Tumor initiating cells in malignant gliomas:

biology and implications for therapy

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Abstract

A rare subpopulation of cells within malignant gliomas, which shares canonical properties with neural stem cells (NSCs), may be integral to glial tumor development and perpetuation. These cells, also known as tumor initiating cells (TICs), have the ability to self-renew, develop into any cell in the overall tumor population (multipotency), and proliferate. A defining property of TICs is their ability to initiate new tumors in immunocompromised mice with high efficiency. Mounting evidence suggests that TICs originate from the transformation of NSCs and their progenitors. New findings show that TICs may be more resistant to chemotherapy and radiation than the bulk of tumor cells, thereby permitting recurrent tumor formation and accounting for the failure of conventional therapies. The development of new therapeutic strategies selectively targeting TICs while sparing NSCs may provide for more effective treatment of malignant gliomas.

Keywords

Central nervous system; Glioblastoma; Cancer stem cells; Brain cancer

Introduction

The hypothesis that tumor growth may be sustained by a rare subpopulation of cells, termed tumor initiating cells (TICs), is currently being explored in different types of cancer [1-6]. These cells have also been referred to as cancer stem cells (CSCs), although this nomenclature has been questioned due to the controversial nature of their "stemness" [7,8]. The terms CSCs and TICs are often used interchangeably. Recently, an attempt at defining TICs from CSCs has been made based on the number of mutations present in these cells and their ability to support tumor growth [9]. TICs are identified in the early stages of tumor development and may not yet have acquired the full tumorigenic potential of CSCs, therefore may carry less mutations and may not grow as well in animal models for tumorigenicity. In this review, we will use the terminology of TICs.

The most common and malignant cancer of the adult central nervous system, known as astrocytoma WHO grade IV or glioblastoma multiforme (GBM), has been shown to contain

TICs [10,11]. Cells with the ability to regenerate a GBM have been isolated from fresh specimens based on their expression of a 120-kDa cell-surface protein known as CD133 (Prominin-1) [10]. CD133 was initially identified as a marker of normal human neural precursors and is also a marker for hematopoietic stem cells [12-14]. CD133+ GBM-initiating cells possess in vitro neural stem-cell like characteristics of extensive self-renewal, multipotency (capability to develop into any cell in the overall tumor population) and the proliferative ability for generation of many progeny. They are able to initiate new tumors in vivo when transplanted into immunocompromised mice at low cell numbers [11].

Glioblastoma continues to be one of the most lethal cancers despite all conventional therapies [15]. These highly vascularized and diffusely infiltrative tumors are radioresistant, chemoresistant, and tend to recur in a local fashion despite surgical resection [16-18]. Complete surgical excision of GBM tumors is impossible, as individual tumor cells can deeply infiltrate adjacent normal brain. Initial findings on GBM-initiating cells provide new explanations for patient relapse. Recent evidence has shown that a subpopulation of TICs resides in a vascular niche and that TICs promote radioresistance [19-21] and chemoresistance [22-24]. Targeted therapy against GBM-initiating cells or their vascular niche may form the basis of new treatments.

Definition and source of neural stem cells in the brain

Neural stem cells and GBM-initiating cells share both phenotypic and functional similarities. The have both been shown to express the CD133 cell surface marker and are self-sustaining and able to self-renew. Reynolds and Weiss were the first to isolate adult neural stem cells from the adult striatum that could proliferate and generate multipotent clones of cells in vitro termed neurospheres [25,26]. Neurosphere culture relies on a serum-free, selective growth factor [epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF-2)] system in which most differentiating or differentiated cells rapidly die [27-30]. Growth-factor-responsive neural stem cells can be passaged and expanded indefinitely with little change in their growth or differentiation characteristics. Removal of growth factors induces the differentiation of the progeny of neural stem cells into neurons, astrocytes, and oligodendrocytes. As a result, adult neural stem cells possess the stem-cell criteria of self-renewal, multipotency, and the generation of many progeny.

Neural stem cells have been isolated from the subventricular zone lining of the lateral ventricles, the dentate gyrus within the hippocampus, and the subcortical white matter [31-34].

The largest of these germinal regions in humans is the subventricular zone, located between the ependymal layer of the lateral ventricles and the parenchyma of the striatum (Fig. 1). The subventricular zone is thought to be the likely source of GBM-initiating cells [35]. A subset of cells in this region display astrocyte features; yet, they can function as full-fledged neural stem cells [32,36]. These astrocytic stem cells express the astroglial marker glial fibrillary acidic protein as do mature astrocytes of the brain parenchyma, which do not possess stem-cell properties. They are able to give rise to neurons, astrocytes, and oligodendrocytes through the generation of uncommitted progenitor cells known as transit-amplifying progenitor cells (TACs) [37-40]. Fast-cycling TACs give rise to committed progenitor cells (neuronal or glial), which retain the capacity of producing progeny along either neuronal or glial lineages (oligodendrocytes and/or astrocytes), but not both (Fig. 2). Committed progenitor cells (PC) can possess replicative ability, but they do not usually have the self-renewal capacity of stem cells. Neural stem cells, committed progenitor cells, and differentiated adult glial cells are all thought to be substrates for neoplastic transformation and ultimate formation of GBM tumors [1].

GBM-initiating cells

Genetic evidence for the existence of a precursor cell population in glioblastoma from which the bulk of the tumor was derived has existed for some time [41]. However, it was only recently that the first experimental evidence of cells with stem-like characteristics was reported in human GBM [42]. Clonogenic, neurosphere-forming precursor cells were isolated from GBM specimens that expressed neuronal and astroglial markers upon differentiation. By applying the same conditions used for the isolation of human neural stem cells, others were later able to isolate clonogenic neurospheres from adult human GBM [10,11,43,44]. Purification of CD133-positive cells from human gliomas by flow cytometry can also allow for the isolation and growth of TICs [45]. These cells are capable of self-renewal, multipotency, and generation of many progeny in vitro (Fig. 2). CD133+ tumor neurospheres under neural stem cell culture conditions express the stem cell marker, nestin, and upon exposure to serum, differentiate into a mixed population of neurons, astrocytes, and oligodendrocytes. A small fraction of CD133+GBM-initiating cells (as few as 100 implanted cells) can initiate tumors in vivo when transplanted into immunocompromised mice. These tumors exhibit many of the key malignant features of GBM, including infiltration and microvascular proliferation. CD133- cells derived from the tumors studied by Singh et al. failed to form orthotopic tumors in mice even following injection of a larger cell inoculum (10^5 per injection) [45]. More recently, several reports suggest a less clear distinction between the ability of CD133+ and CD133- cells to form orthotopic tumors [20,46-48]. CD133- cells isolated from primary GBM tumors have been reported to equally form orthotopic tumors as the CD133+ subpopulation. Implanted CD133- cells have also been shown to form CD133+ cells in orthotopic tumors [49]. The reasons for these discrepancies are currently unclear. This may reflect in part the presence of other types of TICs in GBM, which are devoid of CD133.

Purification of TICs from malignant gliomas was also achieved through the side population technique [50-52]. This technique was originally used to identify adult stem cells in bone-marrow-derived cells. Those cells that did not accumulate an appreciable amount of Hoechst dye 33342 were classified as a Hoechst side population (SP) that remarkably was highly enriched for hematopoietic stem cells. The SP approach represents a valid marker-independent method to identify TICs in various cancers, including brain [51]. SP cells from the rat C6 glioma cell line can be passaged and maintained in vitro by serum-free medium containing platelet-derived growth factor (PDGF) and FGF-2. SP cells can also form tumors in immunocompromised mice. The current lack of a single marker to identify all TICs in malignant gliomas confirms the probable molecular heterogeneity among these cells. Enrichment of TICs relies on either neurosphere culture, isolation of CD133+ cells, or use of the SP approach on glioblastoma tumor cells [53].

GBM-initiating cell niche

An important characteristic of at least one subset of normal neural stem cells is that they are concentrated in regions of the brain that are rich in blood vessels, called "vascular niches" [54-57]. Within these niches, neural stem cells are thought to be sheltered from apoptotic stimuli to maintain a proper balance between self-renewal and differentiation. Endothelial cells are a key component of the vascular niche. They secrete paracrine factors that promote stem cell survival and self-renewal. The high vascularity of glioblastoma tumors is well known. Microvascular proliferation is a pathologic hallmark of glioblastoma [58]; therefore, it was conceivable that the tumor might be an alternative harbor for cells requiring such paracrine factors for their maintenance and survival. Stem cells are also known to thrive in hypoxic regions [59], and the tumor may provide a hypoxic niche [18,60]. Upon examination, GBM-initiating cells, like neural stem cells, were found intimately associated

with the vascular niche in the tumor [19]. CD133+/nestin+ GBM stem cells were frequently found close to capillaries within GBM. When the CD133+ tumor cells were cultured with primary human endothelial cells, they rapidly and selectively associated with endothelial cells, while the majority of tumor cells did not. The endothelial cells were also found to enhance the self-renewal capacity of the CD133+GBM-initiating cells. In vivo, CD133+ tumor cells transplanted with endothelial cells grew more rapidly than cells transplanted alone. Self-renewing rat C6 GBM stem cells have also been maintained in vitro by factors secreted from endothelial cells [61]. This was not due to increased vascular density but rather a reduction in time needed to establish the level of vasculature needed for tumor growth. In addition, tumors established in the presence of endothelial cells contained up to 25 times more CD133+ CSCs. These data suggest that endothelial cells enhance the self-renewal of the TICs in vitro and promote growth of brain tumors in vivo.

Further evidence of a functional relationship between the tumor vasculature and GBM stem cells shows that cancer-initiating cells also sustain vascular development [62]. CD133+GBM stem cells show higher level production of vascular endothelial growth factor (VEGF) than CD133-cells. This likely explains why CD133+ and not CD133-GBM cells readily formed highly vascular and hemorrhagic tumors in the brains of immunocompromised mice.

Genesis of glioblastoma

All forms of cancer are believed to originate from a series of mutations in single cells (clones), which endow them with biological properties that permit their growth into tumors, including disturbances in proliferation, apoptosis, and tissue invasion [63]. A number of genetic aberrations involved in the genesis of glioblastomas have been well described for both primary (de novo) and secondary (progressive) tumors [64,65]. The "cancer-stem cell hypothesis" [2] suggests that a rare subset of cells within GBM tumors may have significant expansion capacity and the ability to generate new tumors. The remainder of tumor cells, which predominantly make up GBM tumors, may represent partially differentiated cells with limited progenitor capacity or terminally differentiated cells that cannot form new tumors.

Mounting evidence supports the observation that transformation of neural stem cells and their progenitors may lead to the formation of GBM-initiating cells and tumors [66-68] rather than from a differentiated cell type. Micro-array studies have revealed considerable overlap between the gene-expression signatures of glioblastomas and progenitor cells of the developing forebrain [69]. Gene expression similarities suggest common signaling pathways present in neural stem cells and GBM-initiating cells [43]. Genetic studies in mice have brought some insight into the genetic alterations (deletion of tumor suppressors and/or activation of oncogenes) that can lead to neural stem cell and progenitor cell transformation [70]. Retroviral transfer of signal transduction proteins, Akt and Ras, in the brain leads to the transformation undifferentiated nestin-expressing progenitor cells into tumors with aggressive features reminiscent of GBM histology. Expression of *ras* and *myc* oncogenes in oligodendrocyte progenitors yields cells that readily form high-grade gliomas when transplanted in vivo [71]. Others have shown transgenic Ink4a-Arf $^{-/-}$ mouse neural stem cells form aggressive GBM-like tumors in vivo when transfected with a constitutively active EGF receptor (EGFR) [72]. In contrast, mature, differentiated mouse astrocytes appear less prone to transformation, although this is still a subject of controversy. In vitro, human astrocytes need three defined transformation events, expression of human telomerase reverse transcriptase, and activation of two oncogenic pathways, for example, ras and Akt [73,74]. Evidence that both astrocytes and neural stem cells can serve as the cell-of-origin for malignant gliomas exists [72]. The combined loss of p16^{INK4a} and p19^{ARF} enables astrocyte

dedifferentiation in response to EGFR activation, while transduction of Ink4a/Arf^{-/-} neural stem cells with constitutively active EGFR induces high-grade gliomas.

Genes involved in cell cycle and apoptosis have been implicated in the proliferation and tumorigenicity of GBM-initiating cells. The HEDGEHOG (HH)-GLI signaling pathway has been shown to control the behavior of human GBM TICs by regulating their self-renewal and tumorigenicity [75]. Another transcription factor, OLIG2, has been shown to promote proliferation of both neural progenitors and GBM-initiating cells, possibly by repressing the p21 tumor suppressor [67]. Jackson and colleagues [76] have shown that amplification of the PDGF signaling in neural stem cells induces the formation of large GBM tumors. Deletion of Nf1 and Trp53 from neural stem cells in mice initiates gliomagenesis in the subventricular zone where neural stem cells reside [77].

Impact of TICs on GBM therapy

Conventional therapies for GBM involving surgery, radiation, and chemotherapy (temozolomide) offer modest benefits to patients with GBM [78]. Virtually, all patients with GBM have a local relapse of their disease at the site of their initial treatment. Current evidence proposes that despite making up a small fraction of tumor cells, GBM-initiating cells are necessary for the initiation and maintenance of tumors (Fig. 3). The presence of a rare subpopulation of cells within GBM possibly responsible for generating the entire mass of tumor cells has important implications for the understanding of the tumor biology and the efficacy of existing therapies. A re-evaluation of our current knowledge needs to be made in light of this new hypothesis.

The last few decades of progress made on the individual parameters that characterize a tumor (proliferation, apoptosis, invasion, angiogenesis, immune resistance, etc.) have all been obtained through analysis of the bulk of the tumor. These studies have been the "guiding principle" for identifying oncogenic pathways for therapeutic targeting. Therapeutic clinical trials that have been performed based on these premises for glioblastoma have largely been a failure. As a result, the question remains as to whether the proper cellular target is missed and whether the signaling pathways driving their transformed state are radically different.

Based on the current TIC hypothesis, the bulk of the tumor is comprised of the differentiated progeny from TICs. Glioblastomas may consist of three broad categories of cells: TICs, cancer progenitor cells (CPGs), and differentiated tumor cells (DTCs) [79,80]. CPGs, also known as progenitor-like glioma cells, may originate from TICs and lead to DTCs by mutational events or differentiation. These are purely speculative observations. The subdivision of the tumor into different cell populations is an attractive hypothesis but needs further substantiation. A hierarchical system of TICs and CPGs may both lead to the development of DTCs. Treatments that target the TIC may be effective at treating the entire tumor mass (Fig. 4). In addition, if the majority of cells contain the genetic alterations that initiated transformation in the neural stem cell population, targeting of these pathways is warranted. Ultimately, targeting GBM-initiating cells may offer better therapeutic strategies that could translate to much better patient survival and quality of life. Several strategies have been shown already to be effective at targeting GBM-initiating cells and causing their demise.

New therapeutic strategies targeting GBM-initiating cells

Recent evidence supports the notion that GBM-initiating cells may be implicated in the resistance of tumors to radiation and chemotherapy [21] (Table 1). Bao et al. suggest that CD133+GBM-initiating cells are resistant to ionizing radiation (IR) because they are more

efficient at inducing repair of damaged DNA than the bulk of tumor cells [20]. They have shown that CD133+GBM-initiating cells escape the lethal DNA-damaging effects of IR by preferential activation of DNA repair checkpoints through phosphorylation of the checkpoint proteins Chk1 and Chk2. In both cell culture and the brains of immunocompromised mice, CD133-expressing glioma cells survive IR in increased proportions relative to most tumor cells, which lack CD133. After irradiation of tumors, CD133+ cells become enriched up to fivefold compared to CD133- cells as a result of apoptosis reduction. The authors further showed that inhibition of the checkpoint kinases, Chk1 and Chk2, with small molecules can reverse the radioresistance of CD133+GBM-initiating cells, suggesting new options for combination radiotherapy using Chk1/2 inhibitors as radio-sensitizers.

Chemoresistance of GBM-initiating cells may also account for glioblastoma tumor progression. Recently, it was shown that GBM-initiating cells are resistant to the currently used adjuvant agent, temozolomide [75]. Several mechanisms of CSC drug resistance have been described in various cancers. Elevated expression of transporters that pump out chemotherapeutic agents may be one important mechanism [24]. In hematopoietic cancers, ATP-binding cassette (ABC) drug transporters have been shown to protect cancer stem cells from chemotherapeutic agents [22]. As mentioned above a distinct SP of cells can be isolated from human tumors and represent a class of CSCs with inherently high resistance to chemotherapeutic agents due to their high drug efflux capacity [23]. SP cells were initially described as a primitive $CD34^{low/neg}$ stem cell population in the normal bone marrow and muscle. This subset of cells was shown to have the unique capacity to efflux lipophilic dyes such as Hoechst 33342 and also to function as stem cells in the tissues from which they were isolated [81,82]. SP cells have been shown to express high levels of the ABC drug transporter genes, ABCG2 and ABCA3, and have been found in glioblastoma cell lines [23].

While these studies highlight the need to develop therapeutic regimens tailored to GBM TIC, such approaches may have unwanted side effects on normal central nervous system (CNS) stem cells. The vulnerability of CNS progenitor cells to chemotherapeutic agents has recently been shown [83]. Clinically relevant concentrations of BCNU, cisplatin, or cytarabine were shown to be more toxic for progenitor cells than glioma cell lines. When administered systemically in mice, these chemotherapeutic agents were associated with increased cell death and decreased cell division in the subventricular zone of the CNS, in the dentate gyrus of the hippocampus, and in the corpus callosum. The vulnerability of normal neural stem cells and their progenitors to chemotherapeutic agents raises the concern of normal neurologic damage when targeting TICs. Novel therapies need to be developed that can penetrate the CNS effectively so as to be able to reach tumor cells deeply infiltrated into the CNS, yet that also show some selectivity in the killing of TIC versus normal CNS stem cell populations so as to avoid induction of neurological deficits.

Differentiation of GBM-initiating cells

Bone morphogenetic proteins (BMPs) are a family of cytokines that can regulate differentiation of normal neural stem cells [44,84,85]. In early embryonic neural stem cells, BMPs can promote both proliferation as well as neuronal differentiation. In contrast, neural stem cells derived from older animals undergo astrocytic differentiation in response to BMPs [86]. The difference in response of neural stem cells appears to be the result of acquisition of a new signaling pathway switch by the older stem cells. Older stem cells express the receptor, BMPR1B, which can activate STAT 3 and promote astrocytic differentiation. Early neural stem cells express BMPR1A and not BMPR1B and therefore are unable to induce activation of STAT3 [44].

Promoting differentiation of GBM-initiating cells by BMPs, specifically BMP4, has been shown to trigger a significant reduction in GBM stem cells [87]. Most CD133+GBM-initiating cells have a functional BMP receptor pathway and exposure to BMP4 resulted in a depletion of these cells in vitro as well as a reduction in their proliferation. Transient BMP4 exposure of engrafted CD133+ tumors in mice reduced tumor growth rate and extended mice survival. The BMP-treated tumor cells engrafted into mice were more mature and less invasive. CD133+ cells could not be recovered from these small tumors, and serial engraftment of mice was unable to be performed with treated tumors. A small number of animals with tumors did not respond to BMP and died 3 months after treatment. More recently, Lee and colleagues [44] have confirmed that BMPs promote glial differentiation of GBM stem cells. However, they were also able to show in one GBM cell line that BMPs failed to induce glial differentiation of GBM-initiating cells, causing instead their proliferation and tumorigenesis. GBM-initiating cells did not induce STAT3-dependent glial differentiation in that cell line due to the epigenetic silencing of BMP1RB by an EZH2-dependent mechanism. In their study, they found that a significant number (20%) of GBM tumor samples had low levels of BMP1RB, and the majority of these samples had hypermethylation of the *BMP1RB* gene promoter. These results provide evidence for epigenetic resistance mechanisms to differentiation therapy and suggest that BMPs in combination therapy with epigenetic modulators may have to be tailored to patients with *BMP1RB* silencing.

GBM-initiating cell niche

Disruption of GBM-initiating cell maintenance by targeting their vascular niche has shown promise in targeted therapy of these cells [56,57]. Clinical trials of the anti-VEGF antibody, bevacizumab, and the pan-VEGF receptor tyrosine kinase inhibitor, cediranib (AZD2171), have demonstrated efficacy in GBM patients [88,89]. The treatment of mice with bevacizumab, after GBM implantation, resulted in a large reduction in the number of GBM-initiating cells and in the growth rate of the tumors [19]. Treatment with bevacizumab did not have much of an effect on the proliferation or survival of most of the cells in the tumors, suggesting that the GBM-initiating cells were targeted. GBM-initiating cells secrete elevated levels of VEGF, which increases endothelial cell migration and tube formation [62]. Treatment of CD133+GBM-initiating cells with bevacizumab blocked their ability to induce endothelial cell migration and tube formation in culture and initiate tumors in vivo. The benefit of anti-angiogenic strategies in combination with conventional cytotoxic drugs has also been shown with rat C6 GBM-initiating cells [61]. Combined, these results suggest that inhibition of blood vessel growth may be an effective method for eliminating GBM-initiating cells.

Perspectives

The discovery of GBM-initiating cells has provided the impetus to rethink our understanding of the growth of glioblastoma and generated interest in developing targeted therapeutics against these cells [19,20,23]. Controversy still exists as to whether the relentless growth of GBM is exclusively driven by the TICs [90] or whether all the cells of a tumor contribute to its growth and regeneration following therapy, including the DTCs [91]. There may well be co-evolution of different cell populations in the tumors, which diverge in their genetic alterations and may co-exist and even be co-dependent (Figs. 4,5, and 6). The picture is further complicated by the recruitment of stromal cells to the tumor, which are known to include vascular cells, immune cells, and even normal neural stem cells [92,93]. Vascular stroma and TIC interactions have been reported, and other TIC stromal interactions remain to be investigated [19]. Such stroma can contribute growth factors and potentially even be modified by adjacent cancer cells through the transfer of oncogenic proteins through microvesicles or exosomes [94-97]. In sum, we are dealing with a very complex multi-

headed target and the heterotypic cell-cell communications between all the tumor elements need to be better understood (Fig. 5). Clearly, the CD133 marker used to define GBM-initiating cells is likely only one of a variety of cell specific markers, and we may have just characterized the tip of the iceberg. If the knowledge acquired with lineage differentiation in blood cancers can serve as a guide, current research needs to develop a nomenclature for GBM-initiating cells and possibly a whole hierarchy of offspring "cancer progenitor" cells (CPG) that is reproducible and consistent. Until a more comprehensive understanding of markers characterizing the different cell populations comprising GBM is acquired, isolation of TICs for study and therapeutic targeting must be performed by harvesting and culturing clonogenic neurospheres with a serum-free culture method utilizing growth factors [43].

The clonal evolution model for tumorigenesis predicts that the genetic alterations in GBM-initiating cells will be all present in the more differentiated tumor cell populations that comprise the bulk of tumors [41]. It also predicts that the cancer cells undergoing terminal differentiation may have acquired more alterations than their precursors (Fig. 4). As discussed above, the initial investigations comparing the biological properties of TICs with the cells derived from the bulk of the tumor have already identified clear differences [20,21,43,44]. These findings highlight the need to reexamine all the biological tenets that we use as current foundation upon which to guide new therapy development. It also brings forth the pressing need to develop new technology to track these cells in patients, as their fate in response to therapy might become a new guiding principle for judging the efficacy of clinical trials (Fig. 4).

Imaging of TICs will be very important in the targeted therapy of these cells in glioblastomas. The ability to monitor the eradication of these cells by magnetic resonance imaging or positron emission tomography imaging will permit a better understanding of the relationship between TICs and tumor response to therapy. Emerging nanotechnologies may permit simultaneous imaging of TICs as well as targeted therapy with conjugated antitumor agents and/or the ability to generate local hyperthermia due to their magnetic properties [98-101].

Immunotherapy may be possible with vaccine peptides that can elicit a natural immune response to TICs [102]. Surface characterization of GBM-initiating cells needs to be further determined to permit the development of peptides derived from TICs that are specific and able to generate an immune response. Use of dendritic cells pulsed with GBM-initiating cells may form the basis of selective immunotherapy against these cells [103]. Such novel approaches will need to carefully examine the cost-benefit ratio of eliminating TIC and possibly normal stem cells in the brain as they are likely to overlap in marker expression.

As a further example, oncolytic therapy agents in the form of viruses [herpes simplex virus 1 (HSV-1), adenoviruses, etc.] may be designed to selectively infect and replicate within GBM-initiating cells [104,105]. Selective uptake of these viruses by TICs will need to be based on viral constructs that can express a ligand specific to TICs.

In summary, the "cancer stem cell" hypothesis has invigorated the neuro-oncology field with a breath of fresh thinking that may end up shaking the foundation of old dogmas, such as the widely held belief that GBM tumors are incurable because of infiltrative disease. If the infiltrated cells are in fact DTCs, their dissemination beyond the surgical boundary may not be the primary cause of tumor recurrence. It is also important to keep in mind that these emerging concepts are still rapidly evolving and questioned. Recent findings ponder the rarity of CSC and suggest that the so-called CSC may simply represent a fraction of tumor cells that can evade the immune system in mice models that are only partially immunocompromised such as the *nu/nu* and NOD/SCID strains [91]. Further work on all

biological fronts will consolidate or deflate the pillars on which we build our current therapies. This is an exciting time for new discoveries and provides new hope for patients who rely on all of us to find a cure for this devastating disease.

Acknowledgments

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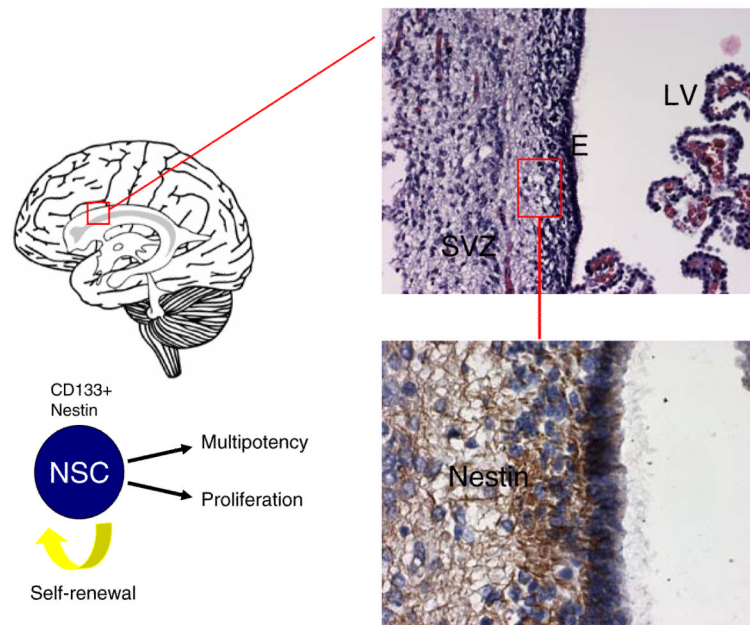
Fig. 1.
Source of neural stem cells and GBM tumors. The subventricular zone (*SVZ*) is found beneath the ependymal (*E*) layer that lines the lateral ventricles (*LV*) of the brain and is believed to be the origin of GBM tumors (upper section). Neural stem cells (*NSCs*) originate from this area of the brain and are characterized by the cell surface markers CD133 and nestin. Immunohistochemistry staining of the SVZ shows the presence of nestin-expressing cells, some of which represent NSCs (lower section). The structures on the right side of the upper section are choroid plexus protrusions from which the CSF filling the ventricles originates
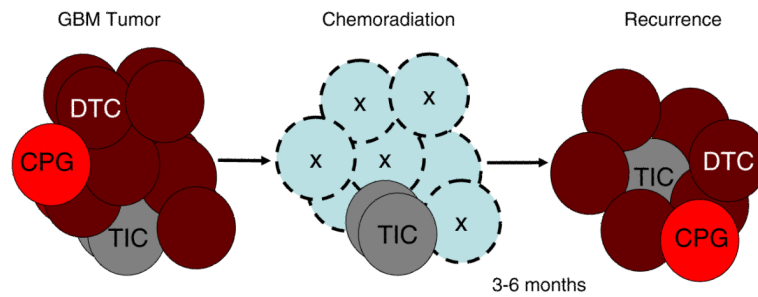
Fig. 2.
Possible lineage relationships for the ontogeny and production of tumor-initiating cells (*TICs*) and generation of GBM tumors. During normal CNS differentiation neural stem cells (*NSCs*) undergo an amplification step to produce transit-amplifying progenitor cells (*TACs*), which then differentiate into committed progenitor cells (*PCs**). PC* (neuronal or glial) retain the capacity of producing progeny along either neuronal or glial lineages (oligodendrocytes and/or astrocytes), but not both. Mutations generating gliomas can occur at all levels within this lineage and produce TICs or differentiated tumor cells (*DTCs*). TICs are thought to be stem-like in behavior by their ability to self-renew, proliferate, and generate DTCs or cancer progenitor cells (*CPG*) within the tumor mass. While this model suggests unilateral progression toward mutated cell populations that become terminally differentiated cancer cells, in reality, there is evidence in the literature for more plasticity, and de-differentiation events may also take place to generate self-propagating cancer cells from astrocytes and oligodendrocytes. It is unknown whether neurons can generate cancer cells

Fig. 3.
TICs as the origin of GBM tumor resistance to therapy. Recurrence of malignant brain tumors in 3-6 months may be related to the resistance of TICs to standard therapies, such as chemoradiation, and their ability to generate new tumors. Note that the bulk of the tumor (shown by *empty circles marked by X*) responds to therapy, but the TICs are unaffected and regenerate the tumor. It is currently unknown whether CPGs may possess the capacity to regenerate the tumor as well. Clearly, this is a simplified model, and different cell populations in the tumor will respond differently to individual therapies, depending on their intrinsic resistance mechanisms; these may include expression of drug transporters, genetic composition, dependence upon specific signaling pathways, aerobic and anaerobic metabolism, and ability to cope with reactive oxygen species (ROS) stress. *TIC* tumor-initiating cell, *CPG* cancer progenitor cell, *DTC* differentiated tumor cell
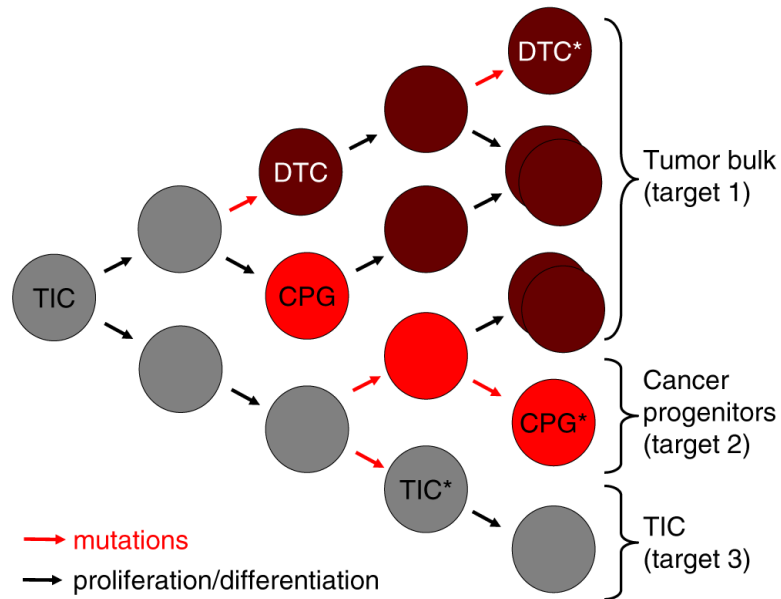
Fig. 4.
Therapeutic intervention for CNS tumors requires targeting of multiple cell types. Treatments for CNS tumors may require targeting of differentiated tumor cells (*DTC*), which likely make up the bulk of the tumor (target 1), cancer progenitor cells (*CPG*; target 2), and TICs (target 3). TICs can give rise to DTCs through mutational events, while TICs may develop CPGs by mutations or differentiation events. CPGs are likely committed to the development of DTCs after proliferation and differentiation. Each of these cell populations may require distinct therapies, either delivered simultaneously or sequentially. Clonal evolution of these cell populations in the tumor is linked to the sequential accumulation of genetic defects (mutations), which will drive cell expansion. Activation of differentiation programs will limit the pluripotency of certain cells but not necessarily their replicative potential. It is currently unknown whether DTC can undergo multiple rounds of cell division or are rapidly terminally differentiated and cease proliferation. This simplified schematic does not exclude the existence of a whole hierarchy of TIC, CPG, and DTC with multiple genetic defects (*asterisk*). It also allows for the coexistence of different cell populations, which may share initial genetic defects but have distinct downstream mutations. Conceivably, such cell populations may even gain a mutual benefit by providing reciprocal cell survival signals. *TIC* tumor-initiating cell, *CPG* cancer progenitor cell, *DTC* differentiated tumor cell
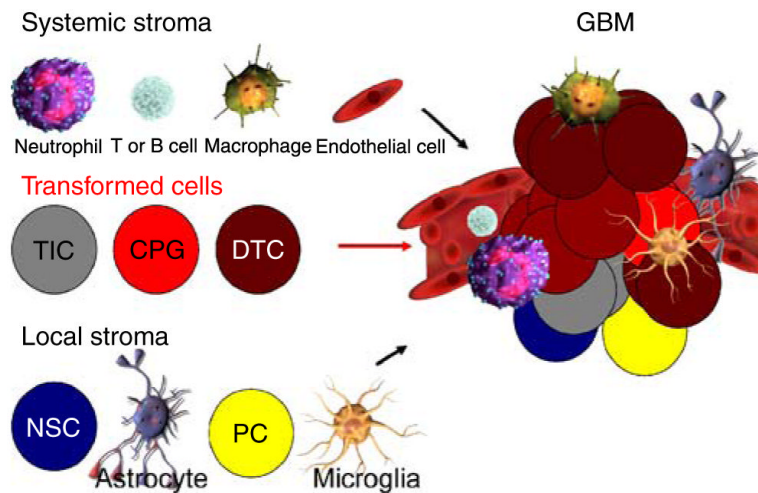
Fig. 5.
Heterogeneous composition of GBM including vascular niche and other cells. The cellular complexity of the tumor is shown, highlighting the fact that any therapy will have to take into consideration all cellular elements of this complex tissue, which likely functions as a self-sustaining and expanding cancer organ growing as a "parasite" of the normal host. Systemic stromal cells (neutrophils, T or B cells, macrophages, and endothelial cells) and local stromal cells (NSCs, astrocytes, PCs, and microglia) are shown to potentially interact with the transformed cells (TICs, CPGs, and DTCs) to form the GBM tumor. The vascular niche provided by endothelial cells is a well-established component to the maintenance and survival of TICs and GBM tumors. It will be the key to determine which stromal cell populations (systemic or local) contribute to tumor initiation and progression and what extracellular signals regulate their recruitment to the tumor. Antagonizing such signals, possibly in the systemic blood circulation, may be a novel approach to therapy. *NSC* neural stem cell, *PC* progenitor cell, *TIC* tumor-initiating cell, *CPG* cancer progenitor cell, *DTC* differentiated tumor cell
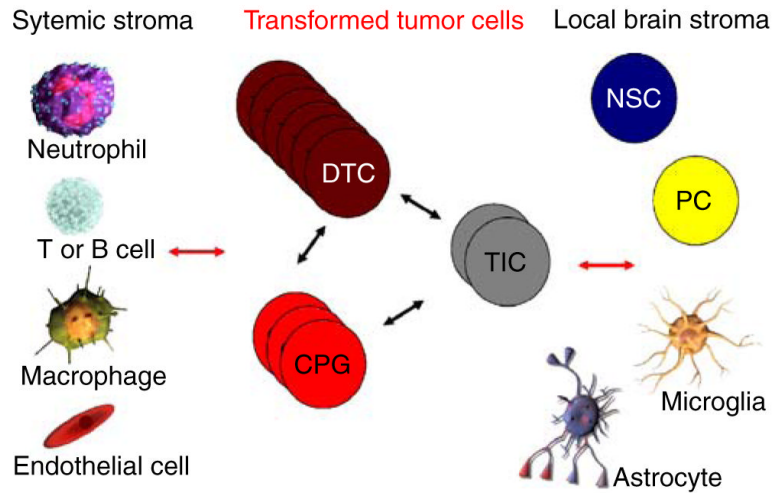
Fig. 6.
Hypothetical interactions between tumor cells and stromal cells. The complexity of heterotypic cell-cell communication in GBM needs extensive study. Are critical signals present which sustain and help the growth of the different tumor cell populations (TIC, CPG, and DTC)? How do these interact with stromal cells, either from the local brain microenvironment or recruited systemically, and are such signals essential for tumor maintenance, progression, and recurrence? Is there transfer of oncogenic proteins from brain cancer cells to normal stroma through secreted vesicles, e.g., oncosomes? Are there tumor suppressor effects from normal stroma onto cancer cells that may be amplified for therapy? *TIC* tumor-initiating cell, *CPG* cancer progenitor cell, *DTC* differentiated tumor cell, *NSC* neural stem cell, *PC* progenitor cell

Table 1

Summary of various therapeutic strategies against TICs in the brain

Technology	Mechanism
BMPs (bone morphogenic proteins)	Promote differentiation of TICs
Anti-angiogenesis molecules (bevacizumab)	Disruption of vascular niche
DNA repair inhibitors (Chk1 and Chk2 inhibitors)	Radiosensitization of TICs
Nanotechnology (magnetic nanoparticles)	Imaging and tracking of TICs; Potential targeted therapy (drug binding or local hyperthermia)
Immunotherapy	Elicit a natural immune response to TICs
Oncolytic therapy (viruses)	Viral constructs specific to TICs may conditionally replicate and lyse cells