Molecular Characteristics and Pathways of Avastin for the Treatment of Glioblastoma Multiforme

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KEYWORDS

• Avastin • Bevacizumab • VEGF • Chemotherapy • Glioblastoma

KEY POINTS

- The overall benefit of bevacizumab remains controversial.
- Although bevacizumab has been shown to extend progression-free survival, it has not been shown to improve overall survival and may facilitate glioma transformation to a more invasive phenotype.
- The mechanism of bevacizumab is still not sufficiently understood and future studies may need to use novel methods of evaluating and visualizing tumor progression to determine the effectiveness of bevacizumab.

INTRODUCTION

Glioblastoma multiforme (GBM) is one of the most common and aggressive primary brain tumors. Despite surgical resection, radiotherapy, and chemotherapy, prognosis for GBM remains poor. The median progression-free survival (PFS) and overall survival (OS) for patients with GBM who undergo surgical resection followed by radiation therapy and temozolomide chemotherapy are 6.9 and 14.7 months, respectively.[1]

GBM is a highly invasive and one of the most angiogenic and vascularized cancer. Altered pathways in GBM include the loss of function of tumor suppressor genes and the activation of oncogenes.[2] GBM is believed to be characterized by tumor progression through the induction of angiogenesis to form new blood vessels via endothelial cell migration and proliferation.[3] GBM is capable of exhibiting endothelial proliferation and rapid formation of tortuous vessels to supply its increasing metabolic needs; as a result, its poor-quality blood vessels are highly permeable and disorganized. Angiogenesis may play a pivotal role beginning in the earliest phase of tumor development and perhaps represents a critical event in the progression of malignant gliomas.[4] Hence, angiogenesis has been targeted in the treatment of recurrent GBM.

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Recurrent Glioblastoma

Nearly all GBM recur after initial therapy, and only 20% to 25% of patients survive beyond 1 year after the diagnosis of recurrent disease.[5,6] Median survival for patients with recurrent GBM ranges from 3 to 9 months.[7] Several potential treatments are being evaluated in ongoing clinical trials for recurrent GBM. bevacizumab (Avastin), a humanized anti-vascular endothelial growth factor (VEGF) antibody, has shown promising results in phase II clinical trials for recurrent GBM; as a result, in May 2009, the Food and Drug Administration (FDA) approved the use of bevacizumab for the treatment of patients with recurrent GBM, which made bevacizumab the third FDA-approved chemotherapy for GBM along with implantable Gliadel wafers and temozolomide.[8]

Bevacizumab

Since its inception, bevacizumab has led a successful yet controversial path. Initially indicated for metastatic colorectal cancer in 2004, bevacizumab was shown in a randomized, double-blind, stage III clinical trial to significantly extend both PFS and duration of survival.[9] Median PFS in a colorectal cancer control group receiving IFL (irinotecan, fluorouracil, and leucovorin) treatment was 6.2 months, compared with 10.6 months in a group receiving IFL with bevacizumab. Median duration of survival increased from 15.6 months in the IFL control group to 20.3 months in the group receiving IFL and bevacizumab.[9] Because of its versatility and success in treating colorectal cancer, bevacizumab was considered as the first anti-angiogenic therapy approved in the United States. Bevacizumab later gained approval for use in several other solid tumors, including lung cancer, breast cancer, renal cell cancer, and in 2009, was granted accelerated approval for GBM.[9–12]

However, in 2010, the FDA rescinded its approval of bevacizumab for metastatic breast cancer based on the lack of evidence for improved OS in phase III clinical trials when compared with standard anti-mitotic chemotherapies, such as docetaxel, 5-fluorouracil, epirubicin, and cyclophosphamide.[13,14] An additional point of concern was the severity of the side effects, including wound dehiscence, gastrointestinal perforation, hemorrhage, and high-grade thrombosis.[15,16] However, the other indications remain and bevacizumab continues to be evaluated as a promising treatment in clinical trial for patients with recurrent GBM.[17,18] Based on these results, only in GBM is bevacizumab approved as a single agent.

Although bevacizumab prolongs GBM PFS, decreases tumor vascularization, and reduces permeability of vessels, it does not prolong the OS. Bevacizumab alone gives a median PFS of 4 months and 6-month PFS (PFS6) of 29%.[8] A meta-analysis of 548 patients showed that bevacizumab given in combination with other drugs gives a higher PFS6 rate of 45%, a 6-month OS rate of 76%, and an OS of 9.3 months.[19] Another study comparing bevacizumab with combination bevacizumab and irinotecan therapy showed that PFS6 is higher when bevacizumab is taken with irinotecan (PFS6 of 50.2% for combination vs 35.1% for bevacizumab alone). Despite the clear benefit of irinotecan plus bevacizumab on PFS, OS for bevacizumab plus irinotecan is 8.9 months, although bevacizumab alone only marginally extends OS to 9.7 months.[20] This lack of a clear benefit in OS for one therapy over another adds to the uncertainty of the benefit of anti-angiogenic therapies.

Subsequently, the FDA's approval of bevacizumab for the treatment of recurrent GBM was accelerated with PFS being relied on as a metric for drug efficacy rather than OS. However, the validity of PFS as an accurate measurement of treatment outcome has been questioned. Although statistical analysis shows that PFS and OS are strongly associated, it has been argued that PFS is not a replacement for OS and cannot be predictive of tumor growth after treatment.[21]

Furthermore, several studies suggest that 1 adverse consequence of this pharmacologic treatment includes hijacking of healthy nontumorous vasculature. Vessels from normal tissue are recruited via tumor infiltration to supply the lesion in a process known as co-option.[22] In short, the mechanism of bevacizumab is still not sufficiently understood[23,24] and must be further elucidated to evaluate its far-reaching effects.

MOLECULAR MECHANISM OF BEVACIZUMAB

In theory, the mechanism of bevacizumab is exquisitely simple. As an antibody for angiogenic factors, bevacizumab cuts off a tumor's life supply by inhibiting the factors required to promote and sustain vessel growth. Angiogenesis has been extensively studied for decades. Tumors should theoretically stop growing if vascularization is absent or insufficient.[25] However, this mechanism has proven to be more difficult in clinical practice than expected,[26,27] and nonangiogenic pathways that may be affected by bevacizumab are also under scrutiny.

Angiogenesis, or sprouting of new vessels from parent vessels, first involves vascular endothelial breakdown and then proliferation of existing vasculature. Extensive investigation has revealed several

VEGF-dependent and VEGF-independent pathways that regulate angiogenesis.[28] VEGF is recognized as one of the most potent stimuli for angiogenesis, making it a key pathway of deregulation in GBM development and proliferation.

VEGF

VEGF, also known as VPF (vascular permeability factor), comprises a family of 5 proteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental-derived growth factor) that regulate vasculogenesis during growth, lymphatic development, wound healing, menstruation, and pregnancy.[29] Hypoxia commonly induces VEGF release, and in particular, VEGF-A is released by GBM as a mitogen that accumulates in nearby blood vessels.[30] After release from hypoxic malignant cells, VEGF binds to both pericytes and vascular endothelial cells.

Pericytes typically wrap around the outside surface of vascular endothelial cells to maintain stability of the blood-brain barrier. In mice without pericytes, extravasation occurs as intravenously administered tracers are found to seep out of vessels and into brain tissue. In fact, the proper functioning of the blood-brain barrier depends on the extent of pericyte coverage of vessels.[31] In the presence of VEGF, pericytes detach from the vessel wall, resulting in a weakened vessel basement membrane[32–34] and contributing to the compromised blood-brain barrier that is often observed in proximity to gliomas.[35]

In addition to binding to pericytes, VEGF-A binds to the surface of vascular endothelial cells through either vascular endothelial growth factor receptor (VEGFR) -1 (also known as Flt-1) or VEGFR-2 (also known as KDR or Flk-1).[36] The binding of VEGF-A to VEGFR-2 produces tyrosine kinase activity that is 10 times more potent than the activity of VEGF-B to VEGFR-1 binding.[28] Not coincidentally, bevacizumab targets VEGF-A and neutralizes its many downstream effects. For endothelial cell proliferation, the VEGFR-2 signal transduces through phospholipase C to C-Raf-MAP kinase.[37] For endothelial cell survival and migration, the VEGFR-2 signal transduces through a tyrosine kinase PI3 K-Akt cascade to activate focal adhesion kinase.[38] One of the first cellular changes observed with VEGF binding is a 4-fold increase in intracellular calcium, which may be used as a marker for VEGF pathway activation.[39]

One of several VEGF downstream pathways leads to the phosphorylation of occludin and zonula occludens-1,[40] key proteins of tight junction function and organization.[41] Phosphorylation ultimately causes the gap junctions between endothelial cells to loosen, resulting in fenestrations, vessel dilation, and additional increase in vascular permeability. Permeability allows proteins to extravasate and extracellular matrix to be laid down as the foundation for new vessels. Endothelial cells recruit to the new extracellular matrix, and the cell at the tip of the growing endothelial cell group (known as the tip cell) is responsible for sensing environmental cues to direct vessel growth. HIF-1α (hypoxia-inducible factor) sensitizes these endothelial cells to angiogenic factors.[42] VEGF-A induces vascular hyper-permeability and sprouting of new vessels from preexisting vasculature. Therefore, the hyperplastic vascularization that is characteristic of GBM may be because of the high levels of VEGF.

Dvorak and colleagues[43–45] argue that movement of fluid between endothelial cell tight junctions and compromised endothelial cells is not sufficient to account for all of the extravasation that occurs in the presence of VEGF. Instead, they report that an intracellular system, the vesicular-vacuolar organelle (VVO), is responsible for VEGF-induced microvascular permeability. More organized than caveolae, VVOs are a system of vesicles or vacuoles that transport fluid and molecules across endothelial cells. Moreover, VVO function upregulates in the presence of VEGF (**Fig. 1**).

Upregulation of VEGF in Glioblastoma

In GBM, VEGF-A is upregulated due to hypoxia and necrosis induced by rapid tumor growth.[46,47] The gene sequence for VEGF shares common elements with erythropoietin, which is also transcribed in an oxygen-level–dependent manner.[48] The stress of unmet perfusion demands (resulting in decreased oxygen, reduced nutrients, and insufficient blood flow) induces the release of VEGF. Cells must always be near nutritional support, otherwise necrosis occurs. Hypoxia stabilizes HIF-1α and HIF-2α, which promote metabolic changes to sustain continued tumor growth. Specifically, HIF-1α and -2α have the following effects: (1) block the proteolysis of VEGF by ubiquitin, therefore allowing VEGF to accumulate[28]; (2) upregulate GLUT-1 receptors to increase uptake of glucose from blood[27]; (3) upregulate carbonic anhydrase IX (CAIX) to stabilize pH in hypoxic conditions, making the environment tolerable for continued proliferation[49,50]; and (4) sensitize endothelial cells to angiogenic signals.[42] One group suggests that different degrees of hypoxia induce different sets of rescue proteins: mild hypoxia induces the production of HIF-1α and VEGF, whereas severe hypoxia induces the production of CAIX.[49,50] Ultimately, this results in growth of additional vessels to supply the malignancy with the nutrients needed to support

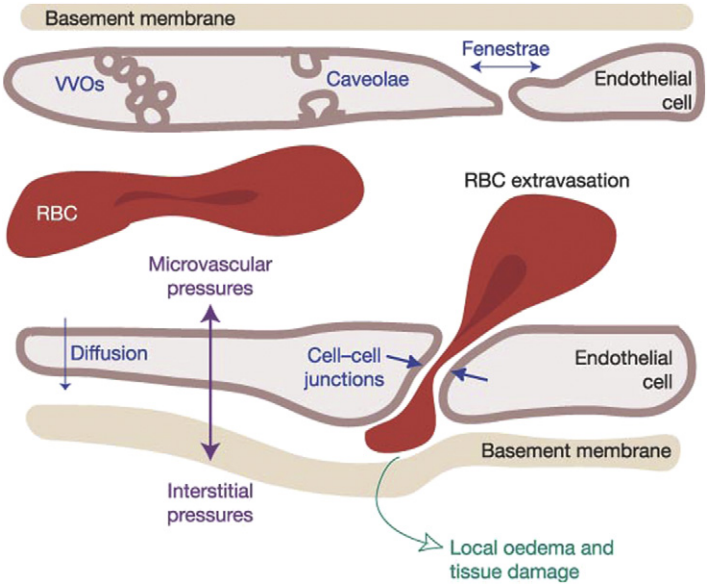

Fig. 1. Mechanisms of VEGF-induced vascular permeability. (*From* Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. Nature 2005;437:497–504; with permission.)

continued growth. All of these adaptive responses make GBM particularly resistant to various treatment modalities.

VEGF AND BEVACIZUMAB

By neutralizing VEGF-A, bevacizumab induces tumor hypoxia and also blocks the mechanism by which new vessels are induced. Although circulating VEGF levels increase after the administration of VEGF antibodies, the compensatory upregulation is not enough to induce substantial angiogenesis. In rats bearing human GBM xenografts, it is observed that the tumor is subjected to hypoxia, resulting in reduced growth, decreased mitochondria, and diminished edema caused by the decreased permeability of vessels.[27]

Tumors treated with bevacizumab do not exhibit the normal histologic characteristics of GBM. Bevacizumab successfully stops proliferation of vessel endothelium and pseudopalisading necrosis. Fewer mitochondria are present within cells, and microareas of cell death, as suggested by the presence of cell lysis, are seen in the core of the tumor. However, the edges of the tumor uniquely comprises loosely connected cells[27] that may suggest increased invasiveness, a potentially harmful consequence of bevacizumab treatment.

Bevacizumab inhibits the VEGF-induced permeability of vessels. Treatment with bevacizumab shows a marked decrease in edema as early as 1 day after treatment according to a phase II trial,[8] therefore replacing the need for corticosteroids given to block transendothelial fluid flow by regulating endothelial tight junctions.[51] Reduction in edema occurs in 50% of patients, equating to a 59% reduction in corticosteroid doses.[8] As a result, minimized corticosteroid administration allows for a decrease in complications, mortality, and morbidity associated with its chronic use. In a phase II study, bevacizumab significantly decreased abnormal fluid attenuated inversion recovery (FLAIR) signal by more than 5% in 77% of patients; 79% of patients had more than 5% volume reduction on T1-weighted magnetic resonance imaging (MRI) with contrast enhancement.[52] Another study reported that 93.2% of patients showed radiographic response (including complete response, partial response, and minimal response) and 34.1% showed either complete or partial response.[53] T2-weighted MRI taken after bevacizumab treatment shows substantial reduction in contrast enhancement. Similar results are seen in FLAIR signals with bevacizumab treatment.[52] However, this apparent improvement according to MRI does not necessarily correspond to reduction in activity of the tumor[54–56] and may simply be because of the reduction in the vascular permeability of gadolinium, which gives an exaggerated impression of tumor reduction.[57] According to a study comparing changes before and after bevacizumab treatment in T2-weighted MRI and contrast-enhancing volume, there was no significant correlation between tumor volume and PFS or OS (**Table 1**).[56]

However, there is a significant linear correlation between proliferation rate and PFS.[56] In addition, infiltrative GBM after bevacizumab treatment is

Table 1
Prediction of patient survival using traditional magnetic resonance estimates of tumor volume in $n = 26$ patients

Tumor Region of Interest	PFS (*P*-Value)	OS (*P*-Value)
Pretreatment T2 volume	0.2909	0.7226
Posttreatment T2 volume	0.8385	0.6421
Change in T2 volume	0.1624	0.5882
Pretreatment CE volume	0.3142	0.7963
Posttreatment CE volume	0.6914	0.5547
Change in CE volume	0.0760	0.4392

Abbreviation: CE, contrast enhancement.

From Ellingson BM, et al. Cell invasion, motility, and proliferation level estimate (CIMPLE) maps derived from serial diffusion MR images in recurrent glioblastoma treated with bevacizumab. J Neurooncol 2011;105:91–101; with permission.

often nonenhancing, making radiographic evaluation difficult and misleading. Overall, pseudoresponse confounds interpretation of imaging after bevacizumab use and may skew the apparent effectiveness of bevacizumab on tumor size and disease progression.[52] As the glioma response to bevacizumab is difficult to assess with traditional computed tomography and MRI,[58] different imaging methods may be necessary to properly evaluate the benefits of this therapeutic approach.

As the concentration of VEGF correlates with vessel density,[53,59,60] anti-VEGF therapy reduces concentrations of active VEGF and leads to a subsequent decrease in vessel density. Staining of endothelial cells after bevacizumab administration shows that large-sized vessels are reduced by 58%, whereas medium-sized vessels are reduced by 17%. However, there is no change in small-sized vessels,[27] indicating that limits exist in vessel remodeling and destruction. This also suggests the role of other angiogenic pathways in maintaining blood flow to the tumor. In addition, bevacizumab works very rapidly; as visualized by CD34 immunohistochemistry, tumor vessels drop to less than 50% of control density by 1 day after administration, and less than 30% of control vessel density by day 7.[61]

Because of the reduction in vessel density, bevacizumab induces a hypoxic state in tumors, resulting in the accumulation of lactate, alanine, choline, myo-inositol, creatine, taurine, mobile lipids,[62] and HIF-1α.[27,63] Although HIF-1α is involved with critical tumorigenic, survival, and angiogenic pathways in GBM, bevacizumab prevents vascularization associated with HIF-1α. As such, tumor upregulation of HIF-1α is not sufficient to induce substantial angiogenesis, yet the tumor finds other pathways for proliferation and invasion.

Although bevacizumab reduces vascularization of tumors, it also results in glioma transformation from an expansive to an invasive phenotype.[64,65] One group was able to induce invasiveness from a xenograft glioma animal model of previously noninvasive tumor by treating with bevacizumab. The borders of bevacizumab-treated tumors are extremely invasive, spreading far beyond landmark vessels, in comparison with untreated controls.[66] In addition, distant recurrences were common in patients treated with bevacizumab, even if they had reduced radiographic enhancement.[53] It is possible that the physiologic stress of hypoxia and reduction in vascularization after anti-VEGF treatment leads to the highly invasive properties of the xenografts. Several other groups[22,46,63] observed that when angiogenesis is inhibited, the tumor activates an alternate pathway that uses preexisting vessels. This bevacizumab-induced vessel co-option occurs with highly invasive tumors, resulting in a significant increase in satellite tumor area when compared with controls.[22] However, primary tumor size does not change with anti-VEGF antibody, and growth rate slows.[22] Therefore, despite slowed primary tumor progression, bevacizumab treatment may activate a separate highly invasive quality in satellite tumors that infiltrate healthy tissue to take over preexisting blood vessels.[22] Co-option provides a VEGF-independent method of vascularization of tumors and therefore serves as a method of escape from anti-VEGF treatment.

Analysis of gene expression after bevacizumab therapy demonstrates an upregulation of the Wnt pathway indicating a potential mechanism for the differentiation and infiltrative nature of these gliomas after treatment.[67] Similarly, bevacizumab induces more than half of the genes related to the PI3 K/Akt pathway to increase,[27] resulting in pathway activation and reduction in apoptosis. In combination, these factors seem to promote tumor proliferation and invasion, despite anti-angiogenic treatment effects.

THE EFFECT OF BEVACIZUMAB ON OTHER TREATMENTS

Anti-VEGF Effect on Surgery

During tumor resection, the high vascularization and abnormal state of vessels from VEGF-induced

angiogenesis increases the risk of bleeding. Therefore, debates exist on the optimal method of approach in the surgical removal of highly vascularized gliomas. Some claim that first finding the edge of the tumor, then progressively moving inwards toward the center (in an "outside-in" en bloc fashion) to remove the mass is more effective than beginning in the center of the tumor and moving outward ("inside-out"). The outside-in method avoids damaging the highly vascular infiltration often found in the center of the growth, thereby minimizing the risk of bleeding from the delicate hyperplastic blood vessels that are characteristic of GBM pathogenesis.[68] The use of anti-angiogenic factors before surgery may be beneficial in normalizing vasculature or promoting the stabilization and return of vasculature to normal healthy states, thereby minimizing the presence of poor-quality vessels and reducing the risk of bleeding. However, a side effect of bevacizumab is altered wound healing, which may complicate surgical recovery.

Anti-VEGF Effect on Radiotherapy and Chemotherapy

Because oxygen sensitizes tissue to radiotherapy, hypoxic tumors such as gliomas have characteristically been less responsive to radiotherapy. Anti-VEGF therapy halts the rapid growth of weak and poorly constructed vessels, sensitizing gliomas to radiation.[69] However, anti-VEGF-induced normalization also attenuates hyperpermeability, therefore decreasing total chemotherapeutic delivery of drugs to the tumor. Vessels seal up and restore the blood-brain barrier, effectively reducing drug movement out of vessels and into tumor tissue. A small window of time exists before further loss of vessels through anti-VEGF treatment will lead again to hypoxia, making delivery of chemotherapy to tumors more difficult.[70] It is believed that hypoxia may select for more aggressive and infiltrative malignant cell types,[71] resulting in recurrence with more invasive and aggressive tumors after bevacizumab therapy.

Dickson and colleagues[61] described phenotypic normalization of GBM vessels after bevacizumab therapy in mice xenografts. Bevacizumab-treated mice had vasculature similar to that of normal skin, whereas control mice had chaotic, dilated, irregular, and unorganized vessels. More importantly, improvement in chemotherapy delivery and efficacy transiently follows bevacizumab treatment. Topotecan penetration into tumor tissue is greater when given 1 day after bevacizumab (51%) than when given after saline control (43%). Topotecan is even more penetrant when given 3 days after bevacizumab (57%) than when given 3 days after saline control (34%). After 7 days; however, drug penetration power is lost (bevacizumab 39% vs saline control 40%) due to either loss in single-dose activity of bevacizumab or treatment-induced normalization of vessels.

Evasive Resistance

Although phase II clinical trials of bevacizumab may seem promising, anti-angiogenic therapies often demonstrate brief intervals of efficacy and are followed by the development of increased tumor growth. This loss of response may be caused by evasion.[72] There are 2 subtypes of bevacizumab evasion: infiltrative bevacizumab evasive gliomas (IBEGs) and nodular enhancing bevacizumab evasive gliomas (NEBEGs).

IBEGs maintain hypoxia but show reduced vascularity, suggesting that the glioma may have relied on invasion to reduce vascular dependence. Through transcription upregulation, IBEGs may be able to overcome the effects of bevacizumab treatment by promoting tumor cell invasion from devascularized areas into those in closer proximity to blood vessels, which results in a subset of bevacizumab-resistant GBM that exhibit an infiltrative radiographic appearance.

NEBEGs result in upregulation of VEGF-A and VEGF-C, but down-regulation of VEGF-B. VEGF-C binds to VEGFR-2 to stimulate angiogenesis.[73,74] The upregulation of VEGF-A and VEGF-C allows NEBEGs to exceed capacity and cross the bevacizumab-mediated VEGF blockade. Subsequently, NEBEGs are capable of reacquiring an increased vascularity and decreased hypoxia status comparable to pretreatment levels. It is therefore possible that VEGF-targeted treatments such as bevacizumab may cause hyperinvasive IBEG or hyperangiogenic NEBEG resulting in bevacizumab evasion.

Other Angiogenic Pathways May Contribute to Unresponsiveness or Adverse Reactions to Bevacizumab

Because GBM is not always responsive to bevacizumab treatment, other angiogenic pathways may be upregulating when VEGF is neutralized. Although VEGF has been shown to be responsible for direct initiation of angiogenesis, other growth factors such as angiopoietin 1 (Ang1), angiopoietin 2 (Ang2), and Delta are responsible for vessel remodeling and maturation.[36] VEGF-independent angiogenesis is regulated by the Angiopoietin-Tie and Delta-Notch pathways. These alternate pathways may be upregulated or adjusted when using anti-VEGF antibodies, resulting in unanticipated

side effects or unresponsiveness to bevacizumab treatment.

Although VEGF increases vessel quantity,[36,75–77] Ang1 increases vessel size.[36,77,78] Ang2 is a growth factor released from host vessels during co-option that binds to the tyrosine kinase receptor Tie to destabilize endothelial cell layers. Ang2 is regulated in part by VEGF; in the presence of high VEGF-A, Ang2 will destabilize endothelial cells, with additional subsequent increases in VEGF promoting angiogenesis. However, in the presence of little or no VEGF-A, Ang2 will simply destabilize vessels through apoptosis of endothelial cells.[28,79] This leads to hypoxia and apoptosis of tumor cells, which then induces the release of VEGF to form new vessels.

A ligand for the Delta-Notch pathway, Dll4 (Delta-like ligand 4), is upregulated in endothelial cells of GBM[80–83] because of the induction by VEGF.[84,85] Dll4 stabilizes vessels, thereby inhibiting sprouting and angiogenesis,[86] while also improving the quality of existing vessels and promoting tumor growth.[84] Dll4 is a negative regulator of angiogenesis even though it is a positive regulator of tumor progression.[81] Dll4 provides a pathway for GBM to grow, despite anti-VEGF therapy.

In addition to VEGFR-2, tumor endothelial cell specimens are often found by immunohistochemistry to have elevated levels of platelet-derived growth factor receptors (PDGFR) α and PDGFRβ.[87] The platelet-derived growth factor (PDGF) pathway is yet to be fully elucidated, but sources report that although PDGFRα is present in all astrocytic malignancies, PDGFRβ is found in tumor vasculature and is involved in angiogenesis. PDGF can stimulate and induce the proliferation of tumors.[88,89] In addition, VEGF and PDGF contain some structural similarities and sequence homology—both are transcribed as monomers, contain cysteine knots, and dimerize to facilitate activity[90] In addition, PDGF is believed to complement angiogenesis via PDGFRβ-mediated synthesis and release of VEGF.[91] One study showed that tumors are able to escape from radiation and anti-VEGF therapy by upregulating PDGF in endothelial cells.[92] This may help explain some of the resistance to bevacizumab therapy.

Side Effects

Several clinical studies have shown a higher toxicity profile in patients with GBM compared with other cancer populations. Patients with recurrent GBM who received single-agent bevacizumab showed several adverse effects including complications in wound healing, intracranial hemorrhage, and venous thromboembolic events.[15,16]

Wound healing

Studies show an increased rate of wound-healing complications in patients treated preoperatively with bevacizumab compared with those without VEGF-targeted chemotherapy.[93] Typically, surgical wounds result in increased expression of VEGF and VEGFR-2 for approximately 24 weeks postoperatively[94] to promote angiogenesis essential for normal wound healing. Thus, when bevacizumab reduces angiogenesis by inhibiting VEGF-A from activating VEGFR-1 and VEGFR-2, patients become predisposed to wound-healing complications as reported by Clark and colleagues[93,95] in their study of craniotomy wound-healing. Bevacizumab's relatively long half-life of approximately 20 days[96] represents a fairly sustained interval until complete elimination from the body. Thus, the timing of VEGF-targeted therapy for both preoperative and postoperative therapy is critical. Sugrue and colleagues[97] reported that 78% of postoperative complications occur when therapy starts within 60 days of surgery. However, postoperative complications still remain low. In fact, the 4% to 6% of wound healing complication rate at the craniotomy site was one of the reasons for the accelerated FDA approval of bevacizumab for GBM.[17,98]

Intracranial hemorrhage

Fatal intracranial hemorrhage is one of the most serious complications of anti-angiogenic treatment of GBM. Clinical trial results show that bevacizumab-administered patients have a risk of severe (grade 3) intracranial hemorrhage of 2% to 5%.[17,99,100] Bevacizumab's inhibition of VEGF causes changes in vascular endothelium, which may play a pivotal role in the mechanism of this complication.[101] Thus, an increased risk of intracranial hemorrhage may be caused by the inability to regenerate the endothelium.

Venous thromboembolic events

Bevacizumab causes abnormal endothelial cell apoptosis, which may result in venous thromboembolic events by exposing subendothelial molecules. A possible mechanism for thrombosis could be the loss of VEGF-dependent production of the platelet inhibitors, such as prostaglandin I-2 and nitric oxide.[102] Risk of venous thrombosis is historically increased with the use of angiogenic inhibitors, such as thalidomide and lenalidomide. As another angiogenic inhibitor, bevacizumab has also been suggested by Nalluri and colleagues[103] to be capable of increasing the risk for venous thromboembolic events in an already susceptible population, with relatively high rates in patients with GBM ranging from 7% to 32%.[8,99]

SUMMARY

The overall benefit of bevacizumab remains controversial. Although it inhibits angiogenesis and sensitizes gliomas to radiotherapy and chemotherapy, these treatment advantages and the extended PFS come at a significant price—tumor expansion may be substituted for tumor invasion into adjacent healthy tissue, and OS is not significantly extended. Future studies may need to assess novel methods of evaluating and visualizing tumor progression to determine the effectiveness of bevacizumab. Phase III clinical trials are currently investigating bevacizumab's potential as a therapeutic option for primary GBM.

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