

## MINIREVIEW

# Brain angiogenesis in developmental and pathological processes: mechanism and therapeutic intervention in brain tumors

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Formation of new blood vessels is required for the growth and metastasis of all solid tumors. New blood vessels are established in tumors mainly through angiogenesis. Brain tumors in particular are highly angiogenic. Therefore, interventions designed to prevent angiogenesis may be effective at controlling brain tumors. Indeed, many recent findings from preclinical and clinical studies of antiangiogenic therapy for brain tumors have shown that it is a promising approach to managing this deadly disease, especially when combined with other cytotoxic treatments. In this minireview, we summarize the basic characteristics of brain tumor angiogenesis and the role of known angiogenic factors in regulating this angiogenesis, which may be targets of antiangiogenic therapy. We also discuss the current status of antiangiogenic therapy for brain tumors, the suggested mechanisms of this therapy and the limitations of this strategy.

## Introduction

Brain tumors are much rarer than tumors of other organs. Specifically, fewer than 2% of all tumors diagnosed each year are primary brain tumors [1]. Furthermore, because of the location and other physiological characteristics of brain tumors, their prognosis is very poor [1]. Whereas primary brain tumors originate in the brain, secondary brain tumors metastasize from other cancers, such as breast, lung and colon cancer, and melanoma. Of the many different types of brain tumors, glioblastoma multiforme (GBM) is the most common, accounting for ~40% of all primary brain tumors and 70% of all malignant gliomas [2–4]. GBM is also one of the most vascular

and deadly cancers, with a very low 5-year survival rate of 5% [5]. Patients with high tumor microvascular densities have shorter postoperative survival durations than do patients with low microvascular densities, suggesting that tumor vasculature is important to brain tumor growth [6,7]. Currently, the majority of the available literature data on vessel formation in brain tumors comes from studies of GBMs. In this minireview, we describe the characteristics of brain tumor angiogenesis and the current status and known mechanisms of antiangiogenic therapy for brain tumors based on published results mainly from studies of primary brain tumors.

**Abbreviations**

BBB, blood–brain barrier; CNS, central nervous system; CSC, cancer stem cell; GBM, glioblastoma multiforme; mTOR, mammalian target of rapamycin; PtdIns3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homologue; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

## Angiogenesis in brain tumors

### Tipping the balance between proangiogenic and antiangiogenic factors

When a solid tumor such as a brain tumor grows larger than a critical size (1–2 mm in diameter), it must recruit new blood vessels to have the oxygen and nutrition supply necessary for its survival and growth. This tumor-induced formation of new blood vessels occurs primarily via angiogenesis, the process of development and growth of new blood vessels from pre-existing vasculature [8].

Researchers now widely accept that angiogenesis is tightly controlled by a balance of pro- and antiangiogenic factors [9–11]. These molecules can be secreted by cancer, endothelial, stromal and blood cells and the extracellular matrix [12,13]. Proangiogenic factors include vascular endothelial growth factor (VEGF), acidic fibroblast growth factor, basic fibroblast growth factor, placental growth factor, angiopoietin-2 and interleukins, whereas antiangiogenic factors include angiostatin, endostatin, thrombospondin 1 and endothelial monocyte-activating polypeptide 2 [14,15]. In addition, the enzymes serine proteinase and metalloproteinase degrade the extracellular matrix, which has an important role in both the induction and suppression of angiogenesis [16]. This biological process, which is essential to not only tumor development, but also normal development and wound repair, is highly regulated. When the expression of proangiogenic molecules is balanced with that of antiangiogenic molecules, the ‘angiogenic switch’ remains off. However, in tumor angiogenesis, the tight regulation of the balance of expression of these molecules is disrupted. Induced expression of proangiogenic molecules leads to uncontrolled and disorganized promotion of angiogenesis [8,17].

### Extracellular signaling promotes brain tumor angiogenesis

Angiogenesis in solid tumors, including brain tumors, is believed to be triggered by low oxygen concentrations (hypoxia) resulting from deficits in the blood supply caused by the tumor’s fast growth. Exposure of brain tumor cells to hypoxia induces expression of hypoxia-inducible factor-1, a transcription factor that regulates the expression of many angiogenesis- and glucose metabolism-related genes. Hypoxia-inducible factor-1 activates the transcription of VEGF and other proangiogenic factors in gliomas, in particular [18–20].

Researchers have found high levels of expression of VEGF mRNA in the hypoxic regions of high-grade but not low-grade gliomas. In addition, the VEGF receptors (VEGFR1 and VEGFR2) are highly expressed in gliomas [21]. Expression of VEGF is correlated with microvascular density in gliomas and meningiomas [22]. basic fibroblast growth factor is a potent mitogen of endothelial cells and is required for glioma angiogenesis *in vivo* [23].

Signaling by neurotrophins and their receptors supports neuronal proliferation, differentiation and synapse formation. The neurotrophin family consists of four structurally related proteins: nerve growth factor, brain-derived neurotrophin factor, neurotrophin-3 and neurotrophin-4 [24]. Nerve growth factor, brain-derived neurotrophin factor and neurotrophin-3 bind primarily to the receptor kinases TrkA, TrkB and TrkC, respectively, to mediate their effects across the cell membrane [25]. Also, nerve growth factor and brain-derived neurotrophin factor enhance endothelial cell survival and proliferation [26–29]. In particular, brain-derived neurotrophin factor can enhance the expression of proangiogenic factors (e.g. VEGF) in brain tumor-derived cells through induction of hypoxia-inducible factor-1 expression [30].

Interleukin-8 (also known as CXCL8) is a chemokine with proangiogenic activity. Authors have reported high levels of expression of hepatocyte growth factor/scatter factor and interleukin-8 in primary and recurrent glial tumors [31,32]. Expression of another chemokine peptide, CXCL12, and its cognate receptors is induced in brain tumors and promotes angiogenesis [33]. In addition, a subset of integrins mediates endothelial-cell spread and migration in response to growth factor signaling in brain tumor angiogenesis [34]. mRNA expression profiles in gliomas from patients have shown expression of many proangiogenic factors including insulin-like growth factor-1 in those tumors [35]. Stem cell factor and its receptor c-Kit pathway play important roles in tumor-induced angiogenesis in the brain, as well [36].

### $\gamma$ -Secretase in brain tumor angiogenesis

Signaling by the transmembrane protein Notch and its ligand Jagged/Delta is indispensable for neural system development and is related to the development of many types of tumors [37]. Notch signaling is activated by VEGF signaling and suppresses angiogenesis [38–40]. Accordingly, researchers found that blockade of Delta-like ligand 4 led to increased blood vessel sprouting in a glioma model [41]. Interestingly, such increased vessel

sprouting does not support, but rather suppresses, tumor growth, suggesting that Notch signaling is required for the negative feedback and fine-tuning of the proangiogenic VEGF signaling to establish functional vessels in brain tumors [41]. Notch signaling also downregulates the expression of VEGFR2 and VEGF in endothelial cells [42]. Notch signaling is mediated by cleavage of the Notch molecule by  $\gamma$ -secretase, a presenilin-dependent protease complex [43]. VEGF increases  $\gamma$ -secretase activity-mediated Notch 1 cleavage in endothelial cells. Inhibition of  $\gamma$ -secretase activity blocks VEGF-induced endothelial cell proliferation, migration and survival, and eventually leads to decreased angiogenesis [44]. In addition, presenilin cleaves the erythroblastic leukemia viral oncogene homologue 4, ErbB-4 [45], which is widely expressed in gliomas and medulloblastomas and enhances tumor angiogenesis [46]. Moreover,  $\gamma$ -secretase cleaves VEGFR1 [47] and insulin-like growth factor-1 receptor, and both of these receptors' signaling promotes angiogenesis in astrocytomas and glioblastomas [35,48]. These results suggest that  $\gamma$ -secretase has important complex, but as yet unidentified, roles in brain tumor angiogenesis.

### **Intracellular machinery of brain tumor angiogenic signaling**

As described above, researchers have made considerable progress in understanding the interactions among cell surface receptors and ligands that regulate angiogenesis. However, the intracellular machinery that governs signaling from the receptors on the cell surface to the nucleus to control the induction of angiogenesis remains poorly understood. Signaling of VEGFR and that of other receptor tyrosine kinases, such as platelet-derived growth factor receptors and epidermal growth factor receptors, have regulatory mechanisms that are similar in many aspects [49]. VEGFR signaling may induce activation of Ras/Raf/mitogen-activated protein kinase [50,51] or phospholipase C- $\gamma$ /protein kinase C signaling [52], which regulates endothelial cell proliferation, migration and permeability [53]. Also, one of the important signaling pathways activated by VEGFR is the phosphatidylinositol-3 kinase/phosphatase and tensin homologue/Akt/mammalian target of rapamycin (PtdIns3K/PTEN/Akt/mTOR) pathway. This PtdIns3K/PTEN/mTOR pathway regulates endothelial cell survival, translation and permeability [53–56]. This pathway is also activated by other proangiogenic stimuli, including platelet-derived growth factor, neurotrophins, insulin-like growth factor, epidermal growth factor and integrins, and plays a critical role in brain tumor

angiogenesis [57]. The pivotal role of this signaling pathway in the proliferation and survival of brain tumor cells strongly suggests the potential use of inhibitors of it to target both brain tumor cells and blood vessel endothelial cells [57].

## **Characteristics of brain tumor vasculature**

### **The blood–brain barrier in brain tumors**

The vasculature in a healthy central nervous system (CNS) tissue is highly specialized and distinguished from the vasculature in other tissues by a unique structure of blood capillaries, the blood–brain barrier (BBB) [58]. Unlike other tissues, in which relatively free diffusion of materials in the blood is allowed through their peripheral capillary walls, the transportation of materials in the blood circulation to the peripheral tissues in CNS is tightly regulated by this barrier. The BBB is an anatomical and physiological barrier that strictly restricts the permeability of blood vessels, suppressing the diffusion of ions, peptides, amino acids and other substances from the bloodstream to the neural system, while supplying the brain with the required nutrients for proper CNS function. This barrier is composed of the walls of vessel endothelia, which are sealed by tight junctions between endothelial cells. Also, the BBB is wrapped with specialized cells (pericytes) and the flattened 'endfeet' of astrocytes. Pericytes are relatively undifferentiated mesenchyme-like cells that support capillary blood vessels. Astrocytes induce the tight junctions of BBB through decreasing VEGF expression and stimulating angiopoietin release [59]. This tight junction prevents the passive diffusion of hydrophilic molecules whose molecular mass is > 500 kDa from the bloodstream to the brain parenchyma. Furthermore, water-soluble materials are not able to pass through the lipophilic membranes of endothelial cells. Thus, the BBB is often the primary obstacle to drug delivery to the CNS [58].

Because the brain is located in a confined space, leakage of fluid into the brain caused by breakage of the BBB results in increased interstitial pressure within the skull and, consequently, vasogenic brain edema. Therefore, keeping the molecules carried in the blood vessels in the brain from breaching the BBB and entering brain tissue is essential for maintaining normal brain physiology. Hence, BBB breakdown is associated with many CNS-associated pathologies, including neurological diseases such as Alzheimer disease.

The BBB in brain tumors is structurally and functionally abnormal [60–63]. Nevertheless, some features

of the normal BBB are retained in brain tumor vasculature [61,63,64]. Researchers found that, in a murine brain tumor metastasis model, the integrity of the BBB was conserved in small tumors (< 0.25 mm in diameter) but not in larger tumors [65]. In addition to loss of BBB integrity, blood vessels in brain tumors exhibit abnormal features similar to those in the vessels in other types of tumors. For example, tumor blood vessels are tortuous, disorganized and highly permeable because of abnormalities in their endothelial walls [61–63,66–69]. Therefore, disruption of the BBB and further increases in the permeability of tumor blood vessels in brain tumors, because of their loosened endothelial structures, result in increased accumulation of fluid peritumorally and in the surrounding brain, and bring about vasogenic brain edema. Vasogenic edema is a major cause of morbidity in patients with brain tumors [70,71]. Hence, tumor angiogenesis must be treated properly to not only prevent brain tumor growth, but also suppress the pathological damage caused by changes in the permeability of brain tissue. The blood vessels associated with brain metastases are dilated and contain highly mitotic endothelial cells [65], which may require high concentrations of VEGF for their growth. Increased leakage from blood vessels in brain tumors causes suppressed and irregular blood flow and leads to heterogeneous and inefficient delivery of oxygen, nutrients and drugs to the brain tumor via the bloodstream [63,71,72].

One of the main causes of increased permeability and loss of BBB integrity in brain tumor blood vessels is increased expression of VEGF by the brain tumor cells. Investigators initially purified VEGF for its ability to induce vascular leakage and permeability, as well as for its role as a mitogenic factor for endothelial cells. Therefore, it was originally known as vascular permeability factor as well as VEGF [73–75]. The effects of VEGF on vascular permeability in the peripheral circulation appear to occur via modulation of calcium influx, nitric oxide, activation of guanylyl cyclase, protein kinase G, vesiculo-vacuolar organelles or increased synthesis of platelet-activating factor in endothelial cells [73–78].

Hypoxia and acidosis are hallmarks of solid tumors, but they are not necessarily correlated with each other [79], and low oxygen and pH levels independently upregulate VEGF transcription in brain tumors *in vivo* [60]. Also, many oncogenes, such as *ras* and *src*, cytokines and growth factor receptor signaling, and tumor suppressor genes, such as *trp53*, regulate transcriptional and translational expression of VEGF in tumors [80,81] and surrounding tissues [12]. In principle, tumor blood vessels, especially brain tumor blood

vessels, are abnormal, which means they are highly permeable and even leaky at many points, resulting in irregular and inefficient blood flow through them. This irregularity and inefficiency are strongly linked with the action of VEGF, the major proangiogenic factor.

## Targeting brain tumor vasculature

### Therapeutic antiangiogenic agents for brain tumors

In the early 1970s, Folkman [9] proposed blockage of tumor vascularization as an approach to treating cancers. Because of the known pivotal role of VEGF in angiogenesis, many antiangiogenic approaches have targeted VEGF and VEGFR signaling. More than 30 years after the seminal findings by Folkman's group, the U.S. Food and Drug Administration approved bevacizumab, a humanized mAb against VEGF, as the first drug used for antiangiogenic therapy for cancer (specifically, colon, lung and breast cancer). Also approved for antiangiogenic therapy for renal carcinoma and other tumors by the U.S. Food and Drug Administration were sorafenib and sunitinib, two small molecules that target VEGFR kinase activity. Unfortunately, none of these agents are currently approved as therapy for brain tumors. However, the number of clinical trials examining the use of antiangiogenic agents to treat brain tumors is increasing. Table 1 summarizes current (as of January 2009) clinical trials targeting angiogenesis in primary and secondary adult brain tumors. More than 70 clinical trials using ~20 anticancer agents that may inhibit angiogenesis in brain tumors are in progress. The majority of these trials are targeting VEGF signaling with mAbs against VEGF [20,68], small-molecule tyrosine kinase inhibitors (TKIs) that inhibit VEGFR2 tyrosine kinase activity [82] and soluble decoy receptors developed from VEGFR1 that selectively inhibit VEGF activity [83]. In addition to VEGFR signaling, platelet-derived growth factor receptor,  $\alpha v \beta 3$  integrins [84] and intracellular mediators of angiogenic signaling (protein kinase C and mTOR) are targets of reagents used in these clinical trials. Also, several clinical trials are studying modulations of BBB integrity designed to modify the permeability of the blood vessels in brain tumors and eventually improve delivery of cytotoxic agents to the brain tumors.

Because Notch signaling suppresses and orchestrates the formation of brain tumor vessel sprouts, treatment with  $\gamma$ -secretase inhibitors may inhibit normal Notch signaling and thus tumor angiogenesis. Indeed, the authors reported that treatment with a  $\gamma$ -secretase

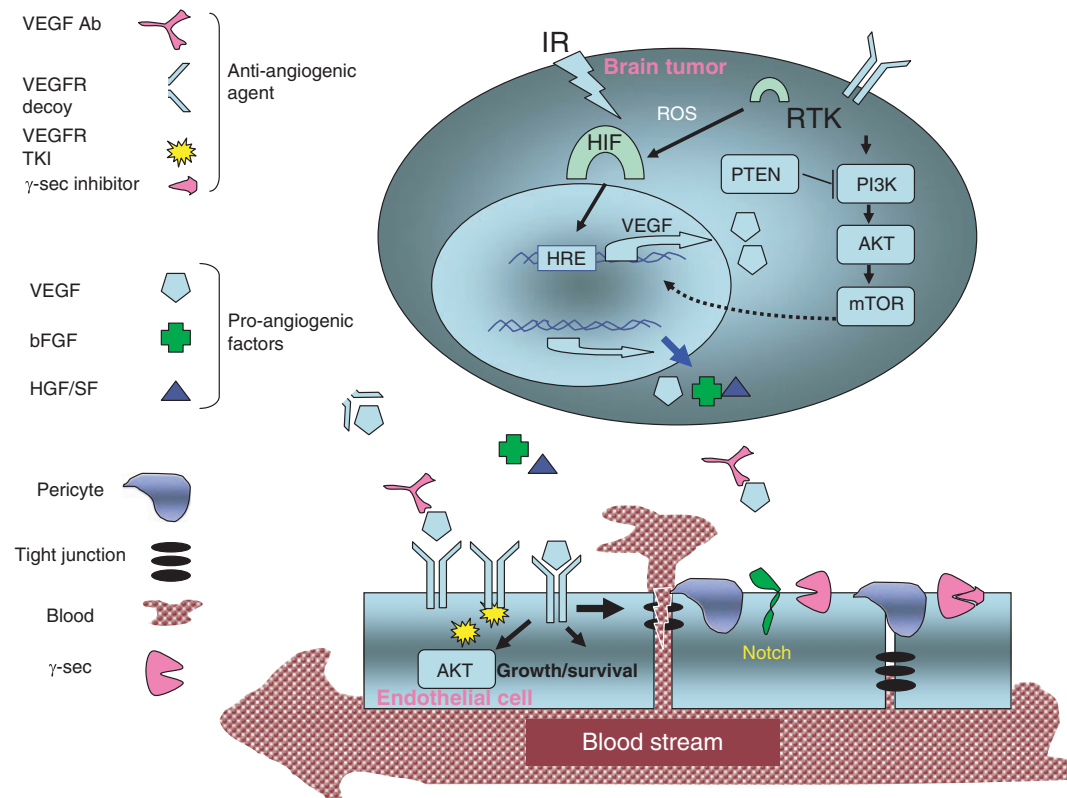
inhibitor suppressed the growth and vascularization of human GBM xenografted into nude mice [85].

The intracellular machinery of angiogenic signaling described above can be an effective target in antiangiogenic therapy for brain tumors. As expected from the observation that the PtdIns3K/Akt/mTOR signaling pathway is essential for endothelial cell survival and blood vessel permeability, inhibition of mTOR signaling by small-molecule inhibitors or RNA interference have proven to be efficacious at antiangiogenic therapy in preclinical models of malignant glioma [86,87]. The signalling in brain tumor angiogenesis and its targeting are summarized in Fig. 1.

### Efficacy of antiangiogenic therapy for brain tumors

The results of the first series of clinical trials (phase I and II) on treatment of GBM with vatalanib, a small-

molecule TKI against of VEGFR2, were disappointing [88,89]. However, recent clinical trials showed that bevacizumab and a TKI of VEGFR were effective in the treatment of brain tumors when combined with standard chemotherapeutic agents [20,82]. Researchers in one of these studies observed a strong antiedema effect of bevacizumab, suggesting that this antiangiogenic therapy decreased the permeability of brain tumor blood vessels [20]. These results suggested that the antiangiogenic agents used augmented cytotoxic drug efficacy by improving blood flow in these tumors [20,82]. The novel hypothesis that 'antiangiogenic therapy normalizes the abnormal tumor blood vessels' may explain these results [90]. As described above, intratumoral vessels are leaky because of high local concentrations of VEGF, a potent vascular permeability factor. Blocking VEGF or VEGFRs in tumors suppresses the function of VEGF signaling; the tumor vasculature permeability then returns to normal, lead-



**Fig. 1.** Angiogenic interaction between brain tumor and endothelial cells and antiangiogenic therapy. VEGF expression is induced in tumor cells by hypoxia [19], radiation, receptor tyrosine kinase signaling and Akt pathways [114]. VEGF and other proangiogenic factors are secreted by brain cancer cells in a paracrine and endocrine manner [83]. These factors support endothelial cell survival. Also, VEGF induces permeability of the BBB [78]. Notch signaling mediated by  $\gamma$ -secretase suppresses angiogenesis but is required for proper vascular development [38–40,44]. The signaling required for angiogenesis in brain tumors can be targeted using an antibody against VEGF [20,68], a decoy receptor to VEGF [115], small-molecule TKIs of VEGFR[82], inhibitors of other kinases in the signaling cascade and inhibitors of  $\gamma$ -secretase [85]. Ab, antibody; HGF/SF, hepatocyte growth factor/scatter factor; IR, ionizing radiation; RTK, receptor tyrosine kinase; PTEN, phosphatase and tensin homologue; PI3K, phosphatidylinositol-3 kinase; HRE, hypoxia-responsive element; ROS, reactive oxygen species;  $\gamma$ -Sec,  $\gamma$ -secretase.

**Table 1.** Clinical trials with antiangiogenic agents targeting brain tumors<sup>a</sup>. bFGF, basic fibroblast growth factor; GBM, glioblastoma multi-form; GS, gliosarcoma; MG, malignant glioma; OG, oligoglyoma; OA, oligoastrocytoma; MB, medulloblastoma; NB, neuroblastoma; Meta, metastasized secondary brain tumors; mAb, monoclonal antibody; PDGFR, platelet-derived growth factor receptor; PKC, protein kinase C; SKI, serine-threonine kinase inhibitor; PtdIns3K, phosphatidylinositol-3 kinase; FKBP-12, FK506 binding protein 1A- 12 kDa.

Drug	Target	Phase	Tumors
XL184 (TKI)	VEGFR, MET	II	GBM
ZK222584 (TKI)	VEGFR, PDGFR	I,II	MG
Pazopanib (TKI)	VEGFR/PDGFR/c-Kit	II	Glioma, Meta
Sorafenib (TKI)	VEGFR/PDGFR/Raf	I,II	GBM, Meta,GS
Sunitinib (TKI)	VEGFR/PDGFR	I,II	GBM,Meta
Thalidomide/ Lenalidomide	VEGF/bFGF/TNF	I,II	GBM,Meta
VEGF Trap (Peptide)	VEGF	II	MG
Ceradinib (TKI)	VEGFR	I,II,III	GBM
ZD6474 (TKI)	VEGFR	I,II	GBM
CT-322 (Peptide)	VEGFR	I,II	GBM
Bevacizumab (mAb)	VEGF	I,II	GBM
Valproic acid	Potential anti-angiogenic	II	Meningioma, GBM
Cilengitide (Peptide)	Integrin	II,III	GBM, MG
Everolimus/ Sirolimus	FKBP-12/mTOR	I, II	MG
		II	GBM
RAD001 (SKI)	mTOR	III	Astrocytoma
Temsirolimus (SKI)	mTOR	I	GBM
Tandutinib (TKI)	PDGFR	I,II	GB, Astrocytoma,Meta
XL765 (SKI)	PtdIns3K/mTOR	I	Oligodendroglioma
Enzastaurin (SKI)	PKC	I,II	GBM
MPC-6827	Microtubule (microvessel)	I,II	GBM
CY997	tubulin (microvessel)	I,II	GBM, Gliosarc, Meta
Sodiumthiosulfate/Mannitol	BBB	I,II	OG,OA,MB,NB,GBM

<sup>a</sup> The information in this table was extracted from the National Cancer Institute Web site (<http://www.cancer.gov>) in a search for currently (January 2009) open clinical trials. Most of the drugs have been used in multiple concurrent clinical trials.

ing to the restoration of effective blood flow [91]. Accordingly, antiangiogenic therapy enhances the delivery and efficacy of concurrently administered cytotoxic agents [90,92–94]. This is supported by the results of studies using a number of preclinical models [63,95–97], including primary and secondary brain tumor models [68,98]. Authors also reported that VEGFR2 blockade by the mAb DC101 temporarily normalized tumor vessel walls, improving vascular function and tumor oxygenation and thus enhancing responses to radiotherapy in a GBM model in nude mice [99]. In human clinical trials, treatment with bevacizumab indeed changed several abnormal characteristics of tumor vessels to resemble the normal characteristics of non-tumor vessels [100]. In patients with recurrent GBM treated with a novel TKI, the VEGFR inhibitor AZD2171, the treatment normalized tumor blood vessels, leading to the significant clinical benefit of alleviation of edema [82]. However, such normalization seems to be only temporal and reversible, suggesting that the optimal window of time during which combined cytotoxic and antiangiogenic therapy for brain cancer provides the

best outcome is limited. It has been reported that during this window, after treatment with VEGFR2 antibody, tumor oxygenation improved and pericyte coverage and angiopoietin expression increased and thus decreased interstitial pressure and vessel permeability in orthotopic GBM [68].

Researchers have suggested that antiangiogenic therapeutic agents have several mechanisms of action in combined treatment with radiotherapy [101]. For example, a study found that blocking VEGF's action enhanced the cytotoxic effect of radiotherapy for tumor cells *in vitro* [102]. Also, authors reported that the response of brain tumors to radiotherapy depended on endothelial cell apoptosis in the tumors in a murine model [103]. This suggested that blockage of VEGF signaling leading to apoptosis of endothelial cells and thus enhanced the efficacy of radiotherapy. Radiotherapy also induced expression of hypoxia-inducible factor-1 and genes downstream of it, including VEGF, in an *in vivo* murine tumor model [104]. In brain tumor models, radiotherapy has induced VEGF expression; this induced expression is essential for endothelial cell

survival [105–107]. Therefore, radiotherapy-induced endothelial cell death would be enhanced by blockage of VEGF action by inhibition of newly expressed VEGF in irradiated cancer cells and endothelial cells.

Compelling evidence suggests that cancer cells are generated by small fractions of self-renewing, multipotent, tumor-initiating cells termed cancer stem cells (CSCs) in brain tumors as they are in other tumors [108]. Calabrese *et al.* [109] observed that brain tumor CSCs are found in vascular niches in the tumors as normal brain stem cells are. These authors reported that antiangiogenic therapy disrupted the tumor vasculature and that the CSC niche microenvironment associated with the tumor blood vessels reduced the CSC population in the brain, suggesting that extirpation of brain CSCs may contribute to the efficacy of antiangiogenic cancer therapy.

### Current limits of and perspectives on antiangiogenic therapy for brain tumors

The use of antiangiogenic therapy for brain tumors carries several concerns [110]. Despite angiogenesis being essential for the growth of all tumors, antiangiogenic therapy usually results in only transitory delays in tumor growth and progression, after which tumors begin to regrow and the cancer progresses. This may result from the adaptation of tumors to antiangiogenic therapy; evasive resistance [111] and intrinsic resistance of tumors to antiangiogenic therapy. Tumor cells self-adjust to antiangiogenic therapy by developing alternative pathways to recruit new blood vessels. Also, tumors may activate and enhance invasion and metastasis of their own cells after antiangiogenic therapy [111]. Therefore, use of antiangiogenic therapy combined with other therapies that may override this adaptive 'self-adjusting' pathway would significantly improve the efficacy of the anticancer treatment.

Hemorrhage and thrombosis are potential complications of antiangiogenic treatment of GBM [20]. Also, authors have reported intracerebral hemorrhages in patients with gliomas treated with bevacizumab in combination with irinotecan, a topoisomerase inhibitor [112]. Rebound cerebral edema caused by the reversibility of normalization of blood vessels in brain tumors after stopping antiangiogenic treatment is another potential problem. In addition, blockage of angiogenesis is potentially cytotoxic to neural stem cells, which reside in perivascular niches in the subventricular zones of the brain and are supported by growth factors generated by the endothelial cells. Therefore, antiangiogenic therapy resulting in VEGF-signaling suppression or endothelial cell death may cause neural stem cell death [113].

When more clinical data on the efficacy and mechanism of action of antiangiogenic therapy for brain and other tumors from many of the ongoing clinical trials of this therapy become available, the resulting improvement in understanding of this mechanism will allow us to optimize combinations of antiangiogenic therapy and other cytotoxic treatments and improve the benefits of this therapy in patients with brain cancer.

### Addendum

After this article was submitted, U.S. Food and Drug Administration (FDA) granted accelerated approval of bevacizumab (antibody to VEGF) for glioblastoma patients with progressive disease following prior therapy, in May 2009. This is the first FDA approved anti-angiogenic agent for brain tumors.

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