



Published in final edited form as:

*Neurosurg Clin N Am.* 2010 January ; 21(1): 159–166. doi:10.1016/j.nec.2009.08.006.

## Glioma Stem Cell Research for the development of Immunotherapy

Jianfei Ji, Keith L Black, and John s Yu

Maxine Dunitz Neurosurgical Institute, Cedars-Sinai Medical Center

### Abstract

Glioma, especially high-grade glioblastoma multiforme (GBM), is the most common and aggressive type of brain tumor, accounting for about half of all the primary brain tumors. Despite continued advances in surgery, chemotherapy and radiotherapy, the clinical outcomes remain dismal. The two-year survival rate of GBM is <30%. Better understanding of GBM biology is desirable to develop novel therapies. Recent studies have demonstrated the existence of a small subpopulation of cells with stem like features cancer stem cells otherwise known as (CSC). These GBM CSCs are self-renewable and highly tumorigenic. They are not only chemo-radio-resistant, but also often multi-drug resistance genes and drug transporter genes. These characteristic enable GBM CSCs to survive standard cytotoxic therapies. Among GBM CSCs, *CD133+* cells are a well-defined population and are prospectively isolated by their cell-surface marker. There are increasing data that *CD133+* CSC presence highly correlates with patient survival. This makes it an ideal immunotherapy target population. In this article, we will review recent studies related with GBM CSCs, particularly *CD133+* + CSCs as well as the novel therapeutic strategies targeting these cells.

### Keywords

cancer stem cell; glioma; *CD133+*; immunotherapy

### Introduction

Human brain tumors are a diverse group of diseases characterized by the abnormal growth of brain cells contained within the skull afflicting both adult and children. According to National Cancer Institute data, there are about 20,000 new cases and 13,000 deaths each year in the US. In children, brain tumors are the leading cause of solid tumor cancer death; all forms of glioma make up about 1/5 of all childhood cancers ([www.cancer.gov](http://www.cancer.gov)). In adults, the most common malignant brain tumor, glioblastoma multiforme (GBM), also the most malignant primary tumor of the brain and associated with one of the worst 5-year survival rates among all human cancers [1,2]. The median survival time is 14.6 months after first diagnosis [3,4]. Despite the advances in conventional treatments, comprised of surgical resection, local radiotherapy and systemic chemotherapy, the incidence and mortality rates for gliomas have changed little in

© 2009 Elsevier Inc. All rights reserved.

John S. Yu M.D., [yuj@cshs.org](mailto:yuj@cshs.org), Cedars-Sinai Medical Center, Maxine Dunitz Neurosurgical Institute, 8631 W. Third St., Suite 800 E, Los Angeles, CA 90048. Keith L. Black, M.D., [Keith.Black@cshs.org](mailto:Keith.Black@cshs.org), Cedars-Sinai Medical Center, Maxine Dunitz Neurosurgical Institute, 8631 W. Third St., Suite 800 E, Los Angeles, CA 90048.

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the past decade. With greater understanding of the cellular and molecular mechanisms cancer initiation and propagation, the cancer stem cell (CSC) hypothesis presents new insights for developing novel treatments that target this group of cells. In this chapter, we will discuss the CSC hypothesis and its application to develop treatments for glioma.

## CSC BTSC and CD133 CSCs

The first conclusive evidence for CSCs came from studies of acute myeloid leukemia (AML) [5,6]. Bonnet and Dick isolated a subpopulation of AML cells that were capable of initiating AML in immunodeficient NOD/SCID mice. These leukemia cells (leukemia stem cells, LSCs) express similar cell surface markers to normal hematopoietic stem cells (HSCs). The AML established from these LSCs recapitulates the morphologic and immunophenotypic heterogeneity of the original tumor. These seminal studies opened the door for the field of CSC study. Besides the properties shared with normal stem cells (self-renewal and the ability to differentiate into other cells), to be considered as CSCs, candidate cells must present the following properties: (a) the unique ability to engraft; (b) the ability to recapitulate the tumor of origin both morphologically and immunophenotypically in xenografts and (c) the ability to be serially transplanted [7]. These criteria are the standard to identify other CSCs not only in hematopoietic tumors, but also in solid tumors.

The first solid tumor CSCs were identified from breast cancer by isolating CD44<sup>+</sup>/CD24<sup>-/low</sup> cells from primary tumor cells [8]. The isolated CSCs can recapitulate the original breast cancer with same morphologic and immunophenotypic features CSCs could be isolated from these grafts and serially transplanted. For gliomas, several groups isolated brain tumor stem cells (BTSCs) from primary tumors based on the above criteria and the ability to form the neuro-spheres as normal neural stem cells do [9–16]. In our study, as few as 100 of these BTSCs could recapitulate the heterogeneity of GBM in immunocompromised rodents [15]. In addition to primary gliomas, we also isolated cancer stem-like cells from the commercial rat gliosarcoma cell line, 9L [17]. This cell line has been cultured in the lab over a long period under neurosphere conditions used for neural stem cell expansion. Similar results were reported by Kondo *et al.* for rat the GBM cell line C6 [18]. These data indicate that glioma cell lines may retain the capacity for a stem-like phenotype even after years of *in vitro* culture. CSCs have also been identified in variety of other malignant primary tumors as well as cancer cell lines by using different cell surface markers (summarized in table 1).

Among the CSCs associated markers, CD133 (prominin-1) is the one of the most important and studied. It is a 120kDa five transmembrane domain glycoprotein (5-TM) with two cytoplasmic loops, two glycosylated extracellular domains and a cytoplasmic C terminal domain [19–22]. Despite mounting evidence show, that CD133 is an important marker for both somatic stem cell and CSCs, its physiologic function is not known. Some studies suggested that CD133 is involved in neural-retinal development and phototransduction [23,24]. Due to its interaction with plasma membrane cholesterol and enrichment in cholesterol-based membrane microdomains, it may play some role in membrane topology [25]. A published study also demonstrated that *CD133+* progenitor cells could promote the healing of diabetic ischemic ulcer through stimulating angiogenesis and activating the Wnt pathway [26]. This observation may suggest a role for *CD133+* CSCs in tumor angiogenesis and in related signaling pathways.

## Glioma CSCs and clinical treatment

CSCs are often resistant to conventional chemotherapy and radiation therapy. Glioma CSCs are resistant to radiotherapy and chemotherapy. *CD133+* glioma CSCs could preferentially activate the DNA damage checkpoint response under irradiation. The activation is Chk1 and Chk2 checkpoint kinase dependent [27]. Maki and colleague also confirmed that *CD133+* glioma cells are more radiation resistant than *CD133-* cells [28]. This study also reported that

CD133 expression is up-regulated 1.6 fold under 2% O<sub>2</sub> hypoxic conditions. Similar results had been reported by other groups as well [29,30]. Because hypoxia conditions exist in most solid tumors including gliomas, this up-regulation of CD133 expression provides enhancement for specific targeting of glioma CSCs rather than NSCs.

*In vitro* study showed that *CD133+* glioblastoma CSCs are more resistant to multiple chemotherapeutic agents treatment than *CD133-* counterparts [31]. Our group demonstrated that *CD133+* glioma CSCs express higher levels of drug transporter gene BCRP combined with up-regulation of the DNA repair protein MGMT mRNA, as well as higher mRNA levels of other genes that inhibit apoptosis, including FLIP, Bcl-2, Bcl-X and some IAP family genes. These cells were significantly resistant to chemotherapeutic agents compared to autologous *CD133-* cells [32].

Glioma CSCs possess an additional property to escape from conventional therapies -- migration. Our group and others reported that over expression of chemokine receptors, such as CXCR4, is a common mechanism related to CSCs migration [32–34]. As reviewed by Lefranc and colleagues, glioma cell migration is a complex combination of multiple molecular processes, including the alteration of tumor cell adhesion to a modified extracellular matrix, the secretion of proteases by the cells, and modifications to the actin cytoskeleton. Intracellular signaling pathways involved in the acquisition of resistance to apoptosis by migrating glioma cells include PI3K, Akt, mTOR, NF-kappaB, and autophagy (programmed cell death type II) [35].

## Targeting signaling pathway in CSCs

Signaling pathways including Wnt, hedgehog, notch, Hox family member, Bmi-1, PTEN, telomerase, efflux transporters are involved in balancing self-renewal and differentiation of NSCs as well as CSCs [36–39]. Recent studies also show that Notch, Hedgehog and BMP pathways are involved in controlling *CD133+* CSCs functions in glioma [40–42]. Bao and colleagues recently showed that glioma CSCs generate vascular tumors through over expression of vascular endothelial growth factor (VEGF) [43]. Since VEGF is a validated therapeutic target for glioma therapy [44–46], this finding may indicate more favorable targeting of CSCs in glioma therapy.

Due to the common pathways and cell surface markers shared by NSCs and CSCs, it is important to develop CSCs specific therapies that avoid potential toxicities to NSCs. Selective targeting of AML CSCs performed by Jordan's group demonstrated the possibility of such selectivity. They showed that LSCs, but not normal hematopoietic stem cells (HSCs), were susceptible to the apoptotic effects of the proteasome inhibitor MG-132 combined with the anthracycline idarubicin through NF-κB activity [47]. NF-κB inhibitors could induce LSCs apoptosis but spare normal HSCs [48]. In a subsequent study, same group also showed that 4-benzyl, 2-methyl, 1, 2, 4-thiadiazolidine, 3, 5 Dione (TDZD-8) treatment could induce oxidative stress and selectively kill LSCs *in vitro* but not HSCs [49]. Other studies demonstrated that AML is phosphatase and tensin homologue (PTEN) pathway dependent. Rapamycin, a PI3K/PTEN signaling pathway inhibitor, could dramatically decrease leukemia burden [50]. In addition, more importantly, this treatment appeared to be specific for the LSCs since normal HSCs were unaffected.

When selective targeting of CSCs becomes possible, another strategy to target CSCs is forcing them to differentiate and become more sensitive to conventional chemo-radiotherapies. Differentiation therapy is based on this concept and a number of agents had been tested in recent years [51,52]. All-trans-retinoic acid (ATRA) is the most studied differentiation therapy molecule. Sell *et al.* reported that about 90% of newly diagnosed patients with acute promyelocytic leukemia (APL) achieve complete remission and over 70% are cured by ATRA

therapy [53]. Differentiation with ARTA had been also reported in early-stage mouse embryonic stem cells [54], rat C6 glioma cells [55], human embryonic NSCs [56]. These studies raised the possibility of using ARTA to induce differentiation of glioma CSCs as a therapy. Besides ATRA, other agents have also been tested for this approach of differentiation therapy. Piccirillo *et al.* Have shown that treating CSCs with differentiation factors can effectively deplete CSCs in human glioma [42]. In this study, researchers reported that bone morphogenic proteins (BMPs), especially BMP4, activate their receptors (BMPRs) and trigger the Smad signaling cascade in cells isolated from human glioblastomas. This activated signaling pathway lead to a reduction in proliferation and increased expression of differentiated neural markers in both *CD133+* CSCs and normal glioma cells. When xenotransplanted BMP4 pretreated glioma CSCs were transplanted into mice, there was no invasive glioma detected. These data provided evidence that differentiation therapy is a promising noncytotoxic strategy to deplete CSCs.

### Targeting CSCs using passive immunotherapy

Antibody therapy (passive immunotherapy) directed against CSCs have resulted in several experimental therapeutic success. Schatton *et al.* Identified melanoma CSCs with chemoresistance mediator ABCB5+ expression [57]. Treatment with anti-ABCB5 antibody for xenografted melanomas resulted in significant reduction of tumor size. Moreover, this direct targeting of CSC antigen induced tumor cell death through antibody-dependent cell-mediated cytotoxicity. Another encouraging result of antibody therapy reported by Dick's group [58]. In their study, CD44 had been identified as an AML CSC surface marker. Although the same marker is also expressed on normal bone marrow HSCs at a lower level, treatment with anti-CD44 antibody before transplant can selectively block engraftment of AML LSCs but not normal HSCs. Treatment of previously engrafted AML with the same antibody led to a significant reduction in disease burden by 83–100%. In vivo treated AML CSCs resulted in lower engraftment, suggesting that anti-CD44 antibody treatment directly altered CSC fate by either inducing differentiation or by inhibiting their repopulation ability. This study provide evidence that passive immunotherapy with antibodies targeting CSCs antigen could be effective even when the same antigen is shared with NSCs. Concurrent with the above study, Krause and colleagues also reported that CD44 is required on leukemic cells that initiate chronic myeloid leukemia (CML)[59]. Anti-CD44 antibody treatment attenuated induction of CML-like leukemia in recipients, suggesting that CD44 blockade may be beneficial in autologous transplantation in CML.

Passive immunotherapy targeting solid CSCs has also been reported. Smith et al. demonstrated antibody-drug conjugates (ADCs) could be used for both hepatocellular and gastric cancers. When an anti-CD133 antibody was conjugated to a potent cytotoxic drug, monomethyl auristatin F (MMAF), this conjugate could effectively inhibited the growth of Hep3B hepatocellular and KATO III gastric cancer cells *in vitro* by inducing apoptosis in *CD133+* CSCs. In vivo administration this ADC also resulted in significant delay of tumor growth in SCID mice.

In addition to directly targeting CSC surface antigens, antibody therapy has also been used as sensitizing agents combined with chemotherapy. Todaro *et al.* showed that treatment of *CD133* + colon CSCs with anti-IL-4 antibody before treatment with oxiplatin, 5-FU or TRAIL resulted in increased cell death [60]. In vivo direct injection of IL-4 neutralizing antibodies followed by oxiplatin could effectively reduced tumor burden.

### Targeting CSCs using active immunotherapy

Active immunotherapy is designed to generate vaccines that could stimulate the host's intrinsic immune response to the tumor. Early stage active immunotherapy vaccines for glioma

treatment utilized irradiated whole tumor cell inoculation, either engineered to secrete cytokines [61] or combined with cytokine secreting cells [62] or cytokine itself [63]. Although promising data has been obtained from those tumor-cell based vaccination strategies, the success of this approach was limited by the poor inherent antigen-presenting capacity of glioma cells themselves. The use of professional antigen-presenting cells, like dendritic cells (DCs), to initiate tumor-specific T-cell responses may be a more promising strategy for cancer vaccination. Emerging evidence showed that DC-mediated antigen presentation might be more effective than using irradiated tumor cells, as DCs abundantly express many of the co-stimulatory molecules that are essential for appropriate activation of naive T cells. Also, they have the ability to efficiently process and present antigenic peptides in combination with cell-surface MHC [64–69]. For glioma immunotherapy with DC vaccines, different tumor-associated antigens, including specific tumor-associated peptides, tumor RNA and cDNA, tumor cell lysate or apoptotic tumor cells all have been tested in various studies [reviewed in 70].

In our phase I study using DC vaccines in patients with newly diagnosed high-grade glioma [71], DC vaccine was generated with patients' PBMC derived DCs pulsed *ex vivo* with autologous tumor cell surface peptides isolated by means of acid elution. Following surgical resection and external beam radiotherapy, nine patients were given DC vaccination intradermally very other week over a six-week period. Four patients, who showed disease progression, underwent repeat surgery after receiving the third DC vaccination. By examining the harvested tumor tissue, two of the four patients samples demonstrated robust infiltration with CD8<sup>+</sup> and CD45RO<sup>+</sup> T cells, which was not apparent in the same patients' tumor specimens prior to the vaccination. More encouragingly, the median survival for the study group was 455 days, which was longer than the 257 days for the matched control population. Given the promising results without observed destructive autoimmune responses, this study was expanded into a phase II trial.

In another Phase I study by using DCs pulsed with tumor lysate as antigen [72], 14 patients with malignant glioma were given three vaccinations over a 6 week period and followed with immuno-monitor assay using an HLA-restricted tetramer staining protocol. Four patients showed that at least one or more tumor-associated antigen (TAA)-specific CTL was activated against specific glioma antigens, including melanoma antigen-encoding gene-1, gp-100 and human epidermal growth factor receptor-2 (HER-2). The median survival of the study group was significantly longer than the control group of recurrent glioblastoma patients by means of 133 weeks vs. 30 weeks.

In a study by Liau and colleagues, 12 glioma patients were treated with DC vaccination by using autologous DCs pulsed with acid-eluted autologous tumor peptides. [73] Results showed six patients generated peripheral tumor-specific CTL post-vaccination without major adverse events and autoimmune reactions. The patients who developed systemic antitumor cytotoxicity had longer survival times compared with negative response patients. And all the patients who had stable disease generated a positive CTL response, whereas those with active progressive disease did not show statistically significant CTL response.

With encouraging data generated from these DC vaccine clinical trails, current studies are attempting to further improve the efficacy of this strategy by not only inducing glioma specific CTL, but by also depleting inhibitory Treg cells [74,75]. Two European group studies show that depletion of Tregs before DC vaccination could boost anti-glioma immune response leading to tumor rejection and long-term immunity. Those studies suggested combination of Treg depletion and DC vaccination is more effective to generate anti-glioma immunity.

## Summary

With emerging evidence showing that glioma CSCs play an important role in tumor initiation, escape from conventional surgical, chemotherapies, targeting glioma CSCs with different therapeutic strategies provide new hope for better glioma therapies. Current immunotherapy targeting glioma studies achieved promising results. But with the complex and divergent mechanisms with which glioma evade immune surveillance, and the genetic instability of CSCs [76], a combination of therapies with two or more immunotherapy strategies may provide more benefit to eliminate gliomas. The advancement with understanding of stem cell biology, especially CSC biology, glioma CSC specific immunotherapy based on the new discovery combined with other therapeutic strategies may eventually provide new approaches to treat gliomas.

## References

1. Walid MS, Smisson HF 3rd, Robinson JS Jr. Long-term survival after glioblastoma multiforme. *South Med J* 2008;101(9):971–972. [PubMed: 18708965]
2. Krex D, et al. Long-term survival with glioblastoma multiforme. *Brain* 2007;130(Pt 10):2596–2606. [PubMed: 17785346]
3. Ohgaki H, et al. Genetic pathways to glioblastoma: a population-based study. *Cancer Res* 2004;64(19):6892–6899. [PubMed: 15466178]
4. Smith JS, Jenkins RB. Genetic alterations in adult diffuse glioma: occurrence, significance, and prognostic implications. *Front Biosci* 2000;5:D213–D231. [PubMed: 10702383]
5. Lapidot T, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994;367(6464):645–648. [PubMed: 7509044]
6. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997;3(7):730–737. [PubMed: 9212098]
7. Park CY, Tseng D, Weissman IL. Cancer stem cell-directed therapies: recent data from the laboratory and clinic. *Mol Ther* 2009;17(2):219–230. [PubMed: 19066601]
8. Al-Hajj M, et al. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A* 2003;100(7):3983–3988. [PubMed: 12629218]
9. Singh SK, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 2003;63(18):5821–5828. [PubMed: 14522905]
10. Singh SK, et al. Identification of human brain tumour initiating cells. *Nature* 2004;432(7015):396–401. [PubMed: 15549107]
11. Galli R, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res* 2004;64(19):7011–7021. [PubMed: 15466194]
12. Ignatova TN, et al. Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. *Glia* 2002;39(3):193–206. [PubMed: 12203386]
13. Hemmati HD, et al. Cancerous stem cells can arise from pediatric brain tumors. *Proc Natl Acad Sci U S A* 2003;100(25):15178–15183. [PubMed: 14645703]
14. Lee J, 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;9(5):391–403. [PubMed: 16697959]
15. Yuan X, et al. Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene* 2004;23(58):9392–9400. [PubMed: 15558011]
16. Uchida N, et al. Direct isolation of human central nervous system stem cells. *Proc Natl Acad Sci U S A* 2000;97(26):14720–14725. [PubMed: 11121071]
17. Ghods AJ, et al. Spheres isolated from 9L gliosarcoma rat cell line possess chemoresistant and aggressive cancer stem-like cells. *Stem Cells* 2007;25(7):1645–1653. [PubMed: 17412894]
18. Kondo T, Setoguchi T, Taga T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. *Proc Natl Acad Sci U S A* 2004;101(3):781–786. [PubMed: 14711994]

19. Corbeil D, et al. The human AC133 hematopoietic stem cell antigen is also expressed in epithelial cells and targeted to plasma membrane protrusions. *J Biol Chem* 2000;275(8):5512–5520. [PubMed: 10681530]
20. Miraglia S, et al. A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood* 1997;90(12):5013–5021. [PubMed: 9389721]
21. Yin AH, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 1997;90(12):5002–5012. [PubMed: 9389720]
22. Bidlingmaier S, Zhu X, Liu B. The utility and limitations of glycosylated human CD133 epitopes in defining cancer stem cells. *J Mol Med* 2008;86(9):1025–1032. [PubMed: 18535813]
23. Maw MA, et al. A frameshift mutation in prominin (mouse)-like 1 causes human retinal degeneration. *Hum Mol Genet* 2000;9(1):27–34. [PubMed: 10587575]
24. Zacchigna S, et al. Loss of the cholesterol-binding protein prominin-1/CD133 causes disk dysmorphogenesis and photoreceptor degeneration. *J Neurosci* 2009;29(7):2297–2308. [PubMed: 19228982]
25. Shmelkov SV, et al. AC133/CD133/Prominin-1. *Int J Biochem Cell Biol* 2005;37(4):715–719. [PubMed: 15694831]
26. Barcelos LS, et al. Human CD133+ Progenitor Cells Promote the Healing of Diabetic Ischemic Ulcers by Paracrine Stimulation of Angiogenesis and Activation of Wnt Signaling. *Circ Res*. 2009
27. Bao S, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 2006;444(7120):756–760. [PubMed: 17051156]
28. Blazek ER, Foutch JL, Maki G. Daoy medulloblastoma cells that express CD133 are radioresistant relative to CD133- cells, and the CD133+ sector is enlarged by hypoxia. *Int J Radiat Oncol Biol Phys* 2007;67(1):1–5. [PubMed: 17084552]
29. Potgens AJ, et al. Monoclonal antibody CD133-2 (AC141) against hematopoietic stem cell antigen CD133 shows crossreactivity with cytokeratin 18. *J Histochem Cytochem* 2002;50(8):1131–1134. [PubMed: 12133915]
30. Griguer CE, et al. CD133 is a marker of bioenergetic stress in human glioma. *PLoS ONE* 2008;3(11):e3655. [PubMed: 18985161]
31. Eramo A, et al. Chemotherapy resistance of glioblastoma stem cells. *Cell Death Differ* 2006;13(7):1238–1241. [PubMed: 16456578]
32. Liu G, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer* 2006;5:67. [PubMed: 17140455]
33. Salmaggi A, et al. Glioblastoma-derived tumorspheres identify a population of tumor stem-like cells with angiogenic potential and enhanced multidrug resistance phenotype. *Glia* 2006;54(8):850–860. [PubMed: 16981197]
34. Dirks PB. Glioma migration: clues from the biology of neural progenitor cells and embryonic CNS cell migration. *J Neurooncol* 2001;53(2):203–212. [PubMed: 11716071]
35. Lefranc F, Brotchi J, Kiss R. Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis. *J Clin Oncol* 2005;23(10):2411–2422. [PubMed: 15800333]
36. Reya T, et al. Stem cells, cancer, and cancer stem cells. *Nature* 2001;414(6859):105–111. [PubMed: 11689955]
37. Lobo NA, et al. The biology of cancer stem cells. *Annu Rev Cell Dev Biol* 2007;23:675–699. [PubMed: 17645413]
38. Huntly BJ, Gilliland DG. Leukaemia stem cells and the evolution of cancer-stem-cell research. *Nat Rev Cancer* 2005;5(4):311–321. [PubMed: 15803157]
39. Krause DS, Van Etten RA. Right on target: eradicating leukemic stem cells. *Trends Mol Med* 2007;13(11):470–481. [PubMed: 17981087]
40. Fan X, et al. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. *Cancer Res* 2006;66(15):7445–7452. [PubMed: 16885340]
41. Clement V, et al. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr Biol* 2007;17(2):165–172. [PubMed: 17196391]

42. Piccirillo SG, et al. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. *Nature* 2006;444(7120):761–765. [PubMed: 17151667]
43. Bao S, et al. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Res* 2006;66(16):7843–7848. [PubMed: 16912155]
44. Vredenburgh JJ, et al. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. *J Clin Oncol* 2007;25(30):4722–4729. [PubMed: 17947719]
45. Vredenburgh JJ, et al. Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. *Clin Cancer Res* 2007;13(4):1253–1259. [PubMed: 17317837]
46. Batchelor TT, et al. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. *Cancer Cell* 2007;11(1):83–95. [PubMed: 17222792]
47. Guzman ML, et al. Preferential induction of apoptosis for primary human leukemic stem cells. *Proc Natl Acad Sci U S A* 2002;99(25):16220–16225. [PubMed: 12451177]
48. Guzman ML, et al. An orally bioavailable parthenolide analog selectively eradicates acute myelogenous leukemia stem and progenitor cells. *Blood* 2007;110(13):4427–4435. [PubMed: 17804695]
49. Guzman ML, et al. Rapid and selective death of leukemia stem and progenitor cells induced by the compound 4-benzyl, 2-methyl, 1,2,4-thiadiazolidine, 3,5 dione (TDZD-8). *Blood* 2007;110(13):4436–4444. [PubMed: 17785584]
50. Yilmaz OH, et al. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature* 2006;441(7092):475–482. [PubMed: 16598206]
51. Sell S. Cancer stem cells and differentiation therapy. *Tumour Biol* 2006;27(2):59–70. [PubMed: 16557043]
52. Sell S. Leukemia: stem cells, maturation arrest, and differentiation therapy. *Stem Cell Rev* 2005;1(3):197–205. [PubMed: 17142856]
53. Sell S. Stem cell origin of cancer and differentiation therapy. *Crit Rev Oncol Hematol* 2004;51(1):1–28. [PubMed: 15207251]
54. Guo X, et al. Proteomic characterization of early-stage differentiation of mouse embryonic stem cells into neural cells induced by all-trans retinoic acid in vitro. *Electrophoresis* 2001;22(14):3067–3075. [PubMed: 11565801]
55. Bianchi MG, et al. C6 glioma cells differentiated by retinoic acid overexpress the glutamate transporter excitatory amino acid carrier 1 (EAAC1). *Neuroscience* 2008;151(4):1042–1052. [PubMed: 18207650]
56. Wang F, et al. [Expression of Notch1 gene in the differentiation of the human embryonic neural stem cells to neurons]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2004;20(6):769–772. [PubMed: 15555458]
57. Schatton T, et al. Identification of cells initiating human melanomas. *Nature* 2008;451(7176):345–349. [PubMed: 18202660]
58. Jin L, et al. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nat Med* 2006;12(10):1167–1174. [PubMed: 16998484]
59. Krause DS, et al. Requirement for CD44 in homing and engraftment of BCR-ABL-expressing leukemic stem cells. *Nat Med* 2006;12(10):1175–1180. [PubMed: 16998483]
60. Todaro M, et al. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. *Cell Stem Cell* 2007;1(4):389–402. [PubMed: 18371377]
61. Herrlinger U, et al. Vaccination for experimental gliomas using GM-CSF-transduced glioma cells. *Cancer Gene Ther* 1997;4(6):345–352. [PubMed: 9408604]
62. Sobol RE, et al. Interleukin-2 gene therapy in a patient with glioblastoma. *Gene Ther* 1995;2(2):164–167. [PubMed: 7719933]
63. Plautz GE, et al. T cell adoptive immunotherapy of newly diagnosed gliomas. *Clin Cancer Res* 2000;6(6):2209–2218. [PubMed: 10873070]
64. Ashley DM, et al. Bone marrow-generated dendritic cells pulsed with tumor extracts or tumor RNA induce antitumor immunity against central nervous system tumors. *J Exp Med* 1997;186(7):1177–1182. [PubMed: 9314567]



65. Heimberger AB, et al. Bone marrow-derived dendritic cells pulsed with tumor homogenate induce immunity against syngeneic intracerebral glioma. *J Neuroimmunol* 2000;103(1):16–25. [PubMed: 10674985]
66. Liao LM, et al. Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. *J Neurosurg* 1999;90(6):1115–1124. [PubMed: 10350260]
67. Ni HT, et al. Immunization with dendritic cells pulsed with tumor extract increases survival of mice bearing intracranial gliomas. *J Neurooncol* 2001;51(1):1–9. [PubMed: 11349874]
68. Okada H, et al. Bone marrow-derived dendritic cells pulsed with a tumor-specific peptide elicit effective anti-tumor immunity against intracranial neoplasms. *Int J Cancer* 1998;78(2):196–201. [PubMed: 9754652]
69. Yamanaka R, et al. Enhancement of antitumor immune response in glioma models in mice by genetically modified dendritic cells pulsed with Semliki forest virus-mediated complementary DNA. *J Neurosurg* 2001;94(3):474–481. [PubMed: 11235953]
70. Soling A, Rainov NG. Dendritic cell therapy of primary brain tumors. *Mol Med* 2001;7(10):659–667. [PubMed: 11713365]
71. Yu JS, et al. Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res* 2001;61(3):842–847. [PubMed: 11221866]
72. Yu JS, et al. Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. *Cancer Res* 2004;64(14):4973–4979. [PubMed: 15256471]
73. Liao LM, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. *Clin Cancer Res* 2005;11(15):5515–5525. [PubMed: 16061868]
74. Maes W, et al. DC vaccination with anti-CD25 treatment leads to long-term immunity against experimental glioma. *Neuro Oncol*. 2009
75. Grauer OM, et al. Elimination of regulatory T cells is essential for an effective vaccination with tumor lysate-pulsed dendritic cells in a murine glioma model. *Int J Cancer* 2008;122(8):1794–1802. [PubMed: 18076066]
76. Lagasse E. Cancer stem cells with genetic instability: the best vehicle with the best engine for cancer. *Gene Ther* 2008;15(2):136–142. [PubMed: 17989699]
77. Ishikawa F, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nat Biotechnol* 2007;25(11):1315–1321. [PubMed: 17952057]
78. Taylor MD, et al. Radial glia cells are candidate stem cells of ependymoma. *Cancer Cell* 2005;8(4):323–335. [PubMed: 16226707]
79. Zeppernick F, et al. Stem cell marker CD133 affects clinical outcome in glioma patients. *Clin Cancer Res* 2008;14(1):123–129. [PubMed: 18172261]
80. Bexell D, et al. CD133+ and nestin+ tumor-initiating cells dominate in N29 and N32 experimental gliomas. *Int J Cancer*. 2009
81. Ricci-Vitiani L, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007;445(7123):111–115. [PubMed: 17122771]
82. O'Brien CA, et al. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007;445(7123):106–110. [PubMed: 17122772]
83. Dallas NA, et al. Chemoresistant colorectal cancer cells, the cancer stem cell phenotype, and increased sensitivity to insulin-like growth factor-I receptor inhibition. *Cancer Res* 2009;69(5):1951–1957. [PubMed: 19244128]
84. Ieta K, et al. Biological and genetic characteristics of tumor-initiating cells in colon cancer. *Ann Surg Oncol* 2008;15(2):638–648. [PubMed: 17932721]
85. Wei XD, et al. In vivo investigation of CD133 as a putative marker of cancer stem cells in Hep-2 cell line. *Head Neck* 2009;31(1):94–101. [PubMed: 18853445]
86. Cox CV, et al. Characterization of acute lymphoblastic leukemia progenitor cells. *Blood* 2004;104(9):2919–2925. [PubMed: 15242869]
87. Yang ZF, et al. Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell* 2008;13(2):153–166. [PubMed: 18242515]

88. Suetsugu A, et al. Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells. *Biochem Biophys Res Commun* 2006;351(4):820–824. [PubMed: 17097610]
89. Ma S, et al. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. *Oncogene* 2008;27(12):1749–1758. [PubMed: 17891174]
90. Ma S, et al. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology* 2007;132(7):2542–2556. [PubMed: 17570225]
91. Yin S, et al. CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity. *Int J Cancer* 2007;120(7):1444–1450. [PubMed: 17205516]
92. Jiang F, et al. Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. *Mol Cancer Res* 2009;7(3):330–338. [PubMed: 19276181]
93. Eramo A, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ* 2008;15(3):504–514. [PubMed: 18049477]
94. Monzani E, et al. Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. *Eur J Cancer* 2007;43(5):935–946. [PubMed: 17320377]
95. Ferrandina G, et al. Expression of CD133-1 and CD133-2 in ovarian cancer. *Int J Gynecol Cancer* 2008;18(3):506–514. [PubMed: 17868344]
96. Hermann PC, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007;1(3):313–323. [PubMed: 18371365]
97. Li C, et al. Identification of pancreatic cancer stem cells. *Cancer Res* 2007;67(3):1030–1037. [PubMed: 17283135]
98. Olempska M, et al. Detection of tumor stem cell markers in pancreatic carcinoma cell lines. *Hepatobiliary Pancreat Dis Int* 2007;6(1):92–97. [PubMed: 17287174]
99. Collins AT, et al. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005;65(23):10946–10951. [PubMed: 16322242]

**Table 1**

identified cancer stem cells from different primary tumors and tumor cell lines

Tumor	type	isolation markers	Reference
AML	primary tumors	CD34 <sup>+</sup> CD38 <sup>-</sup>	[5,6,77]
Breast	primary tumors	CD44 <sup>+</sup> CD24 <sup>-/Low</sup>	[8]
Brain	primary tumors	CD133+	[9,10,13,27,32,78,79]
	Cell lines	CD133+ sphere formation	[17,28,80]
	Cell lines	side population (SP)	[18]
Colon	primary tumors	CD133+	[60,81,82]
	Primary tumors	CD133+cd44+	[83]
	Cell lines	CD133+	[84]
Laryngeal	cell lines	CD133+	[85]
Leukemia	primary tumors	CD34+CD10-	[86]
Liver	primary tumors/ cell line/blood	CD90+CD44+	[87]
	Cell lines	CD133+	[88-91]
Lung	primary tumors	ALDH1	[92]
	primary tumors	CD133+	[93]
Melanoma	primary tumors	ABC5+	[57]
	primary tumors	CD133+ABC2+	[94]
Ovarian	primary tumors	CD133+	[95]
Pancreas	primary tumors	CD133+	[96,97]
	Cell lines	CD133+	[98]
Prostate	primary tumors	CD133+	[99]