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Review

New hope in the horizon: cancer stems cells

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The major goal of researchers and oncologists is to develop promising ground for novel therapeutic strategies to prevent recurrence or relapse of cancer. Recent evidences suggest that a subset of cells called cancer stem cells (CSCs) are present within the tumor mass which possess tumorigenic capacity and may be responsible for propagation, relapse, and metastatic dissemination. These cells have certain stem cell-like properties, e.g. quiescence, self-renewal, asymmetric division, and multidrug resistance which allow them to drive tumor growth and evade conventional therapies. A number of markers and assays have been designed to isolate and characterize the CSC population from the bulk tumor. The objective now is to selectively target the CSCs in order to eliminate the tumor from root, overcoming the emergence of clones capable of evading traditional therapy. This approach may help in increasing the overall disease-free survival in some cancers.

Keywords cancer; stem cells; therapy

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Introduction

The classical model of carcinogenesis suggests that all neoplastic cells within a tumor have tumorigenic capacity. This model assumes that all cells within a tumor maintain an equal ability to sustain tumor growth and to phenotypically recapitulate the original tumor on xenotransplantation. Consistent with this view, the traditional cancer therapies have been designed to eliminate as many cancer cells as possible. However, in recent decades, biomedical research has revealed that cancer comprises a heterogeneous population of cells with marked differences in their proliferative potential as well as the ability to reconstitute the original tumor on xenotransplantation. In this light, it will be imperative to determine which cancer cells have potential to contribute to disease progression in order to devise more effective treatments, and the cancer stem cell (CSC) model proposes that the growth and progression of many cancers are driven by small subpopulations of CSCs [1].

In the early 1990s, clinical observations and genetic studies of a variety of cancers led to the hypothesis that six genetic mutations were necessary to convert a normal somatic cell into a cancer cell [2,3]. These six mutations included: (i) self-sufficiency for growth signals, (ii) insensitivity to antigrowth signals, (iii) evasion of apoptosis, (iv) limitless ability to replicate, (v) sustained angiogenesis, and (vi) tissue invasion and metastasis. As both progenitor cells and mature cells have a very limited lifespan, it is unlikely that all of the mutations could occur during their life. In addition, to maintain the disease, cancer cells must overcome the tight genetic constraints on both self-renewal and proliferation [4]. CSCs must possess the ability to self-renew, it follows that they are derived either from self-renewing normal stem cells that could be transformed by altering only proliferative pathways or from progenitor cells that have acquired the ability to self-renew as a result of oncogenic mutations.

Stem Cells to CSCs

Recent discovery that the adult body harbors small numbers of stem cells offered an alternative possibility for the origin of cancer. It was hypothesized that only one or two mutations, such as self-sufficiency in growth or insensitivity to antigrowth signals, are needed for stem cells to initiate tumorigenesis rather than six mutations, a rare event in any type of cell. Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. The most important potential application of human stem cells is the generation of cells and tissues that could be used for cell-based therapies. Stem cells, directed to differentiate into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases including Alzheimer's diseases, spinal cord injury,

stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis. The most important and useful property of stem cells is to self-renewal and to regulate the relative balance between self-renewal and differentiation [5]. Stem cells sit at the top of the developmental hierarchy, having the ability to self-renew and give rise to all the cell lineages in corresponding tissues. Stem cells divide to produce two daughter cells. One daughter remains a stem cell (self-renewal). The other daughter becomes a progenitor cell that undergoes expansion and further differentiation into mature cells. Stem cells have the highest potential for proliferation and a much longer lifespan compared with their progeny and therefore have a greater opportunity to accumulate genetic mutations [6]. There are two major types of stem cells, adult stem cells and embryonic stem cells. Embryonic stem cells can become all cell types of the body because they are pluripotent, whereas adult stem cells are generally limited to differentiating into different cell types of their tissue of origin [7].

There are similarities between stem cells and cancer cells: tumors may often originate from the transformation of normal stem cells, similar signaling pathways may regulate self-renewal in stem cells and cancer cells, and cancer cells may include 'CSCs'—rare cells with indefinite potential for self-renewal that drive tumorigenesis. A subpopulation of cancer cells have been identified as the CSCs which have the ability to initiate tumorigenesis by undergoing self-renewal and differentiation, like normal stem cells, whereas the remaining majority of the cells are more 'differentiated' and lack these properties [8] (**Fig. 1**). The acquisition of characteristics such as self-renewal, organization into a specific hierarchy, resistance to apoptosis and drugs, and cell migration contribute to the plethora of stem cell-like features that suggest the involvement of stem cells during the process of tumor progression [9]. These similarities led to the emergence of two alternative hypotheses: either that stem cells themselves could be targets of transforming mutations, or dedifferentiation of transformed, terminally differentiated cells results in the emergence of CSCs and thereby, disease manifestation [6]. The deregulation of normal tissue homeostasis despite the existence of checkpoints at various levels, through either adult stem cell transformation, maturation arrest of progenitors, and/or acquisition of a capacity by differentiated cells to re-enter the cell cycle and undergo uncontrolled proliferation, is thus suggested to account for the generation of CSCs [10].

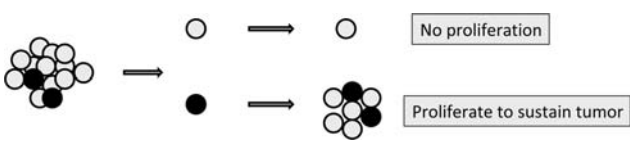

Figure 1 CSCs and tumor proliferation The CSCs (solid black) are the very small number of cells implicated in the tumor population which contribute to disease progression. Gray indicates the bulk tumor population.

The field of solid tumor stem cell biology has re-emerged at the forefront of clinical oncology in recent years due to the identification of these cells. A tumor can be viewed as an aberrant organ initiated by a tumorigenic cancer cell that acquired the capacity for indefinite proliferation through accumulated mutations. If one views a tumor as an abnormal organ, then the principles of normal stem cell biology can be applied to understand better how tumors develop [11]. Both normal stem cells and tumorigenic cells have the extensive proliferative potential and the ability to give rise to new (normal or abnormal) tissues. Both tumors and normal tissues are composed of heterogeneous combinations of cells, with different phenotypic characteristics and different proliferative potentials [12,13]. Because most tumors have a clonal origin, tumorigenic cancer cells must give rise to phenotypically diverse progeny, including cancer cells with indefinite proliferative potential, as well as cancer cells with limited or no proliferative potential. This suggests that tumorigenic cancer cells undergo processes that are analogous to the self-renewal and differentiation of normal stem cells. Many types of tumors contain cancer cells with heterogeneous phenotypes, reflecting aspects of the differentiation that normally occurs in the tissues from which the tumors arise [6]. Thus, tumorigenic cells can be thought of as CSCs that undergo an aberrant and poorly regulated process of organogenesis analogous to the normal stem cells. In other words, CSCs are cancer cells (found within tumors or hematological cancers) that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. CSCs are therefore tumorigenic (tumor forming), perhaps in contrast to other non-tumorigenic cancer cells. CSCs may generate tumors through the stem cell processes of self-renewal and differentiation into multiple cell types. Both stem cells and progenitor cells could act as CSCs, e.g. leukemia induced by the BCR-ABL oncogene includes chronic myeloid leukemia (CML) and B cell acute lymphoblastic leukemia. In the early phase of the disease, BCR-ABL expressing heamatopoietic cells contain leukemia stem cell population [14]. It was reported that during blast crisis of chronic myelogenous leukemia (CML), abnormal granulocyte macrophage progenitors (GMPs) with nuclear β-catenin acquire self-renewal potential and may function as leukemic stem cells [15]. This was further substantiated by *ex vivo* studies where expression of BCR-ABL in the progenitor cells reproducibly stimulated myeloid expansion in culture and generated leukemia-initiating cells specifically in the GMP compartment [16].

The differences between normal stem cells and CSCs are dependent on the stem cell niche, a specialized microenvironment where stem cells reside, proliferate, and differentiate [17]. The niche maintains stem cell homeostasis, serves as a shield from tumorigenesis, and provides regulatory signals for tissue regeneration [18–20]. Niche normally generates dominant signals that inhibit stem cell differentiation and restrict normal stem cells from over-proliferation for self-renewal and multilineage commitment [21]. Disruption of the niche signal can lead to either loss of stem cells or cancer. Such cells are proposed to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors [17]. There is increasing evidence that solid cancers contain cancer-initiating stem cells that are capable of regenerating a tumor that has been surgically removed and/or treated with chemotherapy and/or radiation therapy. Therefore, development of specific therapies targeted CSCs holds hope for improvement of survival and quality of life of cancer patients, especially for sufferers of metastatic disease.

Identifying the CSCs

CSCs' ability to self-renew entails also that they require fewer mutations than differentiated cells to acquire all the canonical properties of cancer cells. In accordance with the new concept of cancer, the majority of tumor cells are derived from an aberrant differentiation process of CSCs, generating a heterogeneous tumor cell population unable to self-renew [22]. This concept clarifies the clinical observation of recurrence of tumors many years after treatment that supposedly eradicated the primary tumor. Scientists now believe that the development, growth, and metastasis in cancer are driven by the CSCs. The isolation and characterization of CSCs represent a revolutionary approach in cancer research with considerable therapeutic implications. The common therapeutic drugs include cytotoxic compounds that can trigger the intrinsic cell death mechanism in cancer cells having high turnover rates. The CSCs, on the other hand, have a slow rate of cell division, overexpression of ATP-binding cassette transporters, constitutive activity of detoxifying enzymes, active DNA repair system, and expression of anti-apoptotic factors. It is therefore postulated that complete disease remission could be obtained only after total elimination of the CSC population. Traditionally, the cancer treatment is based on the ability to kill the proliferating cells indiscriminately. The present therapeutic strategies do not take into account the potential differences in the proliferative capacity, drug sensitivity or target expression between the CSCs and other cells in the tumor bulk. There are certain evidences that the response to therapy in cancer may be influenced by epigenetic differences between tumorigenic cells and their non-tumorigenic progeny [23]. Chronic myeloid leukemia has been reported to be sustained by leukemic stem cells that are more resistant to the drug imatinib than their differentiated progeny [24]. There is also evidence that CSCs in gliomas [25] and breast cancers [26] might be intrinsically more resistant to existing anti-cancer therapies than other cells in these cancers.

CSCs are rare, with plating efficiency ranging from 1/1000–5000 in solid tumors [27] to 1/1,000,000 in leukemia [28]. Identification of stem cell markers CD133 and CD44 and the use of non-obese diabetic mice with severe combined immunodeficiency disease (NOD–SCID mice) xenografts led to the successful isolation of CSCs in many different types of tumors [29–32].

The first characterization of a CSC population able to reconstitute the disease in an immunocompromised mouse dates to late 1990 by Bonnet *et al*. These authors identified $CD34^+CD38^-$ leukemic stem cells from human acute myeloid leukemia and, after comparing them with the $CD34^+CD38^+$ and $CD34^-$ fractions, demonstrated that these stem cells initiated leukemia in NOD–SCID mice [28]. In 2003, $CD24^-CD44^+$ cells were isolated from human breast tumors that could serially propagate in animals and recapitulate their original phenotype [8]. CSCs have since been identified for numerous other epithelial malignancies like melanoma [31], lung [33], head/neck [34], pancreas [35], prostate [36], and colon cancers [37]. Recent advances have unearthed the biologic identity and origin of CSCs in several types of cancers as well as are looking into the mechanisms that lead to transformation of normal cells to CSCs. In order to have a better understanding of the true cellular and molecular picture of CSCs, it is important to identify and isolate the CSC population from the tumor bulk.

Isolation of the CSCs

Different approaches are available to isolate cancer stem population. The side population technique has been used for many years to isolate both normal and tumor stem cells from different organs and species. It is based on the abilities of stem cells to exclude vital dyes such as Hoechst 33342 or Rhodamine 123 from the cells while differentiated cells retain these dyes. Expression of cell surface markers has been widely used to isolate stem cells, but the choice of marker can greatly vary depending on tissues or species. Among the methods that are frequently used for the enrichment and isolation of very small population of cancer progenitor cells with stem cell-like properties, there are fluorescence-activated cell sorting (FACS), using the specific antibodies directed against one or several stem cell-like surface markers, such as CD34, CD138, CD20, CD133, and/or CD44. Flow cytometry methods using cell

surface markers have been successfully applied to mice and human samples to isolate stem cell populations [20,38]. The ALDEFLUOR assay is based on the enzymatic activity of aldehyde dehydrogenase 1 (ALDH1), a detoxifying enzyme responsible for the oxidation of retinol to retinoic acid. ALDH1 has been used for identifying CSC population. CSCs were identified in multiple myeloma and leukemia patients with high capability of engraftment into NOD−SCID mice on the basis of ALDH1 activity [39]. However, the stem cell population identified using the ALDEFLUOR assay has been reported to be heterogeneous, and hence needs to be further verified using additional markers. Xenograft model of patient samples is also a reliable experimental model for study. Unlike cell line-derived xenografts, tumor xenografts maintain the cell differentiation and morphology, the architecture, and molecular signatures of the original tumors [40]. Vasculature, stroma, central necrosis, and peripheral growth occur in tumor-bearing mice in a way that is similar to that of the patient's tumor. Furthermore, tumor xenografts are the most relevant way to test CSC properties such as the ability to form tumors, self-renewal potential, and capacity to differentiate. However, concerns about the adequacy of immunodeficient mice model for stem cell studies still remain [41]. FACS and xenotransplantation of viable single cells from solid tumors require modifications of the approaches used for hematologic cancers because of the need to dissociate solid tissues into single cells, which are larger and more fragile than the hematopoietic cells. In the solid tumors, lack of the markers to characterize CSCs in the tumors have made it difficult to study the origin of these cells; however, there have been identification of cell surface markers in the lung [37], brain [42], prostate [43], and ovary [44] which may allow the separation of the stem or progenitor cells with the tumor-initiating function. Specific markers, typically examined on single cells using flow cytometry, are being used to identify the subpopulation of CSCs. Segregation of tumorigenic and non-tumorigenic population on the basis of marker expression remains the cornerstone of the CSC model. As additional studies are being done to further evaluate the markers, some markers will probably prove more robust than others, so the challenge remains to detect them in clinical samples and to determine their ability to predict outcome and/or response to treatment. The tumorigenic potential of CSCs has been based on the xenotransplantation studies in immunocompromised mice models. Quintana *et al*. [41] have successfully demonstrated that modification of Xenotransplantation assays can affect the detectable frequency of cells with tumorigenic potential. The interpretation of such xenotransplantation studies is complicated by the critical role in tumor growth of interactions with the microenvironment, which are mediated by both soluble and membrane-bound factors like growth factors, e.g. platelet-derived growth factor, epidermal growth factor, or tumor necrosis factor, and receptors e.g. integrin receptors or cytokines. These should be validated in significant number of patients before generalization in cancer. It is important to rigorously test the CSC model in the different above-mentioned conditions with validated assays to detect the full spectrum of cells with tumorigenic potential.

Therapeutic Implications

It is now clear that stem cells play a crucial role not only in the generation of complex multicellular organisms, but also in the development and progression of malignant diseases. It has been shown that most of the tumors contain a subset of distinct CSCs that are responsible for tumor initiation and propagation.

In tumors where the CSCs play roles, three possibilities may exist. First, the mutation of normal stem cells or progenitor cells into CSCs can lead to the development of the primary tumor. Second, during chemotherapy, most of the primary tumor cells may be destroyed; however, if CSCs are not eradicated, these may lead to recurrence of tumor. Third, the CSCs may migrate to distal sites from the primary tumor and cause metastasis [45]. Theoretically, identification of the CSCs may allow the development of treatment modalities that target the CSCs rather than rapidly dividing cells in the cancer. This may cure the cancer as the remaining cells in the cancer growth have limited proliferative capability. If cytotoxic agents spare CSCs, the disease is more likely to relapse. So, identifying and defining the unique properties of CSCs is important for developing early diagnostic and effective therapeutic strategies for human malignancies (**Fig. 2**). The challenge is to identify differences between CSCs and their normal

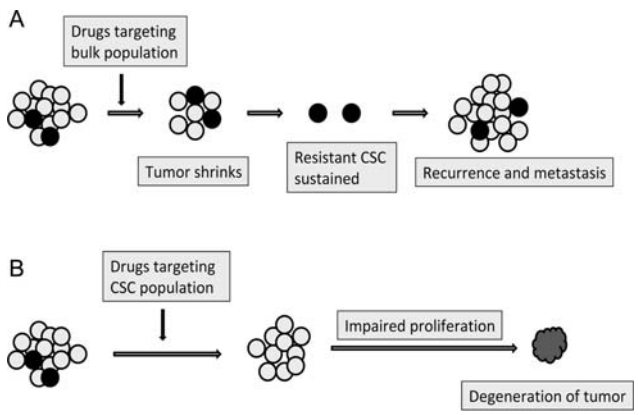

Figure 2 Therapeutic implications of targeting CSCs (A) The drugs that seek to destroy the bulk tumor cells often leave behind the CSCs that are relatively quiescent. These cells may lead to recurrence and metastasis. (B) If drugs are designed to target the CSC population, the chances of complete tumor remission increase.

stem cell counterparts, and demonstrate that complete control of cancer can be achieved by targeting these stem cells. Success of an anti-stem-cell strategy relies on complete inhibition of functions of a gene required for the maintenance of CSCs but not normal stem cells. A number of genes have been found to promote or inhibit cancer cell proliferation, but they also have similar roles in regulating normal stem cells, for example, in pathways involved in signaling through Wnt/β-catenin, Hedgehog and Notch [46,6,47–50], Bim-1 [51], p53 [47], $p16^{INK4a}$ [52], and $p19^{ARF}$ [53]. There may be many aberrantly expressed genes, so it is critical to identify key genes or pathways that are required for initiating and maintaining CSCs and that can be used as targets for inhibiting these cells. For example, inhibition of Notch pathway has been reported to deplete stem-like cells and block engraftment in embryonal brain tumors [54] and Hedgehog signaling is essential for maintenance of CSCs in myeloid leukemia [55]. *Alox5* function has been linked to many important signaling pathways such as p53 [56], NF-κB, and PI3K [57]. Chen *et al*. [58] have identified that loss of *Alox5* gene impaired leukemia stem cells and prevented CML. The work of Zhao *et al*. [50] showed that loss of β-catenin impairs the renewal of CML stem cells in the human system as well as in mouse model collectively, suggesting that Wnt signaling could be proved to be a relevant therapeutic target.

In fact, recently, Gupta *et al*. [59] have identified agents with specific toxicity for epithelial CSCs. These authors have used high-throughput chemical screening method and discovered compounds showing selective toxicity for breast CSCs. They observed that salinomycin reduced the proportion of CSCs by >100 folds relative to paclitaxel, which is a commonly used breast cancer chemotherapeutic drug. Treatment of mice with salinomycin inhibited mammary tumor growth *in vivo* and increased epithelial differentiation of tumor cells. In addition, global gene expression analyses showed that salinomycin treatment resulted in the loss of expression of breast CSC genes previously identified by analyses of breast tissues isolated directly from patients. However, it is important to test the CSC model in every circumstance to detect the full spectrum of cells with tumorigenic potential before its universal application. Additional testing of the CSC model will be required in all cancer types and the validity of markers in each type has to be established.

Conclusions and Future Prospects

We may conclude that the discovery of CSCs in malignancies and tumors has changed the therapeutic approach toward carcinogenesis. This new cancer model will help in designing further studies aimed at our ability to diagnose cancer and identify individuals at risk for metastasis. Thus, through proper combination of therapies, it is possible to treat cancer and convert it from a death sentence to a manageable or even curable disease in future.

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