Cancer Stem Cells: Models and Concepts

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self-renewal, aberrant differentiation, functional heterogeneity of tumor tissues, targeting of antitumor treatments

Abstract

Although monoclonal in origin, most tumors appear to contain a heterogeneous population of cancer cells. This observation is traditionally explained by postulating variations in tumor microenvironment and coexistence of multiple genetic subclones, created by progressive and divergent accumulation of independent somatic mutations. An additional explanation, however, envisages human tumors not as mere monoclonal expansions of transformed cells, but rather as complex tridimensional tissues where cancer cells become functionally heterogeneous as a result of differentiation. According to this second scenario, tumors act as caricatures of their corresponding normal tissues and are sustained in their growth by a pathological counterpart of normal adult stem cells, cancer stem cells. This model, first developed in human myeloid leukemias, is today being extended to solid tumors, such as breast and brain cancer. We review the biological basis and the therapeutic implications of the stem cell model of cancer.

DEFINITION OF "CANCER STEM CELLS"

Many human tissues undergo rapid and continuous cell turnover. In the colonic mucosa or in the peripheral blood, for example, the average life span of a mature, differentiated cell (e.g., a goblet cell in a crypt of the large intestine or a circulating granulocyte) can be measured in days or even hours. Despite the ephemeral nature of most of their individual cell components, human tissues maintain their mass and architecture over time through a tightly regulated process of renovation. Under physiological conditions, this process is sustained by a small minority of long-lived cells with extraordinary expansion potential, known as stem cells. Stem cells are defined by three main properties:

1. differentiation—the ability to give rise to a heterogeneous progeny of cells, which progressively diversify and specialize according to a hierarchical process, constantly replenishing the tissue of short-lived, mature elements;
2. self-renewal—the ability to form new stem cells with identical, intact potential for proliferation, expansion, and differentiation, thus maintaining the stem cell pool;
3. homeostatic control—the ability to modulate and balance differentiation and self-renewal according to environmental stimuli and genetic constraints.

Like their normal tissue counterparts, tumors are composed of heterogeneous populations of cells that differ in their apparent state of differentiation. Indeed, the differentiation features of a tumor, morphological and architectural, are the key parameter used in routine clinical practice by surgical pathologists to define a tumor's primary anatomical origin. This simple observation suggests that tumors are not mere monoclonal expansions of cells but might actually be akin to "abnormal organs," sustained by a diseased "cancer stem cell" (CSC) population, which is endowed with the ability to self-renew and undergo aberrant differentiation (1, 2). This hypothesis is further reinforced by the fact that cancer is known to result from the accumulation of multiple genetic mutations in a single target cell, sometimes over a period of many years (3). Because stem cells are the only long-lived cells in many tissues, they are the natural candidates in which early transforming mutations may accumulate.

Our limited knowledge of normal stem cells, in part due to the overall paucity of experimental assays for their functional study, has made the CSC theory difficult to probe. A new wave of studies, however, has recently begun to address this concept using an innovative, purely empirical approach, based on an in vivo self-renewal assay (4). Starting from whole tumor tissues, cancer cells are purified into single-cell suspensions and subsequently fractioned in different subsets according to the expression of a specific repertoire of surface markers. Once isolated, individual cancer cell subsets are injected into appropriate hosts (in most cases orthotopic tissues of immunodeficient mouse strains), and the subsets are compared with respect to tumorigenic capacity. According to the CSC model, only a specific subset of the cancer cell population (i.e., the long-lived CSC subset) should be able to sustain in vivo tumor growth, whereas all other subsets (i.e., the tumor counterparts of short-lived differentiated cells) should not. Indeed, this assumption has now been repeatedly confirmed in several tumor systems. Three key observations classically define the existence of a CSC population:

1. Only a minority of cancer cells within each tumor are usually endowed with tumorigenic potential when transplanted into immunodeficient mice.
2. Tumorigenic cancer cells are characterized by a distinctive profile of surface markers and can be differentially and reproducibly isolated from nontumorigenic ones by means of flow cytometry or other immunoselection procedures.
3. Tumors grown from tumorigenic cells contain mixed populations of

tumorigenic and nontumorigenic cancer cells, thus recreating the full phenotypic heterogeneity of the parent tumor.

It is important to note that, based on this approach, the term cancer stem cells represents a working definition with a purely operational significance. The term is used to indicate a tumor-initiating cell subset that can give rise to a heterogeneous progeny, similar in composition to the tissue from which it was originally isolated. In most cases, it is currently not possible to define with certainty the "genealogical" relationship between CSCs and normal stem cells of the corresponding tissues (i.e., whether CSCs originate directly from normal stem cells or the early stages of their progeny). Irrespective of the actual origins of CSCs, the identification of a CSC population establishes a functional hierarchy within a tumor tissue and encompasses both the self-renewal and differentiation hallmarks of stem cells. First developed in human myeloid leukemias, the CSC working model is today being progressively extended to several solid tumors, along with several biological and therapeutic implications.

DEVELOPMENT OF THE CANCER STEM CELL MODEL IN HUMAN MYELOID LEUKEMIAS

Normal Hematopoietic Stem Cells

Among mammalian tissues, the hematopoietic system is the first- and best-characterized in terms of hierarchical organization and sequential differentiation of cellular subpopulations. Starting in the 1960s, transplantation experiments in mice demonstrated the existence in the bone marrow of clonogenic precursors capable of remarkable long-term expansion and multipotent myelo-erythroid differentiation (5). These studies allowed progressive functional and phenotypic dissection of blood's distinct cellular lineages and lineage precursors (6). They provided most of the conceptual framework and the technical terminology for the interpretation of adult stem cell biology. They were founded on two main observations:

1. In lethally irradiated mice, where native bone marrow has been completely eradicated, all the different hematopoietic lineages can be fully and permanently reconstituted by transplantation of a very small population of cells, representing as little as 0.05% of total bone marrow. This cell population is characterized by a specific surface marker phenotype that, remarkably, is negative for expression of all lineage-specific differentiation antigens (Lin^{neg}) (7).
2. Cells capable of multi-lineage reconstitution can be further subdivided into two distinct functional groups: long-term reconstituting multipotent progenitors, also defined as true hematopoietic stem cells (HSCs), and short-term or transiently reconstituting multipotent progenitors (MPPs). Both HSCs and MPPs can sustain the production of the full spectrum of blood cells, but reconstitution driven by HSCs is permanent and can last for the entire life of the transplanted animal, whereas reconstitution driven by MPPs is temporary and is usually exhausted after 2–3 months (8). Most importantly, HSCs and MPPs are organized according to a hierarchical lineage: HSCs give rise to MPPs (9).

Taken together, these observations indicate that the long-term, continuous renovation of a complex, functionally heterogeneous tissue (blood) can be sustained by a tiny, undifferentiated population of cells (HSCs), which is capable of both self-renewal and differentiation into a numerically large progeny. According to this model, the differentiation process takes place in two steps: first the loss of self-renewal capacity (the differentiation of HSCs into MPPs), then the actual progressive differentiation into the full spectrum of blood cells, which involves multiple stages and

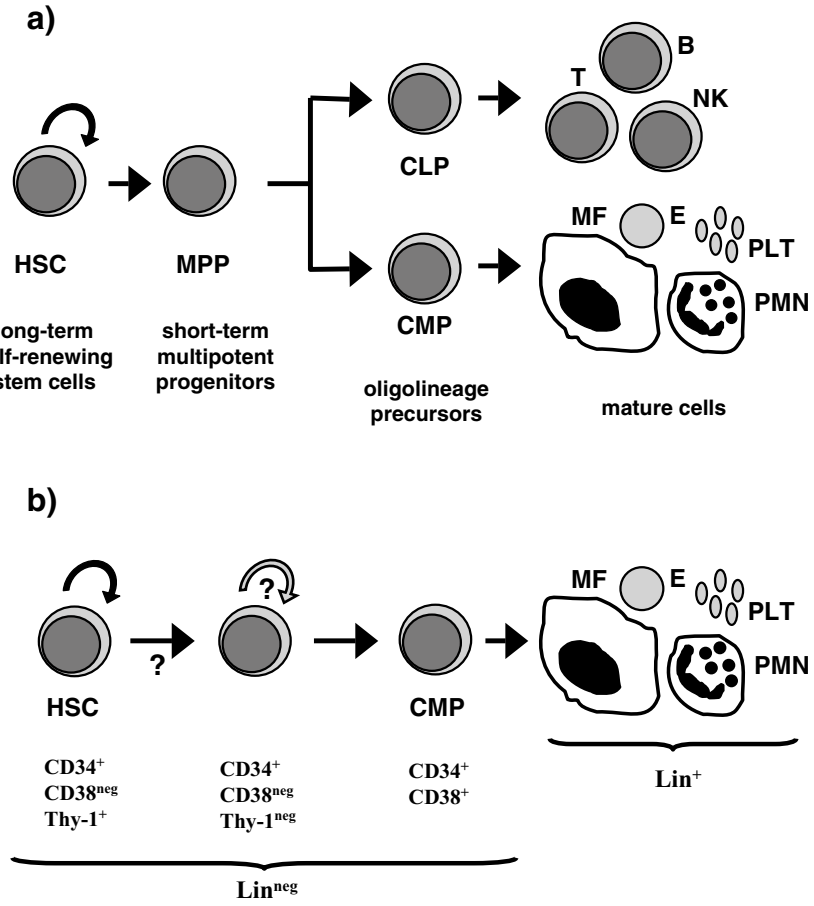

Figure 1

Models of normal and malignant hematopoiesis. (*a*) Blood cells originate from hematopoietic stem cells (HSCs). HSCs differentiate into multipotent progenitors (MPPs), which in turn give rise to oligo-lineage precursors, such as the common lymphoid progenitor (CLP) and common myeloid progenitor (CMP). CLPs differentiate into T, B, and NK cells, whereas CMPs differentiate into macrophages (MF), neutrophils (PMN), erythrocytes (E), and platelets (PLT). (*b*) In humans, the early stages of hematopoiesis are still incompletely characterized. Bone marrow [$CD34^+$, $CD38^{neg}$, Lin^{neg}] cells contain a HSC population, while $CD38^+$ and Lin^+ fractions contain committed progenitors and mature cells. Within the [$CD34^+$, $CD38^{neg}$, Lin^{neg}] population, HSCs are enriched in the Thy-1^+ fraction. The origin of Thy-1^{neg} elements within [$CD34^+$, $CD38^{neg}$, Lin^{neg}] cells is uncertain, as is their capacity to self-renew.

the generation of intermediate oligo-lineage precursors (**Figure 1*a***). The profile of surface markers used to differentiate and purify mouse HSCs has evolved over time, varies among authors, and is undergoing continuous refinement (10). A detailed description of the mouse HSC surface marker profile is beyond the scope of this article but there are several comprehensive reviews of this subject (6, 11).

Initially developed in mice, the stem cell model of hematopoiesis has been adapted to humans, although the impossibility to perform in vivo competitive repopulation assays has limited a detailed functional dissection of all differentiative stages. Most studies on human HSCs have been performed using surrogate in vitro and in vivo studies, such as clonogenic assays on stromal feeder

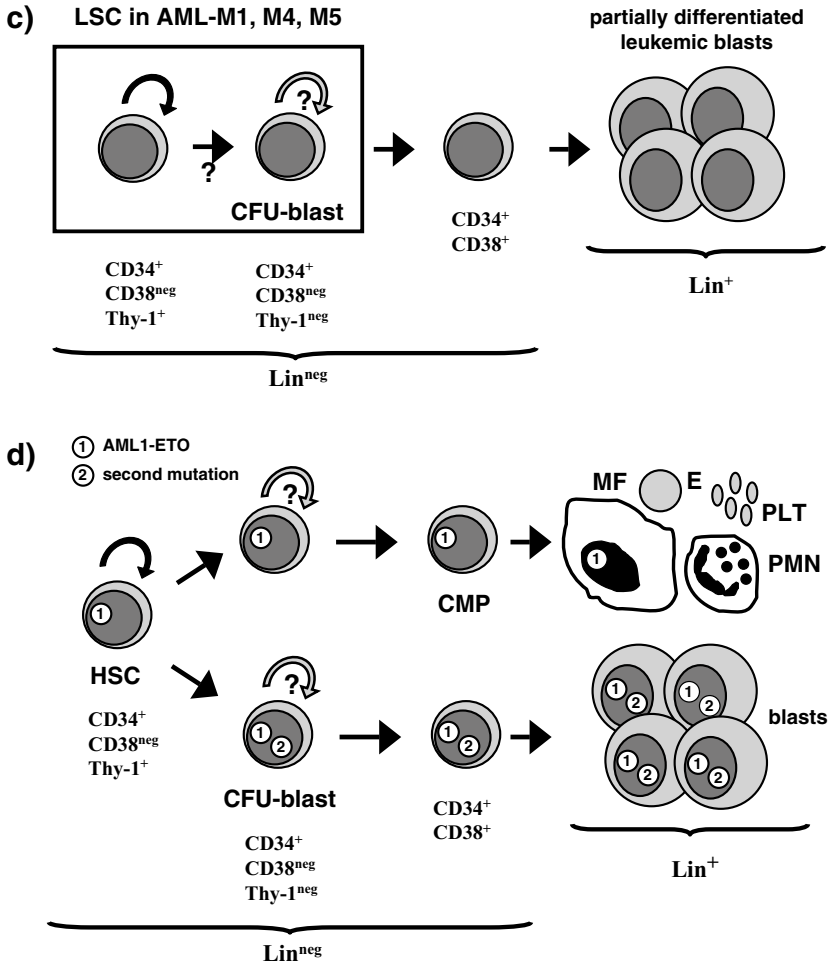

Figure 1 (*Continued*)

(*c*) In several forms of human acute myeloid leukemia (AML), such as the M1, M4 and M5 variants, the [$CD34^+$, $CD38^{neg}$, Lin^{neg}] fraction contains a leukemic stem cell (LSC) population, but it is not clear whether LSCs are Thy-1^+ or Thy-1^{neg}. Contrary to normal HSCs, human AML colony-forming unit (CFU) blast cells are usually Thy-1^{neg}. (*d*) Studies on AML1-ETO^+ AML provide insights into leukemic hematopoiesis. In patients undergoing complete remission, Thy-1^+ HSCs harbor the AML1-ETO chromosomal translocation but undergo normal differentiation, indicating that AML1-ETO is not sufficient to induce leukemia. The bone marrow of the same patients at diagnosis is infiltrated by AML1-ETO^+ CFU-blasts that are [$CD34^+$, $CD38^{neg}$, Lin^{neg}] and Thy-1^{neg}. This observation suggests that full leukemic transformation is likely caused by a second mutation either targeting more differentiated Thy-1^{neg} precursors, or causing loss of Thy-1 expression in LSCs.

layers and repopulation experiments in immunodeficient (SCID, NOD-SCID) mice (6, 12). **Figure 1*b*** illustrates a simplified, tentative consensus model of human myeloid hematopoiesis. It is widely accepted that the [$CD34^+$, $CD38^{neg}$, Lin^{neg}] fraction of human bone marrow cells contains a HSC population, whereas the $CD38^+$ and Lin^+ fractions contain more committed progenitors and mature elements (6, 13–15). Within the [$CD34^+$, $CD38^{neg}$, Lin^{neg}] population, HSCs appear particularly enriched in the Thy-1^+

SCID mouse: severe combined immunodeficiency mouse; an immunodeficient mouse strain, characterized by lack of B and T lymphocytes

NOD-SCID mouse: nonobese diabetic SCID mouse; an immunodeficient mouse strain characterized by lack of B, T, and NK lymphocytes

Hematopoietic stem cell (HSC) mobilization: a procedure by which HSCs are induced to exit the bone marrow and enter blood circulation, usually by cytokine administration

fraction (6, 16), and it is known that transplantation of purified [Thy-1^{+}, CD34^{+}, Lin^{neg}] cells from peripheral blood after HSC "mobilization" is capable of long-term reconstitution of hematopoiesis in human patients (17). The nature of [Thy-1^{neg}, CD34^{+}, CD38^{neg}, Lin^{neg}] cells remains uncertain. It is not clear whether they derive from [Thy-1^{+}, CD34^{+}, CD38^{neg}, Lin^{neg}] cells, nor if they can self-renew in vivo.

Cancer Stem Cells in Human Myeloid Leukemias

In the 1970s, based on the available knowledge of normal hematopoiesis, Fialkow and colleagues began to address the possible relationship between HSCs and human leukemias. They showed that diseases as diverse as chronic myeloid leukemia (CML), acute myeloid leukemia (AML), essential thrombocythemia, and polycythemia vera were all characterized by the expansion of a monoclonal population of cells that contained multiple lineages of differentiated mature blood elements (18–21). In the early 1990s, Dick and colleagues started a series of pioneering investigations to understand whether the functional hierarchy observed in normal hematopoiesis was conserved in blood tumors. These studies showed that in several forms of human AML (M1, M4, M5), the cells with the capacity to engraft in NOD-SCID immunodeficient mice are restricted to a minority subpopulation of the leukemic clone, defined as [CD34^{+}, CD38^{neg}] and thus sharing a surface marker profile with normal immature multipotent progenitors (22, 23). Most interestingly, analysis of leukemic cell populations engrafted in NOD-SCID mice revealed reconstitution of the phenotypic heterogeneity observed in the original donor. This indicates that [CD34^{+}, CD38^{neg}] leukemic stem cells (LSCs) retained differentiative capacity, giving rise to CD38^{+} and Lin^{+} populations. Taken together, these observations provided the first experimental proof that in a human neoplastic disease (i.e., AML), cancer cell populations are organized according to a functional hierarchy mirroring a stem cell system (**Figure 1*c***).

Disease Heterogeneity in Cancer Stem Cell Populations

In addition to providing proof of principle of the CSC model, the study of human AML also shed the first light on its potential complexities. A first observation is that not all forms of AML follow the same paradigm. In patients affected by acute promyelocytic leukemia (APL or AML-M3), the t(15:17) PML/RARα chromosomal translocation is usually detected in [CD34^{+}, CD38^{+}] cells, but not in [CD34^{+}, CD38^{neg}] cells (24). The APL case is somewhat difficult to judge because of its apparent lack of engraftment ability in NOD-SCID mice and the resulting impossibility of transplantation studies (22). Several explanations could account for this discrepancy with other AML forms (e.g., CD38 expression might be induced in LSCs by tumor transformation). However, the absence of t(15:17) in HSCs of APL patients suggests that, in selected cases, AML growth might be sustained by a cell population that does not originate from HSCs.

"Stem Cell Evolution" During Disease Progression

Another layer of complexity is added by investigations of the "evolution" of LSCs during the natural history of leukemic diseases. A first example is provided by studies performed on AML patients whose disease harbored the t(8:21) AML1-ETO chromosomal translocation and who achieved long-lasting clinical remission (25). In these patients, analysis of bone marrow at remission revealed persistence of AML1-ETO fusion transcripts in [Thy-1^{+}, CD34^{+}, CD38^{neg}, Lin^{neg}] cells, suggesting that the AML1-ETO chromosomal translocation is an early genetic event targeting the HSC compartment. In addition, in vitro functional studies performed on the

same cells showed that AML1-ETO$^+$ progenitors were able to regularly differentiate into mature cells of different lineages (**Figure 1*d***). Moreover, AML1-ETO fusion transcripts were detectable in multiple lineages of purified mature cells directly obtained from the same patients. Therefore, although the AML1-ETO translocation is probably an early, necessary event in the natural history of this disease, by itself it is not sufficient to cause leukemic transformation. Additional mutations are probably required to induce a fully transformed phenotype, and most likely arise, as a final transforming event, in a subsequent stage of hematopoietic differentiation. Supporting this hypothesis is the observation that, whereas clonogenic assays performed on the bone marrow of AML1-ETO$^+$ patients in complete remission reveal a normal repertoire of colony-forming units (CFUs) in the [Thy-1$^+$, CD34$^+$, CD38^{neg}, Lin^{neg}] population, those performed on AML1-ETO$^+$ patients at diagnosis reveal only the presence of "blast"-forming units (CFU-blast) restricted to the [Thy-1^{neg}, CD34$^+$, CD38^{neg}, Lin^{neg}] population. This finding, common to most forms of human AML (26), might suggest that the fully transformed LSC, although derived from a mutated preleukemic Thy-1$^+$ HSC, could actually emerge as the consequence of secondary mutations in a Thy-1^{neg} more differentiated progenitor (27) (**Figure 1*d***).

An intriguingly similar scenario can be envisaged in CML when comparing the initial chronic phase (Cp) of the disease with its terminal stage, blast crisis (Bc). During Cp-CML, the leukemic clone undergoes multipotent differentiation, and the t(9:22) BCR-ABL chromosomal translocation, which serves as a diagnostic marker of the disease, is detectable in most circulating mature lineages, especially myeloid cells and B lymphocytes (28, 29). During Bc-CML, as the disease becomes clinically more aggressive, large numbers of undifferentiated BCR-ABL$^+$ blast cells accumulate in the blood and bone marrow. It is not clear which molecular event underlies progression from Cp-CML to Bc-CML. However, it has recently been demonstrated that Bc-CML is characterized by an expansion of oligo-lineage precursors, especially the granulocyte-macrophage progenitor (GMP) (30). Most interestingly, when compared to their normal counterparts, GMPs from Bc-CML show aberrant de novo acquisition of in vitro self-renewal properties, associated with increased nuclear expression of β-catenin (30). Thus, during progression from Cp-CML to Bc-CML, a subpopulation of differentiated leukemic progenitors (i.e., GMPs) appears to acquire stem cell characteristics. This observation suggests that, in CML, disease progression is probably accompanied by both an expansion and a diversification of the leukemogenic stem cell pool owing to the generation of a second class of LSCs (31).

Overall, the picture emerging from studies on human leukemias confirms the CSC working model but also highlights the variety of its potential manifestations. The functional hierarchy of tumor cell populations can vary among disease subtypes and can evolve during their natural history, as a cause or as a result of their progression. In many cases, a detailed understanding of the true hierarchy of human leukemic cell populations is still hampered by the many uncertainties regarding the hierarchy of the early stages of normal human hematopoiesis (11). However, the continuous refinement of the surface marker profiles used to distinguish HSCs from MPPs, such as that recently achieved in the mouse using the differential expression of signaling lymphocytic activation molecules (SLAMs) (10), could shed new light on this issue in the near future.

The Molecular Machinery of Self-Renewal in HSCs and LSCs: Similarities and Differences

At a molecular level, applying concepts and tools first developed for the study of normal HSCs to the study of leukemias is leading to new and provocative insights in basic cancer biology. One interesting example is related to

Colony-forming unit (CFU)-blast: a cell that gives rise to aberrant blast colonies when tested in a clonogenic assay for hematopoietic progenitors

GMP (granulocyte-macrophage progenitor): a committed hematopoietic precursor whose progeny is restricted to the monocytic and granulocytic lineages. GMPs derive from CMPs

Bmi1 (B lymphoma Mo-MLV insertion region 1): oncogene identified as a DNA insertion site of the Moloney murine leukemia virus (Mo-MLV)

the study of the Bmi1 oncogene, a member of the Polycomb group ring finger (PCGF) gene family, shown to be expressed at high levels in HSCs and progressively downregulated during hematopoietic differentiation (32). Bmi1 knockout ($Bmi1^{-/-}$) mice are characterized by progressive bone marrow aplasia and die less than two months after birth (33). In this system, progressive bone marrow aplasia is not associated with differentiation problems but with progressive numerical reduction of bone marrow HSCs. Most interestingly, when transplanted in syngeneic wild-type mice, both fetal liver and bone marrow HSCs obtained from $Bmi1^{-/-}$ mice sustain hematopoiesis in a short-term, transient fashion, thus indicating that $Bmi1^{-/-}$ HSCs lack self-renewal capacity because of an intrinsic cell defect and behave functionally as MPPs (33).

When assessed in a murine leukemia model, the role of Bmi1 in self-renewal has proven to have key implications for tumor biology. In mice, infection of fetal liver HSCs with a retroviral construct simultaneously encoding Hoxa9 and Meis1 oncogenes is sufficient to induce tumor transformation and generation of a leukemic disease resembling human AML (34). When this experiment is performed using $Bmi1^{-/-}$ HSCs, no substantial difference is initially observed: the virus construct induces tumor transformation and generation of a $Bmi1^{-/-}$ AML that rapidly kills the recipient mice (35). However, when the long-term self-renewal of leukemic cells is evaluated by serial transplantation in secondary recipients, only $Bmi1^{+/+}$ AML cells are able to transfer the disease. $Bmi1^{-/-}$ AML cells cannot, thus indicating that lack of Bmi1, while not interfering with the tumor transformation process per se, limits the expansion potential of the leukemic clone (35).

A second molecule that is likely to play a key role in the molecular machinery of both HSC and LSC self-renewal is the protein phosphatase and tensin homologue (PTEN), a known tumor suppressor (36). In mice, conditional deletion of the Pten gene causes progressive depletion of the HSC compartment without substantially interfering with its differentiation capacity, a scenario reminiscent of $Bmi1^{-/-}$ mice (37, 38). However, in contrast to the case of Bmi1, inactivation of Pten is also associated with spontaneous development of acute leukemias, lethal in both primary hosts and secondary transplant recipients (37, 38).

Taken together, these studies show that genes required for self-renewal of normal HSCs can play opposite roles in the development of leukemia. In some cases they are necessary for long-term expansion of the transformed clone (Bmi1), but in others they act as tumor suppressors and prevent leukemic transformation (Pten). Most interestingly, these studies show that two of the common hallmarks of cancer—the endowment of long-term proliferative potential (historically sometimes referred to as "immortality") and the propensity toward uncontrolled, aberrant growth ("transformation")—are two independent properties that can be dissociated, a concept also indirectly demonstrated by in vitro studies on primary cultures of human tumors (39).

CANCER STEM CELLS IN HUMAN SOLID TUMORS

Several tissues share with blood the necessity to undergo a continuous or cyclical renovation. Among them are the skin and all major epithelia of the gastrointestinal (mouth, pharynx, esophagus, stomach, gut), respiratory (larynx, trachea, bronchi, lungs), and reproductive and genitourinary (breast, ovary, vagina, uterus, bladder, prostate) systems. Most of them are known or presumed to be organized according to a hierarchical system based on a dedicated adult stem cell population (40–52). Moreover, many human tissues traditionally considered stable, undergoing only minimal or slow turnover throughout adult life, such as the brain and muscle, are today known to contain specific stem cell populations, which can be mobilized and

activated in specific situations (53, 54). In addition, a stem-like functional hierarchy, although described using a different terminology, is known to exist in the mature lymphoid system: T and B cell populations can be subdivided into long-term ("memory") and short-term ("effector") functional compartments, characterized by specific surface marker expression profiles (55).

On the basis of these observations, our laboratory undertook a new study to test whether a CSC model could be applied to the description of solid tumors, focusing on human breast cancer as a model system (56). The results showed that, in most human breast cancers, only a minority subpopulation of the tumor clone, defined as [$CD44^+$, $CD24^{-/low}$] and representing 11%–35% of total cancer cells, is endowed with the capacity to sustain tumor growth when xenografted in NOD-SCID mice. Most importantly, tumors grown from [$CD44^+$, $CD24^{-/low}$] cells were shown to contain mixed populations of epithelial tumor cells, recreating the phenotypic heterogeneity of the parent tumors. For the first time, this study demonstrated the existence of a functional hierarchy reminiscent of stem cell systems in a solid human epithelial tumor. The limited knowledge of the biology of normal human mammary stem cells and their differentiation programs (57) currently limits our understanding of which lineage, progenitor cell, or differentiation stage of the mammary epithelial tissue is related to [$CD44^+$, $CD24^{-/low}$] human breast CSC. However, development of in vitro culture systems for the functional study of both normal (58) and cancer (59) human mammary epithelial cells could soon provide the experimental means for a phenotypic dissection of breast epithelial differentiation processes.

The CSC working model has also been successfully applied to brain tumors (60–63). Studies performed on glioblastoma multiforme and medulloblastoma have shown that tumorigenic cells are restricted to the $CD133^+$ subpopulation, which usually represents 5%–30% of total tumor cells. As expected from the CSC model, tumors resulting from orthotopic, intracerebral injection of $CD133^+$ cells reproduced the phenotypic diversity and differentiation pattern of the parent tumors (62). In the study of brain tumors, the availability of a well-characterized cell culture system for normal neural stem cells (the "neurosphere" assay) provides a robust tool for the in vitro study of their candidate pathological counterparts. Based on this approach, Galli et al. succeeded in the isolation and serial propagation from human glioblastoma multiforme of "cancer neurospheres," which are highly enriched in long-term self-renewing, multi-lineage-differentiating, and tumor-initiating cells (60). Although the relationship between $CD133^+$ cells and cancer neurospheres still remains to be fully explored, it is safe to assume that ex vivo purification of brain tumor CSCs based on CD133 coupled with in vitro functional studies using neurosphere assays will provide one of the most effective probes for the study of solid-tumor CSCs in the near future.

In addition to breast and brain tumors, interesting results are being accumulated on prostate cancer, where progenitor/tumorigenic subpopulations appear characterized by the expression of CD44 (64, 65). In melanoma, in vitro culture of primary tumor cells with media formulations used for embryonic stem cells allows the isolation and serial propagation of "melanoma spheroids," reminiscent of cancer neurospheres and capable of long-term self-renewal, multi-lineage differentiation and in vivo tumorigenicity (66). Several mouse models for the study of CSC biology in epithelial solid tumors are also currently being investigated, including models for lung (48) and prostate (67) cancer.

The CSC model is also being extended to lymphoid tumors, such as multiple myeloma, where it is becoming apparent that a minority subpopulation of $CD138^{neg}$ cells is preferentially endowed with in vitro clonogenic and in vivo engraftment capacity (68). Studies on multiple myeloma are particularly fascinating because they suggest that the

candidate CSC for plasma cell diseases might be a B cell population in an earlier stage of differentiation, which might reside in a different anatomical compartment (i.e., lymph nodes) than the bulk of the tumor (i.e., mature $CD138^+$ plasma cells in the bone marrow).

IMPLICATIONS OF THE CSC MODEL FOR THE BIOLOGICAL INTERPRETATION OF CANCER

Intratumor Heterogeneity

One conceptual implication of the CSC model is related to the biological interpretation of intratumor heterogeneity, i.e., heterogeneity among cancer cells within the same tumor lesion. Intratumor heterogeneity is a recurrent observation in human tumors, both primary and metastatic (69), and is usually considered the result of variations in the tumor microenvironment and the coexistence of multiple independent cellular subclones originated by progressive accumulation of divergent genetic aberrations within the same cancer cell population (70, 71). The CSC model introduces an additional explanation, suggesting that intratumor heterogeneity can also result from functional diversity among cells in different states of differentiation.

This account has major theoretical and methodological consequences for cancer research because it suggests that the biological significance of observations collected on whole tumor tissues should be judged according to their relation to the CSC subset. For instance, biochemical pathways that are active in the majority of tumor cells might be of little functional relevance for the biology of CSC, whereas biochemical pathways active only on a small minority of cancer cells might play key roles in CSC biology and thus in the overall long-term behavior of a tumor.

Based on this perspective, the CSC model might help shed light on several unexpected observations in cancer biology. An interesting example is the heterogeneous and frequently nonconstitutive pattern of intratumor expression of the human telomerase reverse transcriptase (hTERT) (39, 72). According to current models, based mainly on the study of in vitro cultured cell lines, one of the hallmarks of cancer is the acquisition by cancer cells of a limitless replicative potential, or immortality (73). To become immortal, cancer cells must be endowed with a system for the maintenance of telomere length, in most cases based on telomerase, a ribonucleoproteic enzymatic complex that counteracts the progressive shortening of telomere repeat sequences during in vitro proliferation of normal somatic cells (74). Because telomerase expression is usually not detected in most normal tissues, it is postulated that its expression in cancer cells is the result of tumor-specific genetic and/or epigenetic events that result in ectopic, constitutive hTERT activation (75). Despite extensive investigation, however, the molecular mechanisms underlying telomerase upregulation remain largely undefined (75), and contrary to predictions, the in vivo expression of hTERT has repeatedly proven to be extremely heterogeneous among cancer cells, especially in vivo (39, 72). These observations can be explained in the context of the CSC model if we assume that telomerase expression is restricted to the self-renewing, long-lived, tumorigenic subpopulation of the tumor clone (i.e., CSC). According to this hypothesis, telomerase expression is inherited by CSCs as part of the normal self-renewal machinery of adult stem cells of the corresponding healthy parent tissues and is progressively lost during differentiation processes, both in normal and in tumor tissues (76). Indeed, it has recently been shown that one of the genes involved in the control of stem cell self-renewal, Bmi1, is also able to upregulate hTERT expression in epithelial cells (77). Moreover, it is now well known that telomerase is expressed in normal adult stem cells of healthy tissues, although at levels that are usually below the threshold for routine experimental detection (72, 78). The scenario is probably similar for other proteins that are known to display similar expression patterns

and to play key roles in tumor biology, such as survivin (69, 79).

CSCs and the Study of Tumor Metastasis

The CSC model can also shed new light on the biology of metastases and explain why, despite extensive intratumor heterogeneity (69, 71), comparison of paired samples of primary tumors and autologous lymph node and/or distant-site metastases usually reveals striking similarities over a wide range of parameters, including tissue morphology (69, 71), repertoire of somatic genetic mutations (80–82), expression of tumor-suppressor and immunomodulatory proteins (83), expression of epigenetically controlled genes (84), and overall transcriptional profile as defined by gene-expression arrays (85–87). These observations are in contrast to predictions from traditional cancer models, where metastases are considered to originate from monoclonal expansions of very specific, individual tumor subclones endowed with specific genotypic and phenotypic features, and therefore are postulated to be substantially different from primary tumors (**Figure 2*a***). However, if we take into account the CSC model and we assume that, in each individual tumor, the differentiation pattern is controlled by its specific repertoire of genetic mutations, then we can predict that, if two lesions share identical genetic backgrounds and similar genetic abnormalities, they will also undergo similar differentiation programs and display similar patterns of intratumor heterogeneity in the expression of differentiation antigens (**Figure 2*b***) (69, 87).

IMPLICATIONS OF THE CSC MODEL FOR DESIGN AND EVALUATION OF ANTITUMOR TREATMENTS

Therapeutic Tumor Targeting

The observation that cancer growth can be sustained by a minority subpopulation of tumor cells with unique functional properties (i.e., CSCs) could also assist in the design of new and more effective antitumor treatments. According to the CSC model, therapeutic approaches that do not eradicate the CSC compartment are likely to achieve little success; they might kill the majority of tumor cells and induce temporary regression of gross tumor lesions but fail to prevent disease relapse and metastatic dissemination (**Figure 3*a***) (2). In support of this hypothesis is the finding that, in the hematopoietic system, both normal stem cells and CSCs (i.e., HSCs and LSCs from AML patients) mainly appear to be in a quiescent, nondividing, G_0 state, and therefore inherently resistant to the toxic effect of traditional chemotherapeutic regimens (8, 88, 89). Based on this concept, traditional treatments might be recalibrated and investigational therapies developed, focusing on their ability to target the CSC subpopulation and its specific biochemical pathways (89, 90). Similarly, the CSC model might explain why several experimental therapeutic approaches have shown poor clinical results despite extensive preclinical validation in vitro, and it might provide critical information for the redesign and upgrading of such approaches. A classic example of this situation could be represented by antitumor T cell immunotherapies, where target antigen selection might be re-evaluated on the basis of expression on CSCs (91, 92).

Evaluation of Treatment Efficacy

A second therapeutic implication of the CSC model is related to clinical methodologies for evaluating treatment efficacy. Traditionally, antitumor treatments are screened based on their capacity to induce a clinical response (i.e., a dramatic regression, either complete or partial, of the tumor lesion). This approach, however, tends to select for treatments that are active on the bulk of tumor cell populations but not necessarily on CSCs. From a purely theoretical point of view, antitumor treatments that selectively target the CSC subset might actually be unable to

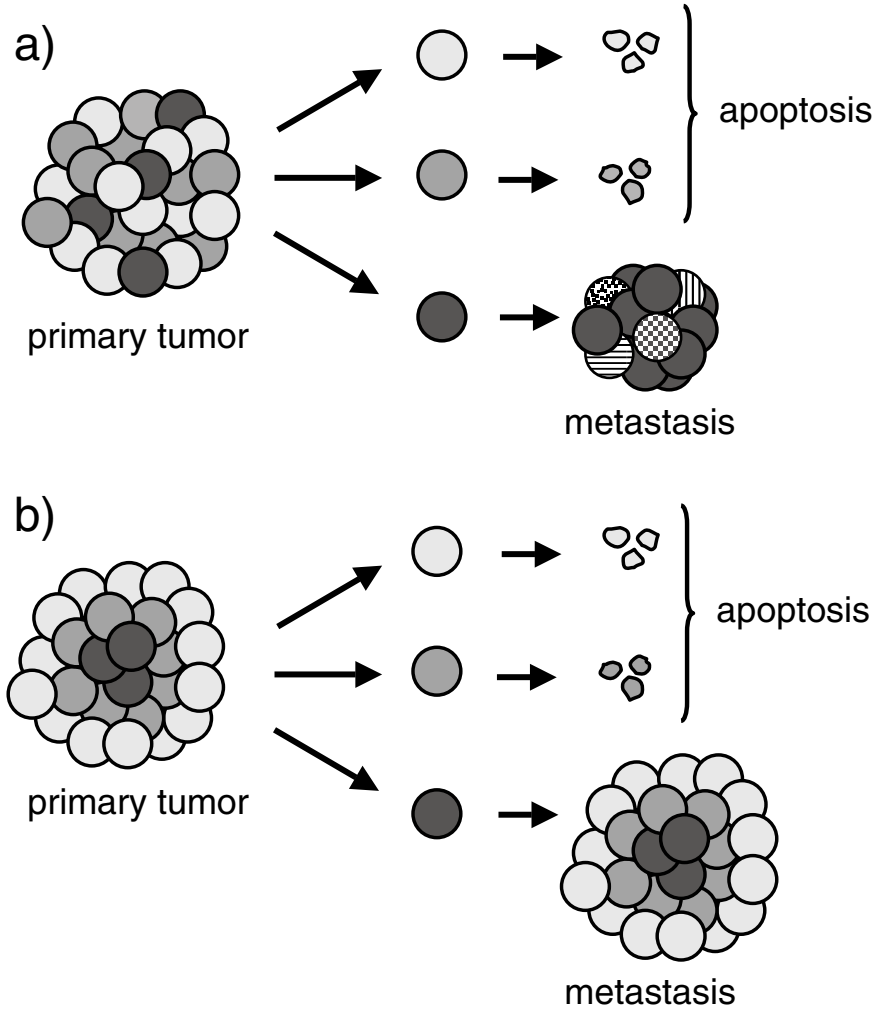

Figure 2

Impact of the cancer stem cell (CSC) model on the origin and biology of metastases. (*a*) According to standard cancer models, tumors are composed of heterogeneous mixtures of independent subclones, originated by divergent genetic mutations; different subclones are endowed with different functional properties, and only selected clones (*dark grey cells*) can migrate and form metastases. The metastasis is predicted to be a homogeneous monoclonal expansion of an individual subclone, which in turn can accumulate further mutations (*striped and variously patterned cells*) and diverge even further from the primary tumor. Overall, the model predicts that primary tumors and corresponding metastases are substantially different. (*b*) The CSC model assumes that intratumor heterogeneity is mainly caused by cell differentiation, and that only CSCs (*dark grey cells*) can migrate and form overt metastases, while differentiated cells (*light grey cells*) undergo apoptosis. In the CSC model, metastatic cancer tissues undergo differentiation programs that closely resemble those observed in the corresponding primary tissues. Recent experimental evidence based on gene-expression microarrays tends to support the CSC model for human epithelial tumors, such as breast and colon cancer. The two hypotheses are not mutually exclusive, and elements of both are probably true.

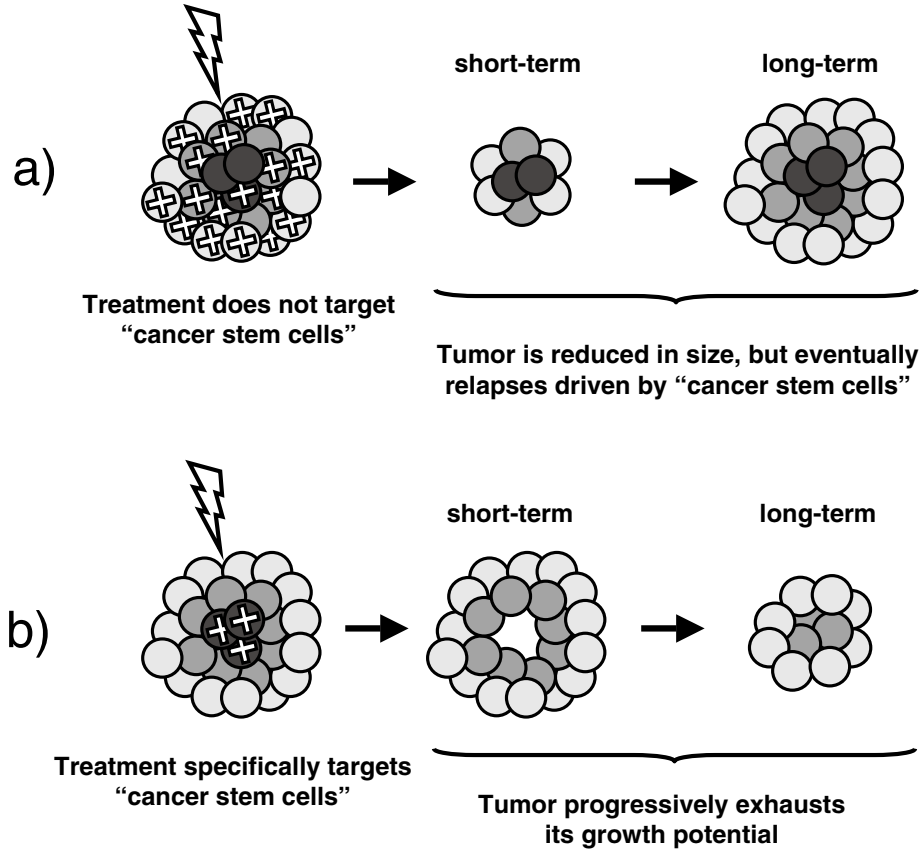

Figure 3

Impact of the cancer stem cell (CSC) model on the design and evaluation of antitumor treatments. (*a*) Antitumor treatments designed and selected for broad cytotoxic activity may kill the majority of cancer cells within a specific tumor tissue and induce dramatic, even complete regression of large tumor masses; however, if any of the CSCs are spared, tumor tissues can be regenerated and cause disease relapse. (*b*) In contrast, antitumor treatments specifically designed to target CSCs, although theoretically unable to cause rapid shrinkage of tumor lesions, might nonetheless achieve long-term disease eradication by exhausting self-renewal and growth potential of cancer tissues.

induce rapid shrinkage of tumor masses but might eliminate their capacity for long-term growth and therefore cause their arrest or slowly reduce their size (**Figure 3*b***). It is therefore likely that, alongside new treatment strategies, new approaches for the preclinical evaluation of their efficacy will need to be devised.

DISCLOSURE STATEMENT

MFC owns stock in Oncomed, a cancer stem cell company.

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