated, maintaining this activation may be simpler than turning it on *de novo* in a more differentiated cell; that is, fewer mutations may be required to maintain self-renewal than to activate it ectopically. Second, by self-renewing, stem cells often persist for long periods of time, instead of dying after short periods of time like many mature cells in highly proliferative tissues. This means that there is a much greater opportunity for mutations to accumulate in individual stem cells than in most mature cell types (Fig. 3).

Even restricted progenitor cells are less likely than stem cells to undergo neoplastic transformation because they proliferate for a much shorter period of time before terminally differentiating. Restricted haematopoietic progenitors of the lymphoid[30] and myeloid lineages all fail to self-renew detectably on transplantation (K. Nankorn, Traver, D., I.L.W. and K. Akashi, submitted). Thus, restricted progenitors would first need to acquire the extensive self-renewal potential of stem cells to have the opportunity to experience additional mutations that would lead to transformation. Nonetheless, restricted progenitors could potentially be transformed either by acquiring mutations that cause them to self-renew like stem cells, or by inheriting existing mutations from stem cells such that only a single mutation is required in the progenitors to cause transformation (Fig. 3).

Stem cells as targets of mutation

For most cancers, the target cell of transforming mutations is unknown; however, there is considerable evidence that certain types of leukaemia arise from mutations that accumulate in HSCs. The cells capable of initiating human acute myeloid leukaemia (AML) in NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mice have a $CD34^{+}CD38^{-}$ phenotype in most AML subtypes, and thus have a phenotype similar to normal HSCs[31]. Conversely,

$CD34^+CD38^+$ leukaemia cells cannot transfer disease to mice in the vast majority of cases, despite the fact that they exhibit a leukaemic blast phenotype. This suggests that normal HSCs rather than committed progenitors are the target for leukaemic transformation.

The most frequent chromosomal abnormalities in AML involve the 8;21 translocation, which results in AML1–ETO chimaeric transcripts in leukaemic cells. In work done on human HSCs from patients in remission, AML1–ETO transcripts were found in a fraction of normal HSCs in the marrow[32]. These prospectively isolated HSCs and their progeny were not leukaemic, and could differentiate to normal myeloerythroid cells *in vitro*. This indicates that the translocation occurred originally in normal HSCs and that additional mutations in a subset of these HSCs or their progeny subsequently lead to leukaemia[32]. In this study, the normal HSCs were $CD34^+CD38^-Thy\text{-}1^+$, whereas the leukaemic blasts were $CD34^+CD38^-Thy\text{-}1^-$. Although the translocation must have occurred in normal HSCs, subsequent transforming mutations might have occurred either in downstream $Thy\text{-}1^-$ progenitors, or in HSCs if one consequence of neoplastic proliferation was the loss of Thy-1 expression. The idea that stem cells are a common target of pre-leukaemic events or leukaemic transformation is also supported by work in lymphoid[33] and chronic myeloid leukaemias[34] where clonotypic leukaemia-associated chromosomal rearrangements have also been found in $CD34^+CD38^-$ cells, a population enriched for HSCs. Thus, a variety of leukaemias may arise from mutations that accumulate in HSCs to cause their malignant transformation at the stage of stem cells or their progeny.

Progenitor cells as targets of transformation

Although stem cells are often the target of genetic events that are necessary or sufficient for malignant transformation, in other cases restricted progenitors or even differentiated cells may become transformed (Fig. 3). By targeting the expression of transgenes specifically to restricted myeloid progenitors using the *hMRP-8* promoter, it is possible to create a mouse model in which myeloid leukaemia arises from restricted progenitors. These leukaemias resemble human leukaemias in many respects, even though the targeted genetic changes cause the leukaemias to arise from restricted progenitors rather than stem cells. For example, we have generated transgenic mouse models for myeloid leukaemias using an *hMRP-8* promoter, which targets the expression of transgenes specifically to myeloid progenitors[35]. The enforced expression of the anti-apoptotic gene *bcl-2* in the myeloid lineage leads to a disease that is similar to human chronic myelomonocytic leukaemia, including monocytosis, splenomegaly and neutropenia, as the mice age. However, these mice rarely develop acute malignancies.

To test whether additional mutations are required to synergize with *bcl-2* to promote AML, *hMRP8–bcl-2* transgenic mice were bred with *lpr*/*lpr* Fas-deficient mice. Remarkably, the loss of these two distinct apoptosis pathways led to the development of AML in 15% of the mice[36]. These mice have an expansion of myeloblasts in all haematopoietic tissues, with a substantially lowered number of granulocytes in the marrow and blood. These studies show that prevention of cell death is a crucial event in myeloid leukaemogenesis and that restricted progenitors can be transformed. As described above, in the case of spontaneously arising human leukaemias it is likely that stem cells accumulate the mutations that are necessary for neoplastic proliferation; however, these mutations may accumulate in stem cells even while the effects of the mutations are expressed in restricted progenitors. That is, mutations that accumulate in stem cells may lead to neoplastic proliferation of primitive progenitors downstream of stem cells.

Perhaps the reason why only 15% of mice progress to AML in mice expressing Bcl-2 and lacking Fas is that the progenitors in these mice also must acquire an additional mutation that causes dysregulated self-renewal (Fig. 3). If a single additional mutation causes transformation then this transforming event is probably a gain-of-function mutation, such as one that promotes constitutive self-renewal. Because stabilized β-catenin can promote the self-renewal of HSCs and other types of progenitors (ref. 25, and T.R. *et al.*, submitted; Fig. 2), we propose that gain-of-function mutations in β-catenin may, in many cases, transform deathless pre-malignant cells to cancer cells by promoting proliferation. In support of this is evidence to show that activation of β-catenin and dysregulation of the Wnt signalling pathway in general is common in cancer[37], and that the targeted overactivation of this pathway can lead to tumours in transgenic mice[38]. It is also possible that mutations in other signalling pathways promote progenitor self-renewal. It is important to study this further, because understanding the molecular basis of the unregulated self-renewal of cancer cells will allow the design of more effective therapies.

In essence, newly arising cancer cells may appropriate the machinery for self-renewing cell divisions that is normally expressed in stem cells. In the haematopoietic system, the only long-term self-renewing cells in the myeloerythroid pathway (Fig. 1, bottom) are HSCs; however, at least two differentiated cell types (Fig. 1, top) can also self-renew. Both T and B lymphocytes undergo clonal expansion on stimulation to produce resting memory lymphocytes. These lymphocytes proliferate again when the antigens are re-encountered. Lymphoid leukaemias can activate these receptor-mediated mitogenic pathways in the course of leukaemogenesis[39–43].

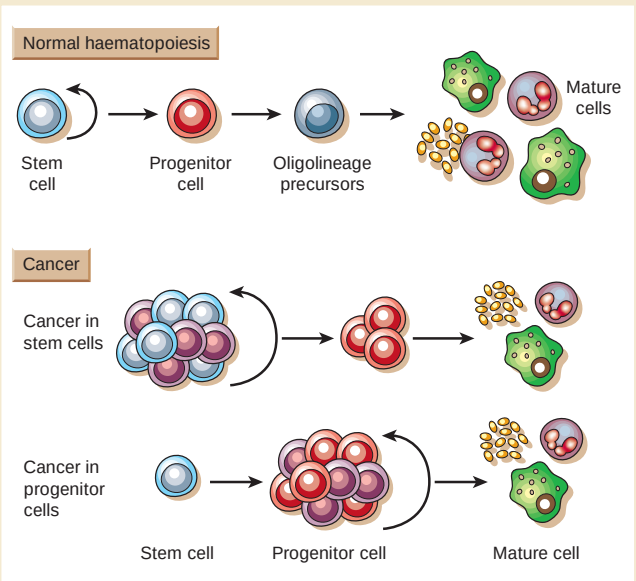

Figure 3 Comparison of self-renewal during haematopoietic stem cell development and leukaemic transformation. Because of their high level of self-renewal, stem cells are particularly good targets of leukaemic transformation. Unlike normal haematopoiesis, where signalling pathways that have been proposed to regulate self-renewal are tightly regulated (top), during transformation of stem cells, the same mechanisms may be dysregulated to allow uncontrolled self-renewal (middle). Furthermore, if the transformation event occurs in progenitor cells, it must endow the progenitor cell with the self-renewal properties of a stem cell, because these progenitors would otherwise differentiate (bottom).

Cancer stem cells and aberrant organogenesis

Basic cancer research has focused on identifying the genetic changes that lead to cancer. This has led to major advances in our understanding of the molecular and biochemical pathways that are involved in tumorigenesis and malignant transformation. But while we have focused on the molecular biology of cancer, our understanding of the cellular biology has lagged. That is, although we understand (to a first approximation) the effects of particular mutations on the proliferation and survival of model cells, such as fibroblasts or cell lines, we

can often only guess what the effects of such mutations will be on the actual cells involved in particular cancers. This has handicapped our ability to translate our identification of mutations into new therapies.

A tumour can be viewed as an aberrant organ initiated by a tumorigenic cancer cell that acquired the capacity for indefinite proliferation through accumulated mutations. If one views a tumour as an abnormal organ, then the principles of normal stem cell biology[12,44] can be applied to understand better how tumours develop (reviewed in ref. 45). In fact, many observations suggest that analogies between normal stem cells and tumorigenic cells may be appropriate. Both normal stem cells and tumorigenic cells have extensive proliferative potential and the ability to give rise to new (normal or abnormal) tissues. Both tumours and normal tissues are composed of heterogeneous combinations of cells, with different phenotypic characteristics and different proliferative potentials[46–49]. Because most tumours have a clonal origin[50–52], tumorigenic cancer cells must give rise to phenotypically diverse progeny, including cancer cells with indefinite proliferative potential, as well as cancer cells with limited or no proliferative potential. This suggests that tumorigenic cancer cells undergo processes that are analogous to the self-renewal and differentiation of normal stem cells.

Although some of the heterogeneity in tumours arises as a result of continuing mutagenesis, it is likely that heterogeneity also arises through the aberrant differentiation of cancer cells. It is well documented that many types of tumours contain cancer cells with heterogeneous phenotypes reflecting aspects of the differentiation that normally occurs in the tissues from which the tumours arise. The variable expression of normal differentiation markers by cancer cells in a tumour suggests that some of the heterogeneity in tumours arises as a result of the anomalous differentiation of tumour cells. Examples of this include the variable expression of myeloid markers in chronic myeloid leukaemia, the variable expression of neuronal markers within peripheral neurectodermal tumours, and the variable expression of milk proteins or the oestrogen receptor within breast cancer.

In other words, both normal stem cells and tumorigenic cells give rise to phenotypically heterogeneous cells that exhibit various degrees of differentiation. Thus, tumorigenic cells can be thought of as cancer stem cells that undergo an aberrant and poorly regulated process of organogenesis analogous to what normal stem cells do. It is perhaps not surprising that tumorigenic cells behave in ways that are analogous to normal stem cells given that cancer cells tend to display functional and phenotypic attributes of the normal cells from which they are derived[28].

Evidence for cancer stem cells

It was first extensively documented for leukaemia and multiple myeloma that only a small subset of cancer cells is capable of extensive proliferation. For example, when mouse myeloma cells were obtained from mouse ascites, separated from normal haematopoietic cells and put in clonal *in vitro* colony-forming assays, only 1 in 10,000 to 1 in 100 cancer cells were able to form colonies[53]. Even when leukaemic cells were transplanted *in vivo*, only 1–4% of cells could form spleen colonies[54–56]. Because the differences in clonogenicity among the leukaemia cells mirrored the differences in clonogenicity among normal haematopoietic cells, the clonogenic leukaemic cells were described as leukaemic stem cells (for example, see ref. 53). But two formal possibilities remained: either all leukaemia cells had a low probability of proliferating extensively in these assays such that all leukaemia cells had the potential to behave as leukaemic stem cells, or most leukaemia cells were unable to proliferate extensively and only a small, definable subset of cells was consistently clonogenic.

To prove the second possibility, it would be necessary to separate different classes of leukaemia cell and show that one subset is highly enriched for clonogenic capacity and all other cells are greatly depleted for clonogenicity. This has been accomplished by Dick and colleagues[57], who showed that human AML stem cells could be identified prospectively and purified as $CD34^{+}CD38^{-}$ cells from patient samples. Despite the fact that these cells represented a small but variable proportion of AML cells (0.2% in one patient), they were the only cells capable of transferring AML from human patients to NOD/SCID mice in the vast majority of cases. This excluded the first possibility that all AML cells had a similar clonogenic capacity, and showed that a small, predictable subset was consistently enriched for the ability to proliferate and transfer disease.

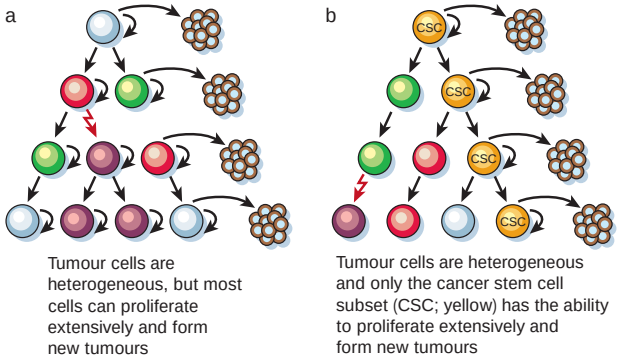

Figure 4 Two general models of heterogeneity in solid cancer cells. **a**, Cancer cells of many different phenotypes have the potential to proliferate extensively, but any one cell would have a low probability of exhibiting this potential in an assay of clonogenicity or tumorigenicity. **b**, Most cancer cells have only limited proliferative potential, but a subset of cancer cells consistently proliferate extensively in clonogenic assays and can form new tumours on transplantation. The model shown in **b** predicts that a distinct subset of cells is enriched for the ability to form new tumours, whereas most cells are depleted of this ability. Existing therapeutic approaches have been based largely on the model shown in **a**, but the failure of these therapies to cure most solid cancers suggests that the model shown in **b** may be more accurate.

It has also been shown for solid cancers that the cells are phenotypically heterogeneous and that only a small proportion of cells are clonogenic in culture and *in vivo*[46–49,58]. For example, only 1 in 1,000 to 1 in 5,000 lung cancer, ovarian cancer or neuroblastoma cells were found to form colonies in soft agar[59]. Just as in the context of leukaemic stem cells, these observations led to the hypothesis that only a few cancer cells are actually tumorigenic and that these tumorigenic cells could be considered as cancer stem cells[59]. But, as explained above, two possibilities remain: either all solid cancer cells have a low probability of proliferating extensively and behaving in clonogenic assays as cancer stem cells, or most cancer cells have only a limited proliferative potential and cannot behave as cancer stem cells, but a small, definable subset of cells is enriched for the ability to proliferate extensively and form tumours.

In both cases, some of the cancer cell heterogeneity would arise as a result of environmental differences within the tumour and continuing mutagenesis. The essential difference between these possibilities is the prediction, according to the second possibility, that whatever the environment or mutational status of the cells, only a small, phenotypically distinct subset of cancer cells has the ability to proliferate extensively or form a new tumour (Fig. 4). It has not been possible to distinguish between these models of solid cancer heterogeneity, because as yet no one has published the identity of purified subsets of uncultured solid cancer cells that are enriched for the ability to form new tumours.

The implications of solid cancer stem cells

If the growth of solid cancers were driven by cancer stem cells, it would have profound implications for cancer therapy. At present, all of the phenotypically diverse cancer cells are treated as though they

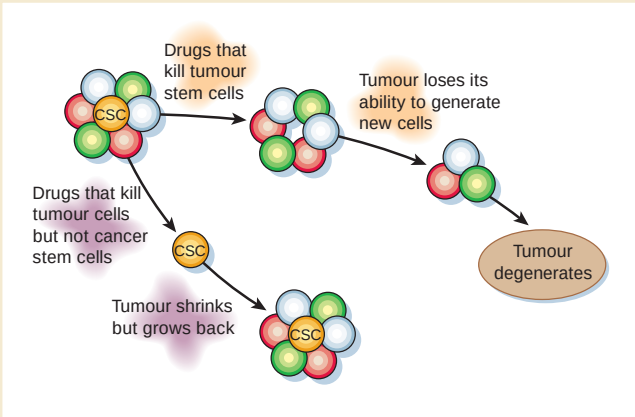

Figure 5 Conventional therapies may shrink tumours by killing mainly cells with limited proliferative potential. If the putative cancer stem cells are less sensitive to these therapies, then they will remain viable after therapy and re-establish the tumour. By contrast, if therapies can be targeted against cancer stem cells, then they might more effectively kill the cancer stem cells, rendering the tumours unable to maintain themselves or grow. Thus, even if cancer stem cell-directed therapies do not shrink tumours initially, they may eventually lead to cures.

have unlimited proliferative potential and can acquire the ability to metastasize. For many years, however, it has been recognized that small numbers of disseminated cancer cells can be detected at sites distant from primary tumours in patients that never manifest metastatic disease[58,60]. One possibility is that immune surveillance is highly effective at killing disseminated cancer cells before they can form a detectable tumour. Another possibility is that most cancer cells lack the ability to form a new tumour such that only the dissemination of rare cancer stem cells can lead to metastatic disease (reviewed in ref. 45). If so, the goal of therapy must be to identify and kill this cancer stem cell population. If solid cancer stem cells can be identified prospectively and isolated, then we should be able to identify more efficiently new diagnostic markers and therapeutic targets expressed by the stem cells.

If tumour growth and metastasis are driven by a small population of cancer stem cells, this might explain the failure to develop therapies that are consistently able to eradicate solid tumours[61]. Although currently available drugs can shrink metastatic tumours, these effects are usually transient and often do not appreciably extend the life of patients[62,63]. One reason for the failure of these treatments is the acquisition of drug resistance by the cancer cells as they evolve; another possibility is that existing therapies fail to kill cancer stem cells effectively.

Existing therapies have been developed largely against the bulk population of tumour cells because they are often identified by their ability to shrink tumours. Because most cells with a cancer have limited proliferative potential, an ability to shrink a tumour mainly reflects an ability to kill these cells. It seems that normal stem cells from various tissues tend to be more resistant to chemotherapeutics than mature cell types from the same tissues[64]. The reasons for this are not clear, but may relate to high levels of expression of anti-apoptotic proteins[65–68] or ABC transporters such as the multidrug resistance gene[69,70]. If the same were true of cancer stem cells, then one would predict that these cells would be more resistant to chemotherapeutics than tumour cells with limited proliferative potential. Even therapies that cause complete regression of tumours might spare enough cancer stem cells to allow regrowth of the tumours. Therapies that are more specifically directed against cancer stem cells might result in much more durable responses and even cures of metastatic tumours (Fig. 5).

Genomics may provide a powerful means for identifying drug targets in cancer cells. Although targeting genetic mutations does not require isolation of the stem cells, there are likely to be differences in gene expression between cancer stem cells and tumour cells with limited proliferative potential. The application of microarray analysis to malignant tumours has shown that patterns of gene expression can be used to group tumours into different categories, often reflecting different mutations[71–74]. As a result, tumour types that cannot be distinguished pathologically, but that can be distinguished on the basis of differences in gene-expression profile, can be examined for differences in treatment sensitivity. However, gene-expression profiling is often conducted on tumour samples that contain a mixture of normal cells, highly proliferative cancer cells, and cancer cells with limited proliferation potential. This results in a composite profile that may obscure differences between tumours, because the highly proliferative cells that drive tumorigenesis often represent a minority of cancer cells. Gene-expression profiling of cancer stem cells would allow the profile to reflect the biology of the cells that are actually driving tumorigenesis. Microdissection of morphologically homogeneous collections of cancer cells is one way of generating profiles that reflect more homogeneous collections of cells[75,76]. The next frontier will be to purify the cancer stem cells from the whole tumour that retain unlimited proliferative potential and to perform gene-expression profiling on those cells. In addition to being a more efficient way of identifying new therapeutic and diagnostic targets, the profiling of cancer stem cells might sharpen the differences in patterns observed between different tumours.

Perspectives

The ideas discussed in this review can be summarized as a set of propositions. First, self-renewal is the hallmark property of stem cells in normal and neoplastic tissues. Second, in the haematopoietic system, long-term self-renewal is limited to rare long-term HSCs and some lymphocytes; other cell types lack this potential. Third, cells that continue to divide over long periods of time are much more likely to accumulate mutations that cause neoplasia. Thus genetic changes that lead to myeloid leukaemias must occur either in long-term HSCs or in progeny that first acquire the ability to self-renew. The fact that normal long-term HSCs in leukaemia patients often have leukaemia-associated translocations strongly supports the idea that leukaemic mutations often accumulate in HSCs. Mutations that lead to certain types of lymphoma may accumulate in lymphocytes, given their ability to self-renew over the long term. Fourth, in other normal tissues that contain self-renewing stem cells, such as the epithelia, the genetic changes that are steps in the progression to solid tumours probably also occur in the stem cells, or in progeny that acquire the potential for self-renewal. Fifth, distinct signalling pathways control stem cell self-renewal in different tissues. But perhaps within individual tissues, the same pathways are used consistently by both normal stem cells and cancer cells to regulate proliferation. For example, Wnt signalling regulates the self-renewal of normal stem cells in the blood and epithelia. Constitutive activation of the Wnt pathway has been implicated in a number of epithelial cancers. The regulation and consequences of Wnt signalling in normal and neoplastic cells need to be further elucidated. Sixth, understanding the signalling pathways that are used by for normal stem cells and neoplastic cells should facilitate the use of normal stem cells for regenerative medicine and the identification of cancer stem cell targets for anticancer therapies. Seventh, within most tumours there may exist cancer stem cells that can self-renew indefinitely, in contrast to most stem cells that may have limited proliferative potential. Finally, in order to cure cancer, it is necessary and sufficient to kill cancer stem cells. To accomplish this it will be necessary to identify and characterize the properties of these cells.

There are many connections between stem cells and cancer that are important to understand. Just as the signals that are known to control oncogenesis are providing clues about the control of

self-renewal of normal stem cells, studies of stem cell biology are lending insight into the origins of cancer and will ultimately yield new approaches to fight this disease. □

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