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Targeting Stem Cells-Clinical Implications for Cancer Therapy

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Abstract

Cancer stem cells (CSC), also called tumor initiating cells (TIC), are considered to be the origin of replicating malignant tumor cells in a variety of human cancers. Their presence in the tumor may herald malignancy potential, mediate resistance to conventional chemotherapy or radiotherapy, and confer poor survival outcomes. Thus, CSC may serve as critical cellular targets for treatment. The ability to therapeutically target CSC hinges upon identifying their unique cell surface markers and the underlying survival signaling pathways. While accumulating evidence suggests cell-surface antigens (such as CD44, CD133) as CSC markers for several tumor tissues, emerging clinical needs exist for the identification of new markers to completely separate CSC from normal stem cells. Recent studies have demonstrated the critical role of the tumor suppressor PTEN/PI3 kinase pathway in regulating TIC in leukemia, brain, and intestinal tissues. The successful eradication of tumors by therapies targeting CSC will require an in-depth understanding of the molecular mechanisms governing CSC self renewal, differentiation, and escape from conventional therapy. Here we review recent progress from brain tumor and intestinal stem cell research with a focus on the PTEN-Akt-Wnt pathway, and how the components of CSC pathways may serve as biomarkers for diagnosis, prognosis, and therapeutics.

Keywords

cancer stem cells; cell surface marker; CD133; PTEN; Akt; Wnt

Introduction

Despite decades of research and many novel therapeutic approaches, cancer remains one of the leading causes of mortality in humans at all ages. We have learned that cancer is a very heterogeneous disease with a variable multitude of differences and similarities between normal and cancer cells [1]. One of the hallmarks of cancer cells is their rapid proliferation rate. A plethora of therapeutics have been developed targeting rapidly proliferating cancer cells, however, few of these treatments provide a durable response, and even fewer can readily eradicate tumors in a small number of malignancies [2]. While it has long been believed that cancer consists of different populations of cells, the first proof that cancer may actually originate from a subset of cancer cells did not emerge until ~10 years ago when a subpopulation of tumor cells were isolated from human acute myeloid leukemia (AML) and were found to be able to initiate leukemia in NOD-SCID mice [3]. This cancer cell

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subpopulation which could recapitulate the human tumor in mice was termed cancer stem cells (CSC) or tumor initiation cells (TIC) [4]. CSC are distinctive from the bulk of tumor cells in that CSC appears to be slow growing and possesses the capacity to initiate and sustain tumor growth [5,6]. The greatest clinical implication evolving around the CSC hypothesis is that one may need to develop targeted treatment regimes against the unique CSC population for the cure of cancer [7]. The most pressing issue is how to target CSC without damaging normal stem cells. The solution is straightforward but daunting: we need 1) to identify accurate biomarkers that distinguish CSC from their normal counterparts and 2) to understand the critical biological processes operating predominantly in the CSC subpopulation.

At the conclusion of the human genome project and the dawn of readily accessible personalized genomes, health care is shifting toward a more personalized approach where an individual's unique molecular characteristics will eventually dictate medical treatment. These molecular characteristics, also known as biomarkers, can be objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers can be found in body fluids, tumors or other tissues and are constituted of molecules such as DNA, RNA, and proteins. A biomarker with predictive properties can provide guidance for physicians to make treatment choices based on the specific characteristics of individual patients and their tumors rather than based on population statistics. It can also be used to indicate how well the body responds to a treatment for a disease or condition [8]. These promising developments have propelled cancer biomarker discovery to the center stage in cancer research, through the application of global genomic and proteomic approaches such as high-throughput sequencing, DNA microarrays, and mass spectrometry (MS) [9,10]. Genetic biomarkers affecting disease susceptibility and drug response (e.g. single nucleotide polymorphisms, SNP) have been identified [11]; distinctive expression patterns of genes and proteins between cancer and normal tissues, or among different stages of disease, have been identified and are currently under investigation as biomarkers that may provide instructive information for cancer diagnosis, progression, treatment, and prognosis [12]. We predict that increasingly more of these applications will be used to identify therapeutically relevant biomarkers in the CSC subpopulation.

To date, cancer stem cells have been isolated from both leukemias and a variety of solid tumors, including breast, brain, pancreatic, and colon cancer, by virtue of either cell surface antigen immunoreactivity (e.g. $CD44^{+}CD24^{-}$, $CD133^{+}$, etc.) or the existence of a dye- efflux side population (SP), in conjunction with cell sorting [13-15]. The sorted cells are then typically subjected to a set of *in vivo* and *in vitro* functional assays for stem cell characteristics by examining clonogenic and tumourigenic properties as well as differentiation potential. Both strategies have been successfully implemented to separate the heterogeneous tumor cells into two populations which exhibit differential tumorigenicity: surface antigen enriched CSC and negative cells; and side population CSC and non-side population cells. Although the surface antigen enriched CSC and the SP CSC usually demonstrate more tumorigenicity, accumulating evidence suggests that the surface antigen negative or non-SP cells do not necessarily lack tumorigenicity [16,17]. Furthermore, some studies have found that $CD133^{+}$ CSC do not reside in the SP [18,19]. Thus, the absolute identification of CSC remains elusive under the current circumstances, exposing the limitations of employing current cell surface markers and sorting schemes to define CSC.

Strategies for the Identification of Better CSC Markers

Further identification of more accurate CSC biomarkers that can better distinguish CSC from non-tumorigenic cells and/or from normal stem cells remains imperative since a

comprehensive understanding of the biological operation system in CSC is required for the development of specific targeted treatment toward CSC [20]. To date, most of the CSC markers are cell surface molecules, *e.g.* CD133, CD34, lineage marker depletion, ATP-binding cassette transporter ABCG2, *ect.* [21]. A combination of these markers has been applied to the isolation and purification of CSC. While these enrichment strategies provide a good starting point for further identification of biomarkers with higher specificity, as stated above, isolation of CSC population solely relying on these cell surface markers has been shown not to be sufficient in some cases. Better strategies need to be developed. In a recent report, Hosen *et al.* described how they identified a leukemic-specific stem cell marker (CD96) using a strategy called signal sequence trap (SST) [22]. The SST PCR screening method can detect signal sequences on cDNA fragments based on their ability to redirect a constitutively active mutant of cytokine receptor to the cell membrane, and thereby inducing IL-3-independent growth of a mouse B cell line Ba/F3 [23]. The researchers applied this strategy to a cDNA library derived from $CD34^+CD38^-$ leukemic stem cell (LSC) purified from acute myeloid leukemia (AML) patients and identified a LSC-enriched cell surface molecule CD96. They subsequently demonstrated that CD96 is expressed on the majority of $CD34^+CD38^-$ LSC; whereas only a few cells in the normal HSC-enriched population ($Lin^-CD34^+CD38^-CD90^+$) exhibited weak CD96 signal. The $CD96^+$ AML but not the $CD96^-$ cells showed significant levels of engraftment in the bone marrow of recipient mice [22]. The implication is that CSC can be initially enriched using currently identified surface molecules in conjunction with a subsequent approach such as SST that can be tagged along for further marker identification.

Another strategy that can be applied to identify cell surface molecules on targeted cells relies on a cell-based aptamer selection, which exploits differences at the molecular level between any two types of cells. The aptamers are first obtained through an *in vitro* selection process known as SELEX (systematic evolution of ligands by exponential enrichment) in which aptamers are selected from a library of random sequences of synthetic DNA or RNA by repetitive binding of the oligonucleotides to target molecules [24]. Previously, the target molecules consisted mainly of pure molecules such as a small protein. More recently, complicated targeting such as red blood cell membrane or even whole cells have also been demonstrated [25-27]. A recent study by Shangguan *et al.* applied cell-SELEX to the T-cell acute lymphoblastic leukemia (T-ALL). After several rounds of selection and counter-selection between T-ALL and a control cells, a DNA pool with high affinity and good selectivity for the T-ALL cells was obtained. The aptamer-binding targets were captured and analyzed by mass spectrometer, which led to the discovery of a new potential biomarker PTK7 for T-ALL [28]. The combined power of cell-SELEX and mass spectrometry holds promise in cancer biomarker discovery. Application of this strategy to CSC should facilitate the discovery of new CSC biomarkers. A CSC surface marker with high specificity will be useful in future cancer diagnosis and more importantly will assist researchers to precisely delineate the differences between CSC and normal stem cells.

Global Genomic Exploration of Stem Cell Properties in Cancers

Although CSC and normal stem cell share many common features, exploiting key difference between them would shed light on new ways of attacking CSC while preserving the functions of normal stem cell [29]. By comparing gene or protein expression patterns between CSC and normal stem cells, specific stem cell markers for certain tissue origins or developmental stages can be identified; differential stem cell properties with regard to self-renewal and/or differentiation can also be delineated. Efforts comparing global gene expression profiles between normal and malignant stem cells of related origins have generated large datasets [30]. Global gene analyses not only enable the identification of individual differentially expressed markers, but also provide a global perspective of

perturbed networks in CSC. Sophisticated bioinformatics analysis allows one to view global alterations by building a network based on high throughput data. By weighing the network with gene expression levels, the distinctive functional modules, which include genes involved in the same pathway or similar regulatory role, may emerge [31]. The coordination among pathways can also be revealed. This network-based approach should provide a deeper understanding of CSC biology as multiple pathways contribute to stem cell self-renewal and differentiation, the dysregulation of which may turn normal stem cells into CSCs, a process which usually involves more than one gene or protein. Supporting evidence comes from a study of BMP1a deficient mice in which the inactivation of either PTEN or BMP signaling activates Akt, which in-turn coordinates with the Wnt signal to fully activate β-catenin in intestinal stem cells (ISC) [32]. Network-based analysis may not pinpoint the activity of a singular protein, but rather provide clues in regard to the connections among pathways leading to new directions for mechanistic studies. A few recent studies demonstrate the power of this approach.

Sperger *et al.* investigated the molecular mechanisms underlie the pluripotency of human embryonic stem (ES) cells by comparing several ES cells to embryonic carcinoma (EC) and seminoma cells via DNA microarray and hierarchical clustering. They found that gene expression patterns of human ESCs are more closely related to EC but more distantly related to seminoma samples; gene candidates involved in the maintenance of pluripotent, undifferentiated phenotype were identified [33].

Another study set to identify hESC properties in different cancers through a comprehensive analysis aimed at identifying the shared and unique molecular modules underlying human malignancies by gene set enrichment analysis [34]. The molecular modules are composed of gene sets which are groups of genes related through a common function, pathway or other property and therefore provide a more global view of a specific biological process. Ben-Porath *et al.* applied the gene set enrichment analysis in a recent study: they first defined the gene sets which represent the core expression signature of ES cells that reflect the activity of the regulatory pathways associated with the ES identity, and then analyzed the enrichment patterns of gene sets associated with embryonic stem (ES) cell identity in the expression profiles of various human tumor types. They found that the embryonic stem cell–like gene expression signatures were enriched in the poorly differentiated aggressive human tumors including breast cancer, gliobalstomas, and bladder carcinomas [35]. The implication of their findings supports the hypothesis that tumors often arise from undifferentiated stem or progenitor cells, and leads to the identification of specific regulators with a role in generating stem-like tumor phenotypes which will eventually benefit cancer therapy.

Majeti *et al.* recently conducted the first genome wide expression analysis directly comparing the expression profile of highly enriched leukemic stem cells (LSC) from patients with acute myeloid leukemia (AML) with normal human hematopoietic stem cells (HSC). Two methods were applied: an unbiased pathway analysis which takes into account all the genes in a given pathway, and a functional groups enrichment analysis which employs only differentially expressed genes (DEGs). The researchers were able to create networks from two independent data sets and identify the perturbed networks in LSC compared to normal HSC. In addition to the Wnt, the Adherens junction and NFκB pathways that are known to play key roles in leukemia and/or leukemic stem cell biology, the network-based analysis also revealed that pathways involve in the interaction of the stem cells with their niche are dysregulated in LSC (Majeti *et al.*, submitted).

Understanding Molecular Pathways Perturbed in CSCs

Complementary to biomarker discovery based on large-scale assays, the in-depth functional elucidation of molecular events that control stem cell proliferation and differentiation adds another layer for the identification of better CSC biomarkers and therapeutic targets [36]. Several key signaling pathways, such as those mediated by PTEN, Akt, Wnt, BMP, FGF, hedgehog, and notch, have been linked with the self-renewal and maintenance of CSC [37,38]. FGFs are growth factors for fibroblast and many other cells. They exert their biological effects in an autocrine or paracrine fashion, by interacting with corresponding receptors. Studies have shown that exogenous FGF-2 is required to maintain hESCs in undifferentiated state in feeder-free condition [39-41]. The importance of FGF signaling in maintaining CSC was also demonstrated by Lee *et al.*, who showed that the human glioblastoma cells cultured in serum-free media with bFGF and EGF closely resemble the primary tumor cells with stem cell property [42]. PTEN is a tumor suppressor gene which plays essential role for the normal development and/or homeostasis of numerous organ systems. It is one of the most frequently mutated genes in human cancers, and loss of PTEN protein expression or activity occurs in approximately half of all tumors [43]. Recent evidences demonstrated that dysregulation of these pathways can disturb the balance between stemness and differentiation, and switch NSC into CSC [44-46]. Understanding where the dysregulation occurs in the cellular signaling cascade that controls stem cell behavior could lead to the development of new anticancer strategies. By using conditional knock-out mouse model, He *et al.* have shown that inactive PTEN (P-PTEN) was present specifically in intestinal stem cells (ISCs) and loss of PTEN activity led to intestinal polyposis in mouse models. The inactivation of PTEN led to activation of PI3K-Akt and resulting in expansion of intestinal crypts, where the ISC resides. This is consistent with findings in embryonic and neural stem cell populations. The study by He *et al.* further demonstrated that the activated Akt induced nuclear accumulation of β-catenin, which possesses a novel specific phosphorylation site at serine 552 by Akt. The cells with nuclear phospho-β-cat-Ser552 were frequently clustered together and were found at sites of crypt fission and budding, suggesting that the phosphorylation events occur only in actively cycling ISC [45,46]. PTEN/Akt/Wnt/β-catenin pathway is also known to be dysregulated in colon cancer, with similar phosphorylation found in colon stem cells [47]. Thus, the level of p-β-cat-Ser552 would be a more promising indicator for the status of the ISC (active or dormant). Inhibition of Ser552 phosphorylation by Akt may be more efficacious than Akt or PI3K inhibitors in restoring PTEN signaling axis.

Signaling pathway like Wnt/β-catenin is crucial for stem cell homeostasis. Studies have shown that even in the presence of an aberrant oncogene, a second signal mediated by β–catenin is required to turn a stem cell into a fully malignant CSC [48]. In a recent study, Malanchi *et al.* isolated cutaneous CSC from a chemical-derived murine skin cancer using a bulge stem cell marker CD34. Transplants derived from these $CD34^+$ CSCs efficiently initiated secondary tumors that recapitulate the organization of the primary tumor. They found that these $CD34^+$ CSCs possess constitutive active Wnt/β-catenin signaling as revealed by the intense nuclear accumulation of β-catenin (a N-terminal non-phosphorylated active form) compared to that in the $CD34^-$ cells. Deletion of β-catenin from murine skin tumor cells resulted in the loss of $CD34^+$ CSCs and complete tumor regression. Similar results were obtained when the researchers knocked-down β-catenin from human squamous cell carcinomas [49]. Since Wnt/β-catenin signaling is not essential for normal epidermal homeostasis [50,51], their findings once again proved that active Wnt/β-catenin is essential for tumorigenesis. Also, there are differential requirements for cellular signaling such as Wnt/β-catenin in cancer versus normal stem cells, which could be exploited for future therapy.

It is generally agreed upon that nuclear localization of β-catenin in response to Wnt is essential for canonical Wnt signaling [52]. However, mechanisms controlling this process differ depending on the tissue or cell types. In parallel with finding by He *et al.* in ISC, a study by Wu *et al.* showed that the nuclear accumulation of β-catenin depends on Rac1 activity and JNK2 mediated phosphorylation at Ser191 and Ser605 during osteoblast differentiation. Mutations of these residues significantly affect Wnt-induced β-catenin nuclear accumulation. Genetic ablation of Rac1 in the mouse embryonic limb bud ectoderm disrupts canonical Wnt signaling and phenocopies deletion of β-catenin in causing severe truncations of the limb [53]. All these studies imply that the dysregulation of the same pathway can occur at different levels within the cascade depending on tissue or cell type. Uncovering where the dysregulation occurs can provide additional and specific targets for therapeutic intervention.

Normal stem cell maintains their population by asymmetric division and differentiation into mature cells during development [54]. In contrast, CSC gain more potential in cell proliferation and lose the ability to differentiate, suggesting dysregulation in differentiation signaling. A recent study by Lee *et al.*, utilized several TIC-like cell lines isolated from primary glioblastomas, human fetal brain-derived neural stem cells (NSCs), and embryonic mouse NSCs isolated from various developmental stages to investigate the differentiation pathway in glioblastoma TIC. They demonstrate that some TICs, which behave similarly to mouse NSC (E11) do not response to BMP-induced differentiation due to the epigenetic silencing of the receptor BMPR1B. Treating the bmpr1b mutated TIC with BMP2 increased cell proliferation and clonogenicity [55]. Similar to this finding, a deficiency in BMPR1a in mice intestinal stem cells resulted in expansion of the stem and progenitor cell populations, eventually leading to intestinal polyposis resembling human juvenile polyposis syndrome [32]. These studies implicate that the dysregulation in BMP-mediated differentiation signaling can alter the status of NSC. Another study by Piccirillo *et al.* showed that treating $CD133^+$ GBM cells with BMP4 reduced cell proliferation *in vitro* and tumorigenicity *in vivo* by directing cells to differentiation, suggesting depletion of TIC can be achieved by inducing cell differentiation rather than manipulate cell proliferation [56]. The study by Lee *et al.* also imply that the level of BMPR1b play a role in the determination of stem cell status and can be a biomarker when conducting a therapy based on the BMP signaling pathway [55].

Clinical Perspective of CSC-Targeting Therapies

The idea of targeting CSC for cancer therapy is relatively new and research remains in progress; preliminary studies are promising. It has been postulated that LSCs are responsible for the persistence and recurrence of leukemia following cytotoxic or targeted therapy [3]. Pearce *et al.* assessed human normal and leukemic stem cells from 59 AML patients and the correlation of LSCs with AML prognosis using the NOD/SCID assay. They found that LSCs from patients with favorable prognosis do not initiate leukemia in NOD/SCID mice [57]. While LSCs were found exclusively in the immature $CD34^+CD38^-$ fraction, recent studies suggest that LSCs ($CD34^+CD38^-$) from most AML patients also express CD33 and a novel antigen C-type lectin-like molecule-1 (CLL-1) [58,59]. Thus, LSCs with positive CD33 are anticipated to be more sensitive to gemtuzumab ozogamicin (GO, Mylotarg, a humanized anti-CD33 antibody conjugated with calicheamicin) [60]. The most recent clinical trial study in this regard is encouraging. Chevallier *et al.* reported that a combination therapy of GO, intermediate-dose cytarabine, and mitoxantrone (MIDAM) in patients with refractory or relapsed $CD33^+$ AML resulted in an increase of 2-year overall survival rate [61]. On the other hand, for AML patients whose LSCs do not express CD33, the presence of genetic markers such as t(8;21), t(15;17), and monosomy 7 exhibited promise in predicting the response to induction chemotherapy [62]. Johnston *et al.* reported that AML patients whose

LSCs ($CD34^+CD33^-$) contained t(8;21), t(15;17) did not give rise to granulocyte/macrophage colony-forming cells *in vitro*, and entered remission at the end of induction chemotherapy. In contrast, AML patients whose LSCs ($CD34^+CD33^-$) contained monosomy 7 were capable of giving rise to granulocyte/macrophage colony-forming cells, and failed to respond to induction chemotherapy.

Glioblastomas are the most lethal primary brain tumor. Since the concept of brain cancer stem cells was introduced, emerging evidences suggest that GBM progression is mediated by cancer stem cells, and that cross-talk of cancer stem cells with their environment is closely associated with angiogenesis-dependent progression and -independent growth [63,64]. A study by Piccirillo *et al.* transplanted BTIC—$CD133^+$ enriched population isolated from human glioma—with injection of BMP4-saturated polyacrylic beads which release BMP4 for 1 week at the site of cells' engraftment immediately or later. The mice implanted with BMP4-releasing beads displayed small, confined lesions, surviving significantly longer. In contrast, animals receiving control beads developed large, malignant tumors and died [56]. Another study by Bao *et al.* demonstrated that BTIC is responsible for the acquisition of radioresistance. The resistance of BTIC to ionic radiation (IR) is due to preferential DNA damaging checkpoint activation. IR-induced DNA damage can lead to the activation of DNA damage checkpoint and subsequent increase in DNA repair capacity and cell survival [65]. Targeting of DNA damaging checkpoint response in BTIC can overcome glioma radioresistance *in vitro* and *in vivo*, which may provide a therapeutic advantage to reduce brain tumor recurrence [66]. A recent clinical study showed how well the CSC correlates to the prognosis of glial tumors. They found high levels of the neural progenitor cell marker nestin in the more aggressive high-grade glioma. High-grade glioma also co-expressed high levels of cathepsin B, a marker for invasion. The combination of both markers indicated a poorer prognosis and significantly shorter survival of glioma patients [67]. These studies have laid the groundwork for developing treatment strategies targeting CSC.

The prognostic value of CSC markers in the clinic has begun to emerge. Recently, Liu and colleagues compared 186-gene expression profiles of $CD44^+/CD24^{-/low}$ putative breast cancer stem cells to that of normal breast epithelium and found a significant association between the gene signature in both overall and metastasis-free survival ($P<0.001$) independent of established clinical and pathological variables. The unique gene signatures could further stratify patients into high risk (10-year survival of 81%) and low risk (10 year survival of 57%, $p = 0.01$) combined with National Institute Health prognostic criteria. The gene signatures are also associated with the prognosis in medulloblastoma ($P=0.004$), lung cancer ($P=0.03$), and prostate cancer ($P=0.01$) [68]. The prognostic value of CD133 has also emerged. High CD133 expression in glioma correlated with poor clinical outcomes [69,70]. Lin and other had also demonstrated prognostic values of elevated CD133 mRNA in the peripheral blood correlated increased risk of death in colon cancer, prostate and head neck cancer [71-73].

Targeting CSC will also depend on achieving a better understanding of the niche and microenvironment in which the CSC resides. Host-tumor interactions have long been recognized to mediate resistance mechanism *in vivo* [73]. Both epithelial-mesenchymal transition (EMT) and tumor angiogenesis are known to mediate drug resistance. Targeting VEGF with bevacizumab leads to a normalization of tumor vasculature, resulting in a disruption of the CSC niche. Mice bearing glioblastoma treated with bevacizumab show depleted vasculature and a dramatic reduction in the number of glioblastoma stem cells without a change in their rate of proliferation.

Colorectal cancer remains as the second most common malignancies in the US [74]. Despite a number of novel agents including bevacizumab, complete response rate remain unchanged at 2-5% [75]. Integrating capecitabine, a 5FU pro-drug and celecoxib (XCEL) with or without radiation, we achieved complete response rate of 18% (12/66) in unresectable metastatic colorectal cancer patients including 7 patients (10%) to undergo curative resections [76]. Nine of 19 patients experienced recurrence (median 13 months after CR), and 4 died with a median follow-up of 31 months after complete response. The median survival for this group of patients reached 73.3 months (95% CI, NR-NR months) from the onset of metastasis which compared favorably to 44-46 months median overall survival for patients who achieved complete response to either chemotherapy or through surgery [77]. The 2-year relapse free survival (RFS) for the unresected and R1-2 resected patients was 71% versus 20% for the R0 resected patients ($p = 0.07$). This paradoxical RFS advantage was due to only maintenance capecitabine and celecoxib ($p = 0.002$). Those who received maintenance capecitabine and celecoxib enjoyed 3-year overall survival of 93% *vs* 38% ($p = 0.04$) for those who did not [78]. It is unlikely that radiation contributed to the distant disease control. Our ongoing work and others in the preclinical model suggested that celecoxib likely mediate its effects through inhibition of the Wnt signaling [79] and other cancer stemness pathways critical in colorectal cancer carcinogenesis. Carefully designed, well powered prospective clinical trials are needed to evaluate therapies that may putatively target the cancer stem cells.

Concluding Remarks

The cancer stem cell hypothesis has had a profound impact on our current view of cancer diagnosis, treatment, and prognosis. To reach the goal of complete eradication of cancers, a panel of biomarkers will be required to distinguish CSC from normal stem cells. This panel of biomarkers should include tissue-specific CSC surface antigens and cytoplasmic or nuclear protein markers, with the former being used to indicate the presence of CSC and the latter being used to indicate the behavior of CSC such as active cycling, loss of differentiation, or invasiveness. A therapeutic regimen including treatments targeting both bulk cancer cells and the rare CSC should significantly improve treatment outcomes. While we strive to exploit differences between normal stem cells and cancer stem cells, it will be important to profile and catalog the molecular overlaps among various stem cell compartments and their niches in order to narrow down “ideal” targets or pathways within these cellular compartments for therapeutic intervention.

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