Correlation of endothelial nitric oxide synthase and vascular endothelial growth factor expression with malignancy in patients with astrocytic tumors

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Summary

Purpose: Many characteristics of malignant brain tumors (increased vascular permeability, vasodilatation, neovascularisation and free radical injury to the tumor and adjacent normal tissues) are believed to be mediated by nitric oxide (NO) synthetized by endothelial NO synthase (eNOS). Overexpression of vascular endothelial growth factor (VEGF) is associated with several central nervous system (CNS) diseases and tumors. Our aim was to study immunohistochemically the coexpression of eNOS and VEGF in astrocytic tumors and to analyse their possible correlation with tumor grade, angiogenesis and proliferation index.

Materials and methods: Sections from 120 randomly selected patients with supratentorial astrocytic tumors [38 glioblastomas (GB), 22 anaplastic astrocytomas (AA) and 20 low-grade astrocytomas (LA)], also including oligodendrogliomas (n=20) and mixed oligoastrocytomas (n=20), were immunostained with monoclonal antibodies for eNOS and VEGF using the avidin-biotin method. The proliferative potential was assessed as the MIB-1 staining index for tumor cells.

Results: There was positive correlation between eNOS and VEGF expressions and histological grade (p < 0.05) in terms of intensity and extent of immunoreactivity distribution. Oligodendrogliomas showed significantly less VEGF and eNOS immunoreactivity compared to pure astrocytomas (p < 0.05).

Conclusion: Overexpressions of eNOS and VEGF in astrocytic tumors were significantly correlated with histological grade, proliferative potential and malignant transformation. The expression of VEGF in a necrotic and ischemic tumor such as GB is more intense and diffuse than low-grade astrocytomas. These findings suggest that eNOS overexpression in tumor vasculature would be precipitated by transformation into an angiogenic phenotype in the process of neovascularisation in astrocytic tumors.

Key words: astrocytic tumor, endothelial nitric oxide synthase (eNOS), immunohistochemistry, vascular endothelial growth factor (VEGF)

Introduction

It is known that angiogenesis plays an important role in growth and spread of some malignant brain and systemic tumors. Many characteristics of malignant tumors such as increased vascular permeability, vasodilatation, neovascularisation and free radical injury to the tumor and adjacent normal tissues are believed to be mediated by NO [1]. It also influences neurotransmission and emerges as an important mediator of neurotoxicity in a variety of disorders of the CNS. The synthesis of NO is catalyzed by NO synthases (NOS) existing in 3 isoforms: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). NO synthase has implications in the pathophysiology of primary glial brain tumors with enhanced expression of nNOS and eNOS in high grade astrocytic tumors, WHO grades III and IV. eNOS plays a crucial role in the regulation of tumor blood flow and vascular permeability [2]. eNOS-derived NO acts as an endogenous inhibitor of TNF-alpha-induced NF-kappaB
activity and COX-2 transcription in the endothelium of the cerebral capillaries [3].

Overexpression of VEGF is associated with several CNS diseases and abnormalities like trauma, ischemic injuries, inflammation and tumors. VEGF and its receptors VEGFR-1 and VEGFR-2 are considered to play a major role in tumor angiogenesis. VEGF has additional effects on endothelial cells, including induction of plasminogen activator and vasodilatation, which occurs by stimulation of NO release from endothelial cells. Microscopically, infusion of VEGF to normal rodent brain also induces vessel dilatation and altered morphology, probably due to increased NO [4]. For astrocytic gliomas, the expression of VEGFR correlated well with tumor malignancy, even better than VEGF content [5]. A recent study showed a correlation existing between the grade of immunoreactivity for VEGF and the grade of p53 protein expression in malignant gliomas [6].

In the English literature, eNOS expression in astrocytic tumors is studied in detail, but only few studies about the relationship between VEGF and NO expressions in astrocytic tumors exist. The aim of this article was to study the coexpression of eNOS and VEGF in astrocytic tumors immunohistochemically and to analyse their possible correlation with tumor grade, angiogenesis and proliferation index.

Materials and methods

Formalin-fixed paraffin-embedded sections from 120 randomly selected patients with supratentorial astrocytic tumors (38 GBs, 22 AAs and 20 low-grade LAs) also including oligodendrogliomas (n=20) and mixed oligoastrocytomas (n=20) were immunostained with monoclonal antibodies for eNOS (anti eNOS mAb, Labvision Corporation, 1:50-1:100) and VEGF (VEGF antigen mAb, Labvision Corporation, 1:100) using the avidin-biotin complex immunohistochemical method. The intensity of immunoreactivity for eNOS in endothelial cells and for VEGF in glial cells was evaluated as slight, moderate or intense staining without any clinicopathologic knowledge. The proliferative potential was assessed as the MIB-1 staining index for tumor cells. For statistical analysis we used the Spearman rank test.

Results

The patients’ mean age was 48.7 years (range 21-74). Expression of eNOS was slight in 17 and moderate in 3 specimens of LAs; slight in 5, moderate in 15 and intense in 2 specimens of AAs; and moderate in 8 and intense in 30 specimens of GBs (p <0.05; Figure 1). Significant correlation between eNOS immunoreactivity and histological grade was found. There was also significant positive correlation between the histological grade and VEGF expression in terms of intensity and extent of distribution of VEGF immunoreactivity (p <0.05) (Table 1; Figure 2). Oligodendroglioma as a group (+, n=14; ++, n=5; ++++, n=1) showed significantly less VEGF and eNOS immunoreactivity compared to pure astrocytomas (p <0.05). No significant difference between oligoastrocytoma group (+, n=4; ++, n=8; ++++, n=8) and pure astrocytic tumor group was found. Greater number of MIB-1 indices were demonstrated in tumors with higher eNOS and VEGF expressions (p <0.05; Table 1).

Discussion

In the English literature there are many studies

<table>
<thead>
<tr>
<th>Histology (no. of patients)</th>
<th>eNOS*</th>
<th>VEGF**</th>
<th>Ki67 (%) Mean***</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA (20)</td>
<td>+ 17</td>
<td>+ 12</td>
<td>12</td>
</tr>
<tr>
<td>AA (22)</td>
<td>+ 5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>GB (38)</td>
<td>+ 0</td>
<td>++ 8</td>
<td>30</td>
</tr>
</tbody>
</table>

*p <0.05, Spearman rank, r=124.3; **p <0.05, Spearman rank, r=253.7; ***p <0.05
LA: low grade astrocytoma, AA: anaplastic astrocytoma, GB: glioblastoma multiforme
which proved that angiogenesis plays a crucial role in the histologically relatively benign tumor, LA. But it is still unknown how neovasularity leads to astrocytic malignant progression. Based on these data Saleem et al. proposed that it is possible to label LAs as “angiogenic” or “nonangiogenic” types, based on their microvascular density and/or VEGF staining pattern [7]. Lamszus et al. detected strongly increased concentrations of VEGFR-1 protein and VEGF-A (the ligand of VEGFR-1) in GBs compared with LAs and normal brain and suggested that inhibition of tumor angiogenesis via VEGFR-1 could have therapeutic potential for a variety of human cancer types [8].

The most recent study by Merrill et al. [4] which is an expanded review of research in humans and data from animal experiments shows every detail about VEGF and NOS-NO and the relationship between these molecules. Increased expression of VEGF is most consistently and dramatically observed in GBs and hemangioblastomas. The VEGF and VEGFR molecules are overexpressed in GBs compared with normal brain. Expression of VEGF correlates with aggressive tumor growth and edema, and with increased microvascular density, a key element in determining glioma grade and a negative prognostic indicator for survival. Upregulation of VEGF correlates with regions of hypoxia, which result from necrosis caused by rapid tumor growth, and hypoxia upregulates VEGF in perinecrotic regions. As a result, VEGF is required for the growth and malignant progression of gliomas/GBs. Although the infiltrative behavior associated with GBs is not angiogenesis-dependent, glioma cells not expressing VEGF do not grow well, nor do they exhibit extensive vascular changes. Upregulation of VEGF itself does not explain all of the vascular abnormalities observed in GBs. The anti-VEGF therapy may delay growth of the primary tumor focus, but it may also encourage migration of tumor cells along the existing cerebral vasculature. So anti-VEGF therapy might be useful as an adjunct to other treatments, but it is unlikely to provide the breakthrough needed to alter the grim prognosis facing a patient with GB [4,9-11]. Also in our study the expression of VEGF in GBs was more intense and diffuse than in LAs and there was a correlation between the expressions of VEGF and eNOS, supporting the mechanism suggested in this study.

When the 3 isoforms of NOS (nNOS, iNOS, eNOS) were studied, Broholm et al. found that nNOS expression may be a putative useful indicator of brain tumor differentiation and malignancy and the enhanced expression of eNOS in vascular endothelial cells of glial neoplasms and metastases raises the possibility that NO production in tumor endothelial cells may contribute to tumor blood flow regulation and possibly brain edema [12-15]. We studied the expression of eNOS in all grades of astrocytic tumors and found that its expression was more intense and diffuse in GBs showing the angiogenic capacity of astrocytic tumors.

Our study demonstrated that overexpression of eNOS and VEGF in astrocytic tumors was significantly correlated with histologic grade, proliferative potential and malignant transformation. Because VEGF expression is increased in hypoxia, the expression of VEGF in a necrotic and ischemic tumor such as GB is more intense and diffuse than LAs. These findings suggest that eNOS overexpression in tumor vasculature would be precipitated by transformation into an angiogenic phenotype in the process of neovascularisation in astrocytic tumors.

References