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# The Role of Brain Vasculature in Glioblastoma

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**Abstract**
For normal functioning, the brain requires an adequate supply of blood. The components of normal brain vasculature are collectively referred to as the neurovascular unit. When the brain develops pathology, the structural and functional components of brain vasculature become compromised. This is evidenced in the case of neoplasia where the integrity of the vasculature is co-opted to further the neoplastic processes that require exponential blood resupply in order to facilitate the diffusion radius of tumor growth. Glioblastoma changes the brain vasculature in such a way that advances the tumor's progress while making it more resistant to standard modes of treatment. While the brain vasculature is changed as a result of glioblastoma growth and progression, it is also changed to advance the invasiveness of the tumor. The diagnostic criteria for glioblastoma is correlated with advanced neovascularization processes that change the previously existing vasculature into that which is morphologically atypical and in a dysfunctional state. Advancing therapies to treat glioblastoma must understand normal brain vasculature and how it is changed as a result of tumor growth.

**Keywords** Antiangiogenesis therapy · Brain vasculature · Blood brain barrier · Edema · Glioblastoma · Glioma · Neovascularization · Neurovascular unit

## Introduction

An adequate blood supply is instrumental to normal brain functioning. The brain's vasculature is composed of a complex network of arterioles, capillaries, venules, and veins that regulate cerebral blood flow (CBF) and maintain the integrity of the blood brain barrier (BBB). Brain pathology compromises the regulation of CBF and BBB integrity, furthering the mechanism of pathologic change. This compromise is evidenced in brain neoplasia where cells undergo uncoordinated cell growth and rapid cellular proliferation that requires blood resupply while also changing the structure and function of the brain's normal vasculature. Neurovasculature dysfunction is an early finding of brain neoplasia and one that develops the neoplastic potential within the tumor milieu, making the malignancy more adept at advancing its pathological progression and more resistant to standard modes of treatment.

Glioblastoma (GBM) is the most common primary brain malignancy and has extremely dismal patient outcomes. GBM has a mean life expectancy of only 14.6 months when treated and has no potential for cure [1]. Characterized by infiltrative growth, nuclear atypia, high proliferation index, microvascular proliferation, pleomorphic vessels, and pseudopalisading necrosis, GBMs are among the most vascular of all solid tumor types. As vascular proliferation is a pathological hallmark of gliomas, the tumor possesses a highly abnormal, dysfunctional vasculature system that differs from normal brain vessels in morphology, functionality, and molecular characteristics. Moreover, GBM malignancy grade is directly correlated with endothelial cell proliferation in forming new vasculature [2].

Extreme yet dysfunctional brain vasculature is essential for gliomagenesis whereupon interactions between tumor cells and blood vessels facilitate tumor growth. The process by which gliomas generate an increasing blood supply to meet an ever-increasing nutrient and oxygen demand is now recognized as a highly complex spectrum of events whereupon neovascularization is critical to glioma growth and survival [2]. Although hypoxia is a well-known driver of neovascularization, evidence exists that non-hypoxia-driven mechanisms exist, including vascular endothelial growth factor (VEGF)-mediated pathways [3]. While the angiogenesis inhibitor, bevacizumab, has extended the progression-free survival of GBM patients by inhibiting blood vessels dependent on VEGF and the vascular permeability of these tumors, it does

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not confer overall survival benefit, suggesting that the tumor vasculature is able to compensate for the loss of VEGF in taking over its role in angiogenesis.

Critical to glioma development and continuous growth is the BBB, whereupon the tumor capitalizes on the membrane's leakage and permeability. The heterogeneous cell population of GBM, which include glioma stem cells (GSCs), allows for extensive proliferation and migration that is only possible through the corrupted vasculature enabled by the afflux of blood via the BBB. Tumor cells under adaptable oxygenation are then able to migrate to different regions of the brain guided by the extension of blood vessels where they can colonize the healthy adjacent tissue, expanding the tumor's growth and asserting dominance in controlling the dysfunctional NVU [4, 5]. Clinically, the formation of new vessels to suit the tumor's needs pose serious implications to patients, foremost in vasogenic brain edema, which dramatically increases the intracranial pressure (ICP) via BBB leakage [6].

The aforementioned resistance of GBM to traditional therapeutics is partly compounded by GSCs that have tumor-initiating functions that are responsible for replenishing and sustaining glioma growth in conjunction with the properties of the NVU. Hypoxia and hypoxia-inducible factors (HIFs) that remain inextricably linked to the aberrant components of the NVU maintain the stem-like cells of the tumor by creating a microenvironment that provides the ideal milieu for GSC survival and self-renewal [7, 8].

The objective of this review is to provide a thorough and comprehensive review of the neurovascular unit (NVU) as it pertains to glioma neovascularization. We aim to describe the NVU as it is impacted by tumor growth and then how it in turn enables gliomagenesis. As neovascularization is a hallmark characteristic of GBM that is responsible for the tumor's survival and aggressiveness, strategies to combat neovascularization in glioma are as undeveloped as much as it is urgently needed. We discuss the five distinct processes of neovascularization in glioma that are now instrumental to understanding GBM biology and translational to therapeutic efficacy: (1) vascular co-option, (2) angiogenesis, (3) vasculogenesis, (4) vascular mimicry, and (5) endothelial cell transdifferentiation.

## Components of the Brain Vasculature

The components of the brain vasculature include a complex, regulated network of arteries, arterioles, capillaries, venules, and veins that regulate CBF and maintain the integrity of the BBB in what is collectively referred to as the neurovascular unit (NVU). The changes in blood supply are according to increases or decreases in neuronal (hyperemia) demand as is accomplished by cells of both vascular and neural origin that encompasses the NVU resulting in a highly efficient system of regulation of CBF [9]. Together with neurons, astrocytes, endothelial cells of the BBB, pericytes, and extracellular matrix (ECM) components, the NVU detects the needs of neuronal blood supply and triggers the necessary responses in the form of vasodilation or vasoconstriction to meet these demands [10–12]. This is accomplished via autoregulation in which adequate delivery of oxygen and nutrients is made through changes to cerebral vascular tone in keeping a constant blood flow to the brain under normal conditions within the range of 50–160 mmHG [13].

The BBB separates blood components from the brain microenvironment, regulating the entry and exit of ions, nutrients, macromolecules, and energy metabolites. It is the regulated interface between the peripheral circulation and central nervous system (CNS). Sensitive to a wide range of chemicals that are ordinarily metabolized and excreted without harm to peripheral organ systems, the CNS cannot process most readily consumable substances in an effect that would cause neurotoxicity. Examples of such neurotoxins may include natural forms of lead or ethanol, for instance, as well as chemotherapeutic agents used against other cancers without adverse effect in the body. Thus, it is imperative that the BBB functions as a dynamic regulator of ion balance, facilitator of nutrient transport, and barrier to potentially harmful molecules [14]. The endothelial cells lining cerebral blood vessels are the core anatomical unit of the BBB by limiting transcellular and paracellular transport mechanisms [15]. United by endothelial tight junctions (TJs), astrocytic endfeet surrounding blood vessels, pericytes embedded in the vessel basement membrane, microglia, and neurons, the BBB is as responsible for all essential roles of CNS homeostasis in a complex manner such that potential mechanisms of drug delivery to the brain remain elusive [16, 17].

Regulating blood flow at the local level is permissive through the mechanism of hyperemia, whereupon the delivery of oxygen and nutrients is adjusted according to the changes in activity of specific brain regions [18, 19]. Hyperemia is triggered in normal physiological conditions as much as it is borne in pathological situations, such as neoplasia or seizures, when the oxygen demand increases in specific brain areas [20]. The regulation of blood flow of the cerebral microvascular endothelium suggests that cerebral endothelial cells are intrinsically unique or that the cellular milieu of the brain somehow induces BBB characteristics mediated by the interplay of gap junctions through adhesion molecules such as cadherins or integrins [14, 21]. Gap junctions, adhesion molecules, and ion channels facilitate the influx and efflux of ions such as $Ca^{+2}$, $K^{+}$, and the action of neuromodulators [18, 19].

As a physical tissue barrier, the choroid plexus (CP) is a collection of thin membranes located anatomically inside the lateral, third, and fourth ventricles that synthesizes the cerebrospinal fluid (CSF) and its major proteins, metabolites, and other molecules with a high degree of vascularization that exceeds that of the brain parenchyma by 10 times [22]. Composed of a monolayer of specialized epithelial cells that

are derived from the ependyma and covers the ventricle walls, the CP coats a highly perfused stroma that contains permeable blood vessels, fibroblasts, dendritic cells, and macrophages [23].

Also closely associated with the endothelial cells of the BBB, astrocytes contribute to the selection and exchange of molecules to the barrier. They interpose in the contacts between microvessels and neurons in coordinating oxygen and glucose transport for neural activity through the regulation of local blood flow, hyperemia [24]. Under normal conditions, this component of the NVU functions according to normal physiological processes providing selective permeability and vascular transport in conjunction with the BBB. The situation becomes discordant when these astrocytes become malignant as in the case of astrocytoma. In conjunction with stem-like cells and glial cells, astrocytes have been found to play a role in the vascularization of glioblastoma [25, 26]. Extensive neovascularization occurs in the tumor microenvironment, contributing immensely to tumor growth, survival, and resistance to therapy. This abnormal and highly unregulated vascularization albeit originating in endothelial cells is complemented and sometimes even recapitulated by tumor cells adapted to this new function as in the case of vascular mimicry.

## How GBM Affects Brain Vasculature

Cerebrovascular dysfunction can be found in CNS neoplasms that are both primary (originating within the CNS) and secondary (metastatic). In both cases, tumor cells localize in the perivascular spaces of the NVU such that they can later remodel, destroy, and neovascularize abnormal, dysfunctional NVU structure and function in order to ensure adequate oxygen and metabolic support through these newly induced vessels [27, 28].

Arising predominantly from glial cells but highly dependent on processes of neovascularization, gliomas demonstrate an extreme degree of NVU pathology in CNS malignancies. Like other CNS tumors but to a larger extent in glioma, tumor cells in the perivascular space migrate within the perivascular space of the NVU in direct contact with the basement membrane(s) of the NVU and its associated soluble factors. This contributes to gliomagenesis by (1) employing proteins such as collagens, fibronectin, and vitronectin to provide scaffolding support for migration, (2) using the activation of TGF-β, cytokine, notch, sonic hedgehog, and extracellular matrix (ECM) signaling pathways to promote multi-potency of tumor cells while supporting their survival and proliferation, and (3) strengthening the tumor's resistance to radio- and chemo-therapy [15, 29–34]. Through the activation of the ECM pathway, gliomas can produce and secrete additional ECM and growth factor proteins to loosen the ECM for invasion [35]. The molecular signaling and crosstalk between cellular components of the NVU and tumor cells have been shown to predict GBM patient survival and disease progression [36–39].

This molecular signaling and crosstalk promotes tumorigenicity while disrupting normal NVU structure and function. The disruption of BBB TJs by pathology or drugs can lead to impaired BBB function and thus compromise the CNS, enabling further vascular dysregulation and irregular neovascularization. Krstic et al. showed that the displacement of normal astrocyte endfeet promotes vascular leakiness and disrupts the regulation of vascular tone by downregulating the expression of TJs [40]. Similarly, Watkins et al. showed that glioma cells within the perivasculature can modulate the regulation of vascular tone in a $K^+$-dependent manner [41]. Evidence now exists that GSCs can generate vascular pericytes that surround blood vessels within the tumor. Cheng et al. observed that GSCs are able to form cells of the vascular pericyte lineage in vitro and showed that such GSCs can yield pericytes in vivo in a mouse xenograft model [42]. As increased pericyte coverage is thought to give glioblastoma cell resistance to bevacizumab, this may be a consequence of hypoxia in accompaniment of VEGF blocking. When tumor cells release pro-angiogenic growth factors such as VEGF, activating VEGF receptors on endothelial cells, they abrogate out such that they can extend into the tumor and promote vascular-mediated growth [43, 44].

The early stages of gliomagnesis are not marked by any apparent disruption of the BBB, as the tumor has not yet destroyed pre-existing vasculature that will eventually be replaced by the tumor's very own vasculature. At this point, the tumor mass is sustained by normal vessels. But, this is markedly changed when the glioma progresses and endothelial cells derived from normal vessels are separated from the main NVU structure where new angiogenic spots are created. Via molecules that disrupt the normal microenvironment, endothlelial cell migration becomes impaired and this is reflected in the changes to the neurovascular unit. This affects the vascular microenvironment through severing the effectiveness of TJs and causing the microvasculature morphology to adapt to changing tumor needs. Microvessel density is a standard prognostic indicator of patients with GBM. As previously mentioned, the strong correlation between abnormal vasculature and tumor aggressiveness results in vasculature that possesses both abnormal morphology and function. This results in vessels that possess irregular diameters and permeability, irregular basal lamina, and abnormal distribution within the tumor mass [45]. The most common explanation to explain irregular vessel formation in GBM is the high amount of VEGF localized in the tumor mass [46]. As a hypoxia-inducible factor, VEGF is most expressed in the necrotic regions of tumor [47]. This overexpression is thought to promote angiogenesis as a mediator of the crosstalk between tumor and endothelial cells.

Clinically, brain-tumor-related edema (BTRE) is one of the primary causes of death in glioma patients. BTRE is induced by severe cerebral edema whereupon there is an abnormal accumulation of water inside the brain parenchyma and leads to brain herniation in up to 60% of GBM patients. The rapid accumulation of water within the fixed skull results in exponentially increased intracranial pressure (ICP) that results in decreased cerebral blood flow and vascular tone, ischemia, brain herniation, and death. In this condition, ischemia ensues when decreased cerebral blood flow results in a depletion of oxygen and essential nutrients [48]. It is associated with increased microvascular permeability [49, 50]. Mark and Davis et al. have shown that hypoxia and hypoxia/reoxygenation in in vitro models of the BBB leads to disruption and increased permeability of BBB TJs. Potentially, this hypoxic stress on BBB integrity may be resultant of transcellular signaling [51]. Mechanistically, BTRE results from (1) vasogenic and (2) cytotoxic processes (Fig. 1). Vasogenic edema is caused by the disruption of the BBB, allowing materials of the blood to permeate into the brain parenchyma [52]. Dysfunctional BBB strength is facilitated by decreased expression of functioning TJs as well as increased endothelial pinocytosis and fenestrations [53]. The cytotoxic process occurs as a result of tumor-induced neuronal cell death and neurodegeneration, which leads to gliosis compounded by increased ICP and other neurological deficits [54]. Bevacizumab possesses potent anti-edema properties in such a way that it has potential use as a steroid-sparing agent [55]. Sole purpose use as a corticosteroid-sparing agent is not presently recommended due to increased risk of intra-cerebral hemorrhage and pulmonary thromboembolism [56].

GBM affects the NVU by destroying previously existing vasculature and remodeling its own vasculature that is suitable for the tumor's needs. This replaced vasculature is highly irregular and dysfunctional. But, it is functional for supporting further cancerous projection and survival. Understanding the increased, yet heterogeneous, permeability of tumor vasculature is essential for optimizing drug delivery platforms to tumors as well as designing effective anti-neovascularization strategies [15, 57].

## How Changes to Brain Vasculature Affect GBM's Pathological Development and Resistance to Treatment

Neoplastic processes need access to circulation to grown beyond the diffusion distance of oxygen. GBM exemplifies this need greater than any other solid tumor type. Correlated with tumor grade and an independent marker of poor patient prognosis, high microvessel density is a hallmark characteristic of astrocytic gliomas. Blood flow to the tumor mass varies according to anatomic position and the temporal oxygen demands of the lesion. This leads to intermittent periods of hypoxia. As already mentioned, work has shown that hypoxia/reoxygenation leads to disruption and increased permeability of BBB TJs [51]. Also known, hypoxia strongly induces tumor progression by supporting immunosuppression, inflammation, apoptotic resistance, metabolic reprogramming, and epithelial-mesenchymal cell transition [58].

When the brain's vascularization changes due to tumorigenesis, abnormal and dysfunctional blood vessels develop. These tumor vessels in glioblastoma are referred to as glomeruloid bodies or vascular tufts. They possess multi-layered endothelial cells, pericytes, smooth muscle cells, and a thick basement membrane [59]. Unique to high-grade gliomas, this diagnostic criterion does not avail itself in low-grade gliomas, thereby setting a potential avenue for targeting neovascularization processes in GBM. Heterogeneity exists within the tumor in vascular morphology. Glomeruloid bodies often coexist with normal-appearing thin-walled vessels. At the edge of the tumor growth radius where invasive, highly migratory tumor cells invade the normal cortex, the BBB remains intact. This demands that therapeutic agents of GBM must be able to cross the barrier in order to effectively target the invasive front that is

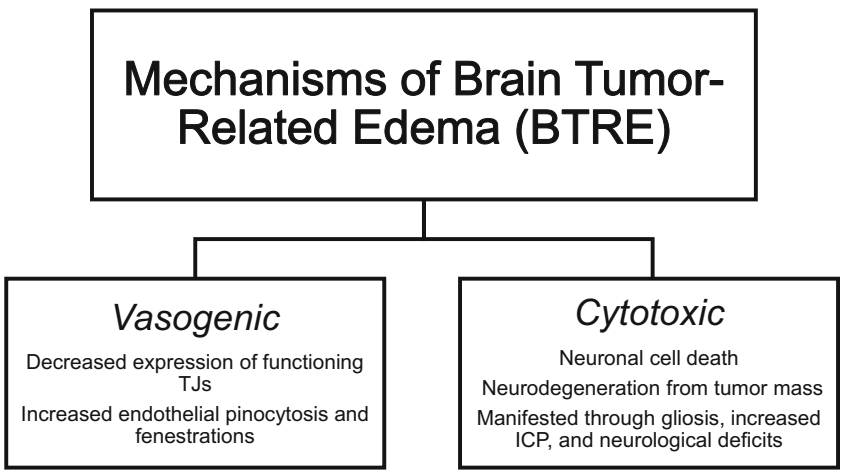


**Fig. 1** Brain tumor-related edema (BTRE) is due to either vasogenic or cyotoxic mechanistic processes. These are described as such in their relation to brain tumor development. (TJs: tight junctions; ICP: intracranial pressure). BTRE is one of the leading causes of death in GBM patients

dynamically changing according to the vascular demands of the tumor in the form of neovascularization.

Five distinct mechanisms of neovascularization in GBM have been identified: (1) *vascular co-option*, (2) *angiogenesis*, (3) *vasculogenesis*, (4) *vascular mimicry*, and (5) *endothelial cell transdifferentiation* (Fig. 2).

First, *vascular co-option* is the mechanism by which the tumor achieves its vasculature after destroying previously existing vasculature and setting the stage for vascular remodeling. Tumor cells organize themselves into cuffs whereupon they surround normal microvessels. This occurs by co-opting normal brain vasculature in the early stages of glioma development as to increase blood flow and vascular tone before the following processes are able to adapt the tumor vasculature microenvironment.

Second, *angiogenesis* is the development of new vessels from pre-existing ones in a mechanism that occurs both under normal physiological conditions as well as that of pathology. It follows *vascular co-option*. It was first described in 1976 when intense neovascularization was observed in rabbit corneas transplanted with GBM, suggesting that the neovascular process seen was mediated by an in vivo production of a "vasoformative substance" that induced new vessel formation [60]. The new formation of vessels devoid of capillary morphogenesis, intussusception, has been observed in other tumor types but not glioma [61]. Studies have suggested that GSCs promote angiogenesis in such a way that supports tumor progression [62]. VEGF and SDF-1$\alpha$ are potent angiogenic factors released by GSCs, and their role is unsurprising as GSCs are known to survive well under hypoxic conditions and their role in hypoxic regions to enhance angiogenic processes to enhance the tumor mass [62, 63]. This results in a highly abnormal vascular network that is characterized by dilated vessels, abnormal branching, arteriovenous shunts, and the potential for abnormal perfusion. This vascular network is notably immature in gliomas, characterized by excessive leakiness that further impedes the normal functioning of the BBB. This is heightened by VEGF-induced leakiness and vesiculovacuolar organelles that contribute to abnormal TJ functioning [64, 65]. This is important to note as immature vasculature is much more permeable than ordinarily mature capillaries likely due in part to the lack of a basal lamina, thus contributing to increased abnormal vasculature from normal brain vasculature and lost integrity of the BBB.

Third, *vasculogenesis* is the differentiation of endothelial progenitor cells (EPCs) by which circulating bone marrow-derived cells differentiate at sites of neovascular pathology into endothelial cells or macrophages through tumor-associated macrophages (TAMs) [66, 67]. It is also evidenced that tumor-derived endothelial cells are more prevalent within the tumor core and less so at the periphery. VEGF contributes to EPC migration and proliferation although the exact mechanism by which EPCs and TAMs contribute to the neovascularization of brain neoplasms has yet to be fully explained.

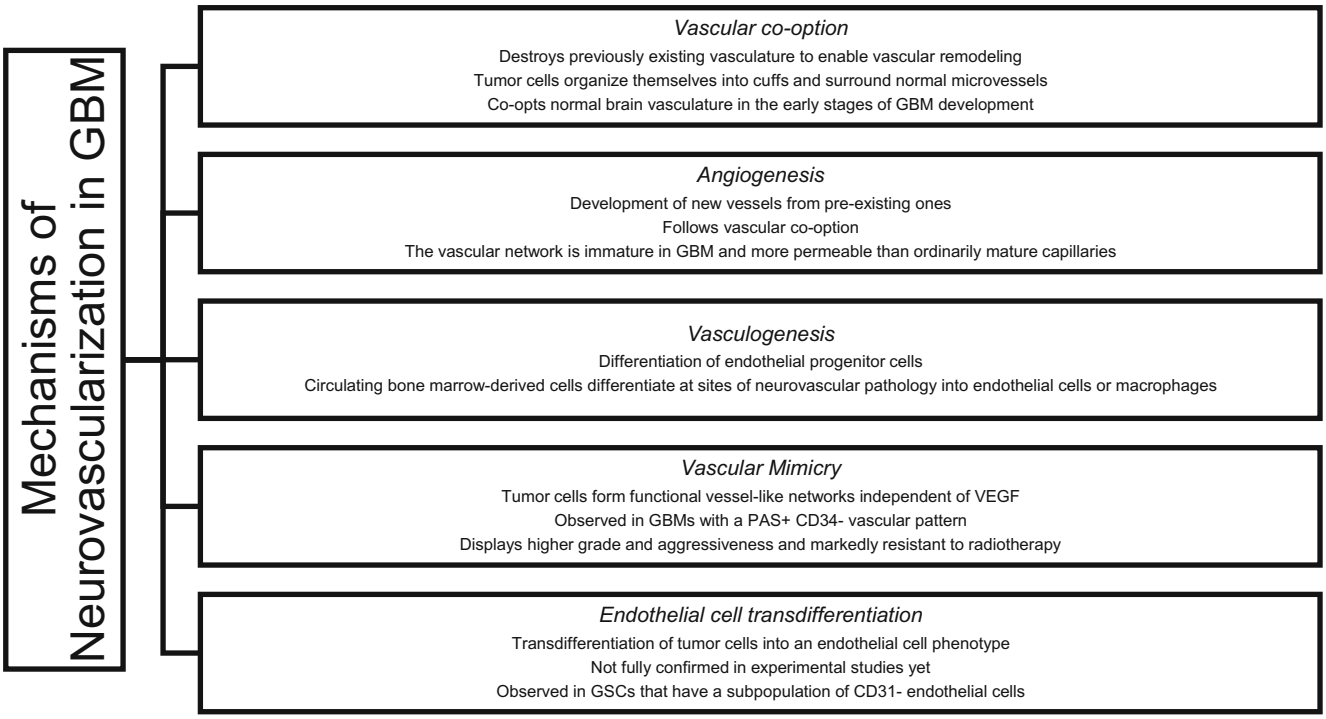


**Fig. 2** Five distinct mechanisms of neovascularization in GBM have been identified: (1) *vascular co-option*, (2) *angiogenesis*, (3) *vasculogenesis*, (4) *vascular mimicry*, and (5) *endothelial cell transdifferentiation*. Neovascularization in GBM is a complex and highly regulated set of processes that are unique but also somehow interlinked mechanisms. These are described with regard to their major characteristics

Fourth, one of the most interesting and increasingly investigated modes of brain tumor vascularization is *vascular mimicry*. In this process, tumor cells form functional vessel-like networks in an alternate mechanism to support tumor growth and survival independent of VEGF. This mechanism has been observed in gliomas as indicated in a PAS+ CD34− vascular pattern [68]. Using PAS as a biomarker of an endothelial cell proliferation phenotype is not suitable despite this, as brain tumor cells have also been observed to express PAS. Liu et al. correlated vascular mimicry (immunohistochemically PAS+ CD34−) with tumor grade, explaining the therapeutic inefficiency of anti-angiogenic therapies against GBM. Tumors with evidence of *vascular mimicry* displayed higher grade and aggressiveness. They also display a marked resistance to radiation therapy. Accordingly, patients who possessed tumors that evidenced *vascular mimicry* exhibited decreased overall survival than those who had tumors that did not facilitate the process. These tumors possessed lower microvascular density than those that did not [69]. This suggests that this process provides an alternative or complementary mechanism to traditional modes of neovascularization, but its contribution, while demonstrated, is not clear in terms of the entirety of tumor growth. Its contribution is even further stratified by tumor type [30]. These tumors may also facilitate vessel formation through the differentiation of endothelial cells or pericytes as mediated by GSCs [70–72].

Fifth and finally, the transdifferentiation of tumor cells into an endothelial cell phenotype is observed in what is called *endothelial cell transdifferentiation*. The process has not been fully confirmed as of yet, but studies that investigate its occurrence are convincing. Ricci-Vitiani et al. observed the process of *endothelial cell transdifferentiation* in GSCs that had a subpopulation of CD31− endothelial cells expressing the very same chromosomal aberrations present in the evaluated tumor samples. A portion of microvascular cells with aberrant endothelial/glial phenotype was GFAP+, indicating that the newly formed endothelial cells may originate from the tumor itself [70]. Shen et al. closely associated endothelial cells with neural stem cells (NSCs) by finding that endothelial cells self-renew NSCs in an integral component of the vascular niche that composes the tumor microenvironment in which GSCs are able to proliferate undifferentiated, unaffected by external factors [73].

## Discussion

Understanding of the cellular contributions to the NVU has seen incredible progress over the past decade. The mechanisms of these cellular components in the process of normal brain functioning as well as disease progression remain incompletely understood. How the brain regulates blood flow under normal physiological conditions as well as pathology remains under investigation. The discovery of glymphatic fluid flow has broadened this understanding in the regulation of CNS clearance pathways that are necessary for vascular processes to contribute to tumor growth [74–77]. Further investigation of how this clearance system is impaired as a key component of tumor progression in the context of the perivascular corridors in glioblastoma is needed in order to understand tumor neovascularization [31, 34, 41].

The NVU facilitates the multiple pathways for which cerebral microvascular permeability can be regulated by drugs or disease. Drug delivery to the CNS remains a foremost challenge to treating brain tumors. Preclinical studies of drugs that show success early on fail to reach therapeutic levels in the brain in vivo on account of the decreased permeability of the BBB and heightened CNS clearance pathways. By increasing the access of therapeutic agents to the NVU or decreasing their clearance, improved drug delivery can be achieved. This can be achieved by transiently opening TJs using a variety of methods including surgically implanted ultrasound methods to allow pharmacological agents to pass endothelial cells in a paracellular fashion [78–80]. Receptor-mediated transport systems can additionally route drugs through endothelial cells such that the therapeutic agents can enter the NVU. Such drugs are rapidly cleared under normal conditions, but therapeutic levels can be maintained by inhibiting perivascular clearance routes or blocking efflux pumps [15].

With this in account, the tumor vasculature has been an attractive target for treating brain tumors such as GBM. High-grade tumors such as these possess a high degree of neovascularization that can be combatted with therapeutics that have less problems crossing the BBB than more traditional chemotherapeutics. The ability to cross the BBB and to reach the brain parenchyma is being exploited as a possible approach for many CNS diseases, including GBM. Additionally, normalization of the vascular mechanisms in the brain has shown to have a synergistic effect with other anti-tumor therapeutic approaches. Targeting the vasculature of gliomas may also allow for dual targeting of GSCs that are believed to be responsible for the tumor resistance to chemo- and radio-therapy. The tumor vessels provide a niche for GSCs that permit self-renewal and maintenance [73]. GSCs are a source of pro-angiogenic factors such as VEGF that thrive in the perivascular niche of the tumor microenvironment such that they may be vulnerable to therapy directed against the tumor vasculature [81].

Even though anti-angiogenic therapy has yielded promising response rates, glioma recurrence is common. A change in the vascular phenotype observed post-therapy is characterized by decreased microvessel density and normalization in vascular architecture as well as atypical expression of CD34 and fascin that results in rebound revascularization [82]. The escape from therapy is attributed to phenotypic/molecular changes in heightened invasion, increased *vasculogenesis* and *vascular*

*co-option*, and up-regulated angiogenic factors aside from VEGF. More worrisome, there is some evidence that such therapy can increase invasiveness and *vascular co-option*. This is especially the case in the second-line treatment of GBM patients with bevacizumab when frontline therapy has failed. The results have been disappointing. Disease progression-free survival is improved in such therapy although no improvement in overall survival is demonstrated. Despite this, patients benefit from reduced BTRE and ICP after therapy although the tumor seems to escape inhibition by invading the brain extracellular matrix [83, 84]. Anti-angiogenic therapy undoubtedly represents important progress in the development of effective targeted therapy in a tumor type such as GBM that is so heavily influenced by increased vascularization. Neovascularization in brain tumors is much more complex than can be explained by angiogenesis factors alone. In these tumors, the newly formed blood vessels are themselves neoplastic.

Continued investigation of the neovascular processes in brain tumors like GBM indicate that the pathways to neovascularization are not simple nor part of a singular mechanism that can be easily understood. Instead, neovascularization in GBM is a complex and highly regulated set of processes that have been identified in five unique but somehow interlinked pathways: (1) *vascular co-option*, (2) *angiogenesis*, (3) *vasculogenesis*, (4) *vascular mimicry*, and (5) *endothelial cell transdifferentiation*. Perturbations in one pathway have been observed to shift the balance of the remaining pathways, thus altering the vascular phenotype and increasing the tumor's resistance to therapy as well as promoting the aggressive and invasive nature of the disease. V*ascular mimicry* and *endothelial cell transdifferentiation* may be at the opposite ends of the GBM neovascularization spectrum with the other mechanisms being somewhere in between such that the tumor is able to fine-tune the vascular processes of neoplastic growth in such a way that enables augmented growth, survival, and resistance to therapy.

An increased understanding of the mechanisms of vessel formation and their regulation in GBM is necessary to the development of novel agents that target the tumor vasculature. While previous strategies have sought to block vessel formation, their efficacy has been limited. VEGF-mediated pathway targeting has been limited as new evidence indicates that neovascularization is able to ensue independent of this pathway as a compensatory mechanism that is not susceptible to the same therapies. While VEGF-mediated targeting of these processes is successful in the short-term, the brain vasculature is able to adapt to this new compensation and thus negates its efficacy over the long-term. New strategies should specifically target the pro-neovascularization function of the tumor vasculature that make GBM the most invasive, resistant to therapy, and deadly tumor there is.

Summarily, the brain vasculature serves as the gateway to the brain and tumor-mediated neovascularization is no exception. Understanding the components of the NVU, how it develops under normal and pathological conditions, and its maintenance and function is necessary to developing the therapeutics to effectively treat essentially all disease of the brain including GBM.

## Compliance with Ethical Standards

**Conflict of Interest** The author declares no conflict of interest.

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