Angiogenesis and Vascular Endothelial Growth Factor in malignant gliomas

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ABSTRACT: VEGF receptors and their ligands are highly expressed in brain tumor cells but not in the adult normal brain cells. Angiogenesis process has been shown to play an important role in brain tumors cells development and survival. Malignant gliomas are the most common brain tumors with a low survival rate, despite the prompt treatment at diagnosis. Standard treatment consists of resection, radiation and chemotherapy with temozolomide, followed by 6 months of chemotherapy. Recurrence after standard treatment is often seen, indicating the high therapeutic resistance of glioblastoma cells. It has also been reported that glioblastomas are among the most angiogenic of all neoplasms in human. This paper will review recent data of the role of VEGF angiogenic growth factors in malignant gliomas angiogenesis.

KEY WORDS: glioblastoma, VEGF, tumor angiogenesis

Introduction

Angiogenesis is a complex process, initiated by many factors such as: hypoxia, wound healing and cancer. The process of angiogenesis is characterized by structural and functional abnormal tumor vessels. These abnormalities include defective endothelium, basement membrane, pericyte coverage leading to decrease levels of oxygen and necrosis that trigger the angiogenic activity of growth factors. The fast tumor growth rate also induces hypoxia, which in turn initiate other pathways involved in new vessels formation, (e.g. intussusceptive angiogenesis, vessel co-option and lymphangiogenesis [1, 2].

A number of tyrosine kinase receptors (PDGFRα, VEGFR1, VEGFR2, EGFR) mediates the response of endothelial cells in adult tumor vessels. Many studies have reported over expression of proangiogenic factors, including basic fibroblast growth factor, vascular endothelial growth factor (VEGF) and platelet derived endothelial growth factor which promotes endothelial cell proliferation/migration in primary brain tumors [3].

The main regulator of endothelial cell proliferation and mobility, VEGF production is activated by the hypoxic conditions. VEGF effect is mediated by two tyrosine kinase receptors, VEGFR-2 (KDR) and VEGFR-1 (Flt-1) [4].

VEGF, VEGFR-1, VEGFR-2 are highly expressed in tumor cells but not in the adult normal brain [5].

Malignant gliomas are the most common brain tumors with a low survival rate (14.6 months), despite the prompt treatment at diagnosis. Standard treatment consist of resection, radiation and chemotherapy with temozolomide, followed by 6 months of chemotherapy. Recurrence after standard treatment is often seen, indicating the high therapeutic resistance of glioblastoma. It has also been reported that glioblastomas cells are resistant to targeted therapy with bevacizumab, a monoclonal antibody against vascular endothelial growth factor (VEGF). Several studies indicate the use of alternative pathways to maintain tumor growth, after the therapy [6].

Tumor metastasis from other cancers, such as lung, breast, colon cancer and melanoma, undergoes the same process, one study demonstrating the importance of VEGF in metastatic brain tumors. The first step in tumor metastasis is tumor cell dissemination, after extravasations, the survival and proliferation is dependent on angiogenesis [7-9].
VEGF system

The VEGF family consists of six factors: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PIGF). While VEGF-A is a key regulator in vasculogenesis, VEGF-C and VEGF-D are essential in lymphatic angiogenesis. VEGF-A is a heparin binding glycoprotein of 45kD [10]. It is important for endothelial cell’s (EC) migration, proliferation, survival, permeability although some studies suggest its mitogenic potential in lymphocytes, Schwann cells, retinal pigment epithelial cells [11;12] also been reported that VEGF-A prevents apoptosis by triggering the expression of anti-apoptotic proteins and B-cell lymphoma 2 (Bcl-2) [13]. Another well documented response of increased VEGF-A is in hypoxia induced angiogenesis. The major protein that mediates the angiogenesis process is hypoxia inducible factor (HIF-1), a glycoprotein hormone that is a heterodimer with two subunits HIF-1α and HIF-1β [14, 15].

In the adult, VEGF-A participates in physiological angiogenesis: wound healing, vascular permeability, haematopoiesis, vascular tone and inflammation. The VEGF system also participates in aberrant angiogenesis such as rheumatoid arthritis, diabetic retinopathy and many types of cancer [1].

In embryos, vascular endothelial growth factor is consequential in the development of functional vessels and in inactivation of VEGF gene results in disruption of vessels and embryonic lethality [5]. Major VEGF isoforms are indicated in table 1.

<table>
<thead>
<tr>
<th>VEGF Isoforms</th>
<th>Binding</th>
<th>Properties</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF121</td>
<td>Does not bind heparan sulfate</td>
<td>Acidic polypeptide</td>
<td>Freely secreted from cells</td>
</tr>
<tr>
<td>VEGF165</td>
<td>Moderate affinity for heparan sulfate</td>
<td></td>
<td>Retained on the cell surface</td>
</tr>
<tr>
<td>VEGF189</td>
<td>High affinity for heparan sulfate</td>
<td>Highly basic</td>
<td>Completely sequestered in the endothelial cell membrane (ECM)</td>
</tr>
<tr>
<td>VEGF206</td>
<td>Highly basic</td>
<td></td>
<td>Completely sequestered in the extracellular matrix</td>
</tr>
</tbody>
</table>

Angiogenesis in brain tumor

Angiogenesis is important in development of tumor, where many types of vasculogenesis were indentified. Tumor vasculogenesis is a process characterized by sequential steps. The activated protease (such as metalloproteinases - MMP) degrades the ECM and basement membrane, vasodilatation mediated by nitric oxide (NO) and integrins mediate the migrations and proliferation of endothelial cells through the matrix. Another form of vasculogenesis, the intussusceptive angiogenesis, is a fast process that expands the capillary plexus in a matter of minutes and the capillary wall splits in two without proliferation. An important role in this process is thought to be played by pericytes and myofibroblast [16, 17].

In brain tumors, especially in glioblastoma, apart from the two types of angiogenesis described above, it has been reported a new vasculogenesis process, named vessel co-option. In vessel co-option process, no angiogenic response is involved and the tumor growth commence as an avascular mass. This mass is saved in later stages, Ang-2 seems to be a regulator in the process, high Ang-2 expression was observed in co-opted vessel [18, 19].

Another type of angiogenesis found in some tumors like astrocytoma, melanoma, osteosarcoma, pheochromocitoma was called vascologenic mimicry [20-23]. That has been associated with an increased expression of laminin5γ2 and metalloproteinase [24].

Besides VEGFs, angiogenesis is controlled by others direct factors, like fibroblast growth
factor (bFGF) family [25]. From the 23 members, FGF-1 and FGF-2 are the most studied with four FGF tyrosine kinase receptors identified. FGF release in the extracellular matrix was observed before the initiation of angiogenesis. Other pro-angiogenic factors are: the placental growth factor (PLGF), the angiopoietin family (angiopoietin-1 and angiopoietin-2) and semaphorins.

Angiopoietin pathway interacts with VEGF system, playing a significant role in vascular remodeling by intervening in the recruitment and proliferation of pericytes. Angiopoietin-1 (ang-1) and angiopoietin-2 (ang-2) bind to and activate tyrosine kinase receptor, Tie-2. While ang-2 is associated with anaplastic astrocytomas and glioblastoma multiforme, ang-1 regulates angiogenesis during progression of astrocytomas [3].

Angiogenesis is also negatively regulated by endogenous inhibitors like endostatin, trombospondin-1, platelet factor 4, interferon γ and interferon α. When the expression of these negative modulators is increased, tumors enter a period of dormancy [26].

**VEGFR and pathway signaling in brain tumors**

VEGF ligands have high affinity for three tyrosine kinase receptors, known as VEGFR-1, -2 and -3. The VEGF ligands also interact with VEGF-binding molecules named co-receptors, which lack established VEGF-induced catalytic function (i.e. heparan sulphate proteoglycans (HSPGs) and neuropilins (NP) [28].

VEGFR-1 and VEGFR-2 are characterized by seven immunoglobulin (Ig) like domains, a single transmembrane region and a constant tyrosine kinase domain, which is interrupted by a kinase-insert domain [28]. VEGFR-1 (Flt-1) is a 180kD glycoprotein, with the highest affinity for VEGFs. VEGFR-1 also binds PIGF and VEGF-B. During early developmental stages VEGFR-1 appear to be a negative regulator of VEGF action. However, VEGFR-1 is implicated in enhancing matrix metalloproteinase expression and chemotaxis in monocyes [28-30].

VEGFR-2 (KDR) is a 200-230 kD with high affinity for VEGF and also for VEGF-C and VEGF-D. VEGFR-2 is expressed in the development of endothelial cells and in hematopoietic stem cells. By comparison to VEGFR-1, VEGFR-2 has a much more efficient response to tyrosine phosphorylation after ligand binding [31-32].

VEGF plays a central role in the pathological process of tumor growth [33]. Several studies have indicated that brain tumors angiogenesis is mediated by VEGF [48]. For this reason, anti-angiogenesis molecules to target VEGF/VEGF receptors are proposed to be effective in brain cancer therapy.

**VEGFR inhibitors in brain tumors**

The modern treatment in high grade gliomas is trying to combine chemotherapy, radiation and surgical resection of the tumor and much newer methods of antiangiogenesis by blocking VEGF and VEGF receptor.

Anti-angiogenic strategies that block VEGF-A/VEGFR2 are the most important approach in clinical therapy of brain tumors (Figure 1). Drugs like Avastin (bevacizumab), are already approved by Food and Drug Administration and have demonstrated an improvement in survival rates in combination with chemotherapy in patients with recurrent glioblastoma and metastatic colorectal cancer [34, 35]. However, for the treatment of recurrent malignant gliomas a new medication, aflibercept, was approved in 2011, and is used in combination with radiation and chemotherapy [36-38]. Other compounds that were used in treating brain tumors are tyrosine kinase inhibitors (TKI) like cediranib, vatalanib, sorafenib or more VEGF-selective newly FDA approved axitinib. The tyrosine kinase inhibitors and VEGF inhibitors can be combine because they have different effects on endothelial cell. While VEGF inhibitors decrease nitric oxide and prostacyclins synthesis, tyrosine kinase inhibitors maximize the antiangiogenic response decreasing blood perfusion in tumor after a single dose of TKI [39, 40].

Some studies are describing a “window of opportunity” when VEGF inhibitors (bevacizumab) and classic chemotherapy (temozolomide) are combined. However, the combined treatment modality did not increase the patient’s survival rate, in all cases studied [41]. It has also been reported that combined therapy with antiangiogenetic drugs and radiation induces antitumor activity in brain tumors [4].

Maybe one of the most important arguments for trying the combination therapy in brain tumors is the decrease in drug toxicity after adding antiangiogenic drugs and reduction in chemotherapeutic drug exposure [42].
The most common adverse effect of antiangiogenesis is hypertension, due to VEGF deprivation. Also, in VEGF inhibitors therapy, thromboembolic events were reported from toxicities. Another reported toxicity was neutropenia, after combination between bevacizumab and chemotherapy [35, 36, 39-41].

A new method (metronomic method) for the administration of antiangiogenic therapy is by lowering the dose of medication and increasing the frequency of drug treatment. Some of traditional drugs such as cyclophosphamide and vinblastine had significant antiangiogenic response. Furthermore, when administered metronomic method cyclophosphamide and bevacizumab were observed better results than classical chemotherapy with bevacizumab.

The monitoring of the patients after therapy was made with contrast enhancement on computer tomography (CT) and magnetic resonance imaging (MRI) scans, new radiographic response criteria are to be made in response to diffusion imaging. Also, tumor markers such as CD31, CD34, CD105, and von Willebrand factor are used to monitor the patient’s response to therapy [43-46].

Neuro-imaging techniques and more important reliable markers are necessary for the evaluation of therapy in patients undergoing antiangiogenesis treatment [46]. Another important point of preclinical studies is the shift toward other growth factors, like PIGF, that was shown to play a significant role in glioma vessels maturation and stabilization.

Resistance to angiogenic treatment by increasing the expression of other angiogenic factors, and increasing the tolerance of tumor to hypoxia was reported in several studies. So, instead of having a positive response after the antiangiogenic therapy a hype in tumor cell invasion and metastasis has been observed [36]. Drugs that block VEGF-A/VEGFR2 are being the subject of several clinical trials for the treatment of malignant brain tumors, although the improvements are more often seen in combination with chemotherapy or radiotherapy, their prolonged administration requires a better comprehension of their antitumor effects and the resistance mechanism to drug treatment that is sometimes seen after antiangiogenic therapy [47]. Even if progress have been made in antiangiogenic treatment of brain tumors, there is still a long way to go until fully understand...
the complex process of angiogenesis, which may lead to a better outcome after drug combination therapy for both primary and metastatic brain tumors.

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References


43. Hylton N. Dynamic contrast-enhanced magnetic resonance imaging as an imaging biomarker, J Clin Oncol 2006;24:3293-3298.


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