The Biology of Cancer Stem Cells

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Annu. Rev. Cell Dev. Biol. 2007. 23:675–99

First published online as a Review in Advance on July 23, 2007

The *Annual Review of Cell and Developmental Biology* is online at http://cellbio.annualreviews.org

This article's doi: 10.1146/annurev.cellbio.22.010305.104154

1081-0706/07/1110-0675$20.00

Key Words

oncogenesis, self-renewal, differentiation, progenitor, signaling

Abstract

Cancers originally develop from normal cells that gain the ability to proliferate aberrantly and eventually turn malignant. These cancerous cells then grow clonally into tumors and eventually have the potential to metastasize. A central question in cancer biology is, which cells can be transformed to form tumors? Recent studies elucidated the presence of cancer stem cells that have the exclusive ability to regenerate tumors. These cancer stem cells share many characteristics with normal stem cells, including self-renewal and differentiation. With the growing evidence that cancer stem cells exist in a wide array of tumors, it is becoming increasingly important to understand the molecular mechanisms that regulate self-renewal and differentiation because corruption of genes involved in these pathways likely participates in tumor growth. This new paradigm of oncogenesis has been validated in a growing list of tumors. Studies of normal and cancer stem cells from the same tissue have shed light on the ontogeny of tumors. That signaling pathways such as Bmi1 and Wnt have similar effects in normal and cancer stem cell self-renewal suggests that common molecular pathways regulate both populations. Understanding the biology of cancer stem cells will contribute to the identification of molecular targets important for future therapies.

Contents

Stem cell: a primitive cell defined by its capacity to self-renew and differentiate into at least one mature cell type

Cancer stem cell: a self-renewing cell within a tumor that has the capacity to regenerate the phenotypic diversity of the original tumor

INTRODUCTION

Stem cell biology has provided a platform to address many questions in developmental biology. Human development follows a predetermined plan that turns a fertilized egg into a complex, multicellular organism. The fertilized egg develops into a totipotent ball of cells that further differentiates into the three germ layers: endoderm, ectoderm, and mesoderm (McClay 1991). These three primitive cell types develop into all tissues in the adult body. Tissues in which malignancies originate, such as the blood, brain, breast, skin, and gut, are organized as a cellular hierarchy with a small population of tissue-specific stem cells responsible for both development and maintenance of tissues for the human lifetime (Cairns 1981). Somatic stem cells are typically slowly cycling cells capable of self-renewing mitotic divisions in which one or both of the daughter cells are faithful reproductions of the parent stem cell. Despite the multitude of regulatory systems that prevent abnormal proliferation during these processes, mutations that result in aberrant mitoses do occur. Most mutations are inconsequential because the abnormal cell is eventually eliminated from the pool of replicating cells, but at some low frequency these mutations accumulate and may lead to cancer. Most of the mutations leading to cancer affect cellular machinery that controls cell division, DNA damage, and signal transduction pathways. Stem cells may be preferential targets of initial oncogenic mutations because in most tissues in which cancer originates they are the only long-lived populations and are therefore exposed to more genotoxic stresses than their shorter-lived, differentiated progeny (Pardal et al. 2003, Reya et al. 2001). The cancer stem cell theory proposes that tumors have a cellular hierarchy that is a caricature of their normal tissue counterpart because they reflect the pluripotency of the originally transformed cell.

Two important observations led to the hypothesis that cancer stem cells may be responsible for growing and maintaining tumors. First, most tumors arise from a single cell, but not all the cells within a tumor are identical. This concept is also known as tumor heterogeneity (Park et al. 1971). In fact, there are many different types of cells in a tumor; some are cancerous, whereas others are infiltrating normal cells that are thought to support the growth of the cancer cells. Although tumors originate from a single transformed cell, the cancer cells within tumors may also display different phenotypes, somewhat reminiscent of the normal tissue from which they originate (e.g., diverse morphology and the expression of proteins expressed by either mature or

immature cells of the organ), and have varying proliferation potential (Fidler & Hart 1982, Fidler & Kripke 1977, Heppner 1984). For example, the most common type of prostate cancer has gland units similar to the normal prostate. The lumens of these units contain normal prostatic secretions. This cancer looks and functions somewhat like its normal counterpart, but it is clearly different because cells from the malignant protastic gland units invade the surrounding stromal tissue. A widely held belief in cancer biology is that all the cellular heterogeneity found in tumors may be attributed to genomic instability and the selection for cells that can adapt to the tumor microenvironment (Grady & Markowitz 2000, Heng et al. 2006), which provides a reasonable explanation for the aforementioned observations. Recent evidence strongly supports the notion that the cancer stem cell model also plays a major role in tumor heterogeneity (Dontu et al. 2003).

The second observation upon which the cancer stem cell theory was built came from studies that demonstrated that a large number of cancer cells were required to grow a tumor (Bruce & Van Der Gaag 1963, Hamburger & Salmon 1977). These observations were seemingly at odds with the traditional stochastic model of cancer development. The stochastic model predicts that every cancer cell has the potential to form a new tumor, but entry into the cell cycle is a stochastic event that occurs with low probability. Under the assumptions that all cancer cells have similar potential to grow tumors and that tumors are usually clonal in origin, one would expect that even a few cancer cells would be able to grow new tumors.

There are two possible explanations for these observations. First, the tumors could contain a cell hierarchy in which only a minority population of tumor stem cells could self-renew and thus was capable solely of regenerating a tumor. The other cancer cells may have had only limited capacity to replicate and thus contribute to tumor bulk but not to tumor maintainance. Alternatively, the assays used in the studies selected for cells that could grow tumors.

RELATIONSHIP BETWEEN NORMAL AND CANCER STEM CELLS

Defining Properties of Normal Stem Cells

To understand the biology of cancer stem cells, we must define the unique properties of normal stem cells. A normal adult stem cell is defined as a somatic cell that can undergo extensive cell division and has the potential to give rise to both stem cells and cells that differentiate into specialized cells. A normal stem cell must possess two qualities to perform its natural function: self-renewal (e.g., the ability to produce more stem cells) and differentiation. Self-renewal, a special cell division that enables a stem cell to produce another stem cell with essentially the same development and replication potential, is perhaps a stem cell's most important capability. The ability to self-renew enables expansion of the stem cell compartment in response to systemic or local signals, which trigger massive proliferation and maintenance of a tissue-specific undifferentiated pool of cells in the organ or tissue. Although some progress has been made recently in understanding the signals that regulate self-renewal, this remains one of the least understood components of stem cell biology. Differentiation is the second function of a stem cell and involves the production of daughter cells that become tissue-specific specialized cells. The hierarchy in which blood stem cells produce progenitor cells that subsequently produce multiple differentiated cells has been known for many years, and numerous in vitro and in vivo assays have been designed to test the developmental potential of stem and progenitor cells. In the blood system, stem cells first differentiate into transiently amplifying progenitor cells. These cells rapidly proliferate for a short time and produce

Self-renewal: specialized mitotic cell division in which a stem cell creates one (asymmetric) or two (symmetric) daughter stem cells

Differentiation: the overall process of progenitor cells activating genetic and epigenetic mechanisms to define the specialized characteristics of mature cells

terminally differentiated cells, such as basophils and macrophages, that are quiescent or die during normal tissue maintenance or damage. Because in many organs and tissues the cells with the longest life span are stem cells, they are more likely to accumulate the initial transforming mutations than transiently amplifying progenitor cells or mature cells of an organ with a shorter life span.

Theory of Cancer Stem Cells

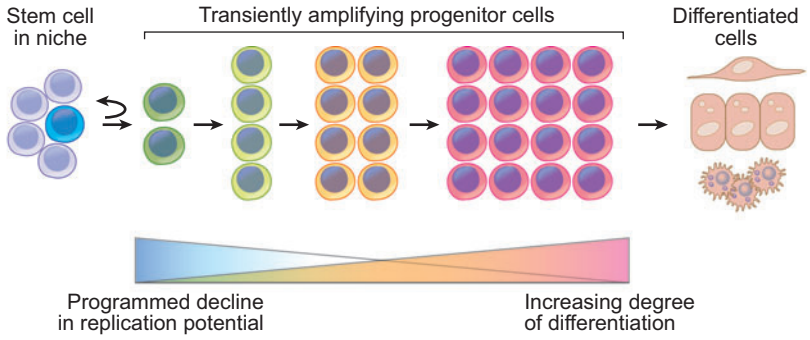
As stated above, malignant tumors arise in tissues containing a cellular hierarchy of long-lived, self-renewing stem cells that differentiate into progenitor cells and mature cells, which have a limited life span. Self-renewal is a specialized type of cell division shared by normal and at least some cancer cells. In normal tissues, this unique replication process allows a stem cell to receive signals from the microenvironment to divide and give rise to either one or two stem cells (Morrison & Kimble 2006). In normal tissues, the expansion of the stem cell pool is restricted to prevent uncontrolled growth (Morrison et al. 2002). This copying mechanism allows for the preservation of the homeostatic pool of stem cells necessary for maintenance of a tissue for the life of an organism. Thus normal tissue is maintained by stem cells. Without stem cells, the tissue or organ would eventually degenerate (**Figure 1*a***).

According to the cancer stem cell theory, a defined subset of cancer cells has the exclusive ability to drive the growth and spread of a tumor, and these cancer stem cells can give rise to cancer cell progeny that are more differentiated and destined to stop proliferating or die because they have limited or no ability to undergo further mitotic divisions. Thus, the cancer stem cell theory holds that some elements of the cellular hierarchy seen in normal tissues are maintained in many tumors. There are two possible ways in which cancer stem cells can arise. Because leukemic cells are capable of differentiating into multiple mature cell lineages and the stem cells in acute leukemia share the expression of some markers with normal stem cells, it was originally postulated that cancer stem cells arose from normal stem cells (Fialkow 1990, Lapidot et al. 1994). As shown in **Figure 1**, this model suggests that mutations allow unregulated expansion of the cancer stem cells derived from normal stem cells. On the basis of the discovery of new markers that allow identification of progenitor cells capable of differentiation into mature blood cells but lacking the ability to self-renew, more recent evidence suggests that in many cancers the cancer stem cells arise from progenitor cells that have gained the ability to self-renew (Cozzio et al. 2003, Huntly et al. 2004, Krivtsov et al. 2006, Lavau et al. 1997, So et al. 2004, Wagner et al. 2006). As shown in **Figure 1*e***, in this refinement of the cancer

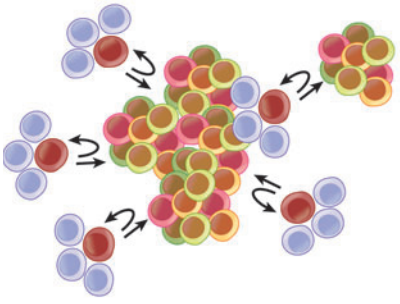
Figure 1

The possible origins of cancer stem cells. (*a*) Normal stem cells. In normal tissues, the niche cells provide signals enabling normal stem cells to self-renew (*curved arrow*). Transiently amplifying progenitor cells do not receive this signal, and their proliferation is constrained by cellular mechanisms that count the number of mitotic divisions. With each cell division, the proliferation capacity of these daughter cells declines, and their degree of differentiation increases. (*b*,*c*,*d*) The possible mechanisms of expansion of cancer stem cells that arise from normal stem cells. (*b*) Expansion of the stem cell niche leads to an expansion of the self-renewing cancer stem cell pool. This pool then gives rise to aberrantly differentiated cancer cells, which are nontumorigenic and comprise the bulk of the tumor. (*c*) Alterations in cancer stem cells enable them to commandeer alternative niche cells to provide self-renewal signals. (*d*) Alterations in cancer stem cells enable them to become niche independent such that they undergo cell-autonomous self-renewal, generating tumors containing self-renewing stem cells and their nontumorigenic progeny. (*e*) Cancer stem cells may arise from progenitor cells. This would occur if multiple oncogenic mutations conferred the ability of self-renewal to progenitor cells. Reprinted from Clarke & Fuller (2006), with permission from Elsevier.

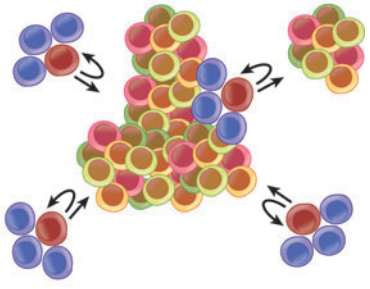
a The dependence of normal stem cells on the niche limits their expansion



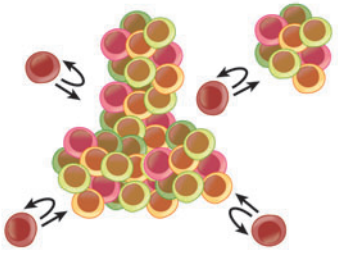
b Expansion of the normal stem cell niche permits the expansion of cancer stem cells that arose from normal stem cells



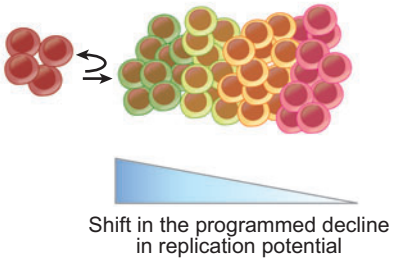
c Cancer stem cells that arose from normal stem cells adapt to a different niche, allowing their expansion

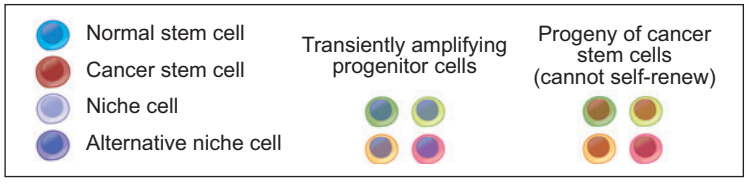


d Cancer stem cells that arose from normal stem cells become niche independent, and self-renewal is cell autonomous



e Cancer stem cells arising from a progenitor cell





HSC: hematopoietic stem cell

stem cell theory, cancer stem cells can also be derived from a progenitor cell that gives rise to self-renewing cancer stem cells, which are capable of generating self-renewing as well as non-self-renewing progeny (Clarke & Fuller 2006, Reya et al. 2001).

Early History of Tumorigenic Stem Cells

Rudolf Virchow, often thought of as the father of modern cellular pathology, was a proponent of the cell theory, which stated that all cells come from other cells, and all organisms are made up of cells. He provided the scientific basis for cancer by correlating clinical outcomes with microscopic findings (Virchow 1858). To explain the origins of "pathological new formations" observed in both tuberculosis and typhoid fever, Virchow compared the observed hyperplasia with differentiating cells in developing epithelium. From his observations, the concept that cancer is a disease that begins from an immature cell was born. This important work seeded the scientific landscape with the concept of cellular hierarchy, a tenet paramount to the cancer stem cell hypothesis.

Till & McCulloch (1961) provided the first experimental models that suggested the existence of normal blood stem cells. The first report was based on a novel method that enabled the detection and counting of multipotent stem cells from the hematopoietic system. Till & McCulloch eradicated the blood system of recipient mice with whole-body ionizing radiation and then injected donor bone marrow cells into the tail veins of irradiated mice to assay how many cells were required to restore blood production. They observed clonally derived clumps (colonies) of blood cells in the spleens of the recipient mice that contained red and white blood cells along with platelets. When individual spleen clones, which were derived from a single cell, were transplanted into secondary recipients, some clones could also give rise to multilineage colonies consisting of all the different mature blood cells. This provided the first evidence for the existence of normal blood, or hematopoietic, stem cells (HSCs). These studies indicated that blood-forming cells were trapped in the spleen of recipients. On the basis of these studies, the hierarchy of the blood stem cell system was established. The eludication of the normal blood system hierarchy provided the framework for investigation of the cancer stem cell theory.

Work performed by Bruce & Van Der Gaag (1963) demonstrated that, like normal bone marrow cells, only a minority of malignant blood cells could form colonies in the spleen of a mouse. Hamburger & Salmon (1977) found that only a small percentage of tumor cells from different epithelial tumors could produce in vitro colonies. Given the similarities of these results from cancer cells with the results of Till and McCulloch, researchers speculated that these clonogenic cancer cells were tumor stem cells. However, one can argue that all the tumor cells were clonogenic in vivo but only some of these tumor cells were capable of growing in a particular assay.

In the 1970s, Pierce & Wallace (1971) showed experimental evidence that cellular hierarchy existed in tumors. By assaying cancer cell proliferation within squamous cell carcinomas in vivo, they found that malignant undifferentiated cells gave rise to benign well-differentiated cells. Potter (1978) championed the concept that "oncology is blocked ontogeny," which arose from the enzymatic similarities of hepatic tumors with fetal liver tissue. By adding halted differentiation into the definition of oncogenesis, researchers took a major step toward establishing tumor cell hierarchy as a fundamental concept in cancer biology.

Mouse teratocarcinoma and human genetic studies provided more definitive evidence for cancer stem cells. The mouse teratocarcimoma cancer model provides a fascinating framework for studying how the cellular microenvironment contributes to oncogenesis. Teratocarcinomas are tumors

that arise from germinal totipotent stem cells upon transplantation (Damjanov 1993). These cells form tumors consisting of immature and mature cell elements upon transplant, which suggests that this model may be particularly useful for studying how the niche affects tumorigenesis. Intriguingly, normally functioning cells may be produced from malignant teratocarcinoma cells if the former are placed into the environment of a normal blastocyst (Illmensee & Mintz 1976, Mintz & Illmensee 1975).

After the initial pathologic studies lost their novelty, cancer etiology remained at the forefront of research efforts. Researchers tried to explain oncogenesis with many competing theories. It was during this time that researchers found a virus that caused a slow-growing neoplasm in chickens, and thus the oncogenic viral field was born. During the latter part of the twentieth century, much attention was placed on physical and chemical carcinogenesis. After this period came a great influx of research that has dramatically expanded our understanding of the genetic mechanisms that underlie cancer development.

Perhaps the first conclusive evidence that a single progenitor cell gives rise to replicating clones that sequentially acquire additional mutations and create a tumor was given by Phillip Fialkow (Fialkow 1990) in studies on chronic myelogenous leukemia (CML) and acute leukemia. CML progresses from a chronic phase characterized by the presence of the Philadelphia chromosome into blast crisis characterized by differentiation arrest, which is often associated with additional genetic and molecular secondary changes (Fialkow et al. 1981) (**Figure 2*b*,*c***). CML initially starts as a relatively indolent disease, called chronic-phase CML, in which patients primarily have increased numbers of mature abnormal white blood cells. However, over time the disease progresses, and patients eventually develop a very aggressive leukemia referred to as blast crisis. The advanced disease resembles an acute leukemia and is characterized by inefficient production of mature blood cells and the accumulation of immature blood cells, called blasts, in the bone marrow. To determine the origin of CML, Fialkow and colleagues used glucose-6-phosphate dehydrogenase (G6PD) isozymes to label and trace the lineage of chronic CML cancer cells (Fialkow 1972). The data suggested that a pluripotent stem cell is initially transformed and produces malignant clonal progeny. In addition, the studies revealed that normal mature differentiated cells, such as red and white blood cells, also contained the leukemic version of the G6PD enzyme, suggesting that the malignant progenitor cell was an individual clone from the normal stem cell pool. During the blast crisis phase, cancerous plasma B cells appeared with the same G6PD isozyme and Philadelphia chromosome mutation as the myelogenous clones (Greaves et al. 1979, Martin et al. 1982, Vogler et al. 1979), suggesting a common malignant cell of origin (**Figure 2*c***). This demonstrated unequivocally the presence of CML cancer stem cells and showed that not all the leukemic cells in those patients could sustain the disease. Similar studies in patients with acute leukemia showed the likely existence of leukemic stem cells (LSCs) in some patients.

CML: chronic myeloid/myelogenous leukemia

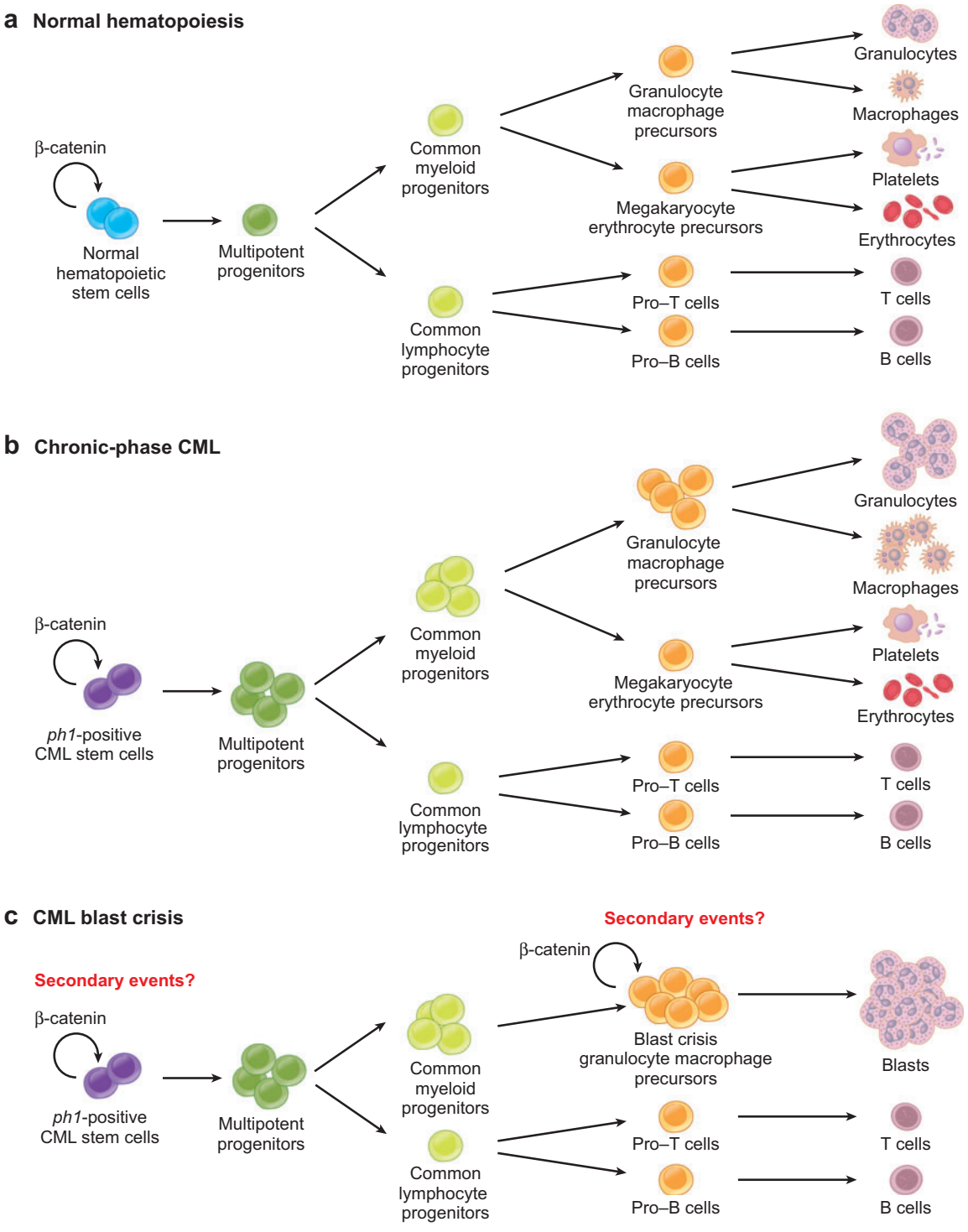
LSCs: leukemic stem cells

AML: acute myeloid leukemia

SCID: severe combined immunodeficiency disease

PROSPECTIVE ISOLATION OF CANCER STEM CELLS

Although LSC isolation and characterization have been reviewed extensively, here we revisit some of the pioneering studies. In 1994, John Dick's group (Bonnet & Dick 1997, Lapidot et al. 1994) reported the prospective isolation of primitive HSCs in acute myeloid leukemia (AML). This initial report utilized the severe combined immunodeficiency disease (SCID) immunodeficient mouse as a model to study the proliferation and self-renewal potential of transplanted human AML cells. On the basis of methods developed by Weissman and colleagues (Baum et al. 1992, Spangrude et al. 1988) to isolate normal blood stem cells,



they employed a phenotypic cell isolation strategy that relied on fluorescently tagged cell surface proteins. This strategy is now widely used in attempting to isolate either normal or cancer stem cells. Consistent with previously reported findings, the authors reported that only a subset of cells were able to transplant AML into recipient mice. These tumorigenic cells were defined as $CD34^+CD38^-$, indicating a presence of CD34 proteins and a lack of CD38 proteins on their surface. The remaining AML cells that grew from the transplanted cells were in various stages of differentiation and contained multiple mature blood cell types. Because the $CD34^+CD38^-$ LSCs comprised a small population, it is unlikely that constraints on self-renewal were corrupted completely. This is another example for which the multiple mature cells found in cancer are an indication of a common transformed progenitor cell. More recent studies have expanded the definition of the LSC in AML to the phenotypic $CD34^+CD38^-CD90^-IL^-3R^+CD71^-HLA^-DR^-CD117^-$ population (Blair & Sutherland 2000; Blair et al. 1997, 1998; Jordan et al. 2000).

Although the authors (Bonnet & Dick 1997, Lapidot et al. 1994) transplanted human AML cells into immune-deficient mice, the leukemias closely resembled the disease of the original patients. Therefore, the transplanted leukemias reflected the effects of the original oncogenic mutations. The authors also reported a 30–100-fold expansion of the $CD34^+CD38^-$ cells within the AML when the cells were transplanted into recipient mice. The expansion may have been caused by selection of tumorigenic clones that adapted to engraftment and growth in mice or acquired mutations that provided a similar growth advantage. These events are not surprising, given the heterogeneity of the LSC population (Hope et al. 2004) and the inherent genomic instability of an uncontrolled population of transforming cells (Bayani et al. 2007). In normal blood development, cells that are $CD34^+CD38^-$ are enriched for HSCs and are different from cells that are $CD34^+CD38^+$, which includes progenitor cells. Further studies from John Dick and colleagues (Hope et al. 2004) used lineage tracing to prove that a single LSC could give rise to the various populations of leukemia cells. They serially transplanted AML cells from patients into NOD/SCID immune-deficient mice. Leukemia-initiating cell heterogeneity reflected the same organizational hierarchy found in normal blood (**Figure 2*a***).

Cellular Origin of Cancer Stem Cells

Various central questions are emerging in the cancer stem cell field: Does cancer always begin from normal stem cells that lose control over their growth and regulatory mechanims? Can progenitor or differentiating cells acquire stem cell attributes through mutations and initiate cancer? Although these questions go to the heart of the cancer stem cell theory, they are very difficult to prove experimentally, mainly because the quantity of normal stem

Figure 2

Cellular hierarchy in normal hematopoiesis and in chronic myelogenous leukemia (CML). Normal hematopoietic stem cells self-renew and differentiate into specialized cells. CML cells contain a cancer stem cell population that self-renews and differentiates. (*a*) Proposed cell compartments in normal hematopoiesis. In normal bone marrow, hematopoietic stem cells give rise to progressively more differentiated progenitor cells, which eventually differentiate into mature blood cells. (*b*) In chronic-phase CML, the size of the cancer stem cell compartment is maintained, and progenitor-cell populations expand. (*c*) In CML blast crisis, secondary events that promote or acquire self-renewing capacity occur in CML stem cells, granulocyte-macrophage precursors, or both. The circular arrows represent self-renewing compartments. *Ph1* denotes the Philadelphia chromosome. Reprinted from Clarke (2004), with permission from *The New England Journal of Medicine*.

and early progenitor cells or cancer stem cells in any given tissue or tumor is usually very small. Because the phenotype of both normal blood stem cells and their leukemic counterparts is $CD34^{+}CD38^{-}$, it was argued initially that the transformation of the stem cell compartment drives AML. However, the phenotype of the LSC population in many patients matches that of progenitor cells, not that of HSCs (Tavil et al. 2006). This suggested that the LSC actually arises from progenitor cells. Alternatively, others suggested that differences between HSC and LSC surface phenotypes reflect a change in surface marker expression in abnormal cells (Blair et al. 1997, Blair et al. 1998, Brendel et al. 1999, Jordan et al. 2000). Recent evidence discussed below strongly favors a progenitor cell origin for many types of LSCs.

Mouse leukemia models provided the first convincing evidence that progenitor cells can give rise to a LSC. As an alternative approach to defining tumor etiology, recent studies have begun gain-of-function assays to assess whether commonly mutated translocations found in human cancer can transform mouse blood progenitor cells, which normally lack the ability to self-renew and thus cannot maintain themselves for long periods of time. These studies try to model cancer by conferring stem cell properties on a cell type that would not normally possess such properties.

Many mutations increase the incidence of leukemia dramatically (Huntly & Gilliland 2005, Miyamoto et al. 2000), which is especially well studied in children who suffer from gross chromosomal or fusion gene translocations (Pine et al. 2003). Researchers identified leukemogenic fusion proteins that could confer self-renewal capability on myeloid progenitor populations. Many chromosomal abnormalities observed in AML involve the mixed-lineage leukemia (MLL) gene in tandem with a variety of other genes. Cozzio et al. (2003) transduced both HSCs and more differentiated granulocyte macrophage progenitors (a subset of myelomonocytic progenitor cells) with a MLL-Erythema Nodosum Leprosum (ENL) fusion minigene known to be strongly associated with AML in human patients. In a transgenic mouse leukemia model, either HSCs or myeloid progenitor cells were transduced with a chimeric minigene encoding MLL-AF9 that resulted from a translocation fusing parts of the MLL and AF9 genes (Krivtsov et al. 2006). In this mouse model, both stem cells and progenitor cells transduced with this leukemia-causing gene could give rise to leukemia, demonstrating that the progenitor cells could indeed give rise to the malignant stem cell. However, compared with HSCs, many more transduced progenitor cells had to be transplanted to establish leukemia. Another study performed by Huntly and colleagues (Huntly et al. 2004) assayed the ability of different leukemogenic genes to transform differentiating progenitor populations. Common myeloid progenitors transduced with a MOZ-TIF2 fusion oncogene could be serially replated in vitro and resulted in transplantable AML in vivo. In contrast, a BCR-ABL fusion gene was not able to transform granulocyte-macrophage cells into leukemia-initiating cells.

These results from experiments using mouse models suggested that progenitor cells need additional mutations to acquire self-renewal ability before they could be transformed into LSCs. However, because HSCs are already capable of self-renewal, it makes sense that it is easier to transform them into LSCs. Nonetheless, not all the leukemia cells in the leukemias arising from these progenitor cells were capable of giving rise to new tumors, suggesting that the LSCs gave rise to both self-renewing and non-self-renewing leukemia cells (Somervaille & Cleary 2006). Hill (2006) has argued that certain phentotypically distinct populations of human cancer cells fail to grow when injected into immunodeficient mice because of differences in the mouse and human microenvironment. Thus, the identification of LSCs in a mouse tumor adds credence to the cancer stem cell model.

New evidence in humans suggests that LSCs can originate from either normal blood stem cells or progenitor cells. Careful lineage analysis of LSCs in human CML by Jamieson, Weissman, and colleagues (Jamieson et al. 2004) provided the strongest evidence for the ability of progenitor cells to give rise to a LSC in human disease. Because the blood stem cell and progenitor cell compartments were extensively characterized, it was possible to isolate different populations of leukemia cells in patients with either chronic-phase or blast-crisis-phase CML. Studying leukemia cells from patients with chronic-phase CML, researchers found that only cells that phenotypically resembled normal blood stem cells were able to form colonies that could be serially transferred in tissue culture. This suggested that the chronic-phase LSC arose from a normal blood stem cell. Surprisingly, in patients with blast crisis CML, leukemia cells resembling a more differentiated blood progenitor cell were the long-lived cell population. These studies suggest that the LSC may change during disease progression. Paradoxically, the LSC in patients with a leukemia dominated by the expansion of a more mature cell population originates from a more immature cell than the LSC in the leukemia dominated by the accumulation of more immature nontumorigenic leukemia cells.

Acute lymphoblastic leukemia (ALL) is the most common cancer in pediatric patients and results from transformed lymphoid progenitor cells. A majority of ALL cases involve the expansion of B cell lymphoid progenitor cells and are characterized by rearrangements of the heavy-chain immunoglobulin gene (Scrideli et al. 1997). A subset of ALL cases are defined by the involvement of the Philadephia chromosome and the BCR-ABL translocation product. Leukemia-initiating cells from Philadelphia chromosome–positive ALL were found exclusively in the $CD34^+CD38^-$ population (Cobaleda et al. 2000). Another study traced T cell receptor rearrangements to identify the origin of leukemia-initiating cells in pediatric patients suffering from ALL (George et al. 2001). The rearrangements were present in both $CD34^+CD38^-$ cells and $CD34^+CD38^+$ cells. Taken together, these data suggest that the leukemia-initiating-cell phenotype is shared with normal HSCs; therefore, the disease originates from the HSC compartment. Another study that analyzed the leukemia-initiating cells in CML found that the LSC compartment may be dynamic: HSCs drive the initial phases of the disease, and other committed compartments are responsible for the disease's progression, presumably because of acquired mutations (Jamieson et al. 2004a).

Multiple myeloma is a human cancer of malignant plasma B cells that expresses CD138, or syndecan-1. Similar to other blood cancers, a minority population of multiple myeloma cells, characterized as $CD138^-$, was enriched for tumor-forming potential over $CD138^+$ cells in vitro and in NOD/SCID mice. This model is interesting because B cells are also a long-lived population in the blood system and, like T cells, are thought of as single-lineage stem cells. Owing to the wealth of information about B cell development and the defined acquisition of surface markers and specification through differentiation, multiple myeloma may be an excellent model with which to study the question, in which stage of cellular differentiation can tumor initiation occur?

CNS: central nervous system

SOLID TUMOR STEM CELLS: TUMOR STEM CELLS IN THE CENTRAL NERVOUS SYSTEM

The brain was long thought to be quiescent tissue, with little turnover during the adulthood of the animal. This dogma was questioned in the 1960s, when neurogenesis was observed in the central nervous system (CNS) of adult rats (Altman 1966, Altman & Das 1965). Stem cells from the human brain were subsequently discovered and isolated (Johansson et al. 1999, Kirschenbaum et al. 1994, Kukekov et al. 1999, Nunes et al. 2003, Pagano et al. 2000, Pincus et al. 1997,

Phenocopy: a regeneration of the parent organ or tumor that represents the phenotypic diversity found in the original

Roy et al. 2000). Successful isolation of human CNS stem cells from both adult and fetal brain has been reported on the basis of CD133 expression (Tamaki et al. 2002, Uchida et al. 2000). The first report of neural stem cells in the context of tissue architecture showed that the subventricular (SVC) zone band in the human brain contains multipotent progenitor cells and these cells display characteristics similar to astrocytes (Sanai et al. 2004). These initial isolations relied on culturing techniques that select cells that grow as nonadherent spheroid colonies, known as neurospheres. Neurospheres can be grown from the expansion of single stem cells and are multipotent; therefore, this assay was used to study self-renewal.

There are a wide variety of brain tumors, ranging from medulloblastomas in children to intraparenchymal neoplasm in elderly patients. Brain cancers originate from germinal and early postnatal CNS zones as well as from the mature zones, where active neurogenesis persists for the lifetime of the animal (Read et al. 2006). For example, gliomas originate from the SVC (Sanai et al. 2005), and medulloblastomas arise from the developing cerebellar external granular layer (Wechsler-Reya & Scott 2001). The hypothesis that an early neural stem or progenitor cell gives rise to tumors is also supported by the observation that glial tumor development arises from a nestin-positive progenitor population rather than from differentiated astrocytes (Holland et al. 2000). Similarly, in a mouse model of glioblastoma, tumors appear to arise from the SVC region, where neural stem and progenitor cells reside (Gil-Perotin et al. 2006).

Taking advantage of neurosphere culture, Dirks and colleagues (Singh et al. 2003) successfully isolated cancer stem cells from different phenotypes of brain tumors. Cancer stem cells were found exclusively in the fraction of cancer cells that expressed CD133 ($CD133^+$ cells). When placed into culture, the $CD133^+$ tumor cells differentiated into cells that phenotypically resembled the original tumor, but the cells that did not express this marker protein ($CD133^-$ cells) failed to do so. The same group tested the ability of $CD133^+$ cells to grow tumors in in vivo xenograft assays. Continuing their studies, the Dirks group (Singh et al. 2004) reported the functional identification of human brain tumor stem cells. By measuring the ability to grow tumors in the brains of NOD/SCID mice, they prospectively identified $CD133^+$ cells as the only ones capable of regrowing a tumor resembling the original human tumor, or phenocopy of the original tumor. The $CD133^+$ population (19–22%) gave rise to a tumor that contained $CD133^+$ and $CD133^-$ tumor cells, showing that $CD133^+$ cells were responsible for repopulating themselves and the $CD133^-$ population. In contrast, the $CD133^-$ tumor cells failed to form tumors, even when 1000-fold more $CD133^-$ cells were injected into the brains of the mice. The xenotransplanted tumors expressed GFAP and nestin, markers of astrocytic and neuronal lineages, respectively. These data showed that the original tumors were initiated by $CD133^+$ cells that could differentiate into $CD133^-$ cells. To test for in vivo self-renewal, the authors performed serial transplants and evaluated the phenotype of the secondary tumors. The data showed that the neural cancer stem cells were always in the $CD133^+$ population. Because both neural stem cells and progenitor cells express CD133, it remains unclear if the target cell of transformation was a stem cell or a progenitor cell.

Another study demonstrated that neurosphere-forming progenitors isolated from glioblastoma multiforme initiate tumors that are phenocopies of the orginal tumor even after two rounds of neurosphere culture (Galli et al. 2004). Interestingly, serial passage of tumors in mice produced neurospheres that grew increasingly faster in culture, indicating that culture conditions or in vitro acquired mutations selected for highly proliferative cells. Recent evidence that radial glial cells may be cultured from human ependymoma-derived neurospheres (Taylor et al. 2005) suggests this technique

may be applied to various neural tumors to isolate neural cancer stem cells successfully.

Cancer Stem Cells in Other Tissues

Cancer stem cells are being identified in a growing list of tumor types. Our report that only a minor fraction of breast cancer cells can form a tumor was the first reported isolation of cancer stem cells from solid tumors. We identified cancer stem cells in breast tumors on the basis of surface marker expression and tumor regeneration potential when these cells were implanted into the mammary fat pads of NOD/SCID mice (Al-Hajj et al. 2003). In eight out of the nine patients examined, a minority population of the breast cancer cells, defined by the $CD44^+CD24^-$ phenotype, was able to form tumors. As few as 100 breast cancer stem cells injected into the breasts of mice formed tumors, although tens of thousands of the other cancer cells were unable to do so. We found that, even after secondary and tertiary transplants, the new tumors were phenocopies of the original cancer, verifying the self-renewal capacity of the tumorigenic population in vivo.

Fang and colleagues (Fang et al. 2005) showed that 20% of the human melanomas evaluated contained a cancer-stem-cell-like fraction. Melanoma stem cells demonstrated self-renewal in vitro and in vivo, retained multipotent potential, grew as spheroid cells, and were enriched for tumor-forming potential. Bapat et al. (2005) identified a single tumorigenic clone from the ascites of a patient afflicted with human ovarian epithelial cancer. The clone grew in an anchorage-independent manner and resembled the original disease when they were serially transplanted. Recently, prostate cancer stem cells have been isolated. The $CD44^+/\alpha_2\beta_1{}^{hi}/CD133^+$ population from both primary and metastatic prostate tumors contained an enriched capacity for tumorigenic properties in vitro (Collins et al. 2005). Although these cells displayed enhanced invasion and proliferation potential in culture, it remains unclear if this population can produce a phenocopy of the original tumor in vivo. The population of $CD44^+/\alpha_2\beta_1{}^{hi}/CD133^+$ cells was reported as 0.1% of the prostate tumors, regardless of the grade of the tumor. Normal prostate epithelial stem cells share the marker $\alpha_2\beta_1{}^{hi}$, indicating that the origin of prostate tumors may be the transformation of stem or progenitor cells (Collins et al. 2001). Building on the principle in in vitro sphere-forming assays, Gibbs and coworkers (Gibbs et al. 2005) were able to isolate a small population of bone sarcoma cells capable of clonal spheroid growth in serum-starved culture conditions. These sarcosphere cells expressed activated STAT3 and the embryonic-stem-cell-associated genes *Oct 3/4* and *Nanog*. A subset of bone sarcoma cells expresses the mesenchymal stem cell markers Stro-1, CD105, and CD44. Taken together, these data suggest that cancer stem cells often share similar markers with progenitors of their original tissue, as exemplified by the above-mentioned blood and brain tumors.

Recently, two groups reported the isolation of colon cancer stem cells on the basis of the expression of CD133. $CD133^+$ cells accounted for approximately 2.5% of the total tumor cells and produced phenocopies of the original tumor when the $CD133^+$ cells were transplanted subcutaneously into NOD/SCID mice (Ricci-Vitiani et al. 2007). The $CD133^-$ population could not form tumors when transplanted. The $CD133^+$ cells grew passageable sphere-forming cells that remained undifferentiated for more than one year and were able consistently to grow in vivo tumors that were similar phenotypically to the original tumor. In a renal capsule xenograft model, $CD133^+$ cells could also generate tumors, but $CD133^-$ cells were unable to do so (O'Brien et al. 2007). Limiting dilution analysis showed the frequency of colon-cancer-initiating cells as 1 in 54,000 tumor cells and 1 in 262 $CD133^+$ cells. These data showed a 200-fold enrichment of colon cancer stem cells through the use of the CD133 marker.

SIGNALING PATHWAYS IN CANCER STEM CELL BIOLOGY

Master Control Signaling Pathways that Regulate Cancer Stem Cell Functions

Because normal stem cells and cancer stem cells must renew themselves, it is reasonable to assume that they share some molecular mechanisms that regulate this critical stem cell function. Stem cells are minority populations; thus, the inherent difficulty of studying molecular pathways with such a small amount of cells has dramatically slowed progress. Even with these drawbacks, multiple crucial pathways have been elucidated in the biology of cancer stem cells and their normal counterparts (**Table 1**). Polycomb-group genes were initially discovered in *Drosophila* as repressors of the Homeobox genes and then discovered in mammals as well. Polycomb-group genes repress the expression of their target genes through chromatin modifications. *Bmi-1* (*PCGF4*), a member of the Polycomb-group protein family, is crucial to the self-renewal of HSCs and neural stem cells (Molofsky et al. 2003, Park et al. 2003) as well as mouse LSCs (Lessard & Sauvageau 2003b). *Bmi-1* is necessary for self-renewal in both HSCs and LSCs, as shown by suppression of the *Ink4a/ARF* locus (Lessard & Sauvageau 2003a). In patients with AML, expression of *Bmi-1* is higher in AML cells than in normal bone marrow (Park et al. 2003, Sawa et al. 2005). Intriguingly, AML can be produced in *Bmi-1*$^{-/-}$ mice but cannot be serially transplanted, suggesting that *Bmi-1* may be important in LSC self-renewal (Lessard & Sauvageau 2003a, Park et al. 2004).

Growing evidence in blood and brain supports the hypothesis that somatic stem cells may be more sensitive to senescence pathways than are more differentiated progeny (Molofsky et al. 2003, Ohtani et al. 2004, Park et al. 2003). Expression of p16^{Ink4a}, a cell cycle inhibitor, is elevated in *Bmi-1* mutant mice. Recent studies showed that p16^{Ink4a} plays a role in stem cell senescence in the blood, brain, and pancreatic islet cells (Janzen et al. 2006, Krishnamurthy et al. 2006, Molofsky et al. 2006).

Sonic hedgehog (Shh) expands human blood progenitors in immunocompromised mice (Bhardwaj et al. 2001). In the brain, Shh appears to activate *Bmi-1* (Leung et al. 2004). The Shh signaling pathway is crucial to the embryonic development of skin, hair follicles, and sebaceous glands (Athar et al. 2006) and is involved in postnatal and adult brain development (Palma et al. 2005). Mutation of *SHH* causes Gorlin's syndrome, whereas activation of *SHH* has been implicated in both skin and brain carcinogenesis (Ruiz i Altaba et al. 2002).

The Wnt/β-catenin pathway, which is associated with many types of cancer, is also implicated in self-renewal (Taipale & Beachy 2001). Secreted Wnt ligands bind to Frizzled receptors and activate a cascade important in development. Wnt inhibitors retard hematopoietic reconstitution in vivo. Wnt signaling increases the expression of *HoxB4* and *Notch-1* genes. Both of these proteins are implicated in the specification and/or self-renewal of HSCs (Reya et al. 2003). In addition, the Wnt/β-catenin pathway is involved in the maintenance of normal intestinal epithelial cells and in regenerative responses during tissue repair (de Lau et al. 2007, Reguart et al. 2005).

Wnt signaling also plays a role in blood diseases and colon carcinoma. Activating mutations of *β-catenin* or inactivating mutations of the *adenomatosis polyposis coli* (*APC*) gene, which targets β-catenin for degradation, occur in a large percentage of colon cancers (Kolligs et al. 1999). In CML, β-catenin accumulates in granulocyte-macrophage progenitor cells when CML progresses to blast crisis, showing that molecular mechanisms can transform committed progenitors into LSC (Jamieson et al. 2004a). β-Catenin accumulation has also been associated with breast cancer, melanoma, sarcoma, myeloid leukemia, multiple myeloma, and brain tumors (Reguart et al. 2005). Chan and

Table 1 Signaling pathways associated with normal stem cells and cancer[a]

Pathway	Normal stem cells	Cancer
Bmi-1	Bmi-1 is required for self-renewal of hematopoietic and neural stem cells	Bmi-1 is upregulated in AML and overexpressed in medulloblastoma
	Bmi-1 downregulates the Ink4a/Arf locus	Overexpressed Bmi-1 and cell proliferation induce self-renewal of leukemic stem cells
Shh	Involved in the maintenance of hematopoietic stem cells and expansion of progenitors	Activation of SHH is implicated in skin and brain carcinogenesis, including basal cell carcinoma of skin and medulloblastoma
	Crucial in embryonic development of skin, hair follicle, and sebaceous gland	Mutation of SHH causes Gorlin's syndrome
	Involved in postnatal and adult brain development	
Wnt/β-catenin	Involved in the maintenance and self-renewal of hematopoietic stem cells and progenitor cells	Overexpression of WNT is seen in many human cancers
	Regulates the maintenance of normal intestinal epithelial cells	Accumulation of β-catenin is associated with breast cancer, melanoma, sarcoma, myeloid leukemia, multiple myeloma, and brain tumors
	Implicated in regenerative responses during tissue repair	Mutations in β-catenin are found in endometrial carcinomas, prostate carcinoma, and hepatocellular carcinomas
		Mutations of both β-catenin and APC genes are common in colorectal cancer
Notch	Mediates the self-renewal of hematopoietic and neural stem cells	Mutations or aberrrant activation of Notch1 are known to cause T-ALL in human and mouse
	Activates Notch target genes involved in T cell differentiation and self-renewal	
Hox family	Involved in the self-renewal of hematopoietic stem cells and the proliferation and differentiation of precursor cells	Overexpression of HOXA9 is found in AML patients with poor prognosis
		Overexpression of HOX11 is described in T-ALL with chromosome translocations
		Hoxb3, Hoxb8, and Hoxa10 are associated with leukemogenesis in a mouse model
Pten	Implicated in the maintenance of hematopoietic stem cells and neural stem cells	Loss of PTEN leads to the formation of a variety of tumors, including myeloproliferative disease, and the emergence of transplantable leukemia
		Mutation and/or LOH cause glioblastoma multiforme, prostate carcinoma, and endometrial carcinoma
Efflux transporters	Marker proteins are found in self-renewing stem cells, such as ABCG family proteins, responsible for the side-population phenotype	Upregulated ABCG2, ABCB1, and CEACAM6 are found in cancer cells from the gastrointestinal system
		Upregulated ABCG is implicated in broad-spectrum chemoresistance of cancer cells, such as AML cells
Telomerase	Expressed at a high level in normal self-renewing populations in the blood	Expressed at a high level in tumor cell populations with upregulated mRNA expression
		hTERT is involved in tumorigenic transformation
		Upregulated telomerase activity is found in glioblastoma

[a]Abbreviations used: AML: acute myeloid leukemia; APC: adenomatous polyposis coli; hTERT: human telomerase reverse transcriptase; LOH: loss of heterogeneity; PTEN: phosphatase and tensin homolog deleted from chromosome 10; Shh/SHH: sonic hedgehog; T-ALL: T cell acute lymphoblastic leukemia; WNT: Wingless-Int.

Side population: a functional definition based on cells' ability to efflux dyes such as Rhodamine 123 or Hoechst 33324

colleagues (Chan et al. 1999) showed that mice expressing activated *β-catenin* developed de novo skin tumors resembling pliomatricomas, and LEF-1 was found in dividing tumor cells. The *APC* gene is mutated early in the development of most colon tumors. Mutations of *β-catenin* are also observed in endometrial, prostate, and hepatocellular carcinomas (Reguart et al. 2005).

The Notch signaling cascade is a transmembrane system widely shared by various animal cells for regulating embryonic development and adult maintenance of homeostasis. The Notch signaling pathway is well conserved from nematodes to humans. In mammals, four Notch receptors (Notch 1–4) physically bind four Notch ligands. A number of studies of gene-modified animal models demonstrated the roles of the Notch signaling pathway in stem cells and early progenitor cells. These gene modifications influence HSC and melanocyte generation, CNS and vasculature development, organogenesis during embryogenesis, adult hematopoietic and immune systems, intestinal mucosal systems, and skeletal muscle, skin, and hair systems (Chiba 2006). Notch signaling regulates neural stem cell expansion in vivo and in vitro (Androutsellis-Theotokis et al. 2006). Mutations or aberrant activation of the Notch signaling pathway causes T-ALL, indicating the contribution of this pathway to both normal development and carcinogenesis.

The *Hox* gene family is important in normal and malignant hematopoiesis. Several translocations in human leukemia involve *HOX* genes such as *HOXA9* in AML (Nakamura et al. 1996). In mouse models, aberrant expression of Hox genes affects the proliferation and differentiation of HSCs (Lawrence et al. 1997). In mouse bone marrow cells, overexpression of several members of the *Hox* gene family results in the expansion of HSCs and myeloid precursor cells. Overexpression of some of these genes, such as *HoxB6*, culminates in AML after approximately seven months (Fischbach et al. 2005), suggesting that genes responsible for stem cell proliferation are directly involved in AML initiation. Overexpression of *HOX11* is described in T-ALL with chromosome translocations. In a mouse model, *Hoxb3*, *Hoxb8*, and *Hoxa10* are associated with leukemogenesis (Huntly & Gilliland 2005).

Other Functional Pathways Affect Cancer Stem Cell Behavior

In mice, loss of expression of Pten drives aberrant self-renewal of HSCs and eventually leads to leukemia (Zhang et al. 2006). Pten normally functions by limiting the activity of inositol triphosphate signaling, resulting in constitutive activation of the inositol triphosphate signaling pathway. Loss of Pten initially promotes adult HSC proliferation, but after a brief period of time the HSC pool is exhausted, suggesting that Pten is important in HSC maintenance (Yilmaz et al. 2006). However, loss of Pten eventually causes myeloproliferative disease and the emergence of a transplantable leukemia. Importantly, inhibition of the inositol triphosphate signaling pathway appears to affect the maintenance of the leukemia cell compartment but does not eliminate the normal HSC pool (Rossi & Weissman 2006). Mutations and/or loss of heterogeneity of Pten can cause glioblastoma multiforme, prostate carcinoma, and endometrial carcinoma (Chow & Baker 2006).

In addition to self-renewal, studies have identified common efflux pathways that both normal and cancer stem cells may share. The ABCG family of transporters is mainly responsible for the side-population phenotype observed in some stem-cell-derived organs. The basis for ABCG family proteins as markers for some, but not all, self-renewing stem cell populations comes from the idea that long-lived cell populations are under constant bombardment from genotoxic chemicals, and thus it is more efficient to efflux these chemicals from the cell rather than process them through cytoplasmic degradation machinery. When both blood and solid organs are dissociated and stained with dyes that are

known to be effluxed, the minor population of cells with high efflux efficiency is revealed. Recent evidence showed that side-population cells from human cancer cell lines have upregulated ABCG2, ABCB1, and CEACAM6, which are all efflux transporters implicated in broad spectrum chemoresistance (Haraguchi et al. 2006). However, it is not clear at this time how common it is for cancer stem cell populations to overexpress these transporters.

Telomere shortening has been implicated in replicative senescence, chromosome instability, and arrest of the cell cycle (Counter et al. 1992). Because stem cells live for long periods of time, in part by activating telomerase, telomerase may play an important role in cancer stem cell biology as well. Telomerase, an enzyme that adds terminal repeats to the end of telomeres, is expressed at high levels in normal self-renewing blood cell populations (Allsopp et al. 2003, Morrison et al. 1996) and tumor cell populations (Kim et al. 1994). The telomerase protein extends the life of somatic human cells (Bodnar et al. 1998), and telomerase mRNA expression is upregulated in tumor cells (Saeboe-Larssen et al. 2006, Xin et al. 2006). Activation of hTERT, the catalytic subunit of telomerase, is involved in tumorigenic transformation of cultured human skeletal muscle myoblasts and mammary cells (Kendall et al. 2005). In addition, telomeric repeat assays revealed that telomerase activity is upregulated in glioblastoma multiforme–derived cultured neural cells as well as in cells from original tumors but is undetectable in human fetal neural stem cells, suggesting that telomerase is also a key component in the cancer stem cell population (Galli et al. 2004). Taken together, these studies point to a molecular model in which the telomerase molecular mechanism that helps to define normal stem cells also defines cancer stem cells.

CLINICAL IMPLICATIONS OF CANCER STEM CELLS

Evidence that many cancers are driven by cancer stem cells has important clinical implications. Clinical treatment regimens operate under the assumption that all cancer cells have equal malignant potential. These treatments suffer from their lack of specificity for only tumorigenic cells. Relatively successful cancer treatments shrink the bulk of tumor cells but often fail to eliminate the cancer stem cells, resulting in the recurrence of tumors. For example, Gleevec, which targets the BCR-ABL tyrosine kinase in CML, blocks the growth signals that the aberrant protein generates. Although this drug helps to maintain long-term remissions of CML, it may not be curative (Copland et al. 2005).

The question of whether a stem cell or a progenitor cell initiates cancer will be crucial for future clinical therapy. If a progenitor acquires the ability to self-renew, this mutation could be therapeutically targeted. Unfortunately, mutations that endow cells with self-renewal remain unidentified. In normal tissues, gene expression and activity of signaling pathways may differ among the stem cells, progenitor cells, and terminally differentiated progeny. Although studies are beginning to profile transcriptional differences between cancer-initiating cells and their progeny, no research that assays the functional significance of these genes has been reported.

The prospective isolation of HSCs has played a central role in the identification of regulators of stem cell self-renewal (Calvi et al. 2003, Cheng et al. 2000, Park et al. 2002, Park et al. 2003). One can infer that the prospective isolation of cancer stem cells should help in better defining pathways important for cancer stem cell functions. Recently, we identified a gene expression signature derived from human breast cancer stem cells. Remarkably, this signature could be used to stratify high-risk and low-risk patients with early-stage breast cancer. Interestingly, if the gene expression profile of a patient's tumor is similar to the cancer stem cell gene signature, the patient has a high risk of relapse (Liu et al. 2007). Furthermore, when the cancer stem cell gene signature is combined with a wound repair signature derived from activated

stromal fibroblasts, the prognostic ability of the two signatures is synergistic. This observation demonstrated for the first time that the interaction of cancer cells with the tumor stroma has clinical relevance.

Refractory tumors may be caused by the resistance of cancer stem cells to commonly used cytotoxic therapies. Jordan and colleagues (Jordan et al. 2000) have shown that two commonly used treatment agents, cytarabine and adriamycin, preferentially kill the leukemic blasts, whereas the LSCs are relatively resistant (Costello et al. 2000). A study of glioblastoma stem cells demonstrated that this is also true for solid tumor stem cells. $CD133^+$ glioblastoma stem cells are relatively resistant to cytotoxic agents compared with their nontumorigenic cancer cell counterparts (Bao et al. 2006). Similarly, brain stem cells are also relatively resistant to γ-irradiation. Both the normal and the malignant stem cells are able to repair double-strand breaks caused by γ-irradiation, probably through increased activity of the ataxia telangiectasia mutated (ATM) DNA repair pathway. Conversely, inhibition of c-src tyrosine kinase (CSK) homologous kinase (CHK), an enzyme involved in the activation of ATM, makes the normal and glioblastoma stem cells more sensitive to radiation (Bao et al. 2006).

CONCLUSIONS

Cancer results from unregulated expansion of a self-renewing cancer stem cell population. The property of self-renewal is shared with normal stem cells. The observation that not all solid cancer cells can establish or maintain tumor growth should help us to define critical pathways that drive the growth and spread of a tumor. The ability to isolate both normal and cancer stem cell populations should enable the identification of differences in self-renewal mechanisms in each cell type. This could provide clues to specific therapeutic targets that can more effectively target and eliminate cancer stem cells while sparing normal stem cells. Such targeted therapies could be much less toxic and more effective than current treatment modalities.

The ability to prospectively isolate cancer stem cells and analyze their growth in xenograft tumor assays could be used to study the biological effects of oncogenic mutations on cancer stem cell functions. This is a powerful tool for better understanding the biological function of a particular gene mutation. It is likely that some oncogenic mutations, such as the activation of *Bmi-1* or the inactivation of some of its downstream targets, play a role in cancer stem cell maintenance. It is also likely that some oncogenic mutations play a role in proliferation but are not required for stem cell maintenance. Studying the function of a particular mutation in cancer stem cells is important because therapies targeting a mutation that disrupts a cancer stem cell maintenance pathway should eliminate the tumor-forming cells and possibly be curative. In contrast, if a therapy targets a mutation that regulates only the rate of proliferation, then it would be at best palliative.

SUMMARY POINTS

1. A tissue stem cell must possess two qualities to perform its natural function: self-renewal (e.g., the ability to produce more stem cells) and differentiation.
2. The cancer stem cell hypothesis predicts that long-lived stem cells are more likely to accumulate the initial mutations leading to cancer than their short-lived differentiated progeny.
3. Transiently amplifying cells are immediate daughters of somatic stem cells. Growing evidence shows these daughter cells inherit parental mutations and may serve as targets for the final transforming events that give rise to a tumor.

4. Transplantable leukemia is not a result of acquired mutations selected for upon transplantation and subsequent proliferation but rather is a reflection of the genetic mutations inherent in the original LSC population.
5. Multiple signaling pathways are crucial in the biology of cancer stem cells and their normal counterparts.

DISCLOSURE STATEMENT

M.F.C. is a member of the paid advisory board of Oncomed Pharmaceuticals, Inc., and owns stock options in the company.

ACKNOWLEDGMENTS

N.L. was supported by funds from the Department of Defense Breast Cancer Research Program Predoctoral Traineeship Award, grant number DAMD17-03-1-0340. Y.S. was supported by funds from the California Breast Cancer Research Program of the University of California, grant number 12FB-0053. M.F.C. was supported by funds from the NIH/NIDDK, grant number DK53074, and the NIH, grant numbers CA100225, CA104987, and CA126524. Additional support was provided by the Breast Cancer Research Foundation.

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