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Understanding glioma stem cells: rationale, clinical relevance and therapeutic strategies

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Abstract

Glioblastoma multiforme is one of the most aggressive brain tumors in adults. Despite the use of the best available multimodal therapeutic approaches, the prognosis remains dismal. The identification of glioma stem cells (GSCs) has offered new hope to affected patients, since it could explain, in part, the highly heterogeneous nature of this tumor and its chemo- and radio-resistance. Although still in its infancy, GSC research has unveiled many of its complexities and the theory itself remains controversial. GSC phenotype can significantly vary between patients and a single tumor may present several distinct GSCs. New therapeutic solutions that effectively target this population are of utmost importance, since they may be able to decrease neoplastic recurrence and improve patient survival. Here, we discuss the mechanisms by which GSCs lead to glioma relapse, the main controversies in this field and the most recent treatments that could successfully target this population.

Keywords

glioblastoma multiforme; glioma stem cells; intratumoral heterogeneity; microenvironment; markers; targeted therapies; therapeutic resistance

Glioblastoma multiforme is one of the most common and aggressive malignant brain tumors in adults. Despite aggressive multimodal therapeutic intervention, patient survival is still restricted to approximately 14 months post-diagnosis [1]. This poor prognosis is mainly due to high rates of tumor recurrence, which is associated with both the extremely infiltrative nature of glioblastoma and its resistance to conventional treatments, such as chemo- and radio-therapy [2]. As an effort to improve patient outcomes, current research has been focused on identifying molecular and cellular elements that have been linked to glioma recurrence [3].

Recent findings have suggested the existence of a small population of therapy-resistant and slow-dividing malignant cells inside the main tumor bulk. These cells have been held

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responsible for glioma formation, maintenance, invasiveness and recurrence [4,5]. Termed tumor-initiating cells or glioma stem cells (GSCs), these neoplastic units possess many common characteristics with normal neural stem cells (NSCs), such as the capacity to self-renew, indefinitely proliferate and differentiate into different cell types that originate from the same lineage (multipotency). They also share common NSC markers, such as CD133 and nestin, and are able to maintain and replenish the neoplastic clone characteristic that is considered liable for tumor relapse [6–8].

Although their nature has not yet been completely unveiled, these GSCs are known to be very different from the rapidly-dividing cells that constitute the rest of the main tumor bulk [9]. One of the major obstacles of the presently available conventional therapies is the inability to efficiently target and eradicate these cells without major toxicity to non-neoplastic tissues. Therefore, existing efforts rely on the study of additional therapeutic approaches that can be used in conjunction with current standard of care in order to selectively target and eliminate this important population. In this review, the main theories that try to explain the origin of cancer stem cells (CSCs) and their mechanisms of self-maintenance and therapeutic resistance will be discussed. A quick overview of the main therapeutic approaches currently under investigation that attempt to address these issues will also be given.

Normal stem/progenitor cells & CSCs

In the developing embryo, stem cells are located in the inner mass of the blastocyst. There, they are known as embryonic stem cells and give rise to the majority of cell types present in the human body, a characteristic referred to as pluripotency. At later developmental stages, embryonic stem cells differentiate into adult stem cells, which are multipotent (i.e., they can give rise to a restricted number of cell types) and are able to form selected tissues and organs [10]. CSCs reportedly share many properties with normal stem cells. It is still not clear whether CSCs derive from mutated tissue-specific stem cells or more differentiated cells that have reinitiated a self-renewal program as part of or following transformation. Regardless of their origin, CSCs have been found to resemble normal stem or progenitor cells that are present in their tumor's derivative tissue [11]. Microscopic analysis of different malignancies reveals a complex heterogeneous picture composed of significant phenotypic diversity. Even though CSCs only make up a minor fraction of a tumor, they are defined by their ability to self-renew through asymmetric cell divisions, producing new tumorigenic CSCs, and their capability to give rise to differentiated and rapidly-dividing cancer cells. Collectively, CSC-descendants contribute to the cellular heterogeneity of the glioblastoma tumor.

GSCs: the operational definition

The designation of 'CSCs' was devised to echo two fundamental properties of normal stem cells: self-renewal capacity and multipotency. There is growing acknowledgement that, although self-renewing, CSCs cannot be contemplated as multipotent because the differentiated progeny derived from transformed precursors is bound to be genetically abnormal. Thus, to be defined as CSCs, glioma cells must be able to self-renew in culture,

propagate phenotypically similar tumors upon *in vivo* secondary transplantation and give rise to neurons and glia-like differentiated progenies both *in vivo* and *in vitro*. Experimentally, glioma-derived CSCs can be defined by their ability to form neurosphere-like structures (known as tumorspheres) in the presence of appropriate growth factors in tissue culture and by their ability to generate tumors when injected, even in low numbers, in immunocompromised mice.

Cell of origin & CSC theory

Even though the CSC theory has gained considerable support in both experimental and clinical fronts over the last decade, the main principles of this hypothesis remain controversial. At the center of this debate lies the question of whether the cell of origin for cancer is a stem cell or any differentiated cell that undergoes specific genetic aberrations. During organ development, a hierarchical organization dictates the process where stem cells first become committed progenitors, which in turn yields differentiated cells that comprise the bulk of the organ. In theory, normal stem cells could be an ideal target for malignant transformation for two main reasons. First, they represent the most primitive cells in a given organ. Second, they generally re-enter cell division to replace the pool of both differentiated progenies and stem cells, thus being responsible for maintaining the stem cell pool in the target organ. Thus, in theory, long-lived stem/progenitor cells could accumulate sequential genetic or epigenetic mutations and initiate oncogenesis.

The existence of a cell of origin for malignancies of the CNS has been investigated through several transgenic animal models. The majority of these models support the notion that neural stem and progenitor cells (NSPCs) in the brain are the primary cellular targets for gliomagenesis [12]. These animal models predominantely utilize NSPC-related cell promoters, such as nestin and glial fibrillary acidic protein, to drive oncogene expression (i.e., activated *Ras*) or to inactivate tumor suppressors (i.e., *PTEN* or *P53*) in specific cellular compartments. This process has proven to be effective in initiating cellular transformation as well as driving oncogenesis. It is important to note that even though the nestin promoter is considered to be selective for the NSPC population, glial fibrillary acidic protein promoter can be active in both NSPCs and mature astrocytes [13]. Such a disparity creates additional complexity that hampers a clear understanding of the results associated with this model. Nevertheless, these studies indicated that NSPCs expressing nestin are more vulnerable for malignant transformation as compared with more differentiated cell types [13–17]. On the contrary, recent reports indicate that differentiated cells have the ability to reinitiate a self-renewal program as part of or following the transformation process. In breast cancer, evidence points toward the notion that CSCs can ascend from transformed mammary epithelial cells, resulting in the acquisition of mesenchymal traits and stem cell markers [18].

The epithelial–mesenchymal transition is a key cellular program during the developmental phase and is often associated with malignant transformation. It has shown to induce the expression of stem cell markers in immortalized human mammary epithelial cells and to increase their ability to form tumorspheres [18]. Spontaneous dedifferentiation, a process where differentiated cells are able to convert into cancer stem-like cells, has also been

observed in a subpopulation of basal-like human mammary epithelial cells both *in vitro* and *in vivo*. Moreover, oncogenic transformation has shown to efficiently enhance such conversion [19]. In gliomas, differentiated cells in the CNS, such as cortical neurons and astrocytes, have also demonstrated the ability to initiate tumorigenesis upon oncogenic transformation. A recent report demonstrated that lentivirus vector-mediated expression of the constitutively active oncogene *H-RasV12* together with simultaneous inactivation of the tumor suppressor p53 by shRNA in NSCs, astrocytes or even mature neurons was able to induce gliomagenesis in murine models [20]. Taken together, these studies clearly demonstrate that any cell in a given organ can serve as a cell of origin for human malignancies. They also reinforce the importance of the interconversion between differentiated cancer cells and CSCs for tumor initiation and maintenance [21]. Therefore, we believe that the CSC theory should consider this possibility as well. In fact, if such a phenotypic plasticity does exist in any given human malignancy, it would explain the observed high frequency of CSCs in some tumors [22]. On the other hand, such cellular plasticity could create a significant challenge in the efforts to develop therapeutic strategies to eliminate CSCs. In order to develop effective anticancer therapies, we must elucidate the underlying molecular mechanisms of such plastic behavior as well as investigate how conventional chemo- and radio-therapies can influence this process. Additionally, we need to find a way to prevent the conversion of differentiated cancer cells into therapy resistant cancer stem-like cells. Only then will we be able to effectively prevent the malignant relapse.

Molecular signature of GSCs

Molecular markers that are used to identify CSC populations mostly rely on the understanding of normal stem cell biology [23]. Advancements in cell sorting technology via fluorescent antibodies and flow cytometry have enabled researchers to reproducibly isolate phenotypically defined rare stem cell populations. Utilizing these tools, John Dick's laboratory isolated the first described CSCs in 1997 from acute myeloid leukemia (AML) patients [23]. In this pioneering work, Bonnet and Dick showed that in human AML, a rare subset of tumor cells with $CD34^{+}/CD38^{-}$ signature possessed the ability to recapitulate the entire original disease over several transplantations [23]. These findings suggested that self-renewal and pluripotency were a characteristic of this small subpopulation, and that this feature was absent within the broader $CD34^{+}/CD38^{+}$ population [23,24]. Such a discovery opened a discussion on the existence of CSCs in other malignancies and on how to optimize their identification. The first CSC associated with a solid tumor was isolated from invasive breast cancer samples in 2003 [25]. Since then, CSCs have been identified in brain [26,27], colon [28], skin [29], pancreatic [30], prostate [31], ovarian [32], lung [33] and gastric cancers [34]. The isolation of many of these CSCs has been carried out using a number of adhesion markers including CD44 and CD24, or other CSC-associated functional markers such as multidrug efflux ABC transporters and prominin1 (CD133). Cell surface markers that have been used in the literature to enrich GSC populations will be discussed furher.

CD133

The initial isolation of GSCs was carried out by using the marker CD133 (AC133; human prominin1), which represents an apical plasma membrane protein predominantly found on normal embryonic stem cells [26,27]. $CD133^+$ cells isolated from glioma patients were capable of forming 'tumorspheres' when cultured in appropriate conditions. In addition, they could generate tumors when as few as 100 $CD133^+$ cells were injected in immunocompromised mice [26,27]. As a result, they were able to fulfill the criteria required to be classified as CSCs. However, not all glioma cells that fit this working definition express the CD133 marker. Approximately 40% of all freshly isolated human-derived glioblastoma specimens did not contain $CD133^+$ tumor cells. Recent reports have also demonstrated that $CD133^-$ cells were able to generate tumors in immunocompromised animal models [35,36]. These findings raised the possibility that CD133 alone might not be a reliable universal marker for GSCs [37]. Moreover, CD133 expression within glioma cells has been shown to depend on both cell cycle and specific stimuli arising from the peritumoral microenvironment [38]. A detailed characterization of CD133 function on GSCs remains necessary. It could help to elucidate its involvement in glioma initiation, maintenance and invasiveness.

Stage-specific embryonic antigen-1

By using a patched (PTC) transgenic mouse model of medulloblastoma, Read and colleagues first identified the stage-specific embryonic antigen-1 (SSEA-1)/CD15 as a marker for CSCs in both murine and human-derived tumors [39]. In glioma, SSEA-1/$CD15^+$ tumor cells demonstrated self-renewal capacity as well as multi-lineage differentiation potential. In patient specimens, $CD133^+$ GSCs also expressed SSEA-1, suggesting that it could also serve as a GSC enrichment marker [37]. In our laboratory, glioma cells sorted by SSEA-1/CD15 marker were able to generate tumors in immunocompromised rodents. However, tumorspheres generated from SSEA-1/$CD15^+$ glioma cells were significantly smaller than the tumorspheres derived from the $CD133^+$ enriched ones. In addition, tumor xenografts derived from SSEA-1/$CD15^+$ enriched glioma cells were positive for Ki-67, indicating that SSEA-1 enriched tumors were more proliferative. Although it supports a deeper characterization of GSCs, this finding contradicts a previously established concept that states that CSCs are predominantly quiescent. It also sustains the idea that multiple markers should be used in order to achieve a reliable identification of glioma-derived CSCs.

Integrin-α6

Integrin-α6 is a member of the integrin family of extracellular matrix receptors for laminin and platelets. Recent reports indicated the selective coexpression of integrin-α6 in glioma cells previously sorted with other conventional CSC markers. Moreover, short hairpin RNA-mediated knockdown of integrin-α6 and functional blocking antibody against integrin-α6 in the GSC population abrogated tumorsphere formation and inhibited both *in vitro* and *in vivo* tumor growth [40]. These observations strongly indicate the role of integrin-α6 in GSCs self-renewal and maintenance. However, such exciting results warrant further investigation.

A2B5

A2B5 is a neural cell surface antigen predominately expressed in glial and neural progenitor cells [41]. In immunocompromised xenograft models, both $A2B5^{+}/CD133^{-}$ and $A2B5^{+}/CD133^{+}$ glioma populations are capable of generating tumors [36]. This result implies the possibility that A2B5 can be used as a GSC enrichment marker.

While the discovery of these functional markers has allowed an appropriate identification and characterization of CSCs, these processes still present some important drawbacks. The first one is that although CD133 has continued to identify tumor cells with self-renewal capacity in a number of other solid tumors, there is an ongoing debate on how robust and specific the universal marker should be [42]. A second important limitation is that most of the cell surface markers used to distinguish stem cells in normal and cancerous tissues are not exclusively expressed in one specific population. Additionally, the same markers used for the isolation of CSCs in one organ cannot directly be used for identification in other organs. This situation underlies the importance of combining phenotypic and functional markers as a signature to identify tissue-specific CSCs. A third shortcoming is that there is little agreement regarding the molecular identity of CSCs in solid tumors and the simple expression of two signature markers is not considered enough to identify most CSCs. Therefore, the combination of multiple markers have been recently employed to allow a better characterization of these populations [43].

CSCs & patient prognosis

According to the CSC hypothesis, these cells are not only responsible for unlimited glioblastoma growth, but also for the maintenance of a minimal residual disease, which commonly leads to post-therapeutic recurrence. Therefore, quantification of the presence of this rare population within such a malignant disease may serve as a prognostic indicator. Generally, it is believed that a high proportion of CSCs within a given tumor would imply a worse patient prognosis. For example, in breast cancer, the most poorly differentiated and invasive tumors present the highest burden of CSCs [44]. Similarly, elevated immunoreactivity of nestin and CD133 in tumor specimens has been associated with a poor prognosis in malignant glioma patients [45]. Other analogous reports have also described the high expression of the CD133 marker as a dismal prognostic factor for progression-free and overall survival in glioma populations [46]. Furthermore, Zeppernick *et al*. have indicated that CD133 could be a reliable marker of tumor regrowth, malignant progression and decreased survival in malignant glioma patients [47]. They observed significant differences in survival estimates between grade III glioma patients containing less than 1% of $CD133^{+}$ cells and those with more than 1% of positivity. Patients bearing tumors with more than 1% of $CD133^{+}$ cells presented with rapid tumor relapse and shorter progression-free and overall survival. Additional findings revealed that the proportion of $CD133^{+}$ cells in glioma samples directly correlated with increased tumor grades. As a consequence, more aggressive and invasive tumors present with significantly higher amounts of $CD133^{+}$ GSCs. A recent report strongly corroborates this idea. Sato *et al*. suggested that CD133 could be a useful molecular marker for the prediction of glioblastoma invasiveness and dissemination [48]. This report

also gives rise to the intriguing idea that $CD133^+$ GSCs may be implicated in the initiation of disseminated lesions.

Contrary to these findings, Kim and colleagues recently examined the three established stem cell markers, nestin, CD133 and CD15 in 88 cases of glioblastoma by immunohistochemical analysis. They reported that there was no correlation between the expression of these stem cell markers and clinical outcomes in glioma patients [49]. However, the overexpression of the CD133 marker still correlated with worse outcomes in other malignant diseases. For instance, elevated expression of CD133 in colon cancer is considered an independent marker of poor prognosis and is associated with liver metastasis [50]. Moreover, high expression of CSC functional markers, such as aldehyde dehydrogenase, has been found to be directly correlated with a poor prognosis in a number of tumors including AML [51], invasive breast cancer [52], prostate cancer [53], and head and neck squamous cell carcinoma [54]. In summary, the above results imply that patients with tumors that express elevated levels of molecular markers related to CSCs tend to present with a worse prognosis than patients with tumors that express low levels of these markers. Nevertheless, considering the inconsistency between individual stem cell markers, there is still a need to define a more precise CSC signature before it can be considered as a predictor of clinical outcome.

CSC biology & mechanisms of therapeutic resistance

Like normal stem cells, CSCs are considered to be relatively quiescent. This characteristic leads to their remarkable resistance towards conventional radio- and chemo-therapy, which predominantly targets rapidly dividing cells [55]. During therapy, the tumor burden may significantly decrease, which results in a false impression of complete neoplastic destruction. However, quiescent CSCs are able to survive the currently available anticancer treatments and eventually give rise to additional tumor cells, which invariably leads to malignant recurrence. In glioblastoma history, there are two described mechanisms that are held responsible for therapeutic resistance. The first relies on the overexpression of DNA damage repair pathways, such as O-6 methylguanine-DNA-methyltransferase (MGMT). The second focuses on the upregulation of transmembrane proteins that are accountable for pumping therapeutic agents outside targeted tumor cells.

DNA damage repair pathways are considered to be the guardians of genomic and chromosomal stability. Because stem cells are at the basis of tissue homeostasis, they appear to have a very efficient DNA repair capacity [56]. Moreover, it has been demonstrated that CNS cells contain lower levels of reactive oxygen species [57] as compared with their mature counterparts due to increased antioxidant defenses [58]. In glioblastoma, increased DNA repair capacity through enhanced *MGMT* expression in $CD133^+$ GSCs appears to be highly correlated with anticancer therapeutic resistance [4,59]. The expression of *MGMT* in GSCs is directly correlated with a decreased efficacy of temozolomide, a widely used chemotherapeutic agent that is currently considered the standard of care for antiglioma therapy. As an alkylating agent, temozolomide methylates the O-6 position of guanine residues in the DNA of cancer cells. This methylation causes severe DNA damage and leads to the destruction of chemosensitive neoplastic tissues. By presenting an enhanced MGMT activity, GSCs are able to resist such a mechanism [60]. In fact, GSCs that present MGMT

activity have been reported to be tenfold more resilient to temozolomide chemotherapy [61]. Contrarily, epigenetic silencing/methylation of the *MGMT* promoter was related to more sensitive cancer cells and a consequently improved chemotherapeutic efficacy [62]. In a recent report, Sato *et al*. demonstrated that the MEK–ERK–MDM2–p53 pathway plays a critical role in the regulation of *MGMT* expression [63]. According to the authors, MEK inhibition rendered chemoresistant GSCs sensitive to temozolomide therapy. In addition, the combination of a MEK inhibitor and temozolomide effectively deprived GSCs of their tumorigenic potential. These results support clinical findings that correlate decreased MGMT methylation status in glioma samples with lower patient survival [62]. However, this theory does not yet explain the mechanism by which some glioblastoma tumors, maintained by GSCs that do not possess enhanced MGMT expression, are still resistant to temozolomide-based chemotherapies. This finding suggests the existence of MGMT-independent mechanisms of GSC chemoresistance.

An increased expression of drug transporters, such as ABC transporters, has been reported in many different types of normal stem cells [64] and also in CSCs [65]. Some studies suggest that the expression of these pumps on the surface of malignant cells identifies a class of CSCs with high drug efflux capacity and an inherently increased resistance to chemotherapeutic agents [66]. Nevertheless, it is still unknown if these transmembrane proteins are capable of transporting antiglioma drugs such as temozolomide [67,68] and if glioblastoma resistance to conventional temozolomide-mediated chemotherapy actually depends on such a mechanism. Other contributions to CSC resistance to currently available anticancer treatments, such as radiotherapy, may arise from the fact that these tumor-initiating cells possibly reside in hypoxic niches [69]. Thus, it seems that CSCs may resist standard anticancer therapies via a combination of molecular mechanisms associated with normal stem cell biology. It is widely believed that in order to prevent relapse, effective targeting of CSCs is likely to be essential.

Therapeutic strategies targeting CSCs

Most currently available cancer therapies are designed to target cells that are highly mitotic and rapidly dividing. However, nearly all malignancies are heterogeneous in nature. Malignant tumors, such as glioblastoma multiforme, have been shown to contain many different subpopulations, including CSCs. These cells, which appear to possess tumor-initiating properties, are able to escape conventional chemotherapy and/or radiotherapy owing to previously discussed evasion mechanisms. After therapy, tumors have a higher population of resistant CSCs, which are capable of replenishing the previously depleted tumor population. Therefore, specific targeting of these tumor-initiating cells is of utmost importance in order to achieve long-lasting therapeutic effects. Here, we highlight the latest strategies to successfully target and eliminate CSCs, the roots of cancer.

Pharmacological targeting

Small molecule inhibitors have shown promising results in the preclinical setting when used alone or in combination with other agents to eradicate slow growing chemo- and radio-resistant CSCs. Several strategies have been investigated including targeting signaling pathways that impart therapeutic resistance to CSCs, thereby enhancing their susceptibility

to conventional treatments. Examples include inhibiting protective vascular niches that shield CSCs from therapeutic insults [70]. In malignant glioma models, Bao *et al*. demonstrated that following conventional radiation therapy, the fraction of $CD133^+$ GSCs is enriched. This increased GSC population essentially contributes to glioma radioresistance through preferential activation of DNA damage checkpoints, which leads to an increase in DNA repair capacity and ultimately provides tumor recurrence. Pharmacological inhibitors that can selectively block the Chk1 and Chk2-dependent DNA damage checkpoints in radioresistant $CD133^+$ GSCs are able to sensitize them to radiotherapy [4]. Similarly, several pharmacological agents have demonstrated promising results in reversing chemoresistance in CSCs. The therapeutic efficacy of temozolomide on chemoresistant GSCs has proven to be enhanced by Notch and Sonic hedgehog homolog pathway inhibition with GSI-I (γ-secretase inhibitors) and cyclopamine [71]. As both Notch and Sonic hedgehog homologs are essential for the maintenance of stem cells, the effective targeting of both pathways has revealed important anticancer proliferative effects.

The majority of GSCs are located in vascular niches or microenvironments that tightly regulate the supply of oxygen and nutrients to these cells, thus maintaining their ‘stemness’ and self-renewal capacity [70]. In glioma xenograft models, the depletion of vascular endothelial cells by human EGF-receptor-2 inhibitors or VEGF signaling inhibitors has proven to efficiently ablate self-renewing malignant cells [11]. Most currently available conventional anticancer drugs preferentially target proliferating tumor cells, which dramatically reduces tumor burden but does not eradicate resistant cancer-initiating cells. In this scenario, malignant recurrence becomes inevitable. Therefore, a successful elimination of CSCs is required to achieve durable therapeutic results. A systematic screening of drugs that would specifically target GSCs could represent a first step for a clinically applicable solution. Nevertheless, this procedure has not yet been widely employed due to the relative instability of GSCs in culture, the paucity of tumor-initiating cells within gliomas and the lack of reliable CSC enrichment markers. To overcome these problems, Gupta and colleagues recently utilized epithelial–mesenchymal transition-induced enrichment of breast cancer cells with stem-like properties in order to enhance their CSC population. They then employed an automated screening technology to identify drugs that could effectively target these CSCs [72]. They were able to successfully identify one compound, salinomycin, that was able to effectively reduce the breast-cancer stem cell population by >100-fold as compared to conventional chemotherapies. A similar approach could be utilized as a blue print to identify drugs that would efficiently target GSCs.

The ability of the microenvironment to promote the maintenance of GSCs provides new avenues for the development of targeted therapeutic interventions. Malignant gliomas display enhanced angiogenesis as well as increased VEGF expression, which promotes endothelial cell survival, migration and proliferation [73]. GSCs have also been associated with the generation of highly vascularized tumors with increased VEGF expression [73]. A number of preclinical and clinical studies have demonstrated the effectiveness of a neutralizing anti-VEGF antibody, bevacizumab, in antiglioma therapy [74,75]. Animals treated with this drug, either alone or in conjunction with other chemotherapeutic agents, have shown increased survival and reduced glioblastoma growth. In addition, mice bearing

GSC enriched xenografts when treated with bevacizumab displayed delayed tumor growth due to decreased vasculogenesis and depletion of GSCs [70,76,77]. The use of other antiangiogenic agents, such as antistromal cell-derived factor-1, has shown similar outcomes [78]. Due to these encouraging results, the US FDA has approved the use of bevacizumab in clinical trials for primary or recurrent malignant gliomas. However, recent data have suggested that the therapeutic efficacy obtained by the use of anti-VEGF treatments may be only temporary [79,80]. There is major concern that bevacizumab may only improve short-term patient outcomes by decreasing the number of leaky vessels. However, in the long term, it could lead to the development of more aggressive and invasive tumors due to increased overall tumor vasculature.

Differentiation-promoting approaches

Instead of eradicating the GSC population, current research has focused on promoting the differentiation of these cells. Piccirillo *et al*. have demonstrated that glioma cells transiently exposed to BMP4 presented a significant reduction in the GSC population and, when transplanted into nude mice treated with BMP4, glioma-initiating cells were unable to establish intracerebral tumors [81]. FOXO3 activation has also been shown to induce differentiation of GSCs and reduce tumorigenicity. In a recent report, Sato *et al*. have identified metformin, a widely used antidiabetic agent, as a FOXO3 activator. Glioma bearing mice treated with systemically administered metformin resulted in depletion of GSC subpopulation, inhibition of tumor formation as well as prolonged median survival in the glioma xenograft model [82]. JNK is another interesting target that is believed to play a critical role in GSC maintenance. By blocking JNK activity with a small-molecule inhibitor, Matsuda *et al*. have shown an important depletion of GSCs within established tumors, decreased tumorigenicity of glioma-initiating cells and substantial survival benefit without adverse reactions [83]. Taken together, these important results have opened a new avenue in antiglioma therapy by revealing new viable and clinically relevant targets that could be effectively used to control the GSC population.

Immunotherapy

CSCs have been associated with immunosuppressive properties, which are a critical part of the mechanism of tumor initiation and maintenance [84]. These cancer-initiating cell lines have shown to be deficient or expressing low levels of MHC-I, MHC-II and NKG2D, which are important antigen-presenting complexes necessary for the activation of the immune system. As a result, the immune surveillance is not able to detect cancer-initiating cells. Understanding the many different immunosuppressive pathways in CSCs allows for a more effective design of anticancer therapeutic strategies. Recent work has demonstrated that the recognition of CSCs by dendritic cells resulted in a massive activation of T lymphocytes. These activated cytotoxic cells expressed elevated levels of IFN-γ, which led to enhanced elimination of identified CSCs [85]. In glioblastoma models, IL-6 signaling supports growth and survival of CSCs. Recent reports have demonstrated that the IL-6 blockade effectively contributes to CSC clearance [86]. In glioblastoma patients, elevated IL-6 is associated with reduced survival. Such a poor outcome indicates the potential clinical utility of IL-6 signaling cascade as a possible antiglioma therapeutic target [86]. Glioma-specific CSCs can also be targeted and killed by cytotoxic T lymphocytes through a perforin-mediated

mechanism. This is supported by recent work by Brown and colleagues, which demonstrates that CSCs derived from high-grade gliomas may be recognized and eliminated by $CD8^+$ cytotoxic T lymphocytes [87]. In addition, autologous T cells can also effectively target HER2-positive GSCs while sparing HER2-negative tumor cells. This resulted in a potent antitumor activity with sustained regression of autologous glioblastoma xenografts [88].

Genetic targeting

miRNAs—By definition, miRNAs are noncoding short nucleotide sequences (~22 nucleotides in length) that are able to block the translation of transcripts with complementary mRNA sequences in both normal and neoplastic cells [89]. This process leads to a precise activation or inhibition of selected genes. miRNAs are frequently deregulated in malignant gliomas and, as such, they have been associated with various aspects of gliomagenesis [90]. For instance, they are able to effectively regulate CSC genomic expression, thus functioning as oncogenes or tumor suppressors. They are also capable of regulating CSC differentiation, being important tools in anticancer therapy. Therefore, the utilization of miRNA-based therapeutic tools to target specific CSC subpopulations shows itself as a very attractive idea.

The number of miRNAs associated with CSCs is continuously expanding. In glioblastoma multiforme, some tumor-suppressor miRNAs, such as miRNA-486, miRNA-451, miRNA-107, miRNA-185 and miRNA-16, have been shown to be down-regulated in $CD133^+$ GSC populations. This characteristic has been correlated to more proliferative and aggressive tumors [91]. Additionally, overexpression of miRNA-128 resulted in efficient *in vitro* inhibition of GSC proliferation and decreased *in vivo* growth of glioma xenografts. By directly targeting BMI-I (poly-comb ring finger oncogene), overexpressed miRNA-128 was also able to effectively block GSC self-renewal [92]. Additional reports demonstrated that *in vivo* transfection of miRNA-34 into GSC-enriched tumors resulted in inhibited growth of glioma xenografts, cell cycle arrest and apoptosis of tumor cells. Such results have been achieved through downregulation of specific oncogene targets, including Notch1/2, CDK6 and c-MET [93]. RNA interference strategies are at the frontline for targeting the aberrant expression of different genes utilizing miRNAs. Using breast-cancer stem cells, Yu *et al*. [94] were able to increase the expression of miRNA let-7, accomplished via a lentiviral vector, leading to a reduced proportion of stem cells and resulting in delayed tumor formation and metastasis. However, targeting such CSC-specific miRNA through RNA interference is yet to be tested in human glioblastoma.

Oncolytic viruses—Oncolytic virotherapy, a therapeutic approach utilizing conditionally replicative viruses, may hold the potential to directly target self-renewing CSCs. In preclinical trials, these viruses have demonstrated functioning independently of common resistance pathways that exist for chemotherapeutic agents. Moreover, it is possible to construct viruses that are able to target both CSCs and other drug-resistant cells. Here, the authors summarize the recent efforts to develop oncolytic viruses that are capable of efficiently eliminating CSCs.

Herpes simplex virus—Attenuated herpes simplex virus (HSV) was used as one of the first gene therapy vectors to target glioma cells. To reduce neurotoxicity, HSV was deleted for RL1, which encodes the ICP34.5 and allows for virus replication even in the presence of the interferon response. Later generations of the HSV construct restored this gene, which then became under the control of specific enhancers expressed in cancer cells. To preferentially target GSCs that expressed high levels of nestin, the ICP34.5 was restored under the control of nestin, creating rQnestin34.5 [95]. GSCs shown to be resistant to conventional therapies were susceptible to the virus. Interestingly, IFN-β treatment inhibited viral replication only in neurospheres, known to be enriched for CSCs [96]. This is especially important in light of the general view that cancer cells have a deficient interferon response and viruses such as vesicular stomatitis virus may not be able to target CSCs.

Adenovirus—Adenoviruses (Ads) are the most commonly used gene therapy vectors. After entry into the cell, the adenoviral early transcription E1A region binds to the cell cycle regulating retinoblastoma (Rb) protein, which is frequently mutated in various human cancers. Tumor-specific conditionally replicative oncolytic Ads were created by the deletion of a 24-nucleotide (Δ24)-specific region in the viral E1A region that binds cellular Rb. As a result, the viral vector replicates only in cells that contain mutant Rb proteins. Another tumor-specific approach comprises the development of adenoviral vectors that express E1A under the regulation of specific promoters found only in cancer cells. Since not all targeted cells express the necessary coxsackie-adenovirus receptor to improve viral entry, the adenovirus serotype 5 has undergone further surface modifications, such as replacing the viral fiber knob with serotype 3 (Ad5/3), or by adding polylysine 7 (Ad.pk7) and RGD motifs (Ad.RGD) to this same knob. GSCs were shown by Ulasov *et al*. to be successfully targeted by genetically engineered Ads, such as Ad5/3, Ad.Δ24.RGD and Ad.Survivin.pk7 [97,98]. These viruses were able to kill not only $CD133^+$ GSCs, but also more differentiated cancer cells. By using stem-cell specific promoters, Cox-2, human telomerase reverse transcriptase and mdr, Bauerschmitz *et al*. were able to show a reduction in breast-cancer stem cell populations after treatment with Ad5/3-mdr-Δ24 [99]. Ads have also shown similar efficacy in other brain-tumor derived CSC models.

The major benefit of the development of new therapeutic approaches that allow better gene targeting in CSCs is the opportunity for discovery of tumor-specific therapies, which would be able to induce long-lasting anticancer results. Although most of these genetic approaches remain to be proven in clinical trials, they appear promising. Their safety profile and no crossresistance with current therapies have made them formidable candidates to combine with conventional anticancer therapies.

Conclusion

The majority of the studies focusing on glioma-derived CSCs consider these tumor-initiating cells as an isolated object. However, instead of only concentrating on the independent study of these cells, researchers should also consider the impact of the tumor microenvironment on the development, maintenance and tumorigenic potential of such entities. Recent findings have determined that the glioma vascular niche truly supports GSC maintenance and nurtures tumor growth by offering all nutrients and signals necessary for an unlimited GSC

proliferation [11]. Thereby, an efficient inhibition of these proangiogenic mechanisms, such as through anti-VEGF therapies, can lead to decreased glioma progression. Nevertheless, there is no explanation as to whether GSCs are a product of the intratumoral microenvironment or if they directly influence it. Additional experiments that will be able to answer these questions are necessary. The resultant responses will give us important clues regarding glioblastoma heterogeneity and the complex mechanisms involved in tumor recurrence. They will also assist in the development of additional therapeutic approaches that will hopefully revert the presently dismal patient prognosis.

Although the currently available CSC markers enable an efficient identification and isolation of these tumorigenic populations, they are not considered a fully reliable measure of CSCs. This is mainly due to high interpatient and intratumoral variation of CSC characteristics. To overcome this problem, new markers need to be discovered and the resulting extensive range of CSC markers will require validation in a large amount of patient samples. Additional corroboration should also be determined for samples derived from different intratumoral locations and functional assessment should be extended for each patient. The consequent acquisition of tumor-specific signatures will enable a better differentiation between CSCs and normal stem cells, facilitating the evaluation of clinically relevant malignant residues. Furthermore, it will allow for the determination of more specific therapeutic windows in which CSCs can be nicely identified and eliminated, while normal NSCs may be finally spared.

The exact relationship between the cell of origin and GSCs remains obscure. Mouse models of gliomagenesis have been vital for answering this important question. However, the choice of transgenic models and lineage-specific promoters/enhancers to generate oncogenic events and recapitulate gliomagenesis may pose a major influence on experimental outcomes. Therefore, the results from these studies must be analyzed carefully. A better optimization of these models could contribute to the development of earlier detection methods of neoplastic burdens and might allow for a more accurate prediction of tumor behavior. The investment on studies that may help to explain the role of cellular plasticity in the CSC theory is also another critical step that needs to be accomplished. If nonCSCs can give rise to CSCs, this could represent a major issue for the development of effective strategies against such populations. As a consequence, understanding CSCs' plastic behavior must be a priority.

Given the highly invasive nature of glioblastoma tumors, it remains extremely improbable that any single therapeutic approach will be able to specifically target and eliminate residual CSCs without producing considerable toxicity in non-neoplastic tissues. Nevertheless, the combination of a single therapy with other treatment options may be able to result in more efficient and long-lasting antitumor effects. Finally, the anticancer therapies described here should be further optimized and evaluated in clinical trials either alone or in combination with other approaches that are able to target relevant CSC signaling pathways. Thus, it may be possible to achieve significant prevention of tumor recurrence as well as improve patient overall and progression-free survival.

Expert commentary

The hierarchical model of cancer originally proposed adult stem cells as the optimal target for sequential oncogenic hits due to their longevity. As a consequence, the transformation of adult stem cells gives rise to human malignancies. These transformed stem cells are considered to be critical for sustaining the continuous abnormal growth of neoplastic tissue. Although several experimental animal models of gliomagenesis provided convincing evidence that glioma tumors originated from the cellular transformation of neural stem/progenitor cells, these models did not exclude the possibility that nonstem cells could be the possible source of such a malignancy. The sole use of such a genetic model of gliomagenesis, together with the over interpretation of the results generated by these models, created a certain confusion surrounding the CSC concept. However, there is no denying that malignant tumors are immortal at the cellular level and CSCs may play a crucial role in this immortality. Indeed, the majority of the controversies surrounding the CSC hypothesis can be resolved by defining such population functionally. The ability to self-renew and differentiate into multiple lineages is a hallmark of adult stem cells and this self-renewing activity can be measured by sphere-formation assays under non-adherent culture conditions. Given the similarity between adult stem cells and CSCs, the same assay is used for defining putative CSCs. Although the sphere-formation assay has the capacity to enrich CSCs from a given tumor population, one should keep in mind that this assay does not provide an exact measurement of self-renewal ability. Thus, CSCs should be defined by *in vivo* functional assays that measure their ability to generate serially transplanted tumors and the implanted tumor should be able to recapitulate the cellular and histological heterogeneity of the parental tumor. Nonetheless, the authors ought to be careful to not overinterpret the results of this assay, which heavily relies on xenotransplantation. In addition, the reported CSCs may not necessarily have the same characteristics as the cells that give rise to tumors derived from patients. This point is critical when considering that *in vivo*, CSCs may vary between different tumors and may continually change as the disease progresses.

Five-year view

Increasing evidence has called into question the rigid cellular hierarchy within a tumor proposed by the initial CSC theory and suggested that the stemness of CSCs could be governed by cellular plasticity. As the maintenance of CSCs niche requires instructive cues from the intratumoral microenvironment, any pro- or antioncogenic therapy may likely affect CSC's properties and promote plasticity in the tumor cell population in order to induce therapeutic resistant CSCs. Understanding how such plasticity can promote the dedifferentiation of cancer cells and increase the overall stemness of the tumor will be critical for developing effective therapeutic strategies to target CSC populations.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. Stupp R, Mason WP, van den Bent MJ, et al. European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005; 352(10):987–996. [PubMed: 15758009]
2. Kang MK, Kang SK. Tumorigenesis of chemotherapeutic drug-resistant cancer stem-like cells in brain glioma. Stem Cells Dev. 2007; 16(5):837–847. [PubMed: 17999604]
3. Liu G, Yuan X, Zeng Z, et al. Analysis of gene expression and chemoresistance of $CD133^+$ cancer stem cells in glioblastoma. Mol Cancer. 2006; 5:67. [PubMed: 17140455]
4••. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006; 444(7120):756–760. First report to demonstrate that $CD133^+$ glioma cells represent the cellular population with elevated DNA repair capacity that confers glioma radioresistance. This may be responsible for disease recurrence postradiotherapy. [PubMed: 17051156]
5. Polyak K, Hahn WC. Roots and stems: stem cells in cancer. Nat Med. 2006; 12(3):296–300. [PubMed: 16520777]
6. Galli R, Binda E, Orfanelli U, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res. 2004; 64(19):7011–7021. [PubMed: 15466194]
7. Dirks PB. Brain tumor stem cells: bringing order to the chaos of brain cancer. J Clin Oncol. 2008; 26(17):2916–2924. [PubMed: 18539973]
8. Vescovi AL, Galli R, Reynolds BA. Brain tumour stem cells. Nat Rev Cancer. 2006; 6(6):425–436. [PubMed: 16723989]
9. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer. 2008; 8(10):755–768. [PubMed: 18784658]
10. Fuchs E, Segre JA. Stem cells: a new lease on life. Cell. 2000; 100(1):143–155. [PubMed: 10647939]
11. Calabrese C, Poppleton H, Kocak M, et al. A perivascular niche for brain tumor stem cells. Cancer Cell. 2007; 11(1):69–82. [PubMed: 17222791]
12•. Visvader JE. Cells of origin in cancer. Nature. 2011; 469(7330):314–322. A comprehensive review discussing the difference between cell-of-origin in cancer and cancer stem cells. [PubMed: 21248838]
13. Alcantara Llaguno S, Chen J, Kwon CH, et al. Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. Cancer Cell. 2009; 15(1):45–56. [PubMed: 19111880]
14. Holland EC, Celestino J, Dai C, Schaefer L, Sawaya RE, Fuller GN. Combined activation of *Ras* and *Akt* in neural progenitors induces glioblastoma formation in mice. Nat Genet. 2000; 25(1):55–57. [PubMed: 10802656]
15. Jacques TS, Swales A, Brzozowski MJ, et al. Combinations of genetic mutations in the adult neural stem cell compartment determine brain tumour phenotypes. EMBO J. 2010; 29(1):222–235. [PubMed: 19927122]
16. Marumoto T, Tashiro A, Friedmann-Morvinski D, et al. Development of a novel mouse glioma model using lentiviral vectors. Nat Med. 2009; 15(1):110–116. [PubMed: 19122659]
17. Wang Y, Yang J, Zheng H, et al. Expression of mutant p53 proteins implicates a lineage relationship between neural stem cells and malignant astrocytic glioma in a murine model. Cancer Cell. 2009; 15(6):514–526. [PubMed: 19477430]
18••. Mani SA, Guo W, Liao MJ, et al. The epithelial–mesenchymal transition generates cells with properties of stem cells. Cell. 2008; 133(4):704–715. Induction of an epithelial–mesenchymal transition in immortalized human mammary epithelial cells results in the acquisition of stem cell markers and gain of epithelial stem cell properties such as enhanced self-renewal capacity and resistance to conventional therapies. [PubMed: 18485877]
19••. Chaffer CL, Brueckmann I, Scheel C, et al. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. Proc Natl Acad Sci USA. 2011; 108(19):7950–7955. Demonstrates that upon oncogenic transformation, noncancer stem cells can spontaneously give rise to cancer stem-like cells *in vitro* and *in vivo*. [PubMed: 21498687]

20. Friedmann-Morvinski D, Bushong EA, Ke E, et al. Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. Science. 2012; 338(6110):1080–1084. [PubMed: 23087000]

21•. Gupta PB, Chaffer CL, Weinberg RA. Cancer stem cells: mirage or reality? Nat Med. 2009; 15(9):1010–1012. Excellent review discussing the possibility of cellular plasticity as a driver for cellular dedifferentiation that can convert noncancer stem cells into cancer stem-like cells. [PubMed: 19734877]

22. Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. Science. 2007; 317(5836):337. [PubMed: 17641192]

23••. 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. First report of the isolation of cancer stem cells from human myeloid leukemia. [PubMed: 9212098]

24. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994; 367(6464):645–648. [PubMed: 7509044]

25. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA. 2003; 100(7):3983–3988. [PubMed: 12629218]

26. Hemmati HD, Nakano I, Lazareff JA, et al. Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci USA. 2003; 100(25):15178–15183. [PubMed: 14645703]

27•. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res. 2003; 63(18):5821–5828. Along with [26], provides evidence for the first time of the existence of cancer stem cells in brain tumors. [PubMed: 14522905]

28. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature. 2007; 445(7123):106–110. [PubMed: 17122772]

29. Fang D, Nguyen TK, Leishear K, et al. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res. 2005; 65(20):9328–9337. [PubMed: 16230395]

30. Hermann PC, Huber SL, Herrler T, 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]

31. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 2005; 65(23):10946–10951. [PubMed: 16322242]

32. Bapat SA, Mali AM, Koppikar CB, Kurrey NK. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res. 2005; 65(8):3025–3029. [PubMed: 15833827]

33. Eramo A, Lotti F, Sette G, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ. 2008; 15(3):504–514. [PubMed: 18049477]

34. Fukuda K, Saikawa Y, Ohashi M, et al. Tumor initiating potential of side population cells in human gastric cancer. Int J Oncol. 2009; 34(5):1201–1207. [PubMed: 19360333]

35••. Beier D, Hau P, Proescholdt M, et al. CD133($^{+}$) and CD133($^{-}$) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Res. 2007; 67(9): 4010–4015. Demonstrates that conventional antiglioma chemotherapy can effectively deplete CD133^{+} glioma stem cells thus contradicting the notion that glioma stem cells represent the cell population within gliomas that can resist conventional chemo- and radio-therapy and initiate clinical relapse. [PubMed: 17483311]

36. Ogden AT, Waziri AE, Lochhead RA, et al. Identification of A2B5+CD133^{-} tumor-initiating cells in adult human gliomas. Neurosurgery. 2008; 62(2):505–514. discussion 514. [PubMed: 18382330]

37. 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; 4(5):440–452. [PubMed: 19427293]

38. Sun Y, Liou B, Quinn B, Ran H, Xu YH, Grabowski GA. *In vivo* and *ex vivo* evaluation of L-type calcium channel blockers on acid beta-glucosidase in Gaucher disease mouse models. PLoS ONE. 2009; 4(10):e7320. [PubMed: 19809509]

39. Read TA, Fogarty MP, Markant SL, et al. Identification of CD15 as a marker for tumor-propagating cells in a mouse model of medulloblastoma. Cancer Cell. 2009; 15(2):135–147. [PubMed: 19185848]
40. Lathia JD, Gallagher J, Heddleston JM, et al. Integrin alpha 6 regulates glioblastoma stem cells. Cell Stem Cell. 2010; 6(5):421–432. [PubMed: 20452317]
41. Shin HY, Hong YH, Jang SS, et al. A role of canonical transient receptor potential 5 channel in neuronal differentiation from A2B5 neural progenitor cells. PLoS ONE. 2010; 5(5):e10359. [PubMed: 20479868]
42. Wu Y, Wu PY. CD133 as a marker for cancer stem cells: progresses and concerns. Stem Cells Dev. 2009; 18(8):1127–1134. [PubMed: 19409053]
43. Alison MR, Lim SM, Nicholson LJ. Cancer stem cells: problems for therapy? J Pathol. 2011; 223(2):147–161. [PubMed: 21125672]
44. Pece S, Tosoni D, Confalonieri S, et al. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell. 2010; 140(1):62–73. [PubMed: 20074520]
45. Laks DR, Masterman-Smith M, Visnyei K, et al. Neurosphere formation is an independent predictor of clinical outcome in malignant glioma. Stem Cells. 2009; 27(4):980–987. [PubMed: 19353526]
46. Zhang M, Song T, Yang L, et al. Nestin and CD133: valuable stem cell-specific markers for determining clinical outcome of glioma patients. J Exp Clin Cancer Res. 2008; 27:85. [PubMed: 19108713]
47. Zeppernick F, Ahmadi R, Campos B, et al. Stem cell marker CD133 affects clinical outcome in glioma patients. Clin Cancer Res. 2008; 14(1):123–129. [PubMed: 18172261]
48. Sato A, Sakurada K, Kumabe T, et al. Tohoku Brain Tumor Study Group. Association of stem cell marker CD133 expression with dissemination of glioblastomas. Neurosurg Rev. 2010; 33(2):175–183. discussion 183. [PubMed: 20135187]
49. Kim KJ, Lee KH, Kim HS, et al. The presence of stem cell marker-expressing cells is not prognostically significant in glioblastomas. Neuropathology. 2011; 31(5):494–502. [PubMed: 21269333]
50. Horst D, Kriegl L, Engel J, Kirchner T, Jung A. Prognostic significance of the cancer stem cell markers CD133, CD44, and CD166 in colorectal cancer. Cancer Invest. 2009; 27(8):844–850. [PubMed: 19626493]
51. Cheung AM, Wan TS, Leung JC, et al. Aldehyde dehydrogenase activity in leukemic blasts defines a subgroup of acute myeloid leukemia with adverse prognosis and superior NOD/SCID engrafting potential. Leukemia. 2007; 21(7):1423–1430. [PubMed: 17476279]
52. Morimoto K, Kim SJ, Tanei T, et al. Stem cell marker aldehyde dehydrogenase 1-positive breast cancers are characterized by negative estrogen receptor, positive human epidermal growth factor receptor type 2, and high Ki67 expression. Cancer Sci. 2009; 100(6):1062–1068. [PubMed: 19385968]
53. Rasheed ZA, Yang J, Wang Q, et al. Prognostic significance of tumorigenic cells with mesenchymal features in pancreatic adenocarcinoma. J Natl Cancer Inst. 2010; 102(5):340–351. [PubMed: 20164446]
54. Chen YC, Chen YW, Hsu HS, et al. Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. Biochem Biophys Res Commun. 2009; 385(3):307–313. [PubMed: 19450560]
55. Rich JN, Bao S. Chemotherapy and cancer stem cells. Cell Stem Cell. 2007; 1(4):353–355. [PubMed: 18371369]
56. Maynard S, Swistowska AM, Lee JW, et al. Human embryonic stem cells have enhanced repair of multiple forms of DNA damage. Stem Cells. 2008; 26(9):2266–2274. [PubMed: 18566332]
57. Smith J, Ladi E, Mayer-Proschel M, Noble M. Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell. Proc Natl Acad Sci USA. 2000; 97(18):10032–10037. [PubMed: 10944195]
58. Ito K, Hirao A, Arai F, et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature. 2004; 431(7011):997–1002. [PubMed: 15496926]

59. Kitange GJ, Carlson BL, Schroeder MA, et al. Induction of MGMT expression is associated with temozolomide resistance in glioblastoma xenografts. Neuro-oncology. 2009; 11(3):281–291. [PubMed: 18952979]
60. Blough MD, Westgate MR, Beauchamp D, et al. Sensitivity to temozolomide in brain tumor initiating cells. Neuro-oncology. 2010; 12(7):756–760. [PubMed: 20388697]
61. Hermisson M, Klumpp A, Wick W, et al. O6-methylguanine DNA methyltransferase and p53 status predict temozolomide sensitivity in human malignant glioma cells. J Neurochem. 2006; 96(3):766–776. [PubMed: 16405512]
62. Hegi ME, Diserens AC, Gorlia T, et al. *MGMT* gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med. 2005; 352(10):997–1003. [PubMed: 15758010]
63. Sato A, Sunayama J, Matsuda K, et al. MEK-ERK signaling dictates DNA-repair gene *MGMT* expression and temozolomide resistance of stem-like glioblastoma cells via the MDM2-p53 axis. Stem Cells. 2011; 29(12):1942–1951. [PubMed: 21957016]
64. Zhou S, Schuetz JD, Bunting KD, et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med. 2001; 7(9):1028–1034. [PubMed: 11533706]
65. Ginestier C, Hur MH, Charafe-Jauffret E, 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; 1(5): 555–567. [PubMed: 18371393]
66. Hirschmann-Jax C, Foster AE, Wulf GG, et al. A distinct ‘side population’ of cells with high drug efflux capacity in human tumor cells. Proc Natl Acad Sci USA. 2004; 101(39):14228–14233. [PubMed: 15381773]
67. Bleau AM, Hambardzumyan D, Ozawa T, et al. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. Cell Stem Cell. 2009; 4(3):226–235. [PubMed: 19265662]
68. Schaich M, Kestel L, Pfirrmann M, et al. A *MDR1* (ABCB1) gene single nucleotide polymorphism predicts outcome of temozolomide treatment in glioblastoma patients. Ann Oncol. 2009; 20(1): 175–181. [PubMed: 18687982]
69. Baumann M, Krause M, Hill R. Exploring the role of cancer stem cells in radioresistance. Nat Rev Cancer. 2008; 8(7):545–554. [PubMed: 18511937]
70. Gilbertson RJ, Rich JN. Making a tumour’s bed: glioblastoma stem cells and the vascular niche. Nat Rev Cancer. 2007; 7(10):733–736. [PubMed: 17882276]
71. Ulasov IV, Nandi S, Dey M, Sonabend AM, Lesniak MS. Inhibition of Sonic hedgehog and Notch pathways enhances sensitivity of CD133($^{+}$) glioma stem cells to temozolomide therapy. Mol Med. 2011; 17(1–2):103–112. [PubMed: 20957337]
72••. Gupta PB, Onder TT, Jiang G, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell. 2009; 138(4):645–659. Authors employed epithelial–mesenchymal transition-induced enrichment of breast cancer cells with stem-like properties in order to enhance their cancer stem cell population and then they employed an automated screening technology to identify drugs that could effectively target these cancer stem cells. [PubMed: 19682730]
73. Bao S, Wu Q, Sathornsumetee 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]
74. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol. 2009; 27(28):4733–4740. [PubMed: 19720927]
75. Vredenburgh JJ, Desjardins A, Herndon JE 2nd, et al. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol. 2007; 25(30):4722–4729. [PubMed: 17947719]
76. Folkins C, Man S, Xu P, Shaked Y, Hicklin DJ, Kerbel RS. Anticancer therapies combining antiangiogenic and tumor cell cytotoxic effects reduce the tumor stem-like cell fraction in glioma xenograft tumors. Cancer Res. 2007; 67(8):3560–3564. [PubMed: 17440065]
77. Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. J Clin Invest. 2010; 120(3):694–705. [PubMed: 20179352]

78. Folkins C, Shaked Y, Man S, et al. Glioma tumor stem-like cells promote tumor angiogenesis and vasculogenesis via vascular endothelial growth factor and stromal-derived factor 1. Cancer Res. 2009; 69(18):7243–7251. [PubMed: 19738068]
79. Thompson EM, Frenkel EP, Neuwelt EA. The paradoxical effect of bevacizumab in the therapy of malignant gliomas. Neurology. 2011; 76(1):87–93. [PubMed: 21205697]
80. Verhoeff JJ, van Tellingen O, Claes A, et al. Concerns about anti-angiogenic treatment in patients with glioblastoma multiforme. BMC Cancer. 2009; 9:444. [PubMed: 20015387]
81. Piccirillo SG, Reynolds BA, Zanetti N, et al. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature. 2006; 444(7120):761–765. [PubMed: 17151667]
82. Sato A, Sunayama J, Okada M, et al. Glioma-initiating cell elimination by metformin activation of FOXO3 via AMPK. Stem Cells Transl Med. 2012; 1(11):811–824. [PubMed: 23197693]
83. Matsuda K, Sato A, Okada M, et al. Targeting JNK for therapeutic depletion of stem-like glioblastoma cells. Sci Rep. 2012; 2:516. [PubMed: 22816039]
84. Di Tomaso T, Mazzoleni S, Wang E, et al. Immunobiological characterization of cancer stem cells isolated from glioblastoma patients. Clin Cancer Res. 2010; 16(3):800–813. [PubMed: 20103663]
85. Weng D, Song B, Durfee J, et al. Induction of cytotoxic T lymphocytes against ovarian cancer-initiating cells. Int J Cancer. 2010; 129(8):1990–2001. [PubMed: 21154809]
86. Wang H, Lathia JD, Wu Q, et al. Targeting interleukin 6 signaling suppresses glioma stem cell survival and tumor growth. Stem Cells. 2009; 27(10):2393–2404. [PubMed: 19658188]
87. Brown CE, Starr R, Martinez C, et al. Recognition and killing of brain tumor stem-like initiating cells by $CD8^+$ cytolytic T cells. Cancer Res. 2009; 69(23):8886–8893. [PubMed: 19903840]
88. Ahmed N, Salsman VS, Kew Y, et al. HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. Clin Cancer Res. 2010; 16(2):474–485. [PubMed: 20068073]
89. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116(2):281–297. [PubMed: 14744438]
90. Zhang Y, Dutta A, Abounader R. The role of microRNAs in glioma initiation and progression. Front Biosci. 2012; 17:700–712.
91. Gal H, Pandi G, Kanner AA, et al. MIR-451 and Imatinib mesylate inhibit tumor growth of Glioblastoma stem cells. Biochem Biophys Res Commun. 2008; 376(1):86–90. [PubMed: 18765229]
92. Godlewski J, Nowicki MO, Bronisz A, et al. Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. Cancer Res. 2008; 68(22): 9125–9130. [PubMed: 19010882]
93. Li Y, Guessous F, Zhang Y, et al. MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. Cancer Res. 2009; 69(19):7569–7576. [PubMed: 19773441]
94. Yu F, Yao H, Zhu P, et al. let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell. 2007; 131(6):1109–1123. [PubMed: 18083101]
95. Kambara H, Okano H, Chiocca EA, Saeki Y. An oncolytic HSV-1 mutant expressing ICP34.5 under control of a nestin promoter increases survival of animals even when symptomatic from a brain tumor. Cancer Res. 2005; 65(7):2832–2839. [PubMed: 15805284]
96. Kurozumi K, Hardcastle J, Thakur R, et al. Oncolytic HSV-1 infection of tumors induces angiogenesis and upregulates CYR61. Mol Ther. 2008; 16(8):1382–1391. [PubMed: 18545226]
97. Ulasov IV, Zhu ZB, Tyler MA, et al. Survivin-driven and fiber-modified oncolytic adenovirus exhibits potent antitumor activity in established intracranial glioma. Hum Gene Ther. 2007; 18(7): 589–602. [PubMed: 17630837]
98. Nandi S, Ulasov IV, Rolle CE, Han Y, Lesniak MS. A chimeric adenovirus with an Ad 3 fiber knob modification augments glioma virotherapy. J Gene Med. 2009; 11(11):1005–1011. [PubMed: 19688792]
99. Bauerschmitz GJ, Ranki T, Kangasniemi L, et al. Tissue-specific promoters active in $CD44^+CD24^-$/low breast cancer cells. Cancer Res. 2008; 68(14):5533–5539. [PubMed: 18632604]

Key issues

- The original cancer stem cells (CSCs) theory, which proposed that only stem-like cells could propagate tumors, has recently been challenged by the fact that 'stemness' can be regulated by cellular plasticity and can be influenced by changes in the intratumoral microenvironment, such as hypoxic conditions, intratumoral pH or activation of epithelial–mesenchymal transition.
- The cancer stem-like state in brain tumors, as well as in other human malignancies, is not restricted to $CD133^+$ cells, but also to the $CD133^-$ cells that can fulfill the criteria of CSCs. These observations suggested that no phenotypic marker such as CD133 could be utilized as a universal marker for CSCs.
- There are considerable controversies over the existing link between adult stem cells and CSCs. Although genetically engineered mouse models provided clues that glioblastoma multiforme originates from the malignant transformation of neural stem/progenitor cells, these results should be inferred judiciously because current animal models do not exclude the possibility that nonstem-cell populations, when manipulated with multiple and sequential mutational hits, can also give rise to glial tumors.
- While the radioresistant properties of glioma stem cells (GSCs) are widely accepted, the question of whether or not GSCs account for the chemoresistance of glioma tumors remains controversial. Some reports describe an increased resistance and others, an increased susceptibility of GSCs toward conventional antiglioma chemotherapies, such as temozolomide.
- Several recent reports indicated a prognostic impact of CSC-related end points. However, prospective powered trials are required for thoroughly understanding the prognostic and predictive importance of CSC-associated parameters.
- The stem cell-centric paradigm of gliomagenesis has challenged the dogma of experimental and medical oncology, and identified novel targets for developing more effective anticancer therapies. However, the majority of the anti-GSC therapies tested in the preclinical setting did not show relevant therapeutic efficacy in clinical trials. These results highlight the need for a more in-depth understanding of the molecular characteristics governing GSCs.