Cancer stem cells and brain tumors: uprooting the bad seeds

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The cancer stem cell (CSC) hypothesis is predicated on the idea that not all cells have equal proliferative potential and that, in brain tumors, the cells with the greatest ability to proliferate and contribute to tumorigenesis have phenotypic and functional properties similar to normal neural stem cells (NSCs). Over the past few years, multiple investigators have shown that CSCs isolated from human brain tumors (glioma and medulloblastoma) undergo self-renewal and multilineage cell differentiation, similar to normal NSCs. In addition, CSCs from these tumors, when implanted into rodent brains, generate tumors histologically identical to the parental tumors, suggesting that progenitor/stem cells can fully recapitulate the neoplastic phenotype *in vivo*. While these seminal studies clearly highlight the central role of stem cells in brain tumors, they also evoke important questions regarding the importance of these unique cells to tumor initiation, maintenance and treatment.

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Brain tumors

Tumors of the nervous system represent the leading cause of cancer-related death in children and the fourth leading cause in adults [1]. Despite decades of research and clinical experience, the life expectancy of an adult diagnosed with a high-grade brain tumor is typically less than 1 year [2]. Our ability to develop new therapies for these deadly cancers is highly dependent on an improved understanding of the molecular and cellular changes that contribute to their development and continued growth.

Brain tumors are currently classified by the WHO according to the normal cell type in the brain that the tumor resembles most [3]. Based on this classification scheme, neuropathologists distinguish between glial cell tumors (e.g., astrocytomas, oligodendrogliomas and ependymomas) and neuronal tumors (e.g., medulloblastomas). While the WHO histologic classification system implies a cell of origin for brain tumors, in fact, the cell of origin has not been unequivocally identified for either glial or neuronal cell tumors. Recent studies have suggested that brain tumors are composed of numerous different cell types, including cells resembling neural stem cells (NSCs), that each might contribute to tumorigenesis and continued tumor growth (maintenance). In this article, we will review what is known about brain tumor stem cells relevant to developing new approaches for treating these clinically challenging cancers.

Heterogeneity of brain tumors

It has long been recognized that cancers are composed of heterogeneous populations of different cell types. In this regard, brain tumors comprise a complex microcosm of both neoplastic and non-neoplastic cells that each have distinct roles in dictating tumor formation and growth (FIGURE 1). The non-neoplastic (stromal) compartment contains immune system cells (microglia), entrapped normal cells (neurons, astrocytes, oligodendrocytes) and tumor-associated blood vessels. Taking a page from studies of other solid tumors, recent reports have shown that important cellular and molecular signals that emanate from the brain tumor microenvironment are critical for regulating neoplastic tumor cell growth. In this regard, high numbers of monocyte-like cells (microglia) are consistently found within

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gliomas [4,5] and the number of these cells has been suggested to correlate with tumor growth [6]. Using both *in vitro* and *in vivo* experimental systems, microglia have been shown to provide critical growth signals that promote brain tumor growth [7]. Microglia are a rich source of growth factors and cytokines, including TNF-α, EGF and chemokines, that increase brain tumor proliferation and migration [8,9]. Support for an important role for microglia in brain tumor proliferation derives from recent studies demonstrating that resident brain microglia contribute to brain tumor growth in a mouse model of neurofibromatosis-1 (NF1)-associated optic glioma [10].

In addition, microglia are important generators of chemokines, including stroma-derived factor-1α (SDF-1α or CXCL12). Studies in both medulloblastoma and glioma cell lines have shown that the chemokine, CXCL12, can regulate brain tumor growth in explant models *in vivo* [11,12]. Moreover, CXCL12 expression in the tumor microenvironment may also dictate where and when optic nerve gliomas grow in patients with NF1 [13]. In these studies, the restricted expression of CXCL12 concentrated along the optic nerve and chiasm in young mice parallels the growth pattern of NF1-associated gliomas within the optic nerve and chiasm in children [14]. While no direct evidence currently exists to support a direct role for microglia in cancer stem cell (CSC) growth or function, CXCL12 promotes NSC proliferation [15] and may serve as a homing signal for stem cells during development and cancer [16].

Similar to microglia, tumor blood vessels have also been proposed to regulate brain tumor growth by providing essential nutrients and oxygen [17]. Recent studies have suggested that malignant glioma CSCs release VEGF and promote angiogenesis in the tumor microenvironment [18]. In addition, the tumor vasculature may create a unique stromal niche that maintains stem cells and progenitor cells [19–21]. The finding that endothelial cells produce novel growth factors, including the novel pigment epithelium-derived factor, which supports normal stem cell self-renewal, provides a molecular mechanism underlying the unique tropism of NSCs for the vascular niche [22].

Cancer stem cells

The neoplastic compartment of brain tumors contains both progenitor (stem) cells and differentiated cells. Recent studies have focused on CSCs, based on experiments that suggest that these cells might be critical for generating or maintaining tumors. CSCs have been successfully identified and isolated in various cancers, including leukemia [23] and breast [24], pancreatic [25], head and neck [26], colon [27] and prostate cancers [28]. Several studies report that CSCs comprise a small percentage of the total cells in brain tumors, ranging from less than 1% in low-grade brain tumors to as many as a quarter of all cells in high-grade brain tumors using sphere formation and clonogenic assays [29–32]. However, only a small population of CSCs in solid cancers is clonogenic, and only one in 1000 to one in 5000 lung cancer, ovarian cancer or neuroblastoma CSCs can actually generate colonies in soft agar [33].

In brain tumors, CSCs, like their non-neoplastic NSC counterpart, are defined by their functional properties. Normal NSCs have the capacity for proliferation, self-renewal and the generation of multiple terminally differentiated cell types. CSCs isolated from human brain tumors can be maintained as neurospheres, similar to NSCs, and can generate new neurospheres from a single CSC (self-renewal) [30–32,34–36]. In addition, CSCs are also capable of differentiating into cellular lineages that arise from normal

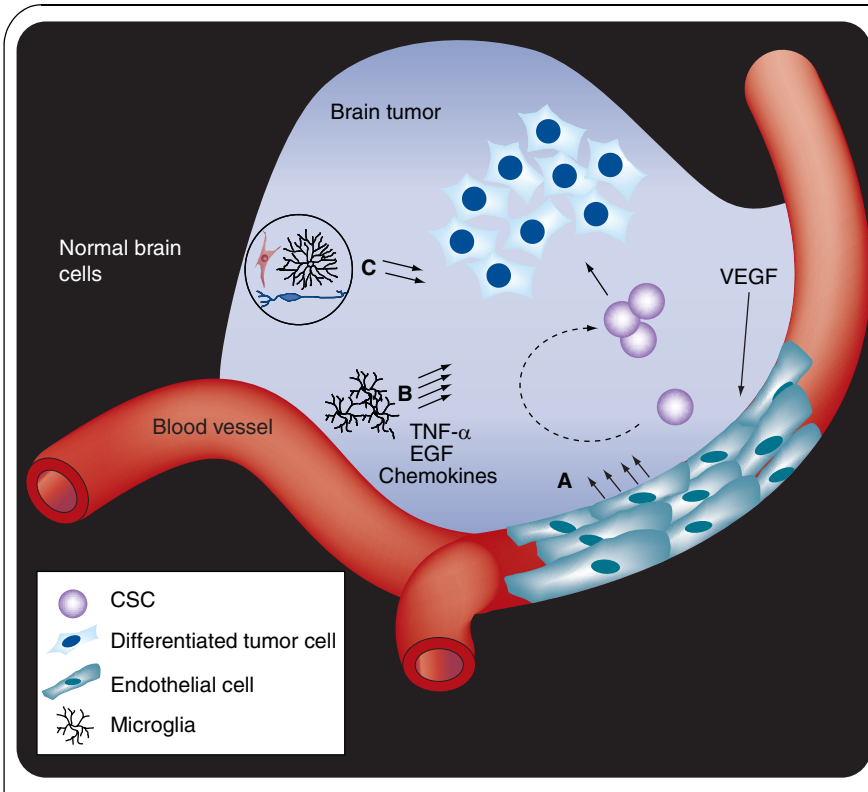

Figure 1. Tumor microcosm. Brain tumors are composed of heterogeneous populations of both neoplastic cells and non-neoplastic cells. The non-neoplastic cell population contains microglia (monocyte-like cells), endothelial cells ('angiogenic niche') and normal brain cells. (A) CSCs in the angiogenic niche receive signals which induce CSC self-renewal (generation of new CSCs) or differentiation into more mature cancer cell types. (B) Microglia increase tumor growth and migration by releasing growth factors (e.g., EGF), cytokines (e.g., TNF-α) and chemokines (e.g., CXCL12). (C) Normal brain cells (neurons, oligodendrocytes and astrocytes) in the tumor microenvironment also promote tumor growth. In addition, brain tumors express high levels of VEGF, which positively regulate endothelial cell proliferation. The dotted line denotes CSC proliferation and self-renewal.
CSC: Cancer stem cell.

NSCs, including neurons, astrocytes and oligodendrocytes *in vitro* (multilineage differentiation), even though they are not functionally normal brain cells [29,30,34,35].

In addition to functional properties, immunocytochemical markers have also been used to identify NSCs and CSCs, including nestin [30,37,38], CD133 [29,30,32], Musashi-1 [30], Sox2 [30,39], brain lipid-binding protein (BLBP) [31,40] and maternal embryonic leucine zipper kinase (MELK) [30,41]. Of all these potential proteins, the most frequently used immunomarker is the cell surface protein, CD133.

Using CD133 to isolate brain tumor CSC populations, several investigators have shown that $CD133^+$ cells exhibit a greater ability than $CD133^-$ cells to form tumors in immunocompromised rodent brains *in vivo* [29,32,42]. $CD133^+$ CSCs were also capable of forming brain tumors at low cell concentrations, consistent with their high self-renewal capacity [36], as well as recapitulating the original tumor *in vivo* even after serial transplantations [30,32,34,43]. However, it is worth noting that a recent study demonstrated that secondary glioblastomas do not contain CD133-expressing cells, and that brain tumor stem cells in these tumors do not express CD133 [42].

Additional evidence for a direct relationship between NSCs and tumorigenesis in the brain derives from elegant studies by Ligon and colleagues [44]. They report that the CNS-restricted transcription factor, Olig-2, regulates the proliferation of neural progenitors as well as glioma-derived CSCs by modulating the function of $p21^{WAF/CIP1}$. Moreover, they demonstrate that Olig-2 is required for glioma formation from NSCs. These exciting findings suggest that CSCs are critical for gliomagenesis.

NSCs live in specialized niches, typically in the subventricular zone, the lining of the ventricles and the hippocampal dentate gyrus [45–47]. These niches are important for maintaining NSCs and for providing extrinsic factors that control cell proliferation and cell fate [22,48,49]. Similar to NSCs, Calabrese and colleagues demonstrated that $nestin^+/CD133^+$ brain tumor CSCs are maintained within vascular niches [19]. They also found that endothelial cell-derived factors in these specialized locations support human brain tumor xenograft growth in mice.

Growth control pathways

Some of the extracellular stromal signals that regulate cell proliferation and fate in normal NSCs are often co-opted during tumor formation to allow the tumor cell to escape normal growth regulation. The most frequently reported mutations involve three important signaling pathways specifying neuroglial cell specification and proliferation: the Sonic hedgehog (SHH), Wnt and receptor tyrosine kinase (RTK) signaling pathways (FIGURE 2).

The identification of SHH pathway signaling abnormalities in brain tumors derives from initial studies of the inherited basal cell nevus cancer predisposition syndrome (Gorlin syndrome) in which a small proportion of affected patients develop medulloblastoma. Gorlin syndrome is caused by a germline mutation in the SHH receptor, PATCHED1 (PTCH) gene. Analysis of sporadic medulloblastomas revealed *PTCH* inactivation in approximately 20% of tumors, and mice engineered to harbor a germline *Ptch* mutation develop medulloblastoma [50,51]. Other proteins involved in SHH signaling are also mutated or deregulated in brain tumors, including smoothened (SMO), the glioma-associated oncogene homologue 1–3 (*Gli1*, *Gli2*, *Gli3*) transcription factors and suppressor of fused (SUFU) [52–55]. During normal cerebellar development, Purkinje cell-derived SHH regulates the proliferation of granule precursor cells in the external granule layer (EGL) and stem cells in the developing neocortex [53,56,57]. The SHH–Gli pathway also influences foliation during cerebellum development by regulating the position and/or size of the lobes [58,59]. Increased *SHH–Gli* pathway signaling not only enhances NSC proliferation and neurogenesis, but is also necessary for brain tumorigenicity and growth [50,60,61].

Similarly, defects in Wnt/β-catenin pathway signaling in brain tumors were initially identified by studying patients with another inherited cancer predisposition syndrome, Turcot syndrome. Patients with Turcot syndrome develop medulloblastoma as a result of a germline mutation in the adenomatous polyposis coli (*APC*) gene [62]. Although mutations in the *APC* gene are rare in sporadic medulloblastoma, some tumors will harbor mutations in the β-catenin or Axin genes [63,64]. The Wnt/β-catenin axis is important for specifying the midbrain–hindbrain boundary during development [65,66], and controls NSC proliferation and self-renewal [67,68].

RTK signaling through the EGF and PDGF receptors is a prime driver of NSC proliferation and differentiation. RTK activation leads to downstream activation of several key signaling molecules, including RAS and AKT. Although activating mutations in RAS are rare in human brain tumors, RAS can be activated by several mechanisms, including increased expression of PDGF receptors, mutational activation of the EGF receptor, and loss of the *NF1* tumor suppressor gene. Mutational activation and overexpression of the EGF receptor is observed in a third of high-grade gliomas, and cooperates with other genetic changes to promote gliomagenesis and malignant progression [69,70]. Similarly, PDGF receptor-expressing cells in the adult subventricular zone are NSCs that can form glioma-like growths in response to ectopic PDGF expression [71]. The importance of PDGF signaling in gliomagenesis is underscored by several additional observations. First, PDGF autocrine signaling regulates glioma survival [72]. Second, PDGF expression in $nestin^+$ progenitor cells results in glioma formation in mice *in vivo* [73]. Finally, a transforming PDGF receptor mutant has been identified in a high-grade glioma [74].

Children with the NF1 inherited tumor predisposition syndrome are prone to the development of low-grade gliomas. The *NF1* tumor suppressor gene encodes a large cytoplasmic protein, termed neurofibromin, which negatively regulates RAS in astrocytes and other neural cell types. Moreover, neurofibromin controls NSC proliferation and self-renewal in a gene dose-dependent fashion *in vitro* [75]. Loss of neurofibromin expression in

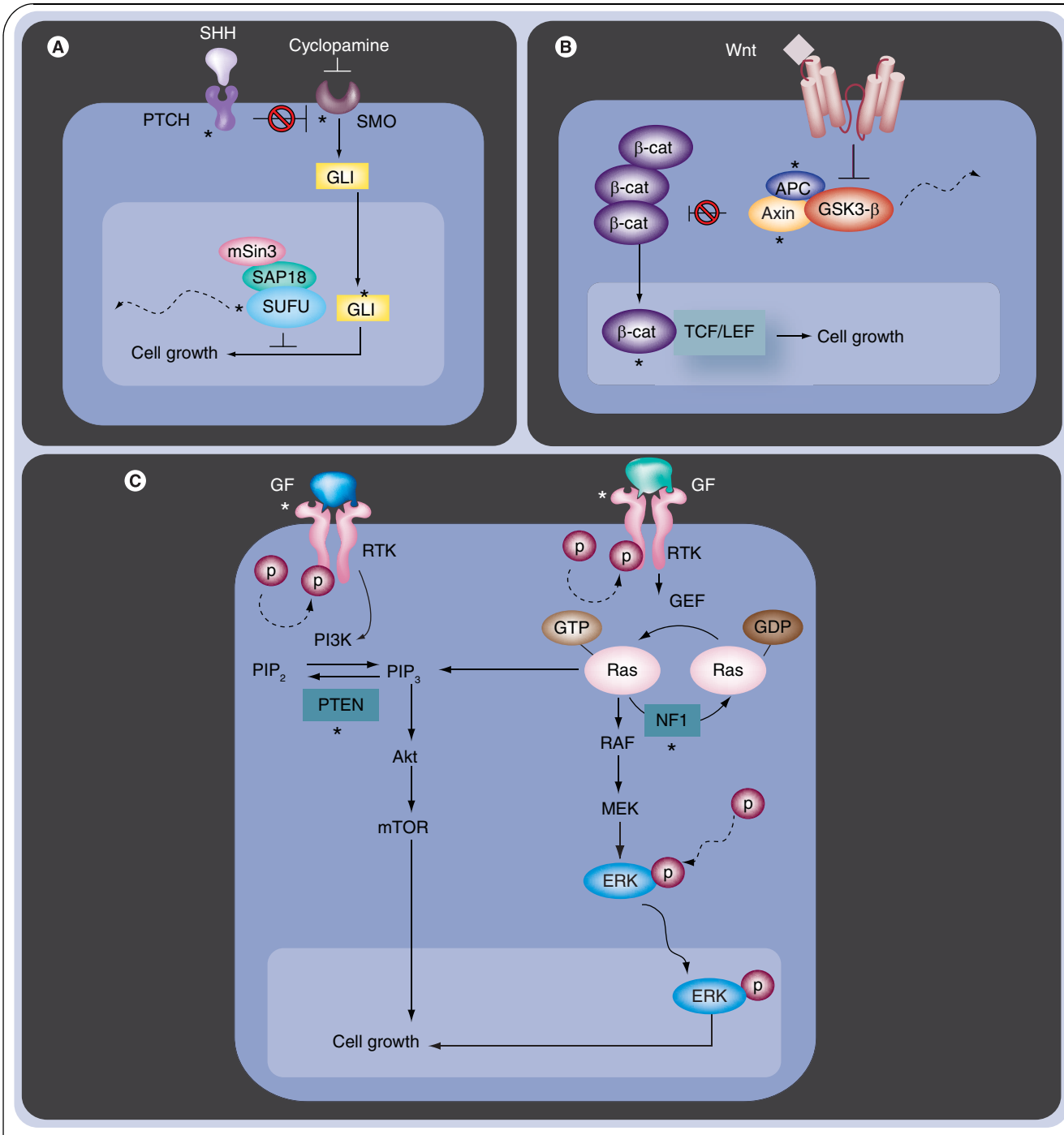

Figure 2. Signaling pathways involved in both neural stem cell proliferation and tumorigenesis. Signaling pathways that involve SHH, WNT and RTKs activated by various GFs (e.g., EGF and PDGF) regulate normal neural stem cell (NSC) self-renewal, as well as brain tumor growth. **(A)** Mutations in the *PTCH*, *SMO* and *SUFU* genes are frequently observed in medulloblastoma (*). PTCH mutations lead to hyperactivation of SMO, resulting in increased GLI expression. SUFU acts as a repressor of GLI, and increased GLI function promotes cell growth. The dotted line denotes dissociation of SUFU from the signaling complex. **(B)** WNT signaling regulates cell growth by controlling β-cat levels and β-cat nuclear translocation. The protein complex containing Axin, APC and GSK-3β acts as a negative regulator of β-cat by promoting β-cat degradation. Mutations in Axin, APC and β-cat have been reported in medulloblastoma (*). The dotted line denotes dissociation of GSK-3 from the signaling complex. **(C)** RTK signaling initiated by the binding of mitogenic GFs (e.g., EGF and PDGF) increase proliferation in both normal NSCs and brain tumor cells. RTK binding by GFs leads to activation of several downstream signaling pathways that regulate cell survival and proliferation, including the Ras, Akt, mTOR and MAPK signaling intermediates. PTEN and NF1 function as tumor suppressors and inhibit signaling through these RTK pathways. Brain tumors (gliomas) have been reported to harbor mutations in the *PTEN*, *NF1* and *EGFR* genes or overexpress the PDGF receptor (*). The dotted lines denote protein phosphorylation.

β-cat: β-catenin; APC: Adenomatous polyposis coli; GEF: GTP exchange factor; GF: Growth factor; GLI: Glioma-associated oncogene homolog; GSK: Glycogen synthase kinase; LEF: Lymphoid enhancer-binding factor; NF: Nuclear factor; NF1: Neurofibromatosis 1 tumor suppressor, neurofibromin; P: Phosphate; PI3K: Phosphoinositide 3-kinase; PIP_2: Phosphatidylinositol-4,5-bisphosphate; PIP_3: Phosphatidylinositol-3,4,5-trisphosphate; RTK: Receptor tyrosine kinase; TCF: T-cell-specific transcription factor.

NSCs results in delayed maturation of both glial and neuronal cell lineages *in vivo* [40]. Moreover, glial progenitor *Nf1* inactivation results in low-grade glioma formation in the optic nerve and chiasm of genetically engineered mice *in vivo* [76,77].

Similarly, AKT activation does not result from mutational activation, but most often reflects loss of the phosphatase and tensin homolog (PTEN) tumor suppressor gene. PTEN regulates NSC proliferation *in vitro* and *in vivo* [78,79], but inactivation or loss of this gene accelerates high-grade glioma development *in vivo* [69,80].

Finally, many intracellular growth control pathways converge on cell-cycle growth regulators, including the p53 and cyclin-dependent protein kinase genes. High-grade gliomas frequently harbor mutations in the *TP53* (p53) tumor suppressor gene and *INK4a* (p16) cyclin-dependent kinase gene [81]. p53 functions as a negative regulator of self-renewal, proliferation and survival of NSCs *in vitro* [82]. Similarly, p16 is important for regulating NSC self-renewal and proliferation in the CNS [83,84]. Cooperativity between the *p53* and *Rb* pathway genes in promoting gliomagenesis has been demonstrated in genetically engineered mice in which loss of *Nf1* and *p53* or *Rb* and *p53* occurs [85–87].

Region-specific neural stem cells

A number of recent studies have suggested that NSCs in the brain are not homogeneous cellular populations, such that NSCs from different brain regions have different biological properties (self-renewal, proliferation and differentiation potential) [88–91]. In this regard, NSCs from the E14.5 mouse midbrain–hindbrain exhibit greater self-renewal and lower neurogenic potential than identical-appearing NSCs isolated from the cortex. Forebrain-derived NSCs exhibit greater growth capacity than those isolated from the spinal cord or cerebellum, while optic nerve NSCs have the lowest capacity for self-renewal.

In addition to these differences in functional properties, NSCs from different brain regions also harbor brain region-specific gene expression patterns [88]. These brain region-specific genetic signatures are also retained in normal glia as well as glial tumors originating from these different brain regions [31,92]. These results suggest that NSCs from different brain regions are unique and give rise to differentiated progeny and tumors that harbor these distinct molecular profiles. These data also support the hypothesis that heterogeneity among pilocytic astrocytomas (PAs) based on brain region might be the result of innate functional differences in NSCs from different brain regions. It is tempting to speculate that these genetic signatures represent hard-wired gene expression programs that modify the ability of normal and preneoplastic cells in specific brain regions to respond to tumor-causing genetic changes and microenvironmental signals [93].

Expert commentary & five-year view

While it is not clear whether brain tumors originate from CSCs or are simply maintained by these progenitor cells, emerging evidence suggests that CSCs have unique properties that might make them particularly resistant to conventional therapy. The current standard of care for many types of brain tumors includes radiation and chemotherapy. However, most high-grade brain tumors recur after treatment or fail to respond to initial therapy. Several groups have suggested that radiation-resistant stem cells in brain tumors may account for the observed resistance to conventional therapy.

One of the unique features of CSCs is an innate resistance to radiation and chemotherapy drugs [18,94–97]. In particular, the $CD133^+$ subpopulation is highly resistant to ionizing radiation in human glioma xenografts and can repair DNA damage more effectively than $CD133^-$ tumor cells [94]. In addition to relative radiation resistance, CSCs exhibit high levels of expression of multiple drug-resistance genes compared with the differentiated bulk tumor [18,96–98] and exhibit significant resistance to temozolomide, etoposide, carboplatin and paclitaxel [97].

As we learn more regarding the function of CSCs in tumorigenesis and maintenance, future therapeutic options may include specifically targeting the CSC by inducing CSC differentiation, eliminating CSCs and/or depriving the CSC of the normal regulatory cues that derive from the brain tumor microenvironment (FIGURE 3). Differentiation therapy could theoretically induce the loss of CSC self-renewal properties in brain tumors [99] and result in loss of the 'tumor-maintaining' cell. One of these anticancer drugs that can induce differentiation in tumors is retinoic acid (RA; vitamin A). All-*trans* RA is an effective drug for acute promyleocytic leukemia [100] and can induce differentiation in teratocarcinomas and melanomas [101]. Similarly, bone morphogenetic protein (BMP)-4 [102] and cannabinoids [103] also promote neural differentiation of stem-like, tumor-initiating precursors in human glioblastomas (GBMs). Another CSC-targeting therapy is to eliminate CSCs and/or target the key molecular signaling pathways that drive self-renewal, proliferation and cell-fate decisions in CSCs. Previous studies have shown that SHH pathway inhibitors can inhibit medulloblastoma growth in mice, whereas inhibition of the Notch pathway with γ-secretase inhibitors blocks CSC self-renewal and decreases mouse medulloblastoma tumor growth [104–106]. Future strategies might also take advantage of targets that derive from the analysis of gene expression profiles of tumorigenic CSCs relative to normal NSCs. Finally, strategies that target cellular and molecular signals that emanate from the tumor microenvironment and promote CSC self-renewal and proliferation might also be effective. In this regard, inhibiting VEGF and its receptor [107] or microglia function may deprive CSCs of the necessary trophic factors required for their maintenance. With continued advances in CSC biology, it is likely that additional treatments will be identified that specifically target the cell(s) most responsible for brain tumor growth.

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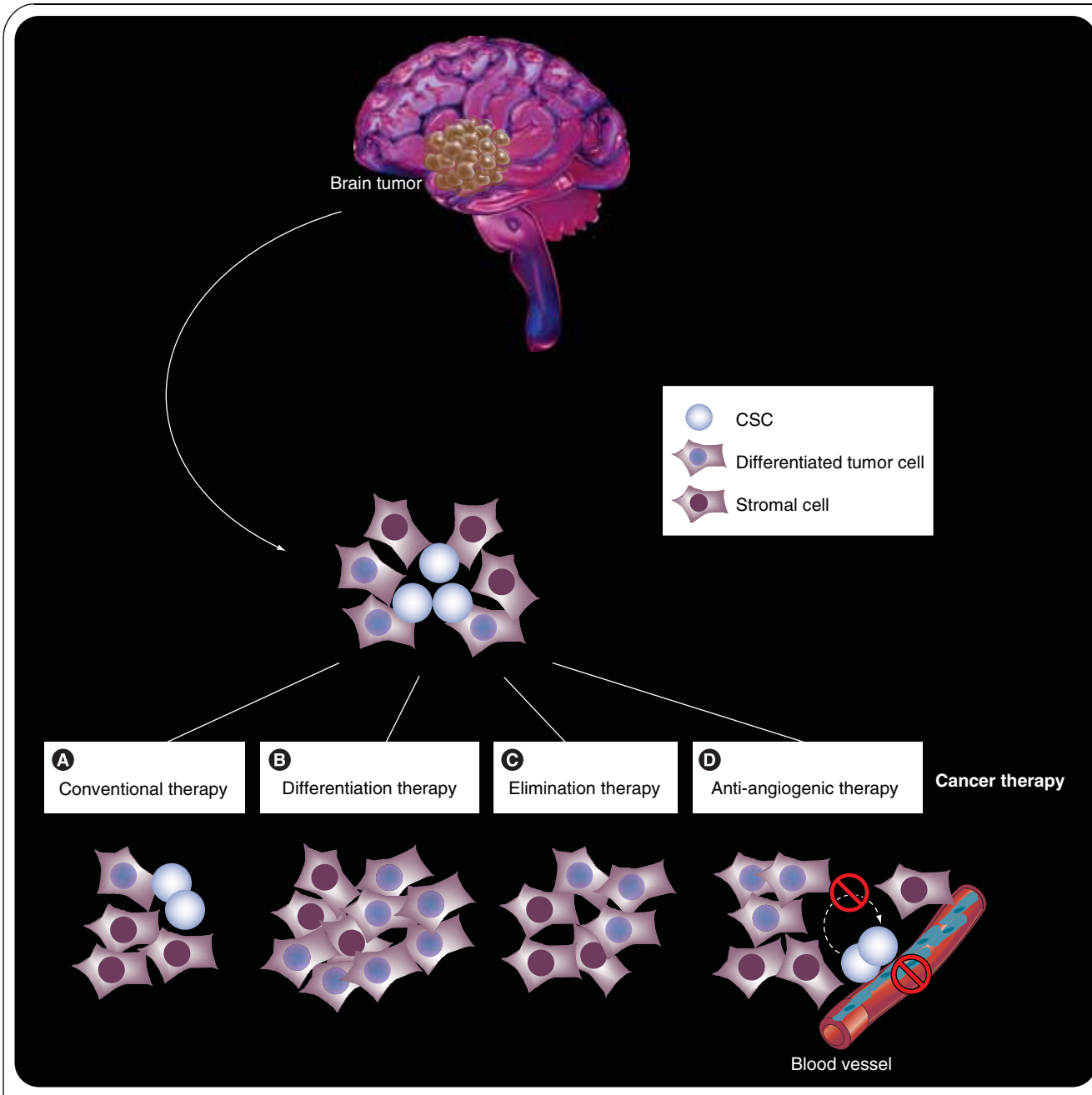

Figure 3. Potential cancer therapies. Future therapies for brain tumors might target other cell types in the tumor microcosm, including CSCs and stromal cells (endothelial cells and microglia). (A) Conventional radiotherapy and chemotherapy result in a reduction in the overall tumor mass by killing cells with limited proliferative potential (e.g., differentiated tumor cells); however, this effect is transient and tumors typically recur when the surviving CSCs give rise to new differentiated tumor cells. (B) Differentiation therapy promotes CSC terminal differentiation resulting in a tumor composed predominantly of differentiated cancer cells that may be more sensitive to conventional therapy. (C) Elimination therapy is designed to kill the CSCs within the tumors. In the absence of these tumor-maintaining CSCs, conventional therapy may provide a more durable response. (D) Anti-angiogenic therapy targets the tumoral blood vessels, disrupting the angiogenic niche in the brain tumor. As a result, the CSCs lose their ability to undergo self-renewal and instead give rise to terminally differentiated tumor cells that are more likely to respond to conventional therapies. Similar strategies that eliminate tumor-promoting signals from microglia might also be effective as adjuvant agents in reducing tumor growth. The dotted line denotes CSC proliferation and self-renewal.
CSC: Cancer stem cell.

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Key issues

- Brain tumors are composed of heterogeneous populations of neoplastic and non-neoplastic (stromal) cell types.
- Cells with stem cell-like properties can be isolated from human brain tumors and can recapitulate the original tumor when explanted into naive recipient rodent brains.
- Cancer stem cells, like normal neural stem cells, are capable of self-renewal, proliferation and multilineage differentiation.
- Cancer stem cells, like normal neural stem cells, reside in specialized niches that regulate their growth and differentiation.
- Signals that control normal neural stem/progenitor cell growth and differentiation are often mutated in human brain tumors.
- Neural stem/progenitor cells from different brain regions have unique biological properties that might dictate their response to cellular and molecular signals.
- A more complete understanding of the cellular components of brain tumors will provide unique opportunities to develop targeted therapies for these deadly cancers.

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