

Twisted tango: brain tumor neurovascular interactions

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The brain is a complicated organ with complexity derived from cellular and microenvironmental interactions. Similarly, brain tumor cells actively modify and are regulated by their microenvironment. Brain tumors are highly heterogeneous and frequently show a cellular hierarchy with self-renewing tumorigenic brain tumor stem cells (BTSCs) at the apex. Although BTSCs are distinct from neural stem cells, they share characteristics, including bidirectional interplay with supportive vasculature critical for maintenance of undifferentiated states and survival. BTSCs stimulate angiogenesis through growth factor secretion and are enriched in perivascular niches. Microenvironmental conditions, including hypoxia, drive expression of stem cell genes and proangiogenic factors, further linking cellular hierarchy regulation and instructive stromal elements. BTSCs may also directly contribute to tumor vasculature through plasticity toward an endothelial lineage. Interrogating the codependence of BTSCs and the perivascular niche may directly inform clinical approaches for brain tumor therapy through targeting of highly angiogenic and tumorigenic cellular subsets.

Research has advanced our understanding of the most prevalent malignant brain tumors (glioma, medulloblastoma and ependymoma), yet clinical translation has not resulted in a substantial change in survival. Among patients with the most frequent adult primary brain tumor, glioblastoma (GBM), median survival with the best available treatments is only 15 months¹. Although these statistics highlight the devastating nature of the disease and the need for new types of treatment, optimism for future development of targeted therapies has experienced a recent surge because of an increased understanding of the complexity of brain tumors. Molecular differences between brain tumor subtypes^{2–5}, among cells within the same tumor^{6–11}, and between tumor microenvironments^{12–16} are becoming increasingly apparent. Subsets of brain tumor cells called brain tumor stem cells (BTSCs) with increased tumor propagation capacity, due in part to the ability to drive angiogenesis and resist therapies, have now been identified^{8–18}. The localization of some BTSCs to a perivascular niche^{12–14} has permitted elucidation of critical interactions and signaling pathways between BTSCs and endothelial cells that can now be targeted. Here we have sought to define the partnership between BTSCs and components of the perivascular niche and discuss how some new therapies in clinical trials against glioma may target these interactions.

Know your partner: defining BTSCs

BTSCs that can selectively give rise to tumors upon transplantation into immunocompromised mice and have some characteristics of normal stem cells, including the expression of progenitor markers, have now been identified by several groups^{6–11}.

These cells have been, and must continue to be, functionally validated to have elevated self renewal, proliferation and tumor propagation in comparison to non-stem tumor cells. In addition to these required functional characteristics, BTSCs may have an increased capacity to promote angiogenesis, survive radio- and chemotherapy, invade normal brain and suppress the immune system when compared to non-stem tumor cells^{10,11,17–20} (Fig. 1).

The characterization of BTSCs has relied on the enrichment and prospective isolation of tumor populations by flow cytometry, with enrichment through neurosphere formation sometimes used as an alternative. Markers including CD133 (refs. 8,10,11), A2B5 (refs. 21,22), CD171 (L1CAM)²³, CD15 (SSEA1)^{24–26}, CD49f (integrin- α_6)²⁷, CD44 (ref. 28) and epidermal growth factor receptor (EGFR)²⁹ have been used to enrich for BTSCs, with subsequent functional assessments including neurosphere formation and tumor propagation capacity. Although experimental results are supportive, controversy regarding the existence of BTSCs is fueled by a lack of consistency with regards to enrichment methods. No single universal BTSC enrichment marker has emerged as the gold standard, which is likely due to differences in cell isolation and propagation, the high degree of inter-tumoral heterogeneity, or differences in glioma subtypes. Data also demonstrate that neurospheres are not always clonal or specific for stem cells and can be difficult to quantify in terms of numbers and proliferation, suggesting that selection based on neurosphere formation is not ideal^{30,31}. However, markers (which often overlap) have been used to segregate functionally distinct tumor cell populations in which one tumor cell fraction has the potential to give rise to a heterogeneous progeny, demonstrating a cellular hierarchy. Evidence that neurosphere formation can be a prognostic factor for clinical outcomes in glioma also suggests that BTSC functional analyses can have clinical relevance³². Together these data demonstrate that, although current methods of BTSC isolation have advantages, there are considerable limitations of relying on only one marker or phenotype (such as neurosphere formation) without further analysis of functional properties, including tumor propagation capacity.

In addition to marker enrichment, several other areas of the BTSC field remain contentious. Whereas the proportion of stem cells

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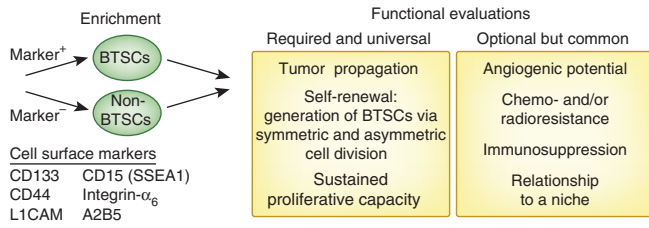


Figure 1 Learning the steps: isolation and characterization of BTSCs. BTSCs from malignant tumors (glioma, medulloblastoma, ependymoma) can be enriched on the basis of cell surface expression using flow cytometry with markers including but not limited to CD133, A2B5, CD171 (L1CAM), CD15 (SSEA1), CD49f (integrin- α_6), CD44 and EGFR. Upon enrichment, hierarchy should be validated by functional assays of tumor propagation. BTSCs often have cellular phenotypes associated with the promotion of angiogenesis, therapeutic resistance, immune evasion and niche interactions that are elevated in comparison to non-stem tumor cells.

remains low in many normal adult somatic tissues and BTSCs have often been found to be a minority of glioma cells, there is no requirement for a limited frequency of BTSCs within a tumor. As a tumor is similar to an aberrant organ with uncontrolled growth, it could be feasible to have a sizable fraction of BTSCs, or an as yet unidentified progenitor cell population, in the tumor. The proliferative nature of this BTSC subset also remains an area of debate and significant interest. Whereas normal stem cells are typically quiescent until signaled to proliferate when needed (such as during injury), tumors are rapidly dividing entities, indicating there may be no requirement for BTSCs to be quiescent. However, recent work has identified a population of dye-retaining BTSCs³³, suggesting that a quiescent population may be present in gliomas and that it may be necessary to further differentiate between stem and progenitor populations within gliomas. It is, finally, important to note that the BTSC theory does not attempt to explain the cell of origin of any brain tumor: a stem cell could be mutated to become a cancer, but this is not a requirement of the BTSC theory. Rather, the theory suggests that activation of growth and self-renewal pathways (often used by normal stem cells) occurs in a portion of tumor cells that have an increased capacity to promote cancer growth. As its natural extension, targeting BTSC signaling or biology should permit greater tumor control.

As the concept of BTSCs has emerged, comparisons have been made to neural stem cells (NSCs). BTSCs and NSCs have similar markers, and both have the capacity to form serially passaged neurospheres in cell culture. Some biological properties of NSCs important for normal physiological responses are also similar to those of BTSCs. For example, BTSCs often drive tumorigenesis by elevating angiogenic factor production, and NSCs produce VEGF and other angiogenic factors to promote revascularization of neural tissue in disease models. Some of these models require minimal immunosuppression, demonstrating that transplanted NSCs do not trigger strong immune responses, and BTSCs are proposed to modulate the immune system to promote tumor growth. BTSCs may also promote tumor recurrence owing to their ability to preferentially survive chemo- and radiotherapy compared to non-stem tumor cells. Similarly, NSCs have been shown to be more resistant to several forms of cellular stress when compared to more differentiated cells³⁴. Although data suggest some properties of BTSCs and NSCs are comparable within their different physiological contexts, there are important differences between BTSCs and NSCs. Unlike NSCs, BTSCs form tumors in animal models and have genetic alterations inherent to cancer. BTSCs have molecular pathways distinct from NSCs, pathways that contribute to tumor growth and could

therefore be targeted for therapeutic interventions. For example, the nitric oxide synthase NOS2 has been found to be highly expressed in BTSCs isolated from human gliomas but to be minimally expressed in NSCs³⁵. Targeting of NOS2 activity with small molecule inhibitors decreases the growth of BTSCs but not NSCs. These results and others strongly suggest that elucidating the similarities and differences between BTSC and NSC molecular pathways and biologies will be critical for developing anti-BTSC therapies.

Perivascular partner: microenvironmental BTSC maintenance

Staining of human GBM specimens indicates that cells positive for BTSC markers are often located adjacent to the tumor vasculature, which is regional within GBM^{13,27,36}. This localization suggests that molecular signals and microenvironmental factors present in the perivascular niche promote BTSC maintenance, and endothelial cells have been shown to promote BTSC self-renewal³⁷. Cell-cell interaction-dependent and paracrine pathways from the vasculature that activate the BTSC phenotype are now being defined. When the effect of these niche signals is combined with the known plasticity of cancer cells, models suggest that the population of BTSCs within a tumor is not fixed but likely fluctuates depending on several factors, including proximity to the perivascular niche.

NSCs rely on interactions with the extracellular matrix (ECM) for stem cell maintenance³⁸, suggesting that the ECM is a critical component of the perivascular niche that maintains BTSCs. The exact composition of the perivascular ECM in gliomas has not been defined, and there are differences between research groups in the exact laminin chains found to be expressed in brain tumors. However, the vessel basement membrane has been shown to express laminin chains including, but not limited to, α_2 , α_4 , β_1 and γ_1 (refs. 39,40). Some data also suggest differences in laminin expression depend on brain tumor grade and are linked to poor patient survival³⁹. Several forms of laminin are recognized by the receptor integrin- α_6 , which is highly expressed by BTSCs³⁸. As targeting of integrin- α_6 decreases BTSC survival and tumorigenic potential³⁸, contact of integrin- α_6 -positive BTSCs with laminin in the perivascular niche is likely to facilitate BTSC maintenance. Indeed, addition of laminins to isolated BTSCs promotes their propagation in cell culture⁴¹. Whether further components of the perivascular ECM similarly support the BTSC phenotype remains to be determined but seems likely.

A paracrine pathway that links endothelial cells to BTSC biology involves nitric oxide. Several studies have suggested a protumorigenic role for NO, and subjects with glioma have elevated levels of endothelial nitric oxide synthase (eNOS)⁴². NO produced by eNOS derived from the perivascular niche has been suggested to promote glioma growth in a mouse model of GBM³⁶. Targeting NO production pharmacologically with a pan-NOS inhibitor or genetically through loss of eNOS increases survival in a mouse GBM model³⁶, suggesting a critical role for paracrine NO from the vasculature in supporting glioma growth. Although we have focused on the interaction of endothelial cells and BTSCs in this review, it is important to note that BTSCs may activate similar autocrine signals to support their own growth. For example, we recently demonstrated that NO produced by inducible nitric oxide (iNOS) in BTSCs is critical for BTSC and glioma growth in xenograft models³⁵. Thus, NO produced by endothelial cells or BTSCs is a vital factor regulating BTSC maintenance.

In the studies of NO production from endothelial cells in mouse models, Notch activity in the perivascular niche was shown to be a major regulator of effects on BTSCs³⁶. Indeed, endothelial cells are known to produce Notch ligands⁴³, and Notch can increase some characteristics of BTSCs^{44,45}. In studies with human BTSCs whose



growth is supported by endothelial cells, targeting Notch signaling decreases BTSC and endothelial cell growth³⁷. Loss of endothelial cell support of BTSCs mimics Notch inhibition, strongly suggesting that endothelial cell-driven Notch signaling mediates BTSC maintenance in the perivascular niche³⁷. However, targeting of Notch in isolated BTSCs also results in decreased self-renewal and increased differentiation, demonstrating the potential benefit of anti-Notch-based strategies in the absence of a perivascular component^{46,47}. Targeting Notch may also increase the sensitivity of BTSCs to radio- and chemotherapy^{48,49}. Thus it is important to determine the pathways (in addition to NO) that modulate Notch signaling both inside and outside the perivascular niche. In addition, the repertoire of Notch ligands and receptors present in the perivascular niche still need to be defined as they could differentially drive Notch signals and cellular outcomes.

Another perivascular BTSC maintenance factor may be Sonic Hedgehog (Shh), a known NSC self-renewal signal. In a mouse model of GBM, tumor-associated endothelial cells and astrocytes express Shh⁵⁰. Shh activates the downstream transcription factor Gli, as determined by a Gli-responsive luciferase reporter, in perivascular tumor cells⁵⁰. Gli activation correlates with tumor grade and is decreased by addition of differentiation-producing culture reagents such as fetal bovine serum⁵⁰, suggesting involvement in the regulation of BTSC biology. Indeed, targeting Shh has been shown to deplete BTSCs and reduce BTSC migration, and may sensitize the cells to chemotherapy^{51–53}. However, not all GBMs have activated Shh signaling as determined by Gli expression⁵¹, indicating there may be subgroups of brain tumors in which targeting of Shh would be ineffective. Among brain tumors dependent on Shh, propagation of Shh signals in BTSCs may require the Polycomb ring finger protein and known oncogene Bmi1 (refs. 54,55). Loss of Bmi1 prevents tumor formation in mice with Shh-driven medulloblastoma⁵⁴, and Bmi1 is a Gli1 target gene in medulloblastoma BTSCs⁵⁵. In glioma BTSCs, Gli1 increases insulin receptor substrate 1 to promote insulin-like growth factor signaling⁵⁶, and elevated Shh signaling is associated with maintenance of the tumor suppressor PTEN⁵⁷. As PTEN represses signals in the PI3K–Akt–mTOR pathway known to regulate BTSC survival in the mouse perivascular niche⁵⁸ and endothelial cell supported BTSC neurosphere formation⁵⁹, activation of Shh or loss of PTEN may represent alternative mechanisms for driving tumorigenesis. It is therefore important to understand the interactions between self-renewal pathways and signals known to be altered in cancer in the context of specific microenvironments found in the perivascular niche (Fig. 2).

Within the tumor, microenvironments develop that generate stresses including nonphysiological levels of oxygen, pH and metabolites. These microenvironments have the capacity to affect the BTSC phenotype, in part through modulation of the above-mentioned signaling pathways. For example, low oxygen tension or hypoxia has been shown to be critical for BTSC maintenance through hypoxia inducible factors (HIFs), with a particularly important role for Hif2 α ^{14,15,60–62}. Non-stem tumor cells can be induced to express self-renewal pathways

and become more tumorigenic upon culture under hypoxic conditions or introduction of a non-degradable form of HIF2 α ¹⁵. Hypoxia increases expression of Notch ligands and targets in BTSCs^{61,62}, demonstrating the ability to contribute to stem cell pathway activation in brain tumors. Although it may be counterintuitive to consider a vascular area susceptible to hypoxia, low oxygen tension may be present in the perivascular niche when considering the often hypoxic conditions of brain tumors and the regions of necrosis that can drive angiogenesis. However, the extent to which hypoxia or other microenvironmental stresses are consistently present in the perivascular niche remains unknown.

Take the lead: BTSC regulation of endothelial cells

Although BTSC-derived tumors can be infiltrative but nonangiogenic⁶³, they are often characterized by a highly vascular nature driven by the greater angiogenic capacity of BTSCs^{10,14,16,17} in comparison with non-stem glioma cells. CD133-enriched BTSCs generate xenografts with an elevated blood vessel density as demonstrated by staining with the endothelial cell marker CD31 (refs. 10,16,64). This increase in microvessels *in vivo* is associated with elevated BTSC production of factors that promote endothelial cell proliferation, migration and tube formation *in vitro*^{10,16}. However, as secreted factor production is not exclusive to BTSCs, non-stem glioma cells can influence endothelial behaviors to contribute to tumorigenesis^{10,16,65}.

One proangiogenic factor produced by BTSCs is the well recognized endothelial growth factor VEGF^{10,16}. Addition of VEGF-neutralizing or VEGFR2-blocking antibodies to BTSC-conditioned medium inhibits endothelial cell proliferation and tube formation^{10,16}, clearly demonstrating a paracrine role for VEGF produced by BTSCs. Anti-VEGF- or anti-VEGFR-based strategies also significantly delay the growth of human glioma xenografts, in association with a decrease in blood vessel density^{10,16}. Although effects on endothelial cells undoubtedly contribute to the effect of VEGF targeting, VEGFRs are also known to be expressed on glioma cells⁶⁶. VEGF can promote proliferation and survival of glioma cells, and targeting of VEGFR inhibits this effect as well as increases sensitivity to radiation-induced cell death⁶⁶. Together, these data suggest that BTSC-driven production of VEGF is an important autocrine and paracrine signal that drives glioma growth.

CXCL12, or stromal-derived factor 1 (SDF1), is a chemokine highly produced by BTSCs that can also promote angiogenesis and has been linked to a shorter time to progression in patients with glioma⁶⁷. CXCL12 can activate its receptor CXCR4 to promote VEGF production in glioma cells⁶⁸. An alternative receptor for CXCL12, CXCR7, which is associated with both cancer and endothelial cells, is expressed in glioma⁶⁹, although its role in BTSC biology remains

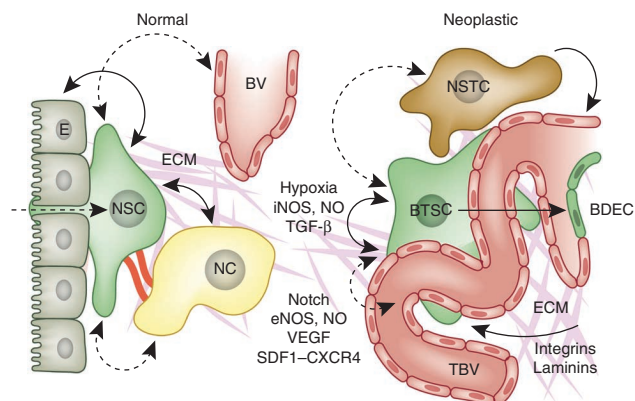


Figure 2 Shall we dance? Coordinated communication between cells in the perivascular niche. Neural stem cells (NSCs) present in the perivascular niche rely on a series of signals between the extracellular matrix (ECM), blood vessels (BV), ependymal cells (E) and other niche cells (NC) to promote their maintenance. BTSCs rely on similar interactions in the perivascular niche, which also consists of ECM, non-stem tumor cells (NSTC) and tumor blood vessels (TBV). BTSC plasticity toward an endothelial lineage and incorporation of these BTSC-derived endothelial cells (BDECs) into the vasculature may also contribute to the perivascular niche. Figure modified from ref. 12.

to be determined. In contrast, CXCR4 is known to be elevated on CD133-enriched BTSCs, indicating a greater capacity for CXCL12-mediated VEGF expression⁷⁰. A CXCR4 antagonist (AMD3100) can prevent endothelial cell proliferation and tube formation induced by BTSC-conditioned medium¹⁶. Knockdown of CXCR4 or treatment with a CXCR4 antagonist also decreases the growth of human glioma xenografts in association with decreased VEGF and blood vessel density *in vivo*^{16,70}. CXCL12 promotes the recruitment of bone marrow-derived cells after irradiation, which can increase vasculogenesis to restore the perivascular niche⁷¹. Targeting CXCR4 prevents this recruitment and blocks tumor vasculature development after irradiation⁷¹. CXCL12 is also produced by endothelial cells⁷², demonstrating that the perivascular niche and BTSCs maintain active CXCL12–CXCR4 loops, which enhances vasculogenesis and angiogenesis.

Although a direct link to BTSC-mediated angiogenesis has not been established for transforming growth factor- β (TGF- β), TGF- β is produced by glioma cells, including those that form neurospheres^{73,74}, and TGF- β -dependent BTSCs are present in the perivascular niche^{28,74}. As glioma cell-conditioned medium promotes endothelial cell tube formation in a TGF- β -dependent manner⁷⁵, TGF- β may be a proangiogenic agent in GBM. TGF- β also contributes to BTSC phenotypes by augmenting self-renewal capacity through induction of the cytokine leukemia inhibitory factor (LIF) and the transcription factor SOX2 and increasing tumorigenicity through regulation of the transcription factors inhibitors of DNA-binding protein (Id)-1 and Id-3 (refs. 28,74,76). Inhibition of TGF- β signaling by targeting the type I TGF- β receptor also reduces tumor propagation potential by decreasing CD44^{hi}Id1^{hi} BTSCs²⁸. Thus, BTSC-produced TGF- β in the perivascular niche, like CXCL12 and VEGF, may increase tumor growth by driving proangiogenic behaviors of endothelial cells.

Become one: BTSC plasticity toward the endothelial lineage

Interactions between tumor and endothelial cells are widely recognized to drive aberrant angiogenesis and vascular proliferation characteristic of GBM, but recent studies suggest that a portion of cells functioning as endothelium within GBM are actually derived from cancer cells. Although these studies may be complicated by the knowledge that normal endothelial cells express the NSC and BTSC marker Nestin⁷⁷, several groups have demonstrated that endothelial cells in brain tumors have genomic alterations consistent with cancer cells. Using DNA fluorescence *in situ* hybridization with CD31 immunofluorescence, substantial numbers of endothelial cells in GBM specimens ($\geq 20\%$) were shown to have the same genomic alterations as the GBM cells themselves⁷⁸. EGFR amplification commonly observed in GBM has also been found on endothelial cells in human GBM specimens^{79,80}. As NSCs can differentiate toward an endothelial lineage⁸¹, the presence of genetic alterations within endothelial cells in GBM was hypothesized to be due to a similar transition of GBM stem cells to endothelial cells^{78–80}. Indeed, human BTSCs enriched through the use of the CD133 marker were able to form vessel-like structures *in vitro* containing cells positive for endothelial cell markers including CD31 (refs. 78,79,82). Xenografts of human GBM cells can contain CD31⁺ cells of human origin, with GFP-labeled human GBM cells present in the tumor endothelium^{78–80}. Endothelial cells in a mouse model of GBM also express tagged oncogenes used to promote tumorigenesis and express a GFP marker⁸⁰. Thus, several laboratories have used similar and complementary methods to demonstrate that BTSCs have the ability to become endothelial-like cells *in vitro* and *in vivo*. Although this change has been designated a transdifferentiation event, transdifferentiation in nontransformed cells is typically restricted

to the transformation of one type of differentiated cell (or lineage-restricted progenitor) into another cell type. As aberrant differentiation is a hallmark of cancer, the acquisition of endothelial cell markers and behaviors by BTSCs may be more accurately described as a high degree of plasticity within these cells.

Considering the potential importance of targeting the tumor vasculature for glioma therapy, the microenvironmental factors and molecular mechanisms driving GBM stem cell acquisition of endothelial cell markers and behaviors need to be fully defined. Some of the initial reports of transitioned BTSCs have already begun to evaluate the contribution of critical angiogenic regulators, including hypoxia and VEGF. Addition of a hypoxia mimetic or culture under low oxygen tension enhances the expression of endothelial cell markers such as CD31 in a culture of mouse glioma tumor-initiating cells⁸⁰. VEGF is secreted by and *VEGFR* (*KDR*) mRNA expressed in BTSCs acquiring endothelial-like characteristics, suggesting the potential for a VEGF–VEGFR autocrine regulatory loop^{79,80}. However, VEGF-neutralizing antibody or *VEGFR2* short hairpin RNA did not block the acquisition of expression of the vascular endothelial cadherin CD144 in GBM stem cells cultured in endothelial medium, but expression of a proliferation-associated marker of active endothelial cells in cancer (CD105) was decreased⁷⁹. Although the biological impact of these changes in gene expression were not evaluated, treatment of GBM stem cells under transdifferentiating conditions with VEGF-neutralizing antibody or VEGFR small molecule inhibitor was not sufficient to prevent tube formation⁸⁰. Treatment of tumor-bearing mice with VEGFR inhibitor also increased the percentage of transdifferentiated endothelial cells *in vivo*, indicating that these cells may be responsible for resistance to anti-VEGF-based therapeutic strategies⁸⁰. Further studies demonstrated that Notch inhibitor prevents the ability of BTSCs to acquire a marker of endothelial progenitors⁷⁹. Notch and hypoxia, but not the hypoxia target gene VEGF, may therefore be critical drivers of BTSC capacity to generate tumor endothelium. However, plasticity of BTSCs towards endothelial cells is controversial, and greater validation will be required to determine the importance of these findings for GBM biology and patient outcomes.

Dancers' demise: collapsing a strong partnership

The most prevalent form of primary brain tumors in adults is GBM, which is treated with maximal safe resection followed by radiation therapy with concurrent and adjuvant temozolomide (TMZ), a DNA-methylating chemotherapy¹. This treatment strategy has led to a median survival of approximately 15 months, with a 5-year survival rate of only 10%¹. Salvage therapies for recurrent disease are generally not effective, with rates of 6-month progression-free survival of less than 20%^{83,84}. To improve this universally dismal outcome, many clinical trials are evaluating new types of therapy directed against underlying genetic and molecular alterations in brain tumors⁶⁵. A few are already targeting the pathways identified in BTSC and perivascular niche-driven tumor growth, providing promise for brain tumor therapy.

Recognition that angiogenesis is a pathologic hallmark of GBM and that antiangiogenic agents demonstrate efficacy in GBM animal models led to clinical testing of bevacizumab (Avastin), a monoclonal antibody against VEGF, in patients with recurrent GBM⁸⁵. Bevacizumab alone or in combination with irinotecan, a topoisomerase I-inhibiting chemotherapy, was associated with an increase in overall survival from the historical average of 6.5 months to 8–9 months^{86,87}. In addition, bevacizumab had antiedema effects resulting in symptomatic benefit, reduction of corticosteroids use, and stabilized or improved neurocognitive function^{87–89}. On the basis of

improved radiographic response and prolongation of progression-free survival rates in phase 2 trials, bevacizumab was granted accelerated approval by the US Food and Drug Administration (FDA) for progressive GBM in 2009. As bevacizumab may target BTSCs by disrupting a perivascular niche and/or neutralizing VEGF secreted from BTSCs^{10,13,90}, bevacizumab may represent the first FDA-approved anti-BTSC therapy.

There are more than 40 ongoing phase 1 and 2 trials using bevacizumab as a backbone in combination with other therapies for recurrent brain tumors, including GBM, medulloblastoma and ependymoma. Further studies are addressing the potential for bevacizumab treatment for newly diagnosed brain tumors in both phase 2 and phase 3 clinical trials. Published phase 2 studies of bevacizumab in combination with radiation therapy and TMZ in newly diagnosed GBM suggest a potential benefit, with a demonstrated overall survival of 20–21 months^{88,91}. Although further methods of targeting VEGF are also being explored^{92–94}, antagonism of VEGF signaling is not sufficient to prevent BTSC plasticity toward endothelial cells, indicating that new, VEGF-independent methods to effectively disrupt the tumor vasculature still need to be identified for use in therapies.

In addition to VEGF signals, there are several other BTSC and endothelial cell interacting pathways that promote angiogenesis and are being targeted in clinical trials for glioma. Targeting of secreted factors includes blockade of CXCL12 signaling through the use of the CXCR4 antagonist AMD3100, and inhibition of TGF- β signaling with the type I TGF- β receptor inhibitor LY2157299. Disruption of tumor cell and perivascular ECM interactions may be achieved with the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin inhibitor cilengitide (EMD121974), which showed modest activity in recurrent GBM⁹⁵ but a promising overall survival of 16.1 months⁹⁶ in newly diagnosed GBM in combination with radiation therapy and TMZ. Inhibitors of self-renewal signals are also in clinical trial for GBM. RO4929097 (Roche) is a γ -secretase/Notch inhibitor undergoing clinical evaluation for newly diagnosed GBM in combination with radiation therapy and TMZ treatment, and for recurrent brain tumors as either monotherapy or in combination with bevacizumab or cediranib. In a recurrent GBM trial being conducted with our participation, RO4929097 pretreatment effects on neurosphere formation will be evaluated as one measure of efficacy against BTSCs. Evaluation of effects on BTSCs is also a component of a clinical trial using the Shh antagonist GDC-0449 (vismodegib) in patients with recurrent GBM, and more trials are recruiting recurrent medulloblastoma patients. The incorporation of measurement of anti-BTSC effects in clinical trials reflects greater understanding of the possible contribution of tumor cell heterogeneity to patient survival and has the potential to considerably improve our ability to interpret clinical outcomes.

Although these studies suggest promise and will continue to be informative for antiangiogenic therapies targeting BTSCs, the potential for stromal and microenvironmental adaptation mechanisms induced by antiangiogenic therapies to contribute to more aggressive tumor phenotypes must be evaluated. Several studies have demonstrated that used of bevacizumab or other anti-VEGF-based treatments increases tumor cell invasion in GBM^{65,97,98}. Owing to the cross-talk between tumor cells and the microenvironment, compensatory mechanisms could result in elevation of proinvasive factors, which could be targeted in combination with antiangiogenics once identified. Alternatively, targeting newly defined proinvasive pathways specific to or increased in BTSCs is likely to provide benefits. These complexities underscore our need to further understand BTSC and microenvironmental interactions in the context of different therapeutic options.

Conclusions

Initial reports characterizing BTSCs often focused on these highly tumorigenic cellular subsets in isolation, but researchers now recognize the importance of understanding the relationships between BTSCs and specific microenvironmental niches. By addressing how the perivascular niche regulates BTSC maintenance and how BTSCs regulate their microenvironment, we can better understand the mechanisms through which BTSCs drive tumor growth. Studies with brain tumor specimens and model systems have already defined several paracrine and autocrine signals critical for BTSC maintenance in the context of components of the perivascular niche. Although these advances in our understanding of brain tumor biology are not yet reflected in substantial improvements in patient outcomes, the many clinical trials targeting pathways implicated in BTSC biology does demonstrate enthusiasm for translation of laboratory findings to patient therapies.

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