# Cancer Stem Cells: An Old Idea—A Paradigm Shift

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## Abstract

**Although the concept that cancers arise from "stem cells" or "germ cells" was first proposed about 150 years ago, it is only recently that advances in stem cell biology have given new impetus to the "cancer stem cell hypothesis." Two important related concepts of this hypothesis are that (*a*) tumors originate in either tissue stem cells or their immediate progeny through dysregulation of the normally tightly regulated process of self-renewal. As a result of this, (*b*) tumors contain a cellular subcomponent that retains key stem cell properties. These properties include self-renewal, which drives tumorigenesis, and differentiation albeit aberrant that contributes to cellular heterogeneity. Recent experimental evidence in a variety of tumors has lent strong support to the cancer stem cell hypothesis that represents a paradigm shift in our understanding of carcinogenesis and tumor cell biology. This hypothesis has fundamental implications for cancer risk assessment, early detection, prognostication, and prevention. Furthermore, the current development of cancer therapeutics based on tumor regression may have produced agents that kill differentiated tumor cells while sparing the rare cancer stem cell population. The development of more effective cancer therapies may thus require targeting this important cell population.** (Cancer Res 2006; 66(4): 1883-90)

## Introduction

In a thought-provoking article published in *Fortune* in 2004, Leaf, a cancer survivor, poses the question, "Are we losing the war on cancer?" (1). In this article, he reviews data on the progress made since the "war on cancer" was declared in 1961. Over this time, there have clearly been dramatic advances in the treatment of such diseases as childhood leukemia, Hodgkin's disease, and testicular cancer. Furthermore, the overall mortality for some of the common epithelial malignances, such as breast cancer and prostate cancer, have been declining recently largely due to advances in early detection and prevention. However, as Leaf points out, for the four most common epithelial malignancies (lung, breast, prostate, and colon cancers), the survival of patients with metastatic disease has not changed significantly over the past several decades. Despite these statistics, there is considerable optimism in the cancer research community that new targeted therapies will significantly improve on the results of empiric-based therapeutics. The ability to specifically target pathways deranged in cancer raises the hope of developing therapies with enhanced specificity and decreased toxicity. However, as our ability to attack specific targets increases, a fundamental question remains, "Are we targeting the right cells"? Evidence is accumulating that most, if not all, malignancies are driven by "a cancer stem cell compartment." Furthermore, these cancer stem cells may be inherently resistant to our current therapeutic approaches. The cancer stem cell hypothesis has fundamental implications for understanding the biology of carcinogenesis as well as for developing new strategies for cancer prevention as well as new therapies for advanced disease. In this commentary, we will discuss the cancer stem cell hypothesis, including recent evidence supporting its validity, and the implications of this model for cancer prevention and therapy.

## The Cancer Stem Cell Hypothesis

All tissues in the body are derived from organ-specific stem cells that are defined by their capacity to undergo self-renewal as well as to differentiate into the cell types that comprise each organ. These tissue-specific stem cells are distinguished from embryonic stem cells in that their differentiation is largely restricted to cell types within a particular organ. The cancer stem cell hypothesis has two separate but related components. The first component concerns the cellular origin of tumors, including the question of whether tumors arise from tissue stem cells. A second related component of this hypothesis is that tumors are driven by cellular components that display "stem cell properties." The concept that cancer might arise from a rare population of cells with stem cell properties was proposed about 150 years ago (2–5). Over 40 years ago, it was postulated that tissue-specific stem cells may be the cell of origin of cancer (6). Over 30 years ago, Pierce (7) proposed that cancers represented a maturation arrest of stem cells. The concept that tumors contain cell populations with stem cell properties was also suggested by *in vitro* "clonogenic assays" that showed subpopulations of tumor cells with increased proliferative capacity as shown by colony formation in *in vitro* assays using cells isolated from tumor specimens (8). A major limitation of these studies, however, was that they measured *in vitro* proliferation rather than true self-renewal. In addition, it has been observed that the production of human tumor xenografts in animal models required a relatively large number of cells. However, it was unclear whether this was due to the inefficiency of these cells in promoting tumor growth or to the existence of rare subpopulations within a tumor that were uniquely tumorigenic in these systems.

Evidence supporting the cancer stem cell hypothesis has gained impetus due to recent advances in stem cell biology and the development of new animal models to measure self-renewal and more directly test the validity of this hypothesis. The concept that cancers arise from the transformation of stem cells is appealing for several reasons. Stem cells by their long-lived nature are subject to the accumulation of multiple mutations that are

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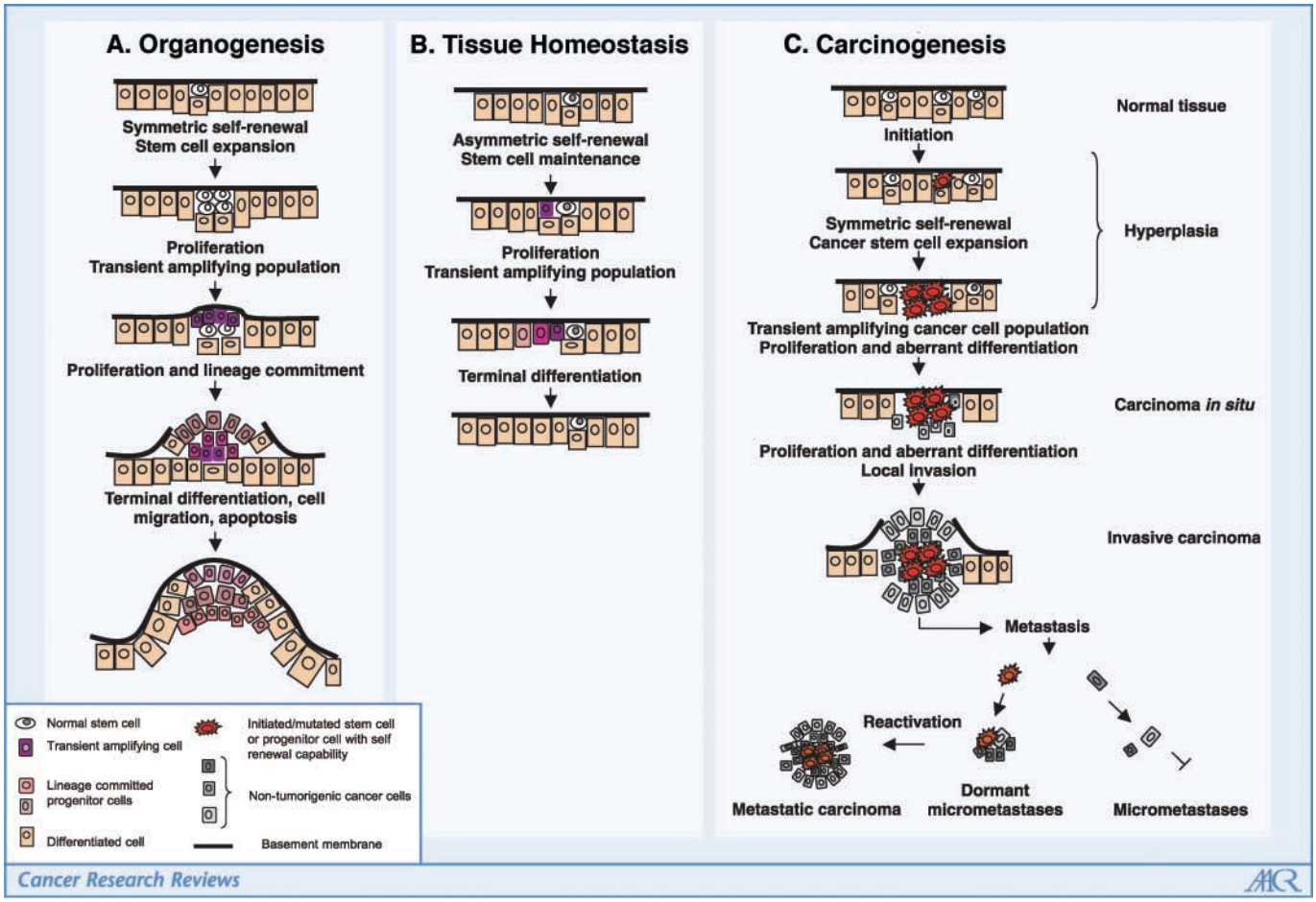


**Figure 1.** Stem cells in normal development, tissue homeostasis, and carcinogenesis. *A,* during normal development, symmetric stem cell self-renewal results in stem cell expansion. This process is tightly regulated by components of the stem cell niche. Stem cells differentiate into a transient amplifying population that undergoes further proliferation and lineage commitment followed by cell migration, terminal cell differentiation, and apoptosis of fully differentiated cells. *B,* during normal tissue homeostasis, asymmetric self-renewal of stem cells results in stem cell maintenance. Proliferation and differentiation of transient amplifying progenitor cells replaces normal cell loss resulting in tissue homeostasis. *C,* carcinogenesis may be initiated by stem cell expansion via symmetric self-renewal. Unlike normal organogenesis, this process is dysregulated resulting in cancer stem cell expansion. Aberrant differentiation of these cells generates tumor heterogeneity. Further mutations or epigenetic changes may accompany tumor invasion and metastasis. Metastases require the dissemination of cancer stem cells that may remain dormant and be reactivated resulting in tumor recurrence. In contrast, dissemination of differentiated tumor cells produces only micrometastasis that do not progress.

required for carcinogenesis. For example, women exposed to atomic bomb radiation in Hiroshima and Nagasaki developed breast cancer approximately 20 to 30 years after exposure (9). Mutations found in these women's breast cancers are consistent with those known to be induced by radiation (9). Furthermore, women exposed to radiation during late adolescents had the highest susceptibility to breast cancer development. This is thought to be the period when the mammary gland has the highest number of stem cells (10). Further evidence that stem cells may play a role in carcinogenesis is the observation that normal stem cells and cancer cells share several important properties. These include (*a*) the capacity for self-renewal, (*b*) the ability to differentiate, (*c*) active telomerase expression, (*d*) activation of antiapoptotic pathways, (*e*) increased membrane transporter activity, and (*f*) the ability to migrate and metastasize. Indeed, properties, such as anchorage independence, which have been thought to be a hallmark of transformed cells, have recently been described by us and others as a property of normal tissue stem cells (11–13). One of the key early events in transformation may be the dysregulation of the normally highly regulated process of self-renewal. Stem cells are the only cells capable of undergoing self-renewal divisions. In the steady state, these divisions are asymmetric in which a stem cell is able to produce an exact copy of itself as well as a daughter cell that undergoes differentiation into the lineages found in differentiated tissues. During stem cell expansion and tumorigenesis, stem cells may undergo symmetric divisions in which stem cells produce two identical stem cell progeny, thus allowing for stem cell expansion (ref. 14; Fig. 1). During normal development, stem cell self-renewal is regulated by signals from the surrounding stem cell "niche." As has been elegantly shown in bone marrow transplantation models, a single hematopoietic stem cell introduced into a lethally irradiated mouse is able to repopulate the stem cell compartment resulting in reconstitution of the entire hematopoietic system. Extensive expansion in the stem cell population stops when this pool is replenished, illustrating the tight control of this process. We and others have hypothesized that deregulation of this self-renewal process leading to stem cell expansion may be a key early event in carcinogenesis. Recently, the pathways that regulate the self-renewal

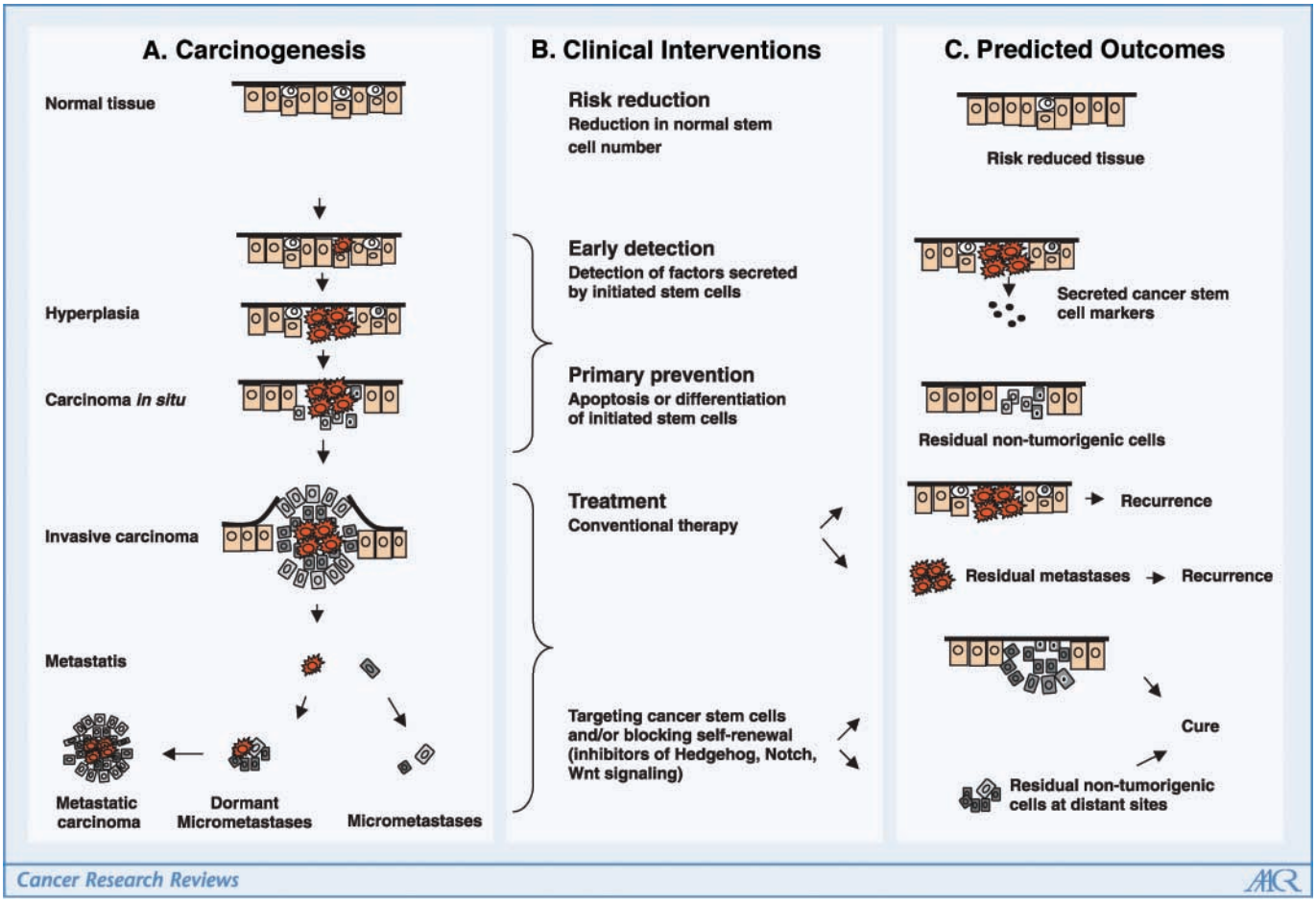


**Figure 2.** Clinical implications of cancer stem cell model. The cancer stem cell model has important implications for cancer risk reduction, early detection, prevention, and treatment. Interventions that reduce normal stem cell number may decrease cancer risk. Detection of factors secreted by initiated stem cells may allow for the earlier detection of cancers. Interventions that induce apoptosis or differentiation of initiated stem cells may be effective in cancer prevention. Conventional cancer therapies, including cytotoxic agents, selectively destroy differentiated cancer cells, sparing the cancer stem cell compartment resulting in cancer recurrence at primary or metastatic sites. Therapies that selectively eliminate cancer stem cells leave residual nontumorigenic cells resulting in potential cancer cures.

of normal stem cells, including Wnt, Notch, and Hedgehog, have begun to be elucidated. These signaling pathways have been implicated in regulating the self-renewal of hematopoietic, neuronal, and mammary stem cells (14, 15). The dysregulation of each of these pathways in rodent models leads to tumorigenesis. Furthermore, there is substantial evidence that dysregulation of these pathways also plays an important role in human carcinogenesis. Defects in the Wnt signaling pathway are seen early in colon cancer carcinogenesis. Alterations in Hedgehog signaling were first shown in human basal carcinomas of the skin (16). More recently, evidence for dysregulation of this pathway has been reported in human pancreatic, gastric, prostate, and breast carcinomas (17, 18). Alterations in Notch signaling have been observed in human T-cell acute lymphoblastic leukemia, cervical cancer, and breast cancer (19–23).

Recent studies have suggested that tumors may arise from progenitor cells and tissue stem cells. Transformation of these cells may require that they acquire the stem cell property of self-renewal. In support of this hypothesis, Jamieson et al. showed that chronic myelogenous leukemia (CML) blast crisis may originate in hematopoietic progenitor cells as a consequence of dysregulated Wnt signaling, allowing these cells to self-renew, a property normally restricted to hematopoietic stem cells (24). Similarly, by transfecting purified populations of hematopoietic progenitor cells, Kelly and Gilliland showed that AML-ETO may induce transformation of myeloid progenitor cells enabling them to acquire the property of self-renewal (25). We have recently proposed that human breast cancers may arise from the transformation of either mammary stem cells or early progenitor cells resulting in production of breast cancers with distinct molecular and clinical phenotypes (26). This concept is also consistent with recent descriptions in transgenic mouse models of mammary tumorigenesis, which suggest that distinct oncogenes may affect different stem and progenitor cells resulting in phenotypic differences in mammary tumors (27).

The second major component of the cancer stem cell hypothesis is that tumors contain and are "driven" by cellular components that display stem cell properties. This concept has gained substantial experimental support recently with the development of animal models that have permitted the direct assessment of stem cell properties of tumor cell subpopulations. These models have shown that prospectively identifiable subpopulations of tumor cells display the defining stem cell properties of self-renewal and differentiation. Self-renewal drives tumorigenesis, whereas differentiation (albeit aberrant in tumors) contributes to tumor phenotypic heterogeneity. In 1997, Dick et al.

showed that the ability to transfer human leukemias into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice was retained in a small population of cancer stem cells that like their normal counterparts displayed the cell surface phenotype $CD34^+CD38^-$. These cells that comprised <1 in 10,000 leukemia cells could transfer the human leukemia into NOD/SCID mice, whereas the introduction of thousands of fold higher numbers of cells that did not bear this phenotype were nontumorigenic. Furthermore, the leukemias that were produced recapitulated the histologic phenotype found in the original tumor (28). More recently, this group used cellular marking studies to show that leukemic stem cells like their normal counterparts are heterogeneous with varying degrees of self-renewal potential. These findings suggest that leukemic stem cells, like their normal counterparts, exist in a hierarchy that is developmentally regulated. This supports the hypothesis that leukemic stem cells originate from the transformation of normal hematopoietic stem cells (29). Using a similar approach, in collaboration with Michael Clarke, we showed that human breast cancers contain a cell population characterized by the expression of the cell surface markers $CD44^+$ $CD24^{low\ lin^-}$ that have stem cell characteristics. As few as 200 of these cells, which comprise between 1% and 10% of the total cell population, are able to form tumors when implanted in NOD/SCID mice (30). In contrast, 20,000 cells isolated from the same tumor that do not display this cell surface phenotype are unable to form tumors. Furthermore, consistent with a stem cell model, cancer stem cells are able to generate tumors that recapitulate the phenotypic heterogeneity found in the initial tumor.

Confirming and extending our findings, Ponti et al. recently reported that, in addition to being tumorigenic, $CD44^+CD24^-$ human breast cancer cells form tumor mammospheres *in vitro*, a property that we described previously for normal mammary stem/progenitor cells (31, 32). Furthermore, the stem cell phenotype of these tumor cells was suggested by their expression of the stem cell markers Oct-4 as well as by the absence of Cx43 expression. Interestingly, these cells also produced vascular endothelial growth factor (VEGF) and were highly angiogenic (31). Lending further support to the cancer stem cell hypothesis and extending its generality, three groups have independently shown the existence of a cancer stem cell compartment in human brain tumors. These cancer stem cells, like their normal counterparts, are able to form neurospheres *in vitro* and express the neural stem cell markers CD133 and nestin. Furthermore, as few as 100 of these cells were able to transfer the tumors when injected intracranially into NOD/SCID mice (33, 34). In contrast, $10^5$ $CD133^-$ cells engrafted but did not produce a tumor. The tumors produced by the $CD133^+$ cells recapitulated the phenotypic heterogeneity found in the initial tumor (33). Evidence for existence of a clonogenic subpopulation of cells in human multiple myeloma was recently reported by Matsui et al. (35). Multiple myeloma cells express syndecan-1 (CD138). However, a small subpopulation resembling postgerminal center B cells were $CD138^-$. Only the $CD138^-$ cells were clonogenic *in vitro* and in NOD/SCID mice (35). In the prostate, Xin et al. showed that stem cell antigen-1 (Sca-1) enriches for a prostate regenerating cell in mouse model and genetic perturbations of PTEN/AKT produced prostate cancer associated with a dramatic increase in $Sca\text{-}1^+$ cells (36). Further evidence for the existence of a cell population with stem cell properties in prostate cancer has been reported by Richardson et al. They found that normal human prostate stem cells expressed CD133 (37). Furthermore, they identified a subpopulation of cells in human prostate cancer characterized as $CD44^+/\alpha_2\beta_1^{hi}/CD133^+$ with stem cell properties. As few as 500 cells with this phenotype that constituted 0.1% of total tumor cells formed tumors in NOD/SCID mice, whereas $5 \times 10^5$ $CD44^-$ cells failed to form tumors (38). Evidence for the existence of cancer stem cells in lung cancer has recently been presented by Kim et al. (39). They identified bronchial alveolar stem cells present at the bronchial alveolar duct junction. These cells exhibited the stem cell properties of self-renewal and multilineage differentiation. These stem cells could be transformed by K-ras *in vitro* and could form tumor in mice (39).

From these studies, it seems that several stem cell markers may be shared by cancer stem cells in multiple tumor types. These include CD44, $\alpha_6$ integrin, $\beta_1$ integrin, and CD133 (Prominin). These cancer stem cells may not only share molecular markers but also display dysregulation of similar self-renewal pathways, such as Wnt, Hedgehog, and Notch. In support of this concept, we have recently found that Hedgehog signaling may regulate the self-renewal of normal mammary stem cells and that this pathway is dysregulated in mammary cancer stem cells.[1] Taken together, these studies suggest that most, if not all, tumors contain a subpopulation of cells that display cancer stem cell characteristics. The relationship between normal and cancer stem cells is depicted graphically in Fig. 1.

## Implications of the Cancer Stem Cell Hypothesis: A Paradigm Shift in Thinking about Carcinogenesis and Our Approach to Cancer Prevention and Therapy

**Models of carcinogenesis.** "Stochastic models" of carcinogenesis hold that transformation results from random mutation and subsequent clonal selection. In this model, any cell may be the target of carcinogenesis. The stem cell model of carcinogenesis, in contrast, suggests that cancers originate in tissue stem or progenitor cells probably through dysregulation of self-renewal pathways. This leads to expansion of this cell population that then may undergo further genetic or epigenetic changes to become fully transformed. In addition, epigenetic changes normally involved in cell differentiation contribute to the cellular phenotypic heterogeneity found in tumors. This model represents a paradigm shift in our thinking and has fundamental consequences for understanding the biology of carcinogenesis as well as important clinical implications for early detection, prevention, and therapy of human malignancies. These implications are summarized in Fig. 2.

**Biological implications.** The cancer stem cell hypothesis has important biological implications for the development of animal models of carcinogenesis as well as for understanding key biological processes, such as stromal-epithelial interactions and metastasis. Although there has been considerable progress in the development of mouse models of human cancer, in many cases, these models fail to recapitulate human disease. Many transgenic models use tissue-specific promoters to drive oncogene expression. However, these tissue-specific genes may be expressed only in differentiated cells. If stem cells or their immediate progeny are

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the true targets of transforming events, then the expression of oncogenes in more differentiated cells may fail to recapitulate actual carcinogenic processes. There is recent evidence that the expression of oncogenes in primitive cells using direct transfection technologies results in a fundamentally different phenotype than expression of the same genes driven by tissue-specific promoters. Welm et al. showed that expression of c-Met and c-Myc driven by the mammary-specific promoter mouse mammary tumor virus fails to produce carcinomas, whereas these genes transduced into primitive cells via a stem cell virus produced mammary carcinomas (40). Kim et al. have developed an animal model that targets normal lung stem cells to produce adenocarcinomas that resemble those found in human lung cancers (39).

The concept of the normal stem cell niche has direct relevance to understanding stromal epithelial interactions that occur during tumorigenesis in addition to understanding such complex processes as tumor metastases. For example, homing receptors found on normal hematopoietic stem cells, such as the cytokine receptor CXCR4, have been shown to play an important role in promoting metastases in a variety of tumors, including human breast and prostate carcinomas (41, 42). Recently, carcinoma-associated fibroblasts were shown to promote angiogenesis, in addition to tumor growth, by secreting SDF-1 that interacts with CXCR4 expressed by tumor cells and endothelial cells (43). In addition, properties, such as induction of angiogenesis, may be inherent in normal stem cells as well as in their transformed counterparts. Supporting this idea, we have recently found that Hedgehog signaling regulates the production VEGF by normal human mammary stem and progenitor cells as well as breast cancer stem cells.[2] The clinical course of micrometastases may also reflect stem cell characteristics of disseminated cells. Up to 30% of women with newly diagnosed breast cancer and men with prostate cancer exhibit micrometastases in their bone marrow as determined by immunochemical staining. However, after 10 years, up to 50% of these have patients have not developed clinically relevant macroscopic disease (44). A potential explanation for this is that although either stem cells or their more differentiated progeny may be capable of forming micrometastases, only stem cells have the self-renewal capacity to create a clinically relevant macroscopic metastases. In addition, the concept of "tumor dormancy" may directly relate to stem cell biology. Stem cells usually exist in a quiescent $G_0$ state and self-renew only when they receive appropriate signals from their niche environment. By analogy, cancer stem cells may remain dormant at metastatic sites until they are activated by the appropriate signals from the microenvironment.

**Implications for cancer risk assessment, early detection, molecular profiling, and prevention.** The cancer stem cell model has important implications for many aspects of cancer risk assessment and prevention. If cancer stem cells or their immediate progeny are the targets for transformation, then cancer risk may be directly related to the number of stem cell targets. Pathways that influence target number may thus influence cancer risk. For example, it has been suggested that a previously unrecognized function of the hereditary breast cancer gene BRCA1 may be in the regulation of normal breast stem cell function (45). An important regulator of stem cell self-renewal of both normal and transformed stem cells is the polycomb gene Bmi-1 (46–48). It has recently been shown that Bmi-1 induced down-regulation of P-16 plays an important role in the regulation of hematopoietic and neuronal stem cell self-renewal (47, 49). Interestingly, recent studies by Holst et al. have suggested that one of the earliest events in carcinogenesis of the breast may be the silencing of P-16 expression by gene methylation (50). Together, these studies suggest that Bmi-1 may regulate normal stem cell self-renewal through down-regulation of P-16. During carcinogenesis, the silencing of this gene through methylation may result in the constitutive expansion of the stem cell population. In a similar manner, dysregulation of Wnt signaling may allow for the expansion of colonic stem cells during early colon cancer carcinogenesis.

The stem cell model also has important implications for the development of markers for the early detection of cancer. Most currently used tumor markers, such as prostate-specific antigen for prostate cancer or CA125 for ovarian cancer, are the products of differentiated cells within tumors. If tumors are to be detected during earlier stages of carcinogenesis, it may be necessary to characterize and detect markers made by the cancer stem cell populations. There has been considerable excitement generated by studies that show that important clinical prognostic and predictive information can be obtained from determining the molecular expression profile of tumors. This is consistent with the hypothesis that these molecular profiles represent the cell of origin as well as the differentiation pattern produced by subsequent oncogenic events. We have proposed previously that the molecular classifications of human breast cancers by gene expression analysis may reflect different cellular origins of these subtypes (51). If tumors are driven by a stem cell component, then elucidation of gene signatures characteristic of these stem cells may provide important prognostic information. In support of this, Glinsky et al. developed an 11-gene signature whose expression was regulated by the stem cell self-renewal gene Bmi-1. Remarkably, expression of this "stem cell gene" signature was associated with a poor prognosis for 10 different types of human malignancies (52). These studies summarized in an accompanying editorial, "Stem Cell-ness: A Magic Marker for Cancer" (53), provide strong evidence for the clinical relevance of the cancer stem cell hypothesis. Despite the important prognostic value of tumor profiling, the cancer stem cell hypothesis predicts that there will be considerably less value in using molecular profiling to identify new therapeutic targets. If cancer stem cells comprise only a minor fraction of total tumor cells and if these cells drive tumorigenesis, then the profiling of purified populations of cancer stem cells may identify more important therapeutic targets than profiling the entire tumor.

The cancer stem cell hypothesis suggests avenues for cancer prevention. If stem cells are the targets of transformation, then strategies that reduce stem cell number might reduce cancer risk. The use of tamoxifen in primary breast cancer prevention might occur through such a mechanism. Furthermore, if early events in carcinogenesis involve expansion of the stem cell pool, then interventions that induce either apoptosis or differentiation with a loss of self-renewal capacity in these cells represent a rational therapeutic approach to cancer prevention. Although the concept of differentiation therapy for cancer is not new (54), development of agents that can specifically target initiated stem cells may provide opportunities to intervene at the earliest stages of

[2] In preparation.

carcinogenesis before significant genetic instability occurs. This highlights the importance of elucidating the pathways that control differentiation and survival in these cells.

**Implications for cancer therapeutics.** The cancer stem cell model has fundamental implications for the development of new cancer therapeutic agents. Antineoplastic agents have largely been developed through testing in animal models as well as phase II human trials. In both of these, the measured outcome has been shrinkage of tumors. Tumor response is usually defined in the clinic as the shrinkage of a tumor by at least 50%. However, if cancer stem cells are inherently resistant to therapeutic agents and if these cells comprise only a minority of the tumor cell population, then shrinkage of tumors may reflect the effects of these agents on the differentiated cells in a tumor rather than the cancer stem cell component. This may explain why in clinical trials for advanced cancers, tumor regression often does not translate into clinically significant increases in patient survival. This has been shown in many tumor types, including solid tumors and multiple myeloma, where patient survival does not correlate with changes in the M-protein levels (55). If the cancer stem cell hypothesis is valid, then we may need to devise new experimental paradigms other than assessment of tumor regression for the evaluation of antineoplastic agents. To develop therapies that target the cancer stem cell population, it will be important to find and validate intermediate end points that predict ultimate patient survival. For instance, future clinical trial design may use such intermediate end points as time to tumor progression following delivery of an agent that can target cancer stem cells.

**Therapeutic resistance of cancer stem cells.** By virtue of their fundamental importance in organogenesis, normal stem cells have evolved mechanisms that promote their survival and resistance to apoptosis. For example, during normal mammary involution following lactation, there is massive apoptosis of differentiated cells, whereas stem cells are spared and regenerate the gland during subsequent pregnancies. Inherent resistance of normal stem cells to apoptosis is also observed in patients undergoing cytotoxic chemotherapy. When patients are given nonmyeloablative doses of cytotoxic chemotherapy, they experience a transient decrease in their WBC counts. This is caused by apoptosis of differentiated neutrophils and myeloid precursors. Stem cells in the bone marrow are not ablated by these doses of chemotherapy and are able to regenerate a normal hematopoietic system after several weeks. Similarly, many of the gastrointestinal side effects of chemotherapy are caused by induction of apoptosis in differentiating colonic epithelial cells. These dying cells are regenerated by gut stem cells that survive these chemotherapeutic insults. Just as normal stem cells may be resistant to the induction of apoptosis by cytotoxic agents and radiation therapy, cancer stem cells may display increased resistance to these agents compared with more differentiated cells that comprise the bulk of tumors. Supporting this concept, Guzman et al. have shown that leukemic stem cells are more resistant to chemotherapy than are the more differentiated myeloblastic cells that constitute the vast majority of cells in leukemia (56). Similarly, Matsui et al. have shown that myeloma stem cells are resistant to many therapies being used to treat myeloma including chemotherapy and the proteosome inhibitor Velcade (35, 57). There are several molecular mechanisms that may account for the resistance to apoptosis of cancer stem cells. These include (*a*) cell cycle kinetics. Many cancer stem cells are not cycling and are in $G_0$ and thus resistant to cell cycle–specific chemotherapy agents (58). (*b*) DNA replication and repair mechanisms. Stem cells may be resistant to DNA-damaging agents by virtue of being able to undergo asynchronous DNA synthesis in addition to displaying enhanced DNA repair (59–63). (*c*) During asynchronous DNA synthesis, the parental "immortal" DNA strand always segregates with the stem cell and not the differentiating progeny. This process may be regulated by P53 (64). This prevents the stem cell compartment from accumulating mutations associated with replication or from being affected by DNA-damaging agents. (*d*) Antiapoptotic proteins. Stem cells express higher levels of antiapoptotic proteins, such as members of the Bcl-2 family and inhibitors of apoptosis, than do differentiated cells (65). (*e*) Transporter proteins. Stem cells express high levels of transporter proteins, such as ABCG2 (BCRP), as well as P-glycoprotein. The development of effective immunologic approaches to cancer therapy may also be affected by the existence of cancer stem cells. Many of these therapies have involved targeting cells that express tumor-specific antigens. These antigens may be selectively expressed on differentiated tumor cells. Cancer stem cells that do not express these antigens may thus be spared by these immunologic interventions.

The concept of cancer stem cells also has implications for the development of targeted therapies. Arguably, the most successful targeted therapy has been the development of imatinib that targets BCR-Abl in patients with CML. The vast majority of patients with early stages of CML are put into a remission by administration of imatinib. However, recent studies have suggested that although imatinib may target differentiated and progenitor CML cells, it does not eliminate CML stem cells that harbor this mutation. Following withdrawal of imatinib in animal models or the development of a resistant clone in patients, the disease reappears with kinetics predicted by a stem cell model (66). These studies suggest that the cure of CML will require the elimination of BCR-Abl containing CML stem cells.

If the ultimate cure of various cancers depends on the elimination of cancer stem cells, one can question why several malignancies, such as testicular carcinoma in men and choriocarcinoma in women, are curable with chemotherapy even in advanced disease, whereas the majority of common epithelial malignancies are not. One might speculate that the stem cell component of testicular carcinoma and choriocarcinoma are inherently different from those in other tissues, because these tumors arise in germ cells. Indeed, chemotherapy treatment of these tumors often produces residual masses that are benign teratomas composed of differentiated cells. An understanding of the inherent differences between stem cells of testicular cancer and choriocarcinoma compared with those from other tumors may provide new clues for the development of therapies for more common tumor types.

**Opportunities for new therapeutics.** The cancer stem cell model suggests that it may be necessary to alter the current paradigm in drug development. Eradication of cancers may require the targeting and elimination of cancer stem cells. Thus, one must devise strategies that can selectively kill these cancer stem cells while sparing normal stem cells, such as those in the gut and bone marrow. This represents a challenge because many pathways, such as those involved in self-renewal, are shared by cancer stem cells and their normal counterparts. However, a variety of recent studies using animal models that have targeted these pathways indicate the feasibility of this approach. For example, Notch signaling requires processing by the enzyme

γ-secretase. An inhibitor of this enzyme has been recently shown to have activity against breast cancers that over express Notch1 (67, 68). Agents targeting Hedgehog signaling have recently been shown to have antineoplastic activity. The Hedgehog inhibitor cyclopamine that specifically inhibits Hedgehog signaling was used to treat animals bearing a variety of tumor xenografts. Cyclopamine caused dramatic regression of tumors that did not recur following cessation of treatment. Furthermore, at least over brief periods, the administration of these agents seemed to be nontoxic (17). A Hedgehog pathway inhibitor, HhAntag, with greater activity than cyclopamine has recently been shown to block medulloblastoma formation in a transgenic mouse model (69). These studies support the feasibility of selectively targeting the cancer stem cell population. The elimination of this key cell population may result in improved therapeutic outcomes for patients with even advanced cancers.

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## Response

In the accompanying article, Hill presents his views that the current evidence is not conclusive regarding the existence of cancer stem cells in solid tumors. Although we agree that much remains to be learned about tumor stem cells, we feel that the substantial biological and clinical implications of this model justify intensive research in this area. Hill points to several theoretical and methodologic questions regarding the experimental evidence for the existence of cells with stem cell properties in solid tumors. He points out that the relative inefficiency of transferring human tumors to xenografts may be due to inherent inefficiencies in the systems rather than tumor subpopulations that differ in their tumorigenicity. We believe that the recent prospective identification of solid tumor stem cells in a variety of malignancies, including breast cancer, brain cancer, and prostate cancer, provide strong evidence that "not all cancer cells are equal." Only a relatively small percentage of cells with characteristic cell surface markers are able to be serially passaged in immunocompromised mice, a demonstration of their self-renewal capacity. We do, however, agree with Hill that tumor stem cells may themselves be heterogeneous with varying self-renewal capacity. Indeed, as stated in our article, this has recently been shown for human leukemias. We also agree with his statement that the microenvironment is important in determining the behavior of transplanted cells. In our review, we stress the importance of the stem cell niche in normal stem cell function and the tumor microenvironment in tumor growth and progression. For this reason, solid tumor xenograft models of breast and brain cancer have used orthotopic installation of tumors to the mammary fat pad or brain, respectively. Hill contends that genetic instability drives tumor development, so that the relationship between stem cell behavior and differentiation might change during tumor progression. Although genetic instability undoubtedly plays an important role in tumor progression, molecular profiling studies suggest that the biological behavior of tumors is inherent in the initial tumor. This is more consistent with a tumor stem cell model in which tumor behavior is largely determined by the cell of origin and its genetic profile. Hill also speculates that differentiated tumor cells that have "lost the ability to manifest as cancer stem cells might regain the ability next week or next month." However, we are unaware of any direct evidence of dedifferentiation of tumor cells. In xenograft models, differentiated tumor cells from tumors fail to form tumors when transplanted even after long periods of observation. Finally, it is important to distinguish between markers that may serve to identify tumor stem cells from molecules that play important roles in stem cell behavior. In many cases, the markers present on tumor stem cells mimic that of their normal stem cell counterparts. Thus, the argument that proteolytic digestion of solid tumors changes cell surface markers has no direct bearing on the behavior of these cells when they are introduced into immunocompromised mice.

In summary, cancer stem cells were first described in human leukemias. Accumulating evidence in a variety of solid tumors suggest that these tumors may also be driven by a subset of cells that display stem cell properties. Further studies should lead to a greater understanding of the biology of these cells with significant implications for cancer treatment and prevention.