Stem Cell Rev (2008) 4:211–216
DOI 10.1007/s12015-008-9037-x

Targeting Cancer Stem Cells in Cancer Prevention and Therapy

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Published online: 29 July 2008
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Abstract The cancer stem cell hypothesis is an attractive framework within which one may think about cancer initiation, recurrence, and metastasis, and methods to devise treatment strategies for cancers. Although all cancers do not appear to sustain themselves with cancer stem cells, but also through a dominant cell population, creating strategies for cancer treatment which include cancer stem cells as targets seems reasonable. In this perspective we discuss possible strategies for controlling the viability and tumorigenecity of cancer stem cells, and extend our discussion to strategies approaching the prevention of cancer.

Keywords Cancer · Stem cell · Breast · Colon · Signal transduction · Prevention · Vaccine · Antibody

Introduction

The developmental model of cancer alludes to the fact that a single transformed stem cell proliferates and/or differentiates to give rise to the bulk mass of the tumor and all the mature and immature cell types which make up the mass, in a manner reminiscent of ontogenic development. Both these processes may be ascribed to a stem cell-like entity, which generates diverse transformed or normal phenotypes from a single cell through cell-intrinsic properties of proliferation and differentiation. The existence of a cancer stem cell (CSC) is thus an extremely attractive framework within which to hypothesize that cancer can be controlled especially at the levels of tumor initiation and metastasis (initiation at distant sites).

Other evidence suggests that the dominant cell population within the cancer sustains the cancer by proliferation and dissemination, and the small number of CSCs within the tumor is not exclusively responsible for this function [1]. It is possible that these two models are not mutually exclusive, and a spectrum of both mechanisms exists in most tumors. Recent reports on mouse models of neurofibromatosis (selective deletion of neurofibromin protein in neural crest stem cells) suggested that although there was an increase in the number of neural stem cells in these animals in the embryonic stage, the neurofibromas which form in the adult animals are composed of and sustained by non-myelinating Schwann cells, a mature phenotype [2, 3]. In the literature the term cancer stem cell has at least two connotations: it refers to the cells which maintain an established tumor, or to the cells which initiated the tumor in the first place (although this is not necessarily the same cell as the CSC within the established tumor).

In general, it appears that tissues which divide actively, including blood, gut (colon), skin, pancreas and breast, have cells which meet the definition of CSCs [4]. In this perspective we will discuss some of the features of CSCs which are relevant to the design of novel therapeutic avenues, and extend the implications of this concept especially in high risk individuals who may be predisposed to familial cancers like those of breast and colon.

Identification of CSCs and their Properties

It is believed that most leukemias are clonal in nature, and thus the extension of the stem cell (SC) hypothesis as a possible explanation of these diseases is logical. Since the

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tumor burden is generated from a single original clone of cells it is possible that a single original 'stem cell', or cancer stem cell, proliferated uncontrollably to yield the excessive population of T or B cells. This CSC could have arisen from a stem cell acquiring transforming mutations, or from or a committed progenitor dedifferentiating into a stem cell. Alternatively a committed progenitor itself may acquire proliferative mutations and cause the tumor burden [5]. However, it is not clear that solid tumors are clonal, even though there is data in the literature detecting parts of tumors having the same methylation and microsatellite markers [6]. The scenario in solid tumors is complicated by the fact that the tumor recruits reactionary events from the connective and immune tissues which although constituting the tumor bulk, is not necessarily a founding portion of the tumor. However one may still speculate that a cancer stem cell may be responsible for founding of the transformed cells of the tumor, and that these stem cells may metastasize to distant locations and form similar tumors. Attempts to prove this in a xenograft model have had some success, where enriched populations of stem cells have formed tumors similar to the initial one when transplanted into SCID (severe combined immunodeficiency) mice.

In order to be defined as such, cancer stem cells would need to possess the following characteristics: they should have the properties of proliferation, differentiation (at least into the mature fates of cells typical of the tissue of their origin), and the ability to form a tumor similar to the one that they were derived from (usually tested in a xenograft model of SCID mice). CSCs have been identified with varying degrees of certainty from several cancers including leukemias, lymphomas, breast cancer, gliomas, meningiomas, colon, prostate, and pancreatic cancers. As is rife in the stem cell literature, there is a paucity of markers with which an unequivocal identification of cancer stem cells may be performed, at least in the case of solid tumors. While leukemia stem cells are isolated by selecting with the cell surface markers $CD34^+$; $CD38^-$, CSCs from solid tumors including breast (markers $CD24^{-/low}$; $CD44^+$), brain ($CD133^+$), colon ($CD133^+$ or $CD44^+$; Lin^-; ESA^+), prostate ($CD44^+$; $\alpha2\beta1^{high}$; $CD133^+$), and pancreas ($CD133^+$ or $CD44^+$; $CD24^+$; ESA) have been enriched for tentative CSC populations, but not purified [7–14]. While it appeared for a while that the cells isolated with the use of these markers were exclusively tumorigenic, and those which did not express these markers were not, recent publications have indicated that the scenario is not as clear cut. At least in brain and colon cancers, it appears that the CD133 negative population can also be tumorigenic contrary to what was originally believed [15, 16].

Other than a functional assay defining the capacity of these cells to form tumors in a xenograft/orthotropic assay, there is a very limited knowledge base by which one may distinguish a CSC from a normal stem cell. While recent proof of the concept of CSCs was rendered in a xenograft transplantation experiment [17], the xenograft assay where human CSCs are serially transplanted into immunocompromised mice to assay the formation of a tumor reminiscent of the original leaves some room for questions. Using this assay the idea that the CSC population is vanishingly small (a maximum of 0.01% of the tumor) was generated. However this low probability of tumor occurrence in xenografts may be due to the alien atmosphere of the mouse that the human cell is expected to generate a tumor within [1]. The necessary stromal, cytokine and chemokine factors which aid tumor formation will be different in a mouse when compared to a natural human host.

Implications of the CSCs in Cancer Treatments

In spite of all the caveats discussed above, the cancer stem cell hypothesis is very attractive if one hypothesizes that the recurrence and/or metastasis of cancer may be controlled if one might target the cancer stem cell. In order to do this effectively one must be able to target exclusively the cancer stem cell and not the normal stem cell. In addition, three other properties of the CSC make it a difficult cell to kill: firstly it exhibits effective efflux of drugs due to efficient expression of ABC (ATP binding cassette) transporters, secondly it is possibly a quiescent cell population thus precluding effective use of chemotherapeutic agents which target dividing cells, and lastly it is probably resistant to radiation therapies and the resulting apoptosis due to DNA damage. Thus one needs to target a cell which is radio- and chemotherapy-resistant and is very similar to the normal stem cell by known physical criteria—a tall order by any means.

Up until now no cell surface markers have been identified which are present exclusively on CSCs. The markers used to identify tissue specific stem cells are mentioned above, and listed in Vermeulen, et al.(2008), and Cho and Clarke (2008) [18, 19]. None of these markers are optimal to exclusively target the CSC, as they are also present on normal stem cells in addition to other normal mature cells in some instances. Steady progress is indeed being made in establishing molecular characteristics which distinguish these cells from their normal counterparts, although an obvious selection of markers still remains elusive. Studies to distinguish CSCs from normal SCs are being done at the level of identifying markers at the cell surface, or discovering functional differences in signaling or structural proteins. CSCs from breast and pancreatic cancers which are capable of metastasizing and giving rise to new tumors could co-express the chemokine receptor CXCR4 [20, 21]. In another report three drugs have been discovered which preferentially kill leukemic stem cells

over the normal stem cell, namely Idarubicin, parthenolide and TDZD-8 [22]. All three inhibit the activity of NFκB.

The niche that the CSC may reside in could be targeted. The concept of a niche which is necessary for the maintenance of stem cells, and in which stem cells reside, has been developed in the hematopoietic and neural stem cell systems [23, 24]. The niche is constituted by fibroblasts, matrix metalloproteinases, adhesion molecules including integrins, and possibly ligands for chemokine receptors such as SDF1 (stromal cell-derived factor 1) [25]. If specific niches for CSCs are defined, differences in the niches of normal SCs and CSCs could be targeted so as to cause apoptosis or promote differentiation of the CSCs. This could be a promising avenue to pursue, as the CSCs themselves are not easily targeted by traditional chemotherapies which include the perturbation of DNA metabolism at various stages (replication, repair, methylation) due to their inherent properties as described above (Fig. 1).

Mechanistic differences between normal and CSCs could be used to design therapeutic agents. There is some evidence which indicates that PTEN may define a difference between hematopoietic stem cells and the leukemia initiating cell, and that these effects may be mediated through the mTOR kinase [26]. Our previous observations have shown that upon BMP (bone morphogenetic protein) activation, the mTOR (mammalian target of rapamycin) kinase mediates glial differentiation in neural stem cells [27], and it has subsequently been shown that BMP reduces the tumorigenic potential of a putative glioma stem cell population [28]. Rapamycin is indeed used in the clinic in some treatment regimens for gliomas [29]. One may thus consider the mTOR kinase and possibly members of this kinase family to be potential targets for selective manipulation of some CSCs. Other pathways which may also be of relevance to this issue are the common developmental regulators shh, wnt and notch. Although the importance of

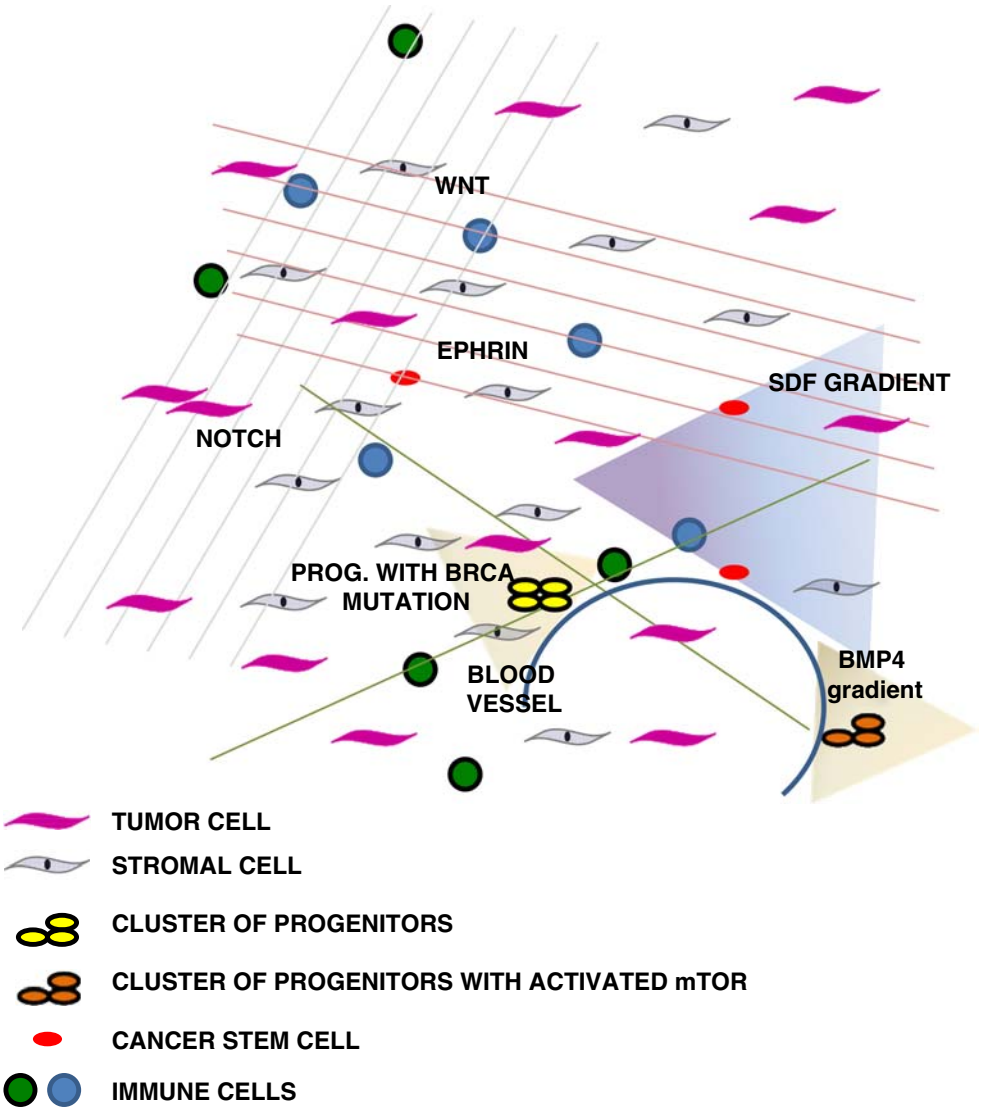

Fig. 1 Schematic of a tumor microenvironment. The tumor includes transformed cells, connective tissue and stromal cells, blood vessels, and the immune cells. Gradients of various factors and matrices exist which could include *wnts*, soluble factors such as SDF1 and EGF. Gradients could form within the stromal components or from the soluble principles such as the blood supply. CSCs and progenitor cells home into the niches most conducive to their survival and/or proliferation

these pathways in stem cell biology is established, methods need to be identified where selective targeting of the CSC is achieved when these pathways are targeted.

The identification of new markers (and possibly targets) specific to CSCs are required. Pharmacogenomic approaches are being used to delineate the signaling pathways which predominate in a particular cancer, so that therapeutic strategies may be designed for individuals, and combinations of chemotherapeutic agents may be tailored [30, 31]. Such high throughput systems may perhaps be used to identify novel markers for progenitors (and possibly the smaller CSC populations), based on differences in tumor expression profiles. The control population may be the enriched CSC populations which have been selected with the current available markers.

Implication of the CSCs in Cancer Prevention

At the present time CSCs are largely invoked with relation to metastasis, and recurrence of the tumor in situ. However, one might extend this concept a further step, and wonder whether they could be targeted to prevent cancer in high risk individuals. A potential precursor population has been identified for multiple myeloma (MM; [32]). While MM cells are CD138 positive, this progenitor/CSC population is CD138 negative but expresses nuclear SOX2 which is considered a marker for this population. A cell and humoral immune response was mounted in patients who were asymptomatic for MM, suggesting that they had mounted an immune response against a 'tumor antigen' on a precursor cell. A better understanding of the status of the normal stem cells in these individuals might yield insights into the CSC-selective targeting of antigens which are present on both normal SCs and on CSCs.

Can this analogy be extended to solid tumors? The generation of vaccines for high risk populations such as familial cohorts is particularly attractive, as this presents a very acceptable alternative to the prophylactic surgeries that some high risk individuals presently resort to. Breast and colon cancers are known to have a familial component where mutation of the breast cancer 1 (BRCA 1) and BRCA 2 genes, and the adenomatous polyposis coli (APC)/β-catenin axis respectively have been implicated [33–35]. The BRCA genes are involved in DNA related functions including DNA repair, transcription and chromatin remodeling. β-catenin, which is stabilized by complexing with APC, is also involved in the transcriptional activation of genes, and is in addition a component of adherence-junctions at the cell surface. Although mutations in these genes were described in specific reference to these two cancers, there is increasing evidence that these mutations are involved in causing cancer in various tissues including blood. Mutations in other tumor suppressor genes such as p53 are also implicated in the formation of cancers. Could epitopes specific to the mutated suppressor gene/s be useful for targeted vaccines and immunotherapies against the CSC/precursor populations in these solid tumors?

A major hurdle in designing strategies targeting CSCs would be the targeting of a quiescent cell among the dividing population of normal and transformed cells. One method to address this is to target molecules in the niche which maintain the quiescence and stemness of the CSC. If quiescence is actively maintained by factors in the milieu of the niche, interfering with their function could force the CSC to undergo apoptosis, differentiate or divide. Reasonable targets might include wnt and SDF1 which could regulate proliferation and homing functions of the CSC niche. Alternative strategies could also be designed where a selective survival advantage could be conferred on all cells which retain a given therapeutic. The efflux of this therapeutic from CSCs could then render them selectively vulnerable to a second drug. Appropriate combinations of these strategies could be designed, depending on the disease and the tissue affected.

However, the question of methods for the surveillance of high risk patients for CSCs remains. In vitro isolation and analysis of quiescent cells could aid greatly with the description of markers. Also the increase in humoral and cellular immune response against a particular CSC marker would be very advantageous as was shown in MM [32]. The results of these studies can also be extended to other cancers which are frequently not detected early such as pancreatic and ovarian cancers.

Immunotherapy for Primary Cancer Initiating Stem Cells

In addition to protecting the host from invading pathogens, the immune system is also believed to protect the host from developing tumors. In looking at cancer immunosurveillance and cancer immunoediting, elegant studies by Schreiber and his colleagues have shown the occurrence of spontaneous tumors as well as a greater susceptibility to carcinogen-induced tumors in immunodeficient mice lacking lymphocytes or efficient IFN signaling (as in $RAG2^{-/-}$ or $STAT^{-/-}$ mice). In addition, tumors in these immune compromised mice are more aggressive than those in their normal counterpart, while often times expressing a different set of antigens compared to tumors in normal mice [36]. There is sufficient evidence that the human immune system can recognize antigens on tumor cells in cancer patients [37]. Patients with cancer demonstrate circulating antibodies or cytotoxic T cells to tumor antigens and in some instances present with spontaneous regression of tumors.

However, in the vast majority of cases such immunological findings have infrequently translated to durable objective clinical responses as the immune response does not prevent a recurrence or eradicate a pre-existing tumor.

Other types of immunotherapy for cancers are being tried in preclinical and clinical trials. These include therapeutic antibodies as well as vaccines that would provide immunological protection from recurrences of tumor following surgical resection. Among the successful biologics which have been developed for the treatment of cancers are antibodies such as Herceptin® for metastatic breast cancer, Avastin® for colon and rectal carcinoma and Erbitux® for advanced colon cancer and Bexxar™ and Zevalin® for Non Hodgkin's Lymphoma, Rituxan™ for CD20 positive, refractory low grade, follicular or transformed B-cell non-Hodgkin's lymphoma. These serve strictly in a therapeutic setting and consecutive treatments over time are required. To generate a more sustained response, vaccination strategies would be preferable. Of the several cancer vaccines that had entered clinical trials, a few (Antigenics, Northwest Biotherapeutics, have received marketing approval for the treatment of kidney and brain cancers, respectively, outside the US (http://www.antigenics.com/news/ceoblog/2008/0408.html; http://www.nwbio.com/press2007.07.09_us.php).

One of the most critical elements in the success of a cancer vaccine is the choice of appropriate antigen target(s) that will allow for recognition and elimination of tumor cells with minimum or undesired autoimmune toxicity. There is some indication that antigens on premalignant lesions are immunogenic, this avenue is less explored as the specific nature of these targets is not as well characterized [38]. MUC1 is a T cell antigen expressed on a variety of cancers such as breast. ovary, pancreas, colon, lung and multiple myeloma as well in normal cells. In cancer it is overexpressed and underglycosylated leading to expression of cryptic sites [39]. In addition there is exposure of the core peptide which is not seen in normal cells, thereby making it immunogenic [40]. Cyclin B1 is another tumor antigen that is overexpressed as a result of non functional p53 [41]. Several premalignant cancers such as lung preneolasia, are associated with aberrant p53 function, which may lead to overexpression of Cyclin B1, making this a candidate target antigen [38].

Some diseases, such as multiple myeloma (MM), have a precursor condition called monoclonal gammopathy of undetermined significance (MGUS) which represents a precursor lesion to myeloma [32, 42]. Patients with this precursor condition mount an antibody and cell response to SOX2, which is a gene normally required for the maintenance of embryonic stem cells and also expressed in some $CD138^{+}$ cells. This spontaneous immunity to SOX2 is lost in patients with MM despite the presence of SOX2 positive cells suggesting that activated T cells may have become anergic or depleted, implying the importance of maintaining a steady pools of these effectors to prevent the probability of the precursor MGUS progressing to MM.

The concept of a vaccine targeting cancer stem cells in contrast to well defined cancer cells is appealing and some key observations obtained from prior vaccine studies can be applied to target stem cells. Firstly, vaccines work best in a setting where tumor burden is at a minimum or with no evidence of disease where tumor induced immunosuppressive mechanisms are kept at a minimum. Cancer stem cells being precursors to a well defined cancer are present in extremely small numbers and could be an appropriate target. Secondly, most antibody therapies that have been successful, whether by passive or active immunotherapy, have targeted those molecules that are necessary for the survival of a cancer cell. In a fully cancerous cell, these are often important signaling molecules such as Her2/neu or EGFR, or a target ligand, such as VEGF, that would sustain cancer growth by inducing angiogenesis. Identification of unique markers among stem cells has been a challenge but with appropriate combination of other therapeutic approaches, identifying and targeting cancer stem cells could well be in the forefront of prevention of cancer recurrence.

Conclusions and Projections

Although tremendous progress has been made in the amelioration of some cancers including childhood cancers, and certain blood cancers, the rate of remission and recurrence of others such as glioblastomas have hardly improved. According to the current thinking initiation, recurrence and metastasis of cancers may be explained at least in part by the presence of CSCs. Thus if these cells could be detected and induced to undergo apoptosis or differentiate, one may envision novel therapeutic options. Furthermore, in the case of high risk individuals it is conceivable that cancer prevention therapies could be employed for disease management rather than, or in addition to, prophylactic surgeries. Can we combine our knowledge of genetic mutations in the familial cancers with our knowledge of stem cells to design immunological and other therapeutic methods to identify and target steady state cancer stem cells in high risk individuals?

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