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TWISTED TANGO: Brain Tumor Neurovascular Interactions

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Abstract

The brain is a remarkably 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 display a cellular hierarchy with self-renewing tumorigenic brain tumor stem cells (BTSCs) at the apex. While 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 pro-angiogenic factors further linking cellular hierarchy regulation and instructive stromal elements. BTSCs may also directly contribute to tumor vasculature through plasticity towards an endothelial lineage. Interrogating the co-dependence of BTSCs and the perivascular niche may directly inform clinical approaches for brain tumor therapy through targeting of highly angiogenic and tumorigenic cellular subsets.

INTRODUCTION

Research has advanced our understanding of the most prevalent malignant brain tumors (glioma, medulloblastoma, 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 fifteen months¹. While these statistics highlight the devastating nature of the disease and the need for novel treatments, optimism for future development of targeted therapies has experienced a recent surge due to 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 current therapies, have now been identified^{8–18}. The localization of some BTSCs to a perivascular niche^{12–14} permitted elucidation of critical interactions and signaling pathways between BTSCs and endothelial cells which can now be targeted. Here we have sought to define the partnership between BTSCs and components of the perivascular niche and discuss how some novel therapies in clinical trials against glioma may target these interactions.

KNOW YOUR PARTNER: Defining BTSCs

BTSCs which 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}. Importantly, 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} (Figure 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^{8,10,11}, A2B5^{21,22}, CD171 (L1CAM)²³, CD15/SSEA1^{24–26}, CD49f (Integrin $\alpha 6$)²⁷, CD44²⁸, and EGFR²⁹ have been used to enrich for BTSCs with subsequent functional assessments including neurosphere formation and tumor propagation capacity. While this experimental evidence is supportive, controversy regarding the existence of BTSCs is fueled by a lack of consistency with regards to enrichment methods. No single universal BTSC 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. Evidence also demonstrates that neurospheres are not always clonal or specific for stem cells and can be difficult to quantify in terms of numbers and proliferation, suggesting neurosphere formation based selection is not ideal^{30,31}. However, markers (which often overlap) have been used to segregate functionally distinct tumor cell populations where one tumor cell fraction had 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 while current methods of BTSC isolation have advantages there are significant limitations of relying on only one marker or phenotype (such as neurosphere formation) without further analysis of functional properties including tumor initiation capacity.

In addition to marker enrichment, several other areas of the BTSC field remain under contention. While the proportion of stem 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, within the tumor. The proliferative nature of this BTSC subset also remains an area of debate and significant interest. While 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 label 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 which have an increased capacity to promote cancer growth. As its natural extension, targeting BTSC signaling or biologies 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 biologic properties of NSCs important for normal physiological responses are also similar to BTSCs. For example, BTSCs often drive tumorigenesis due to elevated angiogenic factor production, while NSCs produce VEGF and other angiogenic factors to promote revascularization of neural tissue in disease models. In some of these models, minimal immunosuppression is required demonstrating 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 due 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. While these data suggest some biologies 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 which contribute to tumor growth and could therefore be targeted for therapeutic interventions. For example, the nitric oxide synthase NOS2 was found to be highly expressed in BTSCs isolated from glioma patient specimens but minimally expressed in NSCs³⁵. Targeting of NOS2 activity with small molecule inhibitors decreased the growth of BTSCs, but not NSCs. These data 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 suggested that molecular signals and microenvironmental factors present in the perivascular niche promote BTSC maintenance, and endothelial cells were shown to promote BTSC self-renewal³⁷. Cell-cell interaction dependent and paracrine pathways from the vasculature which activate the BTSC phenotype are now being defined. When the impact of these niche signals is combined with the known plasticity of cancer cells, current models suggest that the population of BTSCs within a tumor is not fixed but likely fluctuates depending on multiple 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 which maintains BTSCs. The exact composition of the perivascular ECM in gliomas has not been defined, and there are differences in the exact laminin chains found to be expressed in brain tumors between research groups. However, the vessel basement membrane has been shown to express laminin chains including, but not limited to, $\alpha 2$, $\alpha 4$, $\beta 1$, and $\gamma 1$ ^{39,40}. Some data also suggests differences in laminin expression depending on brain tumor grade as well as associations of certain laminins with poor patient survival³⁹. Several forms of laminin are recognized by the receptor integrin $\alpha 6$ which is highly expressed by BTSCs³⁸. As targeting of integrin $\alpha 6$ decreases BTSC survival and tumorigenic potential³⁸, contact of integrin $\alpha 6$ 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 additional components of the perivascular ECM similarly support the BTSC phenotype remains to be determined but appears likely.

A paracrine pathway which links endothelial cells to BTSC biology involves nitric oxide (NO). Multiple studies have suggested a pro-tumorigenic role for NO, and glioma patients have increased levels of endothelial nitric oxide synthase⁴². NO produced by endothelial

nitric oxide synthase (eNOS) derived from perivascular niche was suggested to promote glioma growth in a mouse model of GBM³⁶. Targeting NO production with a pan NOS inhibitor or genetically through loss of eNOS increased survival in a mouse GBM model³⁶, suggesting a critical role for paracrine NO from the vasculature in supporting glioma growth. While we have focused on the interaction of endothelial cells and BTSC, 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 xenografts 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 was supported by endothelial cells, targeting Notch signaling decreased BTSC and endothelial cell growth³⁷. Loss of endothelial cell support of BTSCs mimicked 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 resulted 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 pathway. In a mouse model of GBM, tumor associated endothelial cells and astrocytes within the tumor expressed Shh⁴⁵⁰. Shh produced by endothelial cells and astrocytes activated the downstream transcription factor Gli, as determined by a Gli luciferase responsive promoter, in perivascular tumor cells⁵⁰. Gli activation correlated with tumor grade and was decreased by addition of differentiation producing culture reagents such as FBS⁴⁵⁰, suggesting involvement in the regulation of BTSC biology. Indeed, targeting Shh has been shown to deplete BTSCs, reduce BTSC migration, and may sensitize the cells to chemotherapy^{51–53}. However, not all GBMs have activated Shh signaling as determined by Gli expression⁴⁵¹, indicating there may be subgroups of brain tumors in which targeting of Shh would be ineffective. Among brain tumors dependent upon Shh, propagation of Shh signals in BTSCs may require the polycomb ring finger protein and known oncogene Bmi1^{54,55}. Loss of Bmi1 prevented tumor formation in mice with Shh driven medulloblastoma⁵⁴, and Bmi1 is a Gli1 target gene in medulloblastoma BTSCs⁵⁵. In glioma BTSCs, Gli1 increased insulin receptor substrate 1 to promote insulin-like growth factor signaling⁵⁶, and elevated Shh signaling was associated with maintenance of the tumor suppressor PTEN⁵⁷. As PTEN represses PI3K/AKT/mTOR signals 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 to drive tumorigenesis. These data suggest the importance of understanding 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 (Figure 2).

Within the tumor, microenvironments develop that generate stresses including non-physiological levels of oxygen, pH, and metabolites which have the capacity to impact 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. While it may be counterintuitive to consider a vascular area susceptible to hypoxia, low oxygen tension may be present within the perivascular niche when considering the often hypoxic conditions of brain tumors and the regions of necrosis which 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 non-angiogenic⁶³, BTSC derived tumors are often characterized by a highly vascular nature which has been shown to be driven by elevated angiogenesis^{10,14,16,17} in comparison to non-stem glioma cells. CD133 enriched BTSCs generate xenografts with an increased blood vessel density as demonstrated by staining with the endothelial cell marker CD31^{10,16,64}. This increase in microvessels in vivo is associated with elevated BTSC production of factors which 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 pro-angiogenic 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 media inhibits endothelial cell proliferation and tube formation^{10,16}, clearly demonstrating a paracrine role for VEGF produced by BTSCs. Anti-VEGF or VEGFR based strategies also significantly delayed the growth of human glioma xenografts in association with a decrease in blood vessel density^{10,16}. While 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 which 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 glioma patients⁶⁷. 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 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 prevented endothelial cell proliferation and tube formation induced by BTSC conditioned media¹⁶. Knockdown of CXCR4 or CXCR4 antagonist treatment 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 post 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 which form neurospheres^{73,74} and TGF- β dependent BTSCs are present in the perivascular niche^{28,74}. As glioma cell conditioned media promotes endothelial cell tube formation in a TGF- β dependent manner⁷⁵, TGF- β may be a pro-angiogenic agent in GBM. TGF- β also contributes to BTSC phenotypes by augmenting self-renewal capacity through induction of LIF and SOX2 and increasing tumorigenicity through regulation of inhibitors of DNA-binding protein (Id)-1 and Id-3 transcription factors^{28,74,76}. Inhibition of TGF- β signaling by targeting the type I TGF- β receptor also reduced tumor propagation potential by decreasing CD44^{hi}/Id1^{hi} BTSCs²⁸. Together, these data suggest that BTSC produced TGF- β in the perivascular niche, similar to CXCL12 and VEGF, may increase tumor growth by driving pro-angiogenic behaviors of endothelial cells.

BECOME ONE: BTSC Plasticity Towards 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. While these studies may be complicated by the knowledge that normal endothelial cells express the NPC and BTSC marker Nestin⁷⁷, several groups have demonstrated that endothelial cells within brain tumors have genomic alterations consistent with cancer cells. Using FISH with CD31 immunofluorescence, significant portions of endothelial cells within GBM specimens (~ 20%) were shown to have the same genomic alterations as the GBM cells themselves⁷⁸. EGFR amplification commonly observed in GBM was also found on endothelial cells within human GBM patient specimens^{79,80}. As NSCs can differentiate towards 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^{78,79,82}. Xenografts of human GBM cells contained CD31+ cells of human origin and GFP-labeled human GBM cells were present in the tumor endothelium^{78–80}. Endothelial cells in a mouse model of GBM also expressed oncogenes used to promote tumorigenesis and a GFP marker⁸⁰. Thus, multiple laboratories have utilized similar and complementary methods to demonstrate that BTSCs have the ability to become endothelial-like cells in vitro and in vivo. While this change has been designated a transdifferentiation event, transdifferentiation in non-transformed 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 enhanced the expression of endothelial cell markers such as CD31 in a culture of mouse glioma tumor initiating cells⁸⁰. VEGF was secreted by and VEGFR 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 shRNA did not block the acquisition of expression of vascular endothelial cadherin CD144 in GBM stem cells cultured in endothelial media, although expression of a proliferation associated marker of active endothelial cells in cancer

(CD105) was decreased⁷⁹. While 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 these cells may be responsible for resistance to anti-VEGF based therapeutic strategies⁸⁰. Additional studies demonstrated that Notch inhibitor prevented the ability of BTSCs to acquire a marker of endothelial progenitors⁷⁹. These data suggest that Notch and hypoxia, but not the hypoxia target gene VEGF, is a critical driver of BTSC capacity to generate tumor endothelium.

DANCERS DEMISE: Collapsing a Strong Partnership

The most prevalent form of primary brain tumors in adults is GBM which is currently treated with maximal safe resection, followed by radiation therapy (RT) with concurrent and adjuvant temozolomide (TMZ), a DNA-methylating chemotherapy¹. This treatment strategy has led to a median survival of approximately 15 months, whereas the 5-year survival rate is only 10% following multi-modality treatment¹. 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 novel therapies directed against underlying genetic and molecular alterations in brain tumors⁶⁵. A more limited number are already targeting the pathways identified in BTSC and perivascular niche driven tumor growth, providing promise for anti-brain tumor based strategies.

Recognition of angiogenesis as a pathologic hallmark of GBM and that antiangiogenic agents demonstrated 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 displayed anti-edema effects resulting in symptomatic benefit, reduction of corticosteroids use, and stabilized or improved neurocognitive function^{87–89}. Based on these and additional data indicating improved radiographic response and prolongation of progression free survival rates in phase II trials, bevacizumab was granted accelerated approval by the US 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.

Currently, there are more than 40 ongoing phase I/II trials using bevacizumab as a backbone in combination with other therapies for recurrent brain tumors including GBM, medulloblastoma, and ependymoma. Additional studies are addressing the potential for bevacizumab treatment for newly diagnosed brain tumors in both phase II and phase III clinical trials. Published phase II studies of bevacizumab in combination with RT and TMZ in newly diagnosed GBM suggested a potential benefit with a demonstrated overall survival of 20–21 months^{88,91}. While additional methods of targeting VEGF are also being explored^{92–94}, antagonism of VEGF signaling is not sufficient to prevent BTSC plasticity towards endothelial cells indicating novel VEGF-independent methods to effectively disrupt the tumor vasculature still need to be identified for patient therapies.

In addition to VEGF signals, there are several other BTSCs and endothelial cells interacting pathways which 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 demonstrated modest activity in recurrent GBM⁹⁵ but a promising overall survival of 16.1 months⁹⁶ in newly diagnosed GBM in combination with RT and TMZ. Inhibitors of self-renewal signals are also in clinical trial for GBM. RO4929097 (Roche) is a novel γ -secretase/notch inhibitor undergoing clinical evaluation in newly diagnosed GBM with RT and TMZ treatment or 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 additional trials are recruiting recurrent medulloblastoma patients. The incorporation of measurement of anti-BTSC effects in clinical trial reflects greater understanding of the possible contribution of tumor cell heterogeneity to patient survival and has the potential to significantly improve our ability to interpret clinical outcomes.

While 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 use of bevacizumab or other anti-VEGF based treatments increases tumor cell invasion in GBM^{65,97,98}. Due to the cross-talk between tumor cells and the microenvironment, compensatory mechanisms could result in elevation of pro-invasive factors which may be targeted in combination with antiangiogenics once identified. Alternatively, targeting newly defined pro-invasive pathways specific to or increased in BTSCs is likely to provide benefits. These complexities reiterate our need to further understand BTSC and microenvironmental interactions in the context of different therapeutic options.

CONCLUDING REMARKS

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 specific paracrine and autocrine signals critical for BTSC maintenance in the context of components of the perivascular niche. Although these advancements in our understanding of brain tumor biology are not yet reflected in significant improvements in patient outcomes, the large number of clinical trials targeting pathways implicated in BTSC biology does demonstrate enthusiasm for translation of laboratory findings to patient therapies.

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Figure 1. Learning the Steps –Isolation and Characterization of Brain Tumor Stem Cells

Brain tumor stem cells (BTSCs) from malignant tumors (glioma, medulloblastoma, ependymoma) can be enriched based on cell surface expression using flow cytometry (markers including but not limited to CD133, A2B5, CD171 (L1CAM), CD15/SSEA1, CD49f (Integrin α6), 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 which are elevated in comparison to non-stem tumor cells.

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. Brain tumor stem cells (BTSCs) rely on similar interactions within the perivascular niche, which also consist of ECM, non-stem tumor cells (NSTC), and tumor blood vessels (TBV). BTSC plasticity towards an endothelial lineage and incorporation of these BTSCs derived endothelial cells (BDECs) into the vasculature may also contribute to the perivascular niche. Figure modified from Gilbertson & Rich¹².