Medical Advances

Cancer Stem Cell: The Seed of Tumors?

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Introduction

According to the World Health Organization, cancer is a leading cause of death worldwide (~ 7.9 million people died of cancer in 2007) and will replace heart disease as the number one global killer by 2010. Tumor tissues have long been known to contain heterogeneous massive populations of cancer cells. The most important question for basic researchers and clinicians in oncology field is to determine how many and which tumor cells must be eliminated for treatments to be successful. The traditional therapies to cure cancer patients are designed to kill proliferating cells, based on the long-held concept that tumor formation and growth are due to increased proliferation of cancer cells compared with cells in normal tissues. However, it has been disappointing that these therapies often failed in most cases. Recently, the cancer stem cell (CCS) hypothesis, which suggests that cancers develop from a small subset of cells with self-renewal properties analogous to organ stem cells, has received much attention and attempts to bring new approaches to the development of more effective diagnostics, therapeutics, and prevention strategies in oncology.

The Concept of the Cancer Stem Cell Model

All human tissues contain certain number of stem cells, which are able to self-renew (thus undergo an unlimited number of cell divisions) and to differentiate into all cell types of the tissue. These stem cells proliferate infrequently but form a pool of long-lived cells to continuously supply more differentiated cells to their tissue compartments and thus maintain the mass and architecture of tissues over time through a tightly regulated process of renovation. This process is particular significant for homeostatic control of tissues undergoing rapid and continuous cell turnover, such as blood and epithelium.

Accordingly, the cancer stem cell hypothesis proposes that tumors are actually akin to "abnormal organs," sustained by diseased "cancer stem cells", a small subset of cancer cells, which are endowed with the exclusive capacity to self-renew and thus constitute a reservoir of self-sustaining cells and to aberrantly differentiate into the heterogeneous non-tumorigenic cancer cell types that in most cases appear to constitute the bulk of the cancer cells within the tumor.[12,13,14,36]

The cancer stem cell hypothesis is supported by the observation that, like their normal tissue counterparts, tumors comprise a heterogeneous collection of cell types that differ in apparent state of differentiation. Indeed, clinical practice by surgical pathologists uses the differentiation features of a tumor, morphological and architectural, as the key parameter in routine to define a tumor's primary anatomical origin. This simple observation disfavors the traditional view that tumors are mere monoclonal expansions of cells obtaining tumorigenic mutations (either activation of oncogenes or inactivation of tumor suppression genes), but provides strong support for the cancer stem cell model. 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,[17,18] sometimes over a period of many years. Because stem cells are the only long-lived cells in many tissues, they are the natural candidates in which early transforming mutations may accumulate.

Thus, cancer stem cells are defined as a minority but phenotypic subset of tumor initiating cells that can give rise to a heterogeneous progeny, similar in composition to the tissue from which it was originally isolated and are expected to have three basic properties.[7,12] First, they are usually endowed with tumorigenic potential and able to recapitulate the generation of a continuously growing tumor, when transplanted into immunodeficient mice. Second, tumorigenic cancer stem cells are characterized by a distinctive profile of surface markers and can be differentially and reproducibly isolated from non-tumorigenic ones by means of flow cytometry or other immunoselection procedures. Finally, tumors grown from tumorigenic cancer stem cells contain mixed populations of tumorigenic and nontumorigenic cancer cells, thus recreating the full phenotypic heterogeneity of the parent tumor.

Assays and Markers to Define Cancer Stem Cell

Several tissue specific stem cell markers are used to define and isolate cancer stem cells. For example, cell surface markers including CD44, CD34, and CD133 have been used

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to successfully identify cancer stem cell of leukemia,[3,19] brain tumor,[19,39] myeloma,[29] breast cancer,[1] colon cancer,[31,37] prostate cancer,[8,33] pancreatic cancer,[27] melanoma,[15] and other tumors . Although less frequently applied, SP fraction defined by Hoechst dye efflux properties and label retention (bromodeoxyuridine incorporation) studies have also been proposed as ways of identifying cancer stem cells.[7] Stem cells in different tissues have different properties. However, they share two fundamental hallmarks: self-renewal and lineage capacity. Therefore, as with normal stem cells, cancer stem cells need to be identified and evaluated for their potential to show both self-renewal and tumor propagation. While a few in vitro assays are available to examine activities of cancer stem cells in a rapid way, the gold standard assay that fulfills these criteria is the in vivo serial transplantation in animal models. The assay, although imperfect, is regarded as the best functional assay for identifying cancer stem cells. In such transplantation assays, cells from whole tumor tissues are purified into single-cell suspensions and subsequently fractioned into different subsets based on the expression of certain repertoire of surface markers. Individual cancer cell subsets are then xenografted into an orthotopic site of immunocompromised (typically nonobese diabetic, severe-combined immunodeficient (NOD/SCID) mice that are checked at various time points for tumor formation. To show self-renewal, cells then must be isolated from the tumors and grafted into a second recipient animal. According to the cancer stem cell model, only a specific subset of the cancer cell population (i.e., the long-lived cancer stem cell 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.[1,9,12, 21,27,38,41]

Landmarks of Cancer Stem Cell Research

The basic idea that stem cells are the cells of clonal origin of malignancies has been existing for more than several decades and several approaches had been used to measure stem cells in tumors, but significant progress had not been made over a long period, due to limited knowledge of normal stem cells and the lack of effective experimental assays for isolating putative cancer stem cells and for their functional study. Critical advances occurred in the late 1980s and early 1990s, when distinct cell surface marker profiles for stem cells became known. These advances allowed researchers to isolate normal stem cells and putative cancer stem cells prospectively by fluorescence-activated cell sorting (FACS). Recently, a new wave of studies has begun to address whether these FACS purified subsets of cancer cells are cancer stem cells, using an innovative and empirical approach, based on the abovementioned in vivo transplantation assay, to test the capability of self-renewal and differentiation.

Among mammalian tissues, the hematopoietic system is the first- and best-characterized in terms of hierarchical organization and sequential differentiation of cellular subpopulations.[32] Therefore, it is not surprising that cancer stem cells were initially isolated from blood cancers. Studies of John Dick and colleagues on acute myeloid leukemia (AML) provided the first compelling evidence for the existence of a cancer stem cell subpopulation (Lapidot et al. 1994; Bonnet and Dick 1997). Using FACS to prospectively isolate cells from human AML, they found that only CD34+CD38- cells, but not CD34- or CD34+CD38+ cells, were able to initiate leukemia in irradiated transplanted NOD/SCID mice. Most interestingly, analysis of leukemia cell populations developed in recipient mice revealed reconstitution of the phenotypic heterogeneity observed in the original donor. This study suggests that AML stem cells possess a CD34+CD38- cell surface phenotype, similar to that typical of normal human primitive hematopoietic stem cell (HSC). This study also raises the possibility that the AML stem cells may have originated from normal stem cells rather than arising from more differentiated cells. Later works on lymphoid and chronic myeloid leukemia also support this idea that stem cells are a common target of pre-leukemic-event or leukemic transformation.[6,22]

Relative to hematopoietic system, cells within solid tumors are less accessible, and functional assays and surface markers suitable for detecting and quantifying normal stem cells from many organs have not yet been developed. Therefore, it is more difficult to obtain evidence for the existence of cancer stem cells in solid tumors. In 2003, Clarke and co-workers addressed this issue by analyzing breast tumor, which has been reported to be composed of distinct populations based on the expression of cell surface markers including CD44 and CD24.[1] In this impressive work, fractioned cells have been isolated from human breast tumors and implanted into the mammary pads of NOD/SCID mice. CD44+CD24- cells established tumors in recipient mice when as low as 100 cells were transplanted, whereas tens of thousands of cells from the CD44+CD24+ or CD44- fractions did not form tumors. Further, engrafted tumors exhibited morphologic and immunophenotypic similar to the original specimen, consisting not only CD44+CD24- cells but also CD44+CD24+ or CD44- cells. Finally, engrafted tumors could also be serial transplanted, suggesting a capacity for self-renewal. Similar strategy has been applied to identify cancer stem cells in tumors from brain, prostate, colon, lung, liver and other systems.[3,13,19,33,39]

Implications for Basic Research: The Role of Cancer Stem Cell in Cancer Initiation, Primary Tumor Growth, and Metastases

Decades of oncology research has established one central idea for understanding of human carcinogenesis: most, if not all, cancers result from the accumulation of a series of specific genetic mutations and/or epigenetic changes. These events result in activation or overexpression of proliferation promoting genes (oncogenes) or silencing growth inhibiting genes (tumor suppression genes), and eventually lead to development and unchecked growth of tumors and their progression to metastases. The process for accumulation of these mutations may take years or even decades. Because of

tissue renewal, however, most cells are lost or eliminated in days or weeks. Thus, any mutations they have acquired would be lost with their loss. The cancer stem cell hypothesis provides an attractive model to address this dilemma. The long lifespan of stem cells allows for multiple genetic hits to occur and accumulate. Additionally, stem cells require fewer genetic hits to become cancer initiating cells than more differentiated cells, thanks to their physiologic capacity of self-renewal, which is necessary for tumorigenesis. However, it is also formally possible that more committed progenitor cells or even fully differentiated mature cells may "reacquire" the capability of self-renewal first and then become cancer stem cells (**Figure 1**).

Furthermore, it has been known for long time that tumorigenesis involves aberrant tissue organization and these tumor tissues are composed of heterogeneous populations of cancer cells. The ability to different into progenies of cells of cancer stem cells provides a cellular mechanism that can explain how the genetic and epigenetic changes give rise to the tissue changes during the phase of tumor progression.

Metastasis is one of the most ominous properties of malignant cancer cells and causes death of many, if not most, cancer patients.[16,30] Comparison between paired primary tumors and distant-site metastases revealed striking similarities over a wide range of parameters including genome wide transcription profile[11,42,43] and tissue morphology.[4,5] These observations are in contrast to the traditional monoclonal model of caner but can be well explained by the cancer stem cell hypothesis. Both primary tumors and metastatic tumors are formed by stem cells with identical genetic backgrounds and abnormalities, they should undergo similar differentiation programs and display similar pattern of intratumor heterogeneity and expression profiles. Actually, there is a growing body of evidence that suggests that metastases develop only when distant organs are seeded with cancer stem cells that arise from a primary tumor.[10,27] For example, Hermann and colleagues showed that only the CD133+CDCR4+ subset of the pancreatic cancer cells have the ability to metastasize.[20]

Clinical Implication of the Cancer Stem Cell Hypothesis

Current systemic cancer therapies frequently fail to completely eliminate advanced tumors. The cancer stem cell model could explain the failure of these therapies and may lead to design of novel and more effective anticancer treatments.

Stem cells are relatively quiescent and proliferate only infrequently. In addition, the presence of multi-drug resistance, anti-apoptotic proteins, and enhanced DNA repair mechanisms in stem cells make them be more resistant to chemotherapeutic agents and radiation than are more mature cell types from the same tissues.[2] Since all the features appear to hold true for cancer stem cells as well, those cells would be predicted to be more resistant to current therapies, which are designed to destroy rapidly dividing cells that constitute the majority of tumor population. Consequently, current therapeutic approaches might kill the bulk of tumor cells and induce temporary regression of gross tumor lesions but achieve little success: they do not eradicate cancer stem cells that fuels tumor growth and thus fail to prevent disease relapse and metastatic dissemination (**Figure 2**). Accordingly, the cancer stem cell model also explains why, despite extensive preclinical validation in vitro, quite a few experimental therapeutic approaches have not shown promising clinical results. Thus, traditional treatments should be recalibrated and investigational therapies should be developed to target the cancer stem cell sub-populations as well (**Figure 2**). For example, novel therapies should be designed to target signaling pathways involved in regulation of self-renewal, which are usually either mutated or epigenetically dysregulated in cancer stem cells. These pathways include hedgehog, Notch, PTEN, BMI-1, WNT, and P53 pathways.[28,36] Control of cancer stem cell numbers by targeting cancer stem cell surface antigens would also be an effective strategy to prevent and eradicate tumors. For example, antibodies against CD44, a cancer stem cell surface antigen, effectively eliminated human acute myeloid leukemic cancer stem cells in immunodeficient mice transplanted with human AML.[21,25] Microarray and proteomic profiling of cancer stem cells will also likely lead to identification of potential therapeutic targets.

Finally, cancer stem cell markers could also be used to predict treatment responses and evaluate therapy efficacy for new treatment strategies. For example, an important clinical end point to monitor anti-tumor therapies should be checking the number of cancer stem cell in response to treatments, in addition to the traditional way of checking regression of the bulk tumor.

Challenges and Controversies

Despite the huge growth in the cancer stem cell field, challenges remain. Several recent reports were published to question the universality of the cancer stem cell model.[24,40,44] These works found that in murine leukemia models, almost every cancer cell had cancer initiating activity and there was little evidence of functional heterogeneity. A more recent paper,[35] by using more highly immunocompromised mice, demonstrated that 25% of human melanoma can initiate a tumor. Moreover, this study revealed that the some, but not all, of the tumor initiating cells share many different features, but none of these feature shows a particular association with tumorigenic potential. These reports together have raised the concern that the xenotransplantation experiments do not accurately reflect what happens during cancer development in humans. For example, the rarity of human cancer stem cells observed using xenotransplantation models may be the results of host resistance factors. The interpretation of such xenotransplantation studies is also complicated by that micro-environmental factors, including the soluble cytokines or membrane bound factors presented by non-malignant host-cell populations, can also have a significant role in tumor growth. Thus, the observed rarity of cancer stem cells may also be explained by the absence of cross-species reactivity to

these micro-environmental factors. More carefully designed studies are needed to answer these questions.

Summary and Perspectives

The cancer stem cell theory brings a paradigm shift to the oncology field. This model envisages human tumors as complex tridimensional tissues sustained by a pathological counterpart of normal stem cells, the cancer stem cells. These cancer stem cells have the ability to self-renew and differentiate into functionally heterogeneous populations of tumor cells and therefore drive tumor growth. Like normal stem cells, cancer stem cells are relatively quiescent and drug resistant, therefore account for many treatment failures. Thus, as depicted in **Figure 2**, this model suggests that the only effective approach to cure cancer should include cytotoxic chemotherapies and radiotherapies to kill the proliferating bulk of the tumor (to abolish the damage immediately) as well as CSC-directed therapies to eliminate this critical cell population (to prevent relapse and metastasis). However, as the research into cancer stem cells is still at early stages and is still gathering steam, much preclinical and clinical research efforts are still required to evaluate the effectiveness of cancer stem cell targeted therapy.

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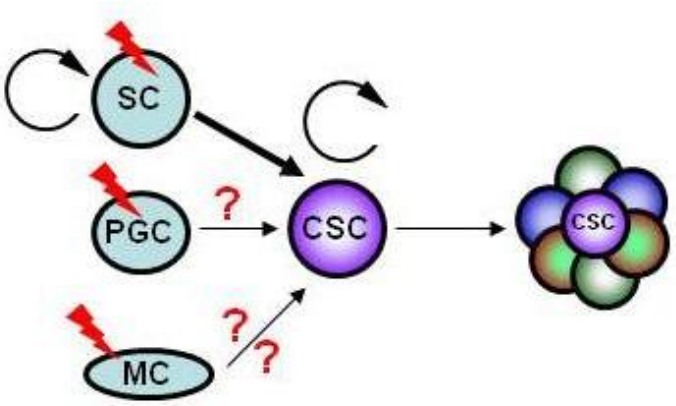

Figure 1. The cancer stem cell model. The cancer stem cells (CSCs) might appear after mutations or epigenetic dysregulation accumulated in stem cells (SC), or, less possibly, from progenitor cells (PGC) or even fully differentiated mature cells (MC). These CSCs have the capability to self-renewal and differentiate into progenies of heterogeneous populations of bulk cancer cells and hence drive the tumor growth.

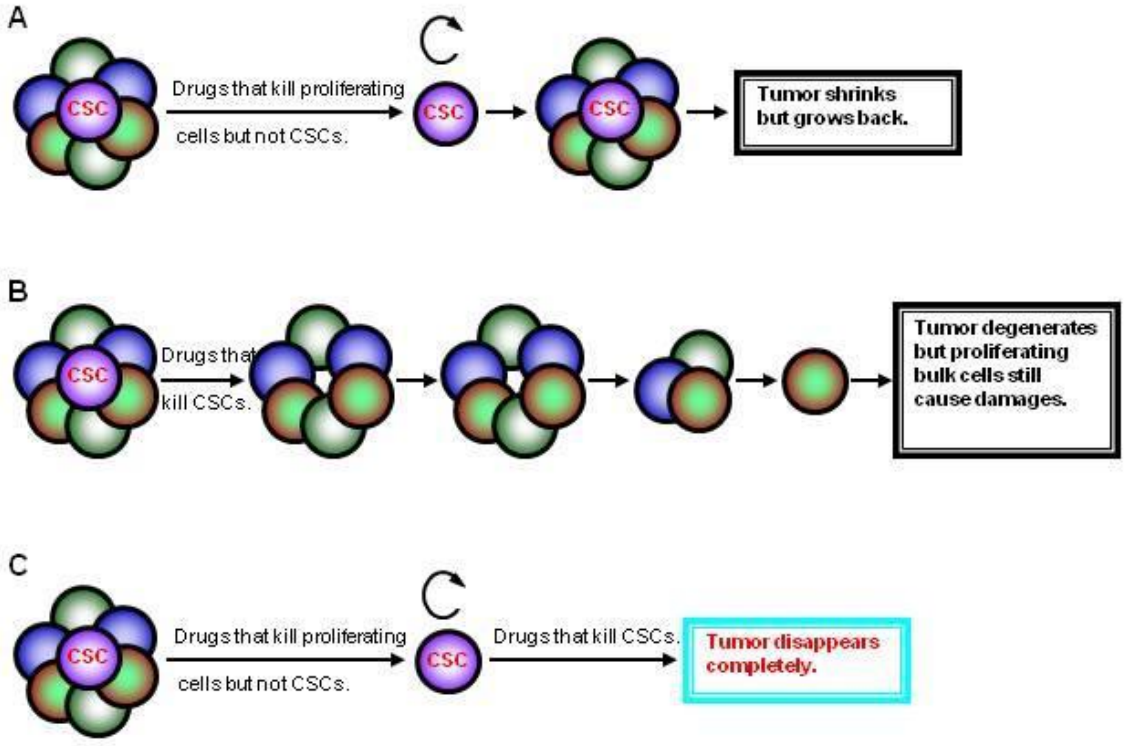

Figure 2. Three ways to design.