Published in final edited form as:

Expert Opin Ther Targets. 2010 June ; 14(6): 621–632. doi:10.1517/14712598.2010.485186.

The Cancer Stem Cell Paradigm: A New Understanding of Tumor Development and Treatment

Johnathan D. Ebben¹, Daniel M. Treisman¹, Michael Zorniak¹, Raman G. Kutty¹, Paul A. Clark^{1,3}, and John S. Kuo^{1,2,3,4,*}

¹Brain Tumor Research Laboratory, Department of Neurological Surgery, School of Medicine and Public Health, University of Wisconsin, CSC K4/879, 600 Highland Ave., Madison, WI 53792

²Department of Human Oncology, School of Medicine and Public Health, University of Wisconsin, CSC K4/879, 600 Highland Ave., Madison, WI 53792

³Stem Cell and Regenerative Medicine Center, School of Medicine and Public Health, University of Wisconsin, CSC K4/879, 600 Highland Ave., Madison, WI 53792

⁴Carbone Comprehensive Cancer Center, School of Medicine and Public Health, University of Wisconsin, CSC K4/879, 600 Highland Ave., Madison, WI 53792

Abstract

Importance of the field—Cancer is the second leading cause of death in the United States, and therefore remains a central focus of modern medical research. Accumulating evidence supports a ‘cancer stem cell’ (CSC) model - where cancer growth and/or recurrence is driven by a small subset of tumor cells that exhibit properties similar to stem cells. This model may provide a conceptual framework for developing more effective cancer therapies that target cells propelling cancer growth.

Areas covered in this review—We review evidence supporting the CSC model and associated implications for understanding cancer biology and developing novel therapeutic strategies. Current controversies and unanswered questions of the CSC model are also discussed.

What the reader will gain—This review aims to describe how the CSC model is key to developing novel treatments and discusses associated shortcomings and unanswered questions.

Take home message—A fresh look at cancer biology and treatment is needed for many incurable cancers to improve clinical prognosis for patients. The CSC model posits a hierarchy in cancer where only a subset of cells drive malignancy, and if features of this model are correct, has implications for development of novel and hopefully more successful approaches to cancer therapy.

Keywords

cancer; stem cell; molecular therapy; therapy resistance

Introduction

Despite substantial research directed towards elucidating the physiological mechanisms of cancer growth, and developing and refining surgery, chemotherapy and radiation techniques,

*Corresponding author, John S. Kuo, MD, PhD, Assistant Professor of Neurological Surgery and Human Oncology, Director, Comprehensive Brain Tumor Program, School of Medicine and Public Health, University of Wisconsin, K3/803 CSC, Mail Code 8660, 600 Highland Ave., Madison, WI 53792-8660, Phone: 608-261-1877, Fax: 608-263-1728, j.kuo@neurosurg.wisc.edu.

only marginal improvements have been made in the overall cancer survival rate from 50% in 1974–1976 to 66% [1]. Cancer remains the second leading cause of death in the United States and will likely soon become number one [2], and has not exhibited the decline in deaths observed with heart disease stemming from modern treatments. The marginal improvements in cancer survival have also been mainly attributed to earlier diagnosis and prevention, and few examples of breakthrough cancer treatments exist from the last three decades of intense research [2]. Survival of patients suffering from several varieties of cancer, including brain, liver, lung and pancreatic cancers, have failed to improve despite modern therapies. A modest increase in mean survival from 12.1 to 14.6 months for patients with glioblastoma multiforme, the most frequently diagnosed and incurable brain neoplasm, was considered a major breakthrough when Stupp and colleagues discovered a new chemoradiation therapy strategy in 2005 [3]. Likewise, chemotherapeutics are practically ineffective in hepatic carcinoma, with a median response below 15% [4]. Even with initially effective treatments, overall survival following a cancer diagnosis remains low because many cancers recur after developing therapeutic resistance and metastasizing throughout the body. Clearly, increased understanding of cancer mechanisms, especially the biology of therapeutic resistance and clinically relevant patient-specific molecular variation in cancers will be crucial for improving outcomes with better therapies.

Interpreting the Mystery

Given the variability of cancers and survival rates, it is clear that certain aspects of cancer are not adequately understood. The stochastic model, a widely accepted theory in the field of oncological research, states that tumors are a homogenous mass, arising from any cell expressing the requisite mutations [5]. New research suggests a cellular hierarchy is involved in tumor development, with only a small subset of cells capable of recapitulating the cancer via tumor initiation and propagation (Figure 1) [6]. A subset of tumor cells can initiate tumors with high efficiency (as few as 100 cells) compared to non-initiating cells from the same tumor specimen that rarely form tumors [7, 8]. These tumor-initiating cells also display stem cell properties such as self renewal and multipotent differentiation, possibly recapitulating and resulting in the heterogeneity of cell phenotypes comprising most cancers [9]. Because of their similarities to normal stem cells, these tumor initiating cells are often referred to as cancer stem cells (CSCs). The origin of CSCs is still being debated; they may result from oncogenic mutation of otherwise normal stem cells, or derive from adult neoplastic cells acquiring stem cell properties via mutations [10]. The total CSC population may also correlate with malignant potential of cancer (Figure 1), although this hypothesis requires experimental verification and is only supported by anecdotal evidence from histologically “undifferentiated” tumors [11, 12]. This model challenges the assumptions of the stochastic theory and suggests that re-examination of cancer mechanisms and cell-targeted therapies would be informative for future therapies.

Leukemia Cancer Stem Cells: Towards a New Model

Although the concept of stem cell involvement in cancer development has been proposed in the past, evidence supporting the hierarchal nature of cancer development originated from experiments carried out with hematopoietic acute myeloid leukemia (AML) cells [13–15]. Hematopoietic cells were extracted from patients afflicted with AML and transplanted into immunodeficient mice with the intention of modeling leukemic disease *in vivo*. Results demonstrated that a core group of cells expressing CD34, a surface marker primarily associated with hematopoietic stem cells, were responsible for generation of leukemia in xenografts [13, 14, 16]. Discovering that only CD34⁺ cancer cells could initiate leukemia suggested that cells expressing stem cell-associated markers and properties play a role in

cancer development. The discovery of cancer cells expressing stem cell properties also raised the possibility that cancers other than AML may be linked to CSCs [14, 15].

Solid Tumor Cancer Stem Cells

After their discovery in hematopoietic cancers, CSCs were hypothesized to exist within solid tumors as well, particularly in tissues subject to rapid self-renewal and proliferation [16]. This theory gained support when Al-Hajj and colleagues (2003) isolated putative CSCs from breast cancer, identifying a small population of CD44⁺CD24⁻ cells, typical cell surface markers of normal mammary stem cells. When these CSCs were re-injected into immunodeficient mice, breast cancer arose in eight out of nine cases [7]. Significant populations of CD44⁺ CSC were not required to cause tumor growth, with as few as 100 CD44⁺ cells recapitulating breast tumors. In contrast, significantly higher numbers of other cell types derived from the tumor mass did not form tumors after injection [7, 17].

The hypothesis that cells sharing many properties of embryonic stem cells were responsible for the development of brain tumors, particularly medulloblastomas, originated from pioneering research conducted by James Homer Wright in the early 1900s [18]. Evidence for the existence of brain tumor stem cells had been growing since the discovery of clonogenic, multipotent cells from brain tumors *in vitro* when cultured under neural stem cell conditions [19, 20]. These findings were verified *in vivo* in 2004 when tumor-initiating cells were isolated from brain tumors [8, 21]. Further study demonstrated the capability of these cells to self-renew and differentiate into multiple neural lineages [22]. The highly tumorigenic nature of these cells was demonstrated through xenografts in NOD/SCID mice, establishing the ability of isolated brain tumor stem cells to generate tumors [8, 23]. Genetic analyses also demonstrated a strong correlation between the original patient tumors and derived brain tumor stem cells, implicating the CSCs as a better model for brain cancer and therapeutic testing [24, 25].

CSCs have also been implicated in other solid tumor cancers, such as colon cancer. Xenografts of human colon cancer in NOD-SCID mice have demonstrated that as few as 100 CD133⁺ cells were required to generate tumor growth, giving additional credence to the CSC theory [26]. These data appear to support the conclusion that a small population of CD133⁺ cells which exhibit stem-like properties serve as the progenitors of colorectal cancer [27].

As further research is conducted and the CSC model becomes better accepted, the list of solid tumor cancers containing putative CSCs has only continued to grow (Table 1). As the list of tumors linked to CSCs has increased, so too has the base of knowledge surrounding CSCs.

A New Therapeutic Approach

Current cancer therapies are often limited by their lack of specificity and high incidence of adverse effects. Chemotherapies usually rely on a “carpet-bombing” strategy that results in toxicity to both cancerous and non-cancerous cells. Likewise, radiation therapy grossly targets tumor masses and cannot target specific tumor cells. Traditional therapies are also hampered by the inability to reliably expunge an entire tumor mass, with individual tumorigenic cells potentially evading destruction. The CSC theory underlies the new strategy of novel therapeutics aiming to overcome many of the limitations of current treatments, by suggesting the more nuanced option of designing therapies for the tumor-initiating cellular compartment, the CSC (Figure 2).

CSCs and Current Therapeutic Options

The efficacy of current chemotherapies and radiotherapies may be effective at destroying the majority of a tumor, but many cancers recur because these therapies are likely ineffective against the CSCs. Cancer stem cells, along with non-cancerous stem cells, express protective drug transport mechanisms that remove cytotoxic chemicals from the cell [28, 29]. Due to the presence of efflux mechanisms, including ATP binding cassette (ABC) and multi-drug resistance (MDR) transporters, CSCs are resistant to traditional chemotherapies compared to tumor mass or non-cancerous cells [30, 31]. Additionally, CSCs demonstrate enhanced radiation resistance through heightened activation of DNA repair mechanisms [32] or increased defenses against reactive oxygen species [33]. Current therapies also fail to take into account the heterogeneous nature of tumors and the differences in tumors between patients, instead applying broad treatment principles rather than personalized treatment regimens.

Neutralizing the Advantages of Cancer Stem Cells

One of the difficulties of targeting CSC is their chemo- and radiation resistance [33–35]. Efforts to target the chemoresistant mechanisms of CSCs have met with some success *in vitro*. Inhibitors have been identified which act on the ABC transporters ABCC1 and ABCG2, both critical to the development and normal function of stem cells. Some of the ABCC1-specific inhibitors are being tested in clinical trials [31]. However, research has also suggested that ABC transporters play a critical role in several physiological processes, including the maintenance of the blood-brain barrier (BBB) [36]. Although such transporter inhibitors might render the CSC population more sensitive to chemotherapeutic agents, it remains to be seen whether they also cause harm by inhibiting the BBB or indirectly by rendering normal stem cells more vulnerable to chemotherapeutic agents.

Interrupting Key Pathways

Pathways which regulate normal stem cell growth and division are also potential cancer therapeutic targets [37]. Up-regulation of the TGF β pathway has been correlated to tumor growth, as well as to regression of differentiated cells into progenitor and stem cell lineages [38]. Bone morphogenic protein is a well-studied TGF β inhibitor; knowledge of the interaction of these two proteins led to the discovery that BMP can limit tumor growth by ‘differentiating’ tumor-initiating brain cells [39]. The Notch developmental pathway is also associated with cancer survival and proliferation, especially in various brain cancers [40, 41]. Inhibition of Notch signaling in medulloblastomas was shown to halt tumor cell proliferation and also to decrease the number of cells positively expressing CD133 both *in vitro* and *in vivo* [42]. Dysregulated gene expression in the Sonic Hedgehog-homolog (SHH) pathway has also been implicated in the development of a wide variety of cancers [43, 44]. Specific small molecular SHH signaling inhibitors have shown success at halting tumor progression in glioblastomas and medulloblastomas [45–47], with some early clinical success [48, 49]. Likewise, the WNT pathway is often up-regulated in cancers [37]. The biological WNT blocker, DKK1, was shown to dramatically increase tumor cell apoptosis levels and synergized with other chemotherapeutic agents [50]. Preliminary work suggests that targeting these pathways for cancer treatment could be accomplished in a feasible and effective manner.

Although many of the initial results seemed promising, there are several limitations to using developmental pathways to target CSC populations. Because these pathways auto-regulate and interact with many other pathways, it is difficult to understand the full effects of inhibiting any one pathway [51–53]. Additionally, targeting these developmental pathways will affect both CSC and normal stem cell populations [44, 54–56]. Nonetheless, therapies

designed to block or to interfere with these pathways may help to minimize CSC proliferation.

Targeting the CSC Niche

Another potential therapy for CSC is to target unique cell surface markers that are expressed in stem cells. Most CSCs are detected and isolated with specific cell surface markers that are typically found in stem cells (Table 1). Early therapy based on this concept targeted CD44, a cell surface marker expressed in AML and critical for residence in a stem cell niche [57]. An antibody specific to CD44 was used to target the cell marker and induced two effects: tumor cells were unable to effectively engraft, leading to inhibition of tumor growth, along with loss of primitive cell types within tumor grafts [58]. This study appears promising; however, a similar study indicated that treatment with antibodies had little effect on tumor formation in xenograft [59]. However, both studies were able to show that blockade of CD44 limited AML CSC proliferation, suggesting that CD44 is a required element of the AML CSC niche [57]. Just as CD44 may prove a useful molecular target in treating AML, several other cell surface markers linked to the stem cell niche have been identified, such as the prostate stem cell antigen (PSCA), a marker expressed in both normal prostate stem cells as well as prostate cancers [60].

Another method for targeting the CSC niche involves blocking the factors that CSCs use to produce and stabilize supporting tissues. Vascular endothelial growth factor (VEGF) has been observed to be up-regulated in tumors, causing angiogenesis that provides the tumor with necessary blood supply [61, 62]. Likewise, the role of VEGF in normal neural stem cell (NSC) function has also been well explored. VEGF and several other factors, involved in cross talk with the endothelium maintain the neural stem cell niche [63]. This discovery has also been applied to CSC, where it was shown that VEGF and several other cell factors are responsible for maintaining the CSC niche [64]. By interrupting the ability of CSCs to interact and regulate supporting cells to maintain the CSC niche, tumor engraftment and growth will be interrupted.

As with targeting the signaling pathways used by CSCs, one of the major risks to all of these therapeutic approaches is the similarities between the CSCs and other adult stem cells in treated patients [65]. Because the targeted markers and factors are present in normal adult stem cells and CSCs, these methods may be inadequately specific and have associated toxicity to normal tissue.

Ongoing Controversies of the CSC hypothesis

Since the conception of the CSC hypothesis, many controversies have arisen that are currently the focus of intense research. Identification of specific surface markers is critical for CSC isolation and subsequent characterization and for future CSC-targeted therapies. Current research is demonstrating that a universal single marker may not exist for any specific cancer or among all patients. One of the earliest markers suggested for brain tumor CSCs was CD133, which was successfully used to enrich for tumor initiating CSCs [8]. Additional work from multiple groups has clearly shown that CD133⁺ populations do not exist in every brain tumor [66, 67] and that CD133⁻ cells are capable of initiating tumors [68]. For this reason, other stem cell markers such as stage-specific embryonic antigen 1 (SSEA-1)/CD15 [66, 69, 70] have been proposed and successfully used to enrich for CSCs, but in each case these newly proposed markers were only expressed in a subset of brain tumors. Similar results have been obtained in breast CSCs, originally defined as CD44⁺CD24⁻ [7]. Upon further characterization, additional surface markers such as aldehyde dehydrogenase 1 (ALDH1), CD201/PROCR, ESA, CD133, and HER2/ERBB2, have been shown to enrich for breast CSCs in certain tumor subsets [71–74]. Accordingly,

varying CSC enrichment markers have been prospectively identified for other forms of cancer as well (Table 1)

Adding to the complexity, it has recently been proposed that multiple CSC populations can exist within any given cancer. For example, distinct CSC subgroups may exist within a tumor that separately drive primary tumor growth and metastasis [75]. Hermann et al. (2007) identified a metastatic CSC subgroup within the CD133⁺ cells of pancreatic cancer expressing chemokine (C-X-C motif) receptor 4 (CXCR4). Depletion of CXCR4⁺ cells within this population abrogated the metastatic phenotype without affecting tumorigenic potential [76]. The existence of specific recurrence CSCs has also been recently postulated [77]. In general, CSCs are likely a dynamic population subject to change and adaptation through clonal evolution [78] or epigenetic plasticity [78]. The diversity of CSCs in any given tumor may be especially evident in highly malignant cancers or after chemotherapeutic and radiation interventions [78, 79]. It is likely that successful cancer treatments must eradicate all CSC sub-populations along with associated tumor cells.

The origin of CSCs and contribution to cancer growth remains a topic of hot debate. Some evidence suggests that cancer can arise from aberrant stem cells that acquired oncogenic characteristics [18, 80]. Human adult stem cells can rarely transform into cancerous cells following long-term culturing in an *in vitro* environment for 4–5 months [81]. Expression of oncogenes driven by stem cell-specific promoters (Sca1 in HSCs, CD133 in intestinal goblet cells, nestin in multiple brain tumors) in mice consistently results in tumors that faithfully recapitulate the human condition [82, 83]. However, in certain instances, more differentiated progenitor cells may initiate and drive tumor progression as CSCs. Jaiswal et al. (2003) restricted BCR/ABL oncogene expression specifically to myeloid progenitors leading to AML in mice [84]. Similarly, Krivtsov et al. (2006) described conversion of a committed granulocyte macrophage progenitor to leukemic stem cell through expression of MLL-AF9 fusion protein [85]; similar leukemia driving progenitors have been inferred in human leukemias through surface marker analysis [86]. It is also likely that not every cancer is driven by stem cell-like cells. Joseph et al. (2008) analyzed the development of malignant peripheral nerve sheath tumors (MPNSTs) in NF1-deficient mice and found that although the neural crest stem cells hyperproliferate extensively in the fetal peripheral nervous system, these stem cells do not persist into adulthood and the MPNSTs are driven by cancerous differentiated glia [87]. An interesting view on these results is that some forms of cancer may be stem cell driven following a reprogramming-like process. Oncogene expression from primitive HSCs (Sca1⁺) led to CML in mice and recapitulated the molecular and cellular diversity of the disease [82]. Similar reprogramming has been demonstrated in the conversion of fully mature fibroblasts to embryonic stem-like cells, also termed induced pluripotent stem cells [88, 89], and in direct conversion of mature pancreatic exocrine cells to insulin-producing cells [90]. In many of these instances, the initial reprogramming events are turned off again as the cells re-differentiate to more mature phenotypes; a similar process could occur in cancer where initial oncogenic / reprogramming hits occur in stem cells but further mutations or epigenetic modifications are required later during differentiation to initiate tumorigenesis. In these cases, the cell-of-origin of the cancer (the true CSC) may not be tumorigenic on its own and would differ from the identified CSC of the disease. Eradication of the originating CSC would still halt the disease, but attacking the tumorigenic progeny would do little [82]. Some evidence for reprogramming exists in human childhood leukemia in which only 1 of a pair of monozygotic twins developed leukemia despite the shared altered self-renewal and survival properties of the twins' preleukemic cells [91]. The importance of the stem cell microenvironment, or niche, and supporting stromal cells in regulating oncogenic processes must also be acknowledged. CSC induction through disruption of the niche can lead to deregulated proliferation and tumor initiation by adult stem cells [10]. Obviously, much

research needs to be done into the origin and mechanisms of propagation of CSCs, focusing on clinical relevance of CSC biology to improved therapeutics.

Finally, CSCs initially isolated were described as rare subsets of cells that drive cancer propagation and recurrence, with only approximately 1 CSC per every couple hundred thousand cells [13]. Recent work using human melanoma and transplantation between syngeneic mice have challenged this assumption that CSC sub-populations are always rare. Kelly et al. (2007) in leukemia and Quintana et al. (2009) in melanoma have each demonstrated abundance of CSCs in these cancers, with CSCs estimated at 1 in 20 cells or lower [92, 93]. These authors point out currently used xenograft models into NOD/SCID mice as inefficient, and improved the rates of CSCs seen through improvements on this traditional model. Despite the increased frequency of CSCs found, the overall CSC hypothesis is not refuted, it is just important to remember that, especially in highly malignant cancers, a larger sub-population of CSCs may need to be extinguished than originally thought. Clinically, increased staining for stem cell markers in various forms of cancer has been correlated with aggressiveness and malignancy [11, 94], so in advanced cases or after non-CSC specific therapies self-renewing CSCs may have overtaken any differentiated tumor cells.

Expert Opinion

Although relatively new, evidence for the CSC hypothesis (Figure 1) is rapidly accumulating, and putative CSCs have been isolated from many human cancers (Table 1). The highly efficient tumor-initiating ability of CSCs in xenograft animal models [7, 8] strongly supports a critical role for CSCs in human cancer initiation, progression, and recurrence. *In vitro* and *in vivo* evidence also have demonstrated increased radiation and chemotherapeutic resistance of CSCs [32, 33, 95, 96]. Therefore, it is imperative that new therapeutic strategies focus on ablating the CSC sub-population, whether this is achieved through targeting key molecular pathways, overcoming CSC resistance mechanisms, or via other methods. Ideally, many of these targeted treatments would also result in fewer side effects, although rigorous testing of new treatments will still be required to ensure safety and efficacy.

Although the study of CSCs offers an array of new therapeutic strategies, current efforts have been limited by the lack of an identified, unique marker for CSCs. Currently, efforts to localize CSCs rely on normal stem cell surface markers such as CD133 [8, 26] or CD44 [7], among others. As discussed above, even this list of surface markers continues to grow, and since these markers are expressed in normal, adult human stem cells, it is likely that targeted therapies directed to these markers would ablate the normal as well as cancerous stem cell populations. These directed therapies may be particularly dangerous to the pediatric patients. Identification and validation of specific CSC markers through genetic, proteomic, or metabolomic screens may enable substantial progress in the development of targeted therapeutics, and possibly antibody-mediated therapy or CSC-specific vaccine-based therapies. Because of the multiple isolated populations of CSCs in various cancers and the potential for neoplastic transformation of progenitors as well as stem cells, universal markers of CSCs may not exist. Therefore, study of the shared signaling pathways of CSCs, such as Wnt, TGF β , Notch, and Hedgehog as described above, may be a more useful focus and more efficient for drug targeting opportunities [79]. Many of these pathways have already been demonstrated critical to CSC self-renewal and propagation [45, 47, 74, 79, 97, 98]; however, rigorous *in vivo* as well as *in vitro* tests may be performed as certain proteins, such as the polycomb BMI-1, may have profoundly different effects [99]. It is important to note that proof-of-principle evidence in animal models and anecdotal clinical evidence already exists for the efficacy of killing CSCs to eradicate cancer. Unique elimination of the

CSC population through conditional knockdown or drug treatment in mouse models of CML eradicated the CML, whereas drug treatments not eliminating the CSC population had little effect [82, 100]. Short-term treatment of glioblastoma multiforme (GBM, brain cancer) CSCs with Notch or Hedgehog inhibitors or treatment with BMPs depletes the CSC population and prevents engraftment in immunodeficient mouse models [39, 45, 101]. In certain forms of breast cancer, the HER2-expressing cells have CSC properties, and blockade with the pharmaceutical trastuzumab depletes these CSCs preventing primary and serial engraftment into mice. These results may help to explain the better than expected results seen in some patients [74, 102]. However, much of the complexity of targeting and eradicating CSCs must still be worked out before more broad successful clinical therapies.

Further characterization of adaptive and/or resistance mechanisms of CSCs to current treatment regimens will also be required for developing more effective future therapies. Although molecular-targeted therapies show early promise in treating cancer and its stem cells [48, 103, 104], CSCs are likely a dynamic and changing population through clonal evolution [78] or adaptive/resistance responses. For many complex cancer types, such as GBM, it is likely that molecular monotherapy will be inefficient, and knowledge of the resistance mechanisms of CSCs will inform rational selection of CSC-combinatorial medicine using molecular therapeutics combined with other modalities. Blockade of specific CSC resistance mechanisms to traditional therapies is also a useful approach, with early successes of IL-4 blockade to sensitize colon CSCs to chemotherapy [105] and CHK kinase inhibition to radiosensitize GBM CSCs [32]. Different cancer CSCs may exhibit varied response mechanisms, such as breast vs. GBM CSCs in radioresistance [32, 33], so tissue-specific resistance mechanisms may need to be determined in many cases.

Lastly, the heterogeneity of cancer and rapid improvement in diagnostic methods is making possible an age of customized medicine for individual patients. The power of genetic and other global arrays has demonstrated repeatedly the molecular variation among cancers, and genetic signatures can often predict outcome and treatment response better than histological analysis alone [106, 107]. Molecular variation is also quickly being demonstrated in the CSCs within cancer as well [67, 72, 108]. Ideally, CSCs will be rapidly isolated and screened for molecular defects sensitizing them to specific drugs or subjected to a direct drug screen (Figure 3). Some research has focused on developing such methods [109, 110]; however, much work must be done to optimize these methods and establish feasible protocols for clinical application as the time and effort required currently make them impossible to broadly apply. Isolation techniques of CSCs must also be further perfected before such protocols can be enacted in the clinic. One example pertains to GBM CSCs, which can be isolated from operating room specimens via flow sorting, neurosphere formation, or tissue culture on laminin [8, 109, 111, 112]; if these different techniques isolate the same or unique CSC populations is unknown and as discussed above all populations of CSCs must be eliminated for successful clinical therapies. Much of the improvement in cancer survival over the past decades is attributed to improved preventive and diagnostic measures [2]; along these lines, improved biomarker identification of CSC transformation either by non-invasive imaging or blood draw could help early detection and make it easier to eliminate CSCs before multiple and highly aggressive populations can arise. With these diagnostic tools in hand, doctors may eventually be able to create a tailored prescription for oncogenic pathway components from a wide array of options, achieving individualized, targeted therapies with enhanced efficacy to patient's specific tumor with a lower toxic side effect profile.

The CSC field is a relatively new one with a great deal of research and exploration still necessary to yield greater practical benefits towards improved cancer treatment. The cancer stem cell hypothesis offers a new perspective for understanding tumorigenesis, and will

likely lead to rational development of refined and novel therapies that yield tangible clinical benefits to patients.

Acknowledgments

We apologize to colleagues whose work was not cited due to space constraints. PAC was partially supported by a NIH T32 postdoctoral fellowship from the University of Wisconsin Stem Cell Program. MZ was supported by the NIH-supported Neuroscience Training Program at the University of Wisconsin – Madison. RGK is a University of Wisconsin Letters and Sciences Honors Scholar. JSK was supported by the HEADRUSH Brain Tumor Research Professorship, Loff Memorial Fund for GBM Research, AANS-NREF Young Clinician Investigator Award, and funding from the Dept. of Neurological Surgery, Graduate School, and Medical School at the University of Wisconsin.

References

1. ACS. Cancer stats and figures. 2006
2. Leaf C. Why We're Losing the War on Cancer (And How to Win It). FORTUNE magazine. 2004; 149:76–86.
3. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005 Mar 10; 352(10): 987–996. [PubMed: 15758009]
4. Pastorelli D, Carlei G, Zustovich F, Marchese F, Artioli G, Zovato S, et al. Gemcitabine and liposomal doxorubicin in biliary and hepatic carcinoma (HCC) chemotherapy: preliminary results and review of the literature. Ann Oncol. 2006 May; 17(Suppl 5):v153–v157. [PubMed: 16807446]
5. Pandal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. Nature reviews. 2003 Dec; 3(12):895–902.
6. Lobo NA, Shimono Y, Qian D, Clarke MF. The Biology of Cancer Stem Cells. Annu Rev Cell Dev Biol. 2007 Jul 23.
7. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003 Apr 1; 100(7):3983–3988. [PubMed: 12629218] ** First paper to demonstrate cancer initiating cells in solid tumors (breast cancer) by sorting for CD44⁺CD24^{-low} cells.
8. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. Nature. 2004 Nov 18; 432(7015):396–401. [PubMed: 15549107] ** Identification of CD133⁺ cells as tumor initiating in brain tumors.
9. Maitland NJ, Collins A. A tumour stem cell hypothesis for the origins of prostate cancer. BJU international. 2005 Dec; 96(9):1219–1223. [PubMed: 16287434]
10. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer Stem Cells-- Perspectives on Current Status and Future Directions: AACR Workshop on Cancer Stem Cells. Cancer Res. 2006 Oct 1; 66(19):9339–9344. [PubMed: 16990346] * Very good review on cancer stem cell hypothesis and terminology.
11. Dahlstrand J, Collins VP, Lendahl U. Expression of the class VI intermediate filament nestin in human central nervous system tumors. Cancer Res. 1992 Oct 1; 52(19):5334–5341. [PubMed: 1382841]
12. Almqvist PM, Mah R, Lendahl U, Jacobsson B, Henderson G. Immunohistochemical detection of nestin in pediatric brain tumors. J Histochem Cytochem. 2002 Feb; 50(2):147–158. [PubMed: 11799134]
13. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994 Feb 17; 367(6464):645–648. [PubMed: 7509044] ** First paper to demonstrate that only a subset of cancer cells have initiating properties in leukemia.
14. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997 Jul; 3(7):730–737. [PubMed: 9212098]

15. Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. *Nat Immunol.* 2004 Jul; 5(7):738–743. [PubMed: 15170211]
16. Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. *Annu Rev Med.* 2007; 58:267–284. [PubMed: 17002552]
17. Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. *Cancer research.* 2005 Jul 1; 65(13):5506–5511. [PubMed: 15994920]
18. Read TA, Hegedus B, Wechsler-Reya R, Gutmann DH. The neurobiology of neurooncology. *Ann Neurol.* 2006 Jul; 60(1):3–11. [PubMed: 16802285]
19. Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA. Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. *Glia.* 2002 Sep; 39(3):193–206. [PubMed: 12203386] * First paper using neurosphere assay to isolate human cancer stem cells from glioblastoma multiforme *in vitro*.
20. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res.* 2003 Sep 15; 63(18):5821–5828. [PubMed: 14522905]
21. Dirks PB. Cancer: stem cells and brain tumours. *Nature.* 2006 Dec 7; 444(7120):687–688. [PubMed: 17151644]
22. Singh SK, Clarke ID, Hide T, Dirks PB. Cancer stem cells in nervous system tumors. *Oncogene.* 2004 Sep 20; 23(43):7267–7273. [PubMed: 15378086]
23. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer research.* 2004 Oct 1; 64(19):7011–7021. [PubMed: 15466194]
24. Pomeroy SL, Tamayo P, Gaasenbeek M, Sturla LM, Angelo M, McLaughlin ME, et al. Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature.* 2002 Jan 24; 415(6870):436–442. [PubMed: 11807556]
25. Lee J, Kotliarova S, Kotliarov Y, Li A, Su Q, Donin NM, et al. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer Cell.* 2006 May; 9(5):391–403. [PubMed: 16697959] * Cancer stem cells grown as neurospheres may be a better in vitro model of glioblastoma multiforme than traditionally grown cell lines.
26. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature.* 2007 Jan 4; 445(7123):106–110. [PubMed: 17122772] * CD133⁺ cells as tumor initiating cells in colon cancer.
27. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature.* 2007 Jan 4; 445(7123):111–115. [PubMed: 17122771] * Colon cancer stem cells can be isolated and expanded by cancer sphere culture in vitro.
28. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer.* 2005 Apr; 5(4): 275–284. [PubMed: 15803154]
29. Blagosklonny MV. Target for cancer therapy: proliferating cells or stem cells. *Leukemia.* 2006 Mar; 20(3):385–391. [PubMed: 16357832]
30. Kruger GM, Morrison SJ. Brain repair by endogenous progenitors. *Cell.* 2002 Aug 23; 110(4): 399–402. [PubMed: 12202029]
31. Lou H, Dean M. Targeted therapy for cancer stem cells: the patched pathway and ABC transporters. *Oncogene.* 2007 Feb 26; 26(9):1357–1360. [PubMed: 17322922]
32. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature.* 2006 Dec 7; 444(7120):756–760. [PubMed: 17051156] ** First paper to demonstrate increased resistance of cancer stem cells to radiation therapy.
33. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature.* 2009 Apr 9; 458(7239): 780–783. [PubMed: 19194462]

34. Chakravarti A, Chakladar A, Delaney MA, Latham DE, Loeffler JS. The epidermal growth factor receptor pathway mediates resistance to sequential administration of radiation and chemotherapy in primary human glioblastoma cells in a RAS-dependent manner. *Cancer Res.* 2002 Aug 1; 62(15):4307–4315. [PubMed: 12154034]
35. Donnenberg VS, Donnenberg AD. Multiple drug resistance in cancer revisited: the cancer stem cell hypothesis. *J Clin Pharmacol.* 2005 Aug; 45(8):872–877. [PubMed: 16027397]
36. Hermann DM, Kilic E, Spudich A, Kramer SD, Wunderli-Allenspach H, Bassetti CL. Role of drug efflux carriers in the healthy and diseased brain. *Annals of neurology.* 2006 Nov; 60(5):489–498. [PubMed: 17048260]
37. Clark PA, Treisman DM, Ebben J, Kuo JS. Developmental signaling pathways in brain tumor-derived stem-like cells. *Dev Dyn.* 2007 Dec; 236(12):3297–3308. [PubMed: 18000980]
38. Sharif A, Legendre P, Prevot V, Allet C, Romao L, Studler JM, et al. Transforming growth factor alpha promotes sequential conversion of mature astrocytes into neural progenitors and stem cells. *Oncogene.* 2006 Oct 23.
39. Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, et al. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. *Nature.* 2006 Dec 7; 444(7120):761–765. [PubMed: 17151667] ** Proof-of-concept is shown in this paper for 'differentiation' therapy, in which agents are administered to differentiate cancer stem cells to a state in which they no longer initiate tumors. These agents, such as BMPs in this paper, may be the same as used to differentiate normal stem cells.
40. Purow BW, Haque RM, Noel MW, Su Q, Burdick MJ, Lee J, et al. Expression of Notch-1 and its ligands, Delta-like-1 and Jagged-1, is critical for glioma cell survival and proliferation. *Cancer Res.* 2005 Mar 15; 65(6):2353–2363. [PubMed: 15781650]
41. Kanamori M, Kawaguchi T, Nigro JM, Feuerstein BG, Berger MS, Miele L, et al. Contribution of Notch signaling activation to human glioblastoma multiforme. *J Neurosurg.* 2007 Mar; 106(3):417–427. [PubMed: 17367064]
42. Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, et al. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. *Cancer Res.* 2006 Aug 1; 66(15):7445–7452. [PubMed: 16885340]
43. Corcoran RB, Scott MP. A mouse model for medulloblastoma and basal cell nevus syndrome. *J Neurooncol.* 2001 Jul; 53(3):307–318. [PubMed: 11718263]
44. Karhadkar SS, Bova GS, Abdallah N, Dhara S, Gardner D, Maitra A, et al. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. *Nature.* 2004 Oct 7; 431(7009):707–712. [PubMed: 15361885]
45. Bar EE, Chaudhry A, Lin A, Fan X, Schreck K, Matsui W, et al. Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. *Stem Cells.* 2007 Oct; 25(10):2524–2533. [PubMed: 17628016]
46. Berman DM, Karhadkar SS, Hallahan AR, Pritchard JI, Eberhart CG, Watkins DN, et al. Medulloblastoma growth inhibition by hedgehog pathway blockade. *Science.* 2002 Aug 30; 297(5586):1559–1561. [PubMed: 12202832]
47. Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr Biol.* 2007 Jan 23; 17(2):165–172. [PubMed: 17196391]
48. Rudin CM, Hann CL, Laterra J, Yauch RL, Callahan CA, Fu L, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. *N Engl J Med.* 2009 Sep 17; 361(12):1173–1178. [PubMed: 19726761]
49. Yauch RL, Dijkgraaf GJ, Alicke B, Januario T, Ahn CP, Holcomb T, et al. Smoothened Mutation Confers Resistance to a Hedgehog Pathway Inhibitor in Medulloblastoma. *Science.* 2009 Sep 2.
50. Vibhakkar R, Foltz G, Yoon JG, Field L, Lee H, Ryu GY, et al. Dickkopf-1 is an epigenetically silenced candidate tumor suppressor gene in medulloblastoma. *Neuro-oncol.* 2007 Apr; 9(2):135–144. [PubMed: 17329407]
51. Niimi H, Pardali K, Vanlandewijck M, Heldin CH, Moustakas A. Notch signaling is necessary for epithelial growth arrest by TGF-beta. *J Cell Biol.* 2007 Feb 26; 176(5):695–707. [PubMed: 17325209]

52. Attisano L, Labbe E. TGFbeta and Wnt pathway cross-talk. *Cancer Metastasis Rev.* 2004 Jan-Jun; 23(1-2):53-61. [PubMed: 15000149]
53. Bhardwaj G, Murdoch B, Wu D, Baker DP, Williams KP, Chadwick K, et al. Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation. *Nat Immunol.* 2001 Feb; 2(2):172-180. [PubMed: 11175816]
54. Alexson TO, Hitoshi S, Coles BL, Bernstein A, van der Kooy D. Notch signaling is required to maintain all neural stem cell populations--irrespective of spatial or temporal niche. *Dev Neurosci.* 2006; 28(1-2):34-48. [PubMed: 16508302]
55. Machold R, Hayashi S, Rutlin M, Muzumdar MD, Nery S, Corbin JG, et al. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. *Neuron.* 2003 Sep 11; 39(6):937-950. [PubMed: 12971894]
56. Palma V, Lim DA, Dahmane N, Sanchez P, Brionne TC, Herzberg CD, et al. Sonic hedgehog controls stem cell behavior in the postnatal and adult brain. *Development.* 2005 Jan; 132(2):335-344. [PubMed: 15604099]
57. Williams DA, Cancelas JA. Leukaemia: niche retreats for stem cells. *Nature.* 2006 Dec 14; 444(7121):827-828. [PubMed: 17167463]
58. Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nature medicine.* 2006 Oct; 12(10):1167-1174. ** Established proof-of-concept for niche targeting drugs to eradicate cancer stem cells and prevent leukemia initiation.
59. Krause DS, Lazarides K, von Andrian UH, Van Etten RA. Requirement for CD44 in homing and engraftment of BCR-ABL-expressing leukemic stem cells. *Nat Med.* 2006 Oct; 12(10):1175-1180. [PubMed: 16998483]
60. Tran CP, Lin C, Yamashiro J, Reiter RE. Prostate stem cell antigen is a marker of late intermediate prostate epithelial cells. *Mol Cancer Res.* 2002 Dec; 1(2):113-121. [PubMed: 12496358]
61. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature.* 1993 Apr 29; 362(6423):841-844. [PubMed: 7683111]
62. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. *Nature.* 1992 Oct 29; 359(6398):845-848. [PubMed: 1279432]
63. Li Q, Ford MC, Lavik EB, Madri JA. Modeling the neurovascular niche: VEGF- and BDNF-mediated cross-talk between neural stem cells and endothelial cells: an in vitro study. *J Neurosci Res.* 2006 Dec; 84(8):1656-1668. [PubMed: 17061253]
64. Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, et al. Stem Cell-like Glioma Cells Promote Tumor Angiogenesis through Vascular Endothelial Growth Factor. *Cancer Res.* 2006 Aug 15; 66(16):7843-7848. [PubMed: 16912155]
65. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature.* 2005 Apr 14; 434(7035):843-850. [PubMed: 15829953]
66. Son MJ, Woolard K, Nam DH, Lee J, Fine HA. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. *Cell Stem Cell.* 2009 May 8; 4(5):440-452. [PubMed: 19427293]
67. Gunther HS, Schmidt NO, Phillips HS, Kemming D, Kharbanda S, Soriano R, et al. Glioblastoma-derived stem cell-enriched cultures form distinct subgroups according to molecular and phenotypic criteria. *Oncogene.* 2007 Nov 26. * Demonstrates heterogeneity among cancer stem cells isolated from different glioblastoma multiforme patients.
68. Beier D, Hau P, Proescholdt M, Lohmeier A, Wischhusen J, Oefner PJ, et al. CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Res.* 2007 May 1; 67(9):4010-4015. [PubMed: 17483311]
69. Ward RJ, Lee L, Graham K, Satkunendran T, Yoshikawa K, Ling E, et al. Multipotent CD15+ cancer stem cells in patched-1-deficient mouse medulloblastoma. *Cancer Res.* 2009 Jun 1; 69(11):4682-4690. [PubMed: 19487286]

70. Mao XG, Zhang X, Xue XY, Guo G, Wang P, Zhang W, et al. Brain Tumor Stem-Like Cells Identified by Neural Stem Cell Marker CD15. *Transl Oncol.* 2009 Dec; 2(4):247–257. [PubMed: 19956386]
71. Charafe-Jauffret E, Ginestier C, Iovino F, Tarpin C, Diebel M, Esterni B, et al. Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer. *Clin Cancer Res.* Jan 1; 16(1):45–55. [PubMed: 20028757]
72. Hwang-Verslues WW, Kuo WH, Chang PH, Pan CC, Wang HH, Tsai ST, et al. Multiple lineages of human breast cancer stem/progenitor cells identified by profiling with stem cell markers. *PLoS One.* 2009; 4(12):e8377. [PubMed: 20027313]
73. Croker AK, Goodale D, Chu J, Postenka C, Hedley BD, Hess DA, et al. High aldehyde dehydrogenase and expression of cancer stem cell markers selects for breast cancer cells with enhanced malignant and metastatic ability. *Journal of cellular and molecular medicine.* 2008 Aug 4.
74. Magnifico A, Albano L, Campaner S, Delia D, Castiglioni F, Gasparini P, et al. Tumor-Initiating Cells of HER2-Positive Carcinoma Cell Lines Express the Highest Oncoprotein Levels and Are Sensitive to Trastuzumab. *Clin Cancer Res.* 2009 Mar 10.
75. Li F, Tiede B, Massague J, Kang Y. Beyond tumorigenesis: cancer stem cells in metastasis. *Cell research.* 2007 Jan; 17(1):3–14. [PubMed: 17179981]
76. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell.* 2007 Sep 13; 1(3):313–323. [PubMed: 18371365]
77. Dittmar T, Nagler C, Schwitalla S, Reith G, Niggemann B, Zanker KS. Recurrence cancer stem cells—made by cell fusion? *Med Hypotheses.* 2009 Oct; 73(4):542–547. [PubMed: 19564079]
78. Shackleton M, Quintana E, Fearon ER, Morrison SJ. Heterogeneity in cancer: cancer stem cells versus clonal evolution. *Cell.* 2009 Sep 4; 138(5):822–829. [PubMed: 19737509]
79. Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB. Tumour-initiating cells: challenges and opportunities for anticancer drug discovery. *Nat Rev Drug Discov.* 2009 Oct; 8(10):806–823. [PubMed: 19794444]
80. Shiras A, Chettiar ST, Shepal V, Rajendran G, Prasad GR, Shastry P. Spontaneous transformation of human adult nontumorigenic stem cells to cancer stem cells is driven by genomic instability in a human model of glioblastoma. *Stem Cells.* 2007 Jun; 25(6):1478–1489. [PubMed: 17332509]
81. Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC, et al. Spontaneous human adult stem cell transformation. *Cancer research.* 2005 Apr 15; 65(8):3035–3039. [PubMed: 15833829]
82. Perez-Caro M, Cobaleda C, Gonzalez-Herrero I, Vicente-Duenas C, Bermejo-Rodriguez C, Sanchez-Beato M, et al. Cancer induction by restriction of oncogene expression to the stem cell compartment. *EMBO J.* 2009 Jan 7; 28(1):8–20. [PubMed: 19037256]
83. Alcantara Llaguno S, Chen J, Kwon CH, Jackson EL, Li Y, Burns DK, et al. Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. *Cancer Cell.* 2009 Jan 6; 15(1):45–56. [PubMed: 19111880]
84. Jaiswal S, Traver D, Miyamoto T, Akashi K, Lagasse E, Weissman IL. Expression of BCR/ABL and BCL-2 in myeloid progenitors leads to myeloid leukemias. *Proc Natl Acad Sci U S A.* 2003 Aug 19; 100(17):10002–10007. [PubMed: 12890867]
85. Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature.* 2006 Aug 17; 442(7104):818–822. [PubMed: 16862118] ** This paper shows that under certain conditions progenitor-like cells can obtain self-renewal properties and drive cancer.
86. Jamieson CH, Ailles LE, Dylla SJ, Muijtens M, Jones C, Zehnder JL, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med.* 2004 Aug 12; 351(7):657–667. [PubMed: 15306667]
87. Joseph NM, Mosher JT, Buchstaller J, Snider P, McKeever PE, Lim M, et al. The loss of Nf1 transiently promotes self-renewal but not tumorigenesis by neural crest stem cells. *Cancer Cell.* 2008 Feb; 13(2):129–140. [PubMed: 18242513] * Suggests that not all cancers need to be driven by cancer stem cells.

88. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007 Nov 30; 131(5): 861–872. [PubMed: 18035408]
89. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007 Dec 21; 318(5858): 1917–1920. [PubMed: 18029452]
90. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature*. 2008 Oct 2; 455(7213):627–632. [PubMed: 18754011]
91. Hong D, Gupta R, Ancliff P, Atzberger A, Brown J, Soneji S, et al. Initiating and cancer-propagating cells in TEL-AML1-associated childhood leukemia. *Science*. 2008 Jan 18; 319(5861): 336–339. [PubMed: 18202291]
92. Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. *Science*. 2007 Jul 20; 317(5836):337. [PubMed: 17641192]
93. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. *Nature*. 2008 Dec 4; 456(7222):593–598. [PubMed: 19052619] ** Challenges idea that cancer stem cells are rare by demonstrating efficient tumor initiation by unsorted human melanoma cells in optimized xenograft systems.
94. Li T, Su Y, Mei Y, Leng Q, Leng B, Liu Z, et al. ALDH1A1 is a marker for malignant prostate stem cells and predictor of prostate cancer patients' outcome. *Lab Invest*. 2009 Dec 14.
95. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer*. 2006; 5:67. [PubMed: 17140455]
96. Bleau AM, Hambardzumyan D, Ozawa T, Fomchenko EI, Huse JT, Brennan CW, et al. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. *Cell Stem Cell*. 2009 Mar 6; 4(3):226–235. [PubMed: 19265662]
97. Armstrong F, Brunet de la Grange P, Gerby B, Rouyez MC, Calvo J, Fontenay M, et al. NOTCH is a key regulator of human T-cell acute leukemia initiating cell activity. *Blood*. 2009 Feb 19; 113(8):1730–1740. [PubMed: 18984862]
98. Espada J, Calvo MB, Diaz-Prado S, Medina V. Wnt signalling and cancer stem cells. *Clin Transl Oncol*. 2009 Jul; 11(7):411–427. [PubMed: 19574199]
99. He S, Iwashita T, Buchstaller J, Molofsky AV, Thomas D, Morrison SJ. Bmi-1 over-expression in neural stem/progenitor cells increases proliferation and neurogenesis in culture but has little effect on these functions in vivo. *Dev Biol*. 2009 Apr 15; 328(2):257–272. [PubMed: 19389366]
100. Ito K, Bernardi R, Morotti A, Matsuoka S, Saglio G, Ikeda Y, et al. PML targeting eradicates quiescent leukaemia-initiating cells. *Nature*. 2008 Jun 19; 453(7198):1072–1078. [PubMed: 18469801]
101. Fan X, Khaki L, Zhu TS, Soules ME, Talsma CE, Gul N, et al. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells*. 2010 Jan; 28(1):5–16. [PubMed: 19904829]
102. Korkaya H, Wicha MS. HER-2, notch, and breast cancer stem cells: targeting an axis of evil. *Clin Cancer Res*. 2009 Mar 15; 15(6):1845–1847. [PubMed: 19276254]
103. Soeda A, Inagaki A, Oka N, Ikegame Y, Aoki H, Yoshimura S, et al. Epidermal growth factor plays a crucial role in mitogenic regulation of human brain tumor stem cells. *J Biol Chem*. 2008 Apr 18; 283(16):10958–10966. [PubMed: 18292095]
104. Hambardzumyan D, Becher OJ, Rosenblum MK, Pandolfi PP, Manova-Todorova K, Holland EC. PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. *Genes Dev*. 2008 Feb 15; 22(4):436–448. [PubMed: 18281460]
105. Francipane MG, Alea MP, Lombardo Y, Todaro M, Medema JP, Stassi G. Crucial role of interleukin-4 in the survival of colon cancer stem cells. *Cancer Res*. 2008 Jun 1; 68(11):4022–4025. [PubMed: 18519657]
106. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell*. 2006 Mar; 9(3):157–173. [PubMed: 16530701]

107. Liu R, Wang X, Chen GY, Dalerba P, Gurney A, Hoey T, et al. The prognostic role of a gene signature from tumorigenic breast-cancer cells. *N Engl J Med*. 2007 Jan 18; 356(3):217–226. [PubMed: 17229949]
108. Okamoto OK, Carvalho AC, Marti LC, Vencio RZ, Moreira-Filho CA. Common molecular pathways involved in human CD133+/CD34+ progenitor cell expansion and cancer. *Cancer Cell Int*. 2007; 7:11. [PubMed: 17559657]
109. Pollard SM, Yoshikawa K, Clarke ID, Danovi D, Stricker S, Russell R, et al. Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. *Cell Stem Cell*. 2009 Jun 5; 4(6):568–580. [PubMed: 19497285]
110. Gal H, Makovitzki A, Amariglio N, Rechavi G, Ram Z, Givol D. A rapid assay for drug sensitivity of glioblastoma stem cells. *Biochemical and biophysical research communications*. 2007 Jul 6; 358(3):908–913. [PubMed: 17512905]
111. Kelly JJ, Stechishin O, Chojnacki A, Lun X, Sun B, Senger DL, et al. Proliferation of human glioblastoma stem cells occurs independently of exogenous mitogens. *Stem Cells*. 2009 Aug; 27(8):1722–1733. [PubMed: 19544433]
112. Chaichana K, Zamora-Berridi G, Camara-Quintana J, Quinones-Hinojosa A. Neurosphere assays: growth factors and hormone differences in tumor and nontumor studies. *Stem Cells*. 2006 Dec; 24(12):2851–2857. [PubMed: 16945995]
113. Kong Y, Yoshida S, Saito Y, Doi T, Nagatoshi Y, Fukata M, et al. CD34+CD38+CD19+ as well as CD34+CD38-CD19+ cells are leukemia-initiating cells with self-renewal capacity in human B-precursor ALL. *Leukemia*. 2008 Jun; 22(6):1207–1213. [PubMed: 18418410]
114. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell*. 2007 Nov; 1(5):555–567. [PubMed: 18371393]
115. Hemmati HD, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M, et al. Cancerous stem cells can arise from pediatric brain tumors. *Proc Natl Acad Sci U S A*. 2003 Dec 9; 100(25):15178–15183. [PubMed: 14645703]
116. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, et al. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer research*. 2005 Oct 15; 65(20):9328–9337. [PubMed: 16230395]
117. Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, et al. Identification of cells initiating human melanomas. *Nature*. 2008 Jan 17; 451(7176):345–349. [PubMed: 18202660]
118. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, et al. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci U S A*. 2007 Jun 12; 104(24):10158–10163. [PubMed: 17548814]
119. Huang EH, Hynes MJ, Zhang T, Ginestier C, Dontu G, Appelman H, et al. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Res*. 2009 Apr 15; 69(8):3382–3389. [PubMed: 19336570]
120. Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. *Cancer research*. 2007 May 15; 67(10):4827–4833. [PubMed: 17510412]
121. Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell death and differentiation*. 2008 Mar; 15(3):504–514. [PubMed: 18049477]
122. Gibbs CP, Kukekov VG, Reith JD, Tchigrinova O, Suslov ON, Scott EW, et al. Stem-like cells in bone sarcomas: implications for tumorigenesis. *Neoplasia*. 2005 Nov; 7(11):967–976. [PubMed: 16331882]
123. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proceedings of the National Academy of Sciences of the United States of America*. 2007 Jan 16; 104(3):973–978. [PubMed: 17210912]

124. Ma S, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, et al. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology*. 2007 Jun; 132(7):2542–2556. [PubMed: 17570225]
125. Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, et al. Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell*. 2008 Feb; 13(2):153–166. [PubMed: 18242515]
126. Xu Q, Yuan X, Tunici P, Liu G, Fan X, Xu M, et al. Isolation of tumour stem-like cells from benign tumours. *British journal of cancer*. 2009 Jul 21; 101(2):303–311. [PubMed: 19568241]
127. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. *Cancer Res*. 2007 Feb 1; 67(3):1030–1037. [PubMed: 17283135]
128. Hermann. Distinct Populations of Cancer Stem Cells Determine Tumor Growth and Metastatic Activity in Human Pancreatic Cancer. *Cell Stem Cell*. 2007; 1:313–323. [PubMed: 18371365]

Article highlights

- Cancer treatments and outcomes have improved little over the last few decades
- Cancer stem cell (CSC) model hypothesizes that only a small, defined subset of cancer cells can drive tumor progression and recurrence, in contrast to the classical stochastic theory that all cancer cells are the same
- The CSC model predicts that stem cell-targeted therapies will be a more effective in treating cancer; such strategies include CSC-targeting therapy, blockade of CSC-specific pathways, or removal of CSCs from their protective microenvironment
- Many controversies and unanswered questions about the CSC model remain
- Rapidly improving isolation and culture techniques may make possible personalized CSC therapies

Figure 1.

Cancer stem cell hypothesis. Cancer stem cells (CSCs) are organized into a hierarchy much like normal stem cells, with long-term self-renewing CSCs differentiating into cells with decreasing self-renewal potential. Clinically, tumor malignancy may be correlated to the number of CSCs, which drive tumor growth and recurrence.

Figure 2.

Therapeutic relevance of cancer stem cells (CSCs). Tumors contain a mix of CSCs, progenitor-like cells, and differentiated tumor mass. Conventional therapies kill mostly progenitor-like and tumor mass cells, while therapy-resistant CSCs survive and continue to proliferate leading to recurrence. CSC-specific therapies kill or differentiate the CSCs. Without the self-renewing CSCs to drive tumor progression, the tumor shrinks and eventually dies.

Figure 3.

Personalized CSC-specific therapies. Increasing evidence is revealing that drug sensitivity of CSCs may be patient as well as tumor specific, which will require personalized therapies. CSCs are isolated via surface marker or functional assays and subjected to high-throughput drug screening. The identified CSC-specific drug(s) are then administered to the patient, resulting in tumor shrinkage and death.

Table 1

Cancers with identified stem cells and cells surface markers expressed

Cancer	Cell Surface Markers	References
Leukemia	CD34 ⁺ , CD38 ⁻ , CD19 ⁺	[14, 15, 113]
Breast	CD44 ⁺ , CD24 ⁻ , Lin ⁻ ALDH1 ⁺	[7, 114]
Brain	CD133 ⁺	[8, 115]
Melanoma	CD20 ⁺ ABCB5 ⁺	[116, 117]
Colorectal	CD133 ⁺ EpCAM ⁺ , CD44 ⁺ , CD166 ⁺ ALDH1 ⁺	[26, 27, 118, 119]
Lung	CD24 ⁺ , CD44 ⁺ CD133 ⁺	[120, 121]
Sarcomas	CD105 ⁺ , CD44 ⁺ , Stro1 ⁺	[122]
Head and Neck Squamous Cell Carcinoma	CD44 ⁺	[123]
Liver	CD133 ⁺ CD90 ⁺ , CD44 ⁺	[124, 125]
Pituitary adenoma (benign)	None	[126]
Pancreatic	CD44 ⁺ , CD24 ⁺ , ESA ⁺ CD133 ⁺	[127, 128]

ESA: epithelial specific antigen; EpCAM: epithelial cell adhesion molecule; ALDH1: aldehyde dehydrogenase 1; ABC: ATP-binding cassette