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Vascular endothelial growth factor (VEGF) is elevated in brain tumor cysts and correlates with tumor progression

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Abstract Vascular endothelial growth factor (VEGF), a key regulatory protein in neoangiogenesis, is strongly expressed in a variety of primary brain tumors, particularly malignant gliomas. In previous studies, high levels of VEGF were also reported in tumor cysts of glioblastomas. Using an ELISA method we measured the concentration of VEGF in matched samples of aspiration fluid from tumor cysts and serum. Samples were collected from 14 patients with primary brain tumors of various histology (six glioblastomas, one protoplasmatic astrocytoma, two pilocytic astrocytomas, one ependymoma, one meningioma, and three craniopharyngiomas) and two patients with solitary cystic brain metastases from adenocarcinomas of the lung. Aspiration fluids of tumor cysts from all patients revealed high VEGF levels ranging between 882 and 1,263,000 pg/ml, which were 2 to more than 2,000 times higher than the corresponding serum levels. Maximum VEGF levels were detectable in cyst fluids from recurrent glioblastoma. Serum VEGF levels ranged between 125 and 716 pg/ml and did not differ from serum levels in 145 healthy volunteers. In a single patient with metastatic lung cancer the concentration of VEGF in serum and cyst fluid was determined during disease progression. During 60 days of follow-up VEGF concentrations in the cyst fluid collected by puncture of an Ommaya reservoir increased 650-fold, while serum levels remained rather constant. These findings indicate that immunoreactive VEGF is produced at the tumor site and abundantly released into the cyst fluid of primary and metastatic brain tumors. Interestingly, this abundant local release is not reflected in serum VEGF levels, even in the case of very high VEGF concentrations in tumor cysts. Thus, VEGF may be biologically relevant for the formation of tumor cysts in brain tumors and correlates with local disease progression.

Key words Brain tumors · Tumor cysts · Angiogenesis · Vascular endothelial growth factor

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Introduction

Vascular endothelial growth factor (VEGF), a 46-kDa glycosylated homodimeric protein, has been implicated in the neovascularization of a variety of tumors [11]. In addition, VEGF has been identified as an important regulator of vascular permeability [3, 4, 16, 19]. VEGF can be expressed and secreted by tumor cells and acts on endothelial cells in a paracrine fashion [14]. Among others, this angiogenic growth factor is expressed in various primary brain tumors [2, 5, 9, 13, 14, 15, 20, 22] and solid tumors, which frequently metastasize to the brain, such as lung cancer [10]. A number of studies indicate the bifunctional role of VEGF in the biology of brain tumors, regulating both angiogenesis and vascular permeability [2, 3, 8, 9, 13, 14, 19, 20, 22]. VEGF expression in glioblastomas and meningiomas was shown to also be associated with the peritumoral edema [3, 15]. VEGF has been shown to be highly expressed not only in glioblastoma tissue, being most abundant in the perinecrotic palisading cells, but also in cyst fluids from glioblastomas [2, 3, 20, 22]. In ad-

dition, high levels of VEGF mRNA transcripts have been demonstrated in the tumor cyst wall of pilocytic astrocytoma when using in situ hybridization [8]. These data suggest that VEGF, besides its angiogenic capacity, may be important for vascular permeability, the production of cyst fluid and the formation of cysts within brain tumors. In fact, the formation of tumor cysts is not only a typical feature of glioblastomas but of other brain tumors as well, including low-grade astrocytomas, meningiomas, craniopharyngiomas, cerebellar hemangioblastomas, and brain metastases [3, 5]. Fluid-filled macrocysts are most likely caused by microvascular extravasation [3, 19]. To assess whether VEGF is locally produced and detectable in cysts formed by various brain tumors, we measured VEGF concentrations in matched samples of cyst fluids and sera from 16 patients with brain tumors of various histologies. In addition, in a patient with a solitary brain metastasis from lung adenocarcinoma the concentration of VEGF in cyst fluid and serum was determined during disease progression.

Materials and methods

Patients

Fourteen patients with primary cystic brain tumors of various histologies (six glioblastomas, one protoplasmatic astrocytoma, two pilocytic astrocytomas, one ependymoma, one meningioma, and three craniopharyngiomas) and two patients with solitary cystic brain metastases from bronchogenic adenocarcinomas were investigated for VEGF levels in aspiration fluids from tumor cysts. Corresponding serum VEGF levels were determined in 14 of these 16 patients. The patients' characteristics are summarized in Table 1. Serum samples from 145 healthy blood donors were taken as a control. Brain tumor samples, obtained during open surgery, were routinely processed for histology and diagnosed according to the WHO classification of brain tumors [6]. In all except two patients cyst fluid was collected by intraoperative puncture of the tumor cyst during open surgery. In cases 10 and 15 (Table 1), cyst fluid was drawn via a catheter stereotactically inserted into the tumor cyst and connected to a subcutaneous Ommaya reservoir.

A 57-year-old male patient suffering from a solitary brain stem metastasis from a lung adenocarcinoma (case 15; Table 1) was treated with an Ommaya reservoir for repeated tumor cyst aspirations. The patient was diagnosed with lung cancer in October 1995. After primary tumor resection the patient received local radiotherapy. After 2 years in complete remission the patient developed neurological symptoms with a sixth nerve palsy on the right and hemiparesis on the left side. MRI showed a brain stem mass lesion, suggestive for metastasis. The lesion was partially resected. Histology confirmed the diagnosis of adenocarcinoma metastasis. For the residual tumor lesion the patient received local radiotherapy with 40 Gy. After 6 months of remission the patient again deteriorated clinically as well as on MRI with a growing tumor mass. The patient was treated with chemotherapy using topotecan and experienced a partial response. Eight months later deterioration was again observed clinically and radiographically. MRI scan showed an increasing mass lesion, predominantly caused by a tumor cyst encased by relatively little solid tumor (Fig. 1a). To decompress the space-occupying cyst the catheter of an Ommaya reservoir was placed into the cyst. The cyst was decompressed by repeated fluid draws from the reservoir. Initially, this resulted in relief of symptoms for 6 weeks. During clinical deterioration the patient underwent follow-up MRI showing progressive growth of the solid tumor (Fig. 1b). Three weeks later the patient died from pneumonia. Autopsy showed the brain stem cyst surrounded by solid tumor and confirmed the correct position of the reservoir catheter. No recurrence of the primary tumor in the lung and no other metastases were found.

Sample processing

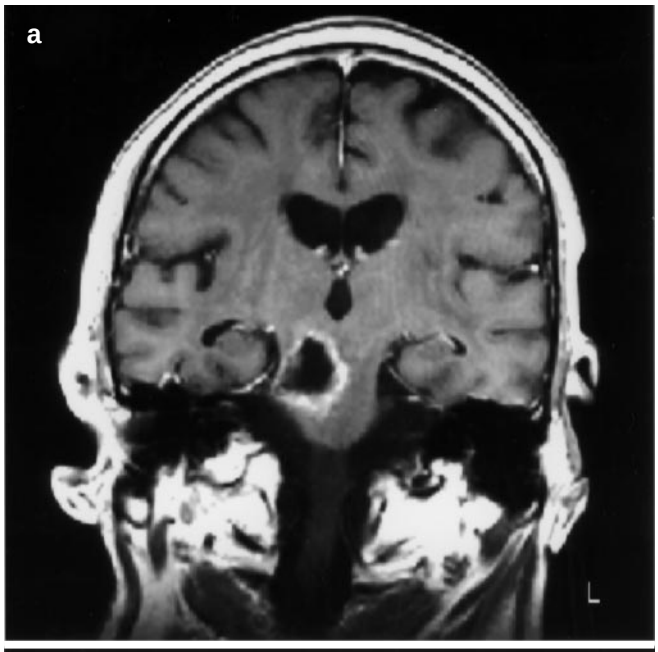
Cyst fluids in each individual patient and serum samples were collected at the same time. Serum and cyst fluid samples were centrifuged at 1,700 *g* for 10 min at 4 °C. Cell-free supernatants were collected, separated in aliquots and stored frozen at –80 °C until assaying.

Immunoassay for human VEGF

To determine VEGF concentrations in sera and cyst fluids a commercially available sandwich ELISA (Quantikine Kit, R and D Systems, Minneapolis, Minn.) was used according to the manufacturer's guidelines. A standard curve was generated using human recombinant $VEGF_{165}$. In our hands the detection limit for recombinant human VEGF was 25 pg/ml. Values of intra- and inter-assay

Table 1 Patient characteristics, tumor histology and VEGF concentrations in tumor cyst fluid and matched serum specimens. VEGF levels were determined by means of ELISA (*VEGF* vascular endothelial growth factor, *protopl.* protoplasmatic, *Rec.* recurrent, *NT* not tested)

Case no.	Sex	Age (years)	Histology	VEGF cyst fluid (pg/ml)	VEGF serum (pg/ml)
1	M	45	Glioblastoma WHO grade 4	8,925	125
2	F	38	Glioblastoma WHO grade 4	2,252	244
3	F	40	Rec. glioblastoma WHO grade 4	814,600	711
4	F	54	Rec. glioblastoma WHO grade 4	194,200	236
5	F	69	Rec. glioblastoma WHO grade 4	986,300	374
6	M	52	Rec. glioblastoma WHO grade 4	1,263,000	510
7	F	28	Protopl. astrocytoma WHO grade 2	24,191	129
8	M	36	Pilocytic astrocytoma WHO grade 1	1,886	NT
9	F	15	Pilocytic astrocytoma WHO grade 1	94,831	716
10	M	8	Craniopharyngioma	14,433	376
11	F	2	Craniopharyngioma	39,010	322
12	M	12	Craniopharyngioma	9,128	201
13	M	30	Ependymoma WHO grade 2	44,461	228
14	F	73	Meningioma	10,998	140
15	M	57	Lung cancer metastasis	882	441
16	F	58	Lung cancer metastasis	177,100	NT



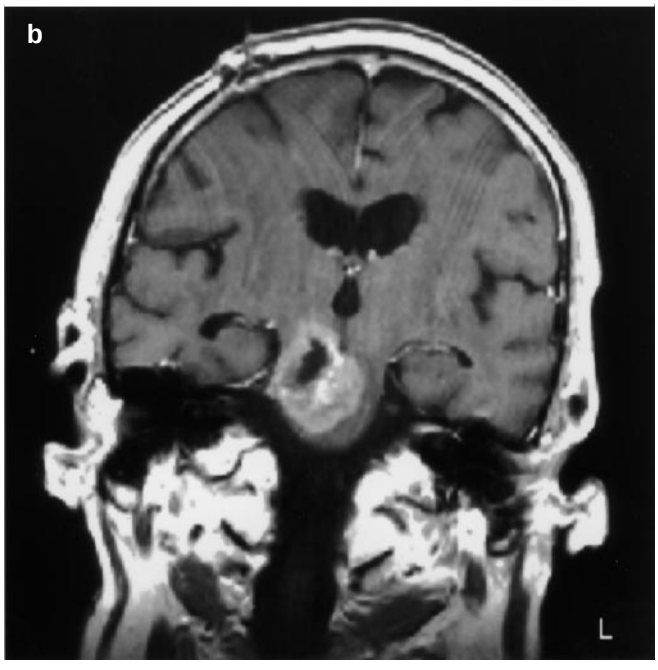


Fig. 1 MRI of case 15 with cystic brain metastasis from an adenocarcinoma of the lung before (**a**) and 6 weeks after placement of the Ommaya reservoir (**b**). **a** The coronal T1-weighted MR image (TR = 550 ms, TE = 15 ms, 4-mm slice thickness) after intravenous contrast enhancement (0.1 mmol/kg Gd-DTPA) shows brain stem metastasis with a large cystic portion encased by relatively little solid tumor. **b** The follow-up MRI (identical parameters to Fig. 1a) shows considerable progression of the solid tumor component (*MRI* magnetic resonance imaging)

variation were essentially within the range given by the manufacturer, i.e., 3.5–6.5% and 5–8.5%, respectively. This assay detects $VEGF_{121}$ and $VEGF_{165}$, the two low-molecular weight isoforms of human VEGF [15]. Tumor cells are known to predominantly secrete $VEGF_{165}$ [15].

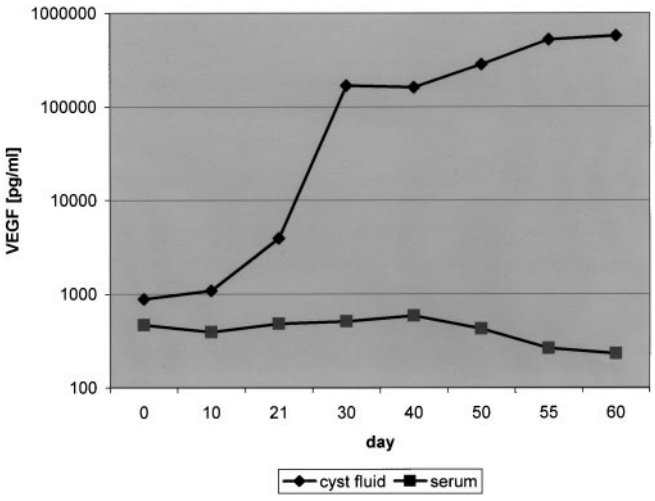


Fig. 2 Plot graph of VEGF concentrations [pg/ml] determined by ELISA in cyst fluid and matched serum samples in case 15 suffering from progressive cystic brain metastasis (*VEGF* vascular endothelial growth factor)

Results

VEGF concentrations in serum and cyst fluid

The VEGF concentrations in cyst fluid and serum of each individual patient are summarized in Table 1. Aspiration fluids of tumor cysts revealed a median VEGF concentration of 31,600 pg/ml (range 882–1,263,000 pg/ml). The VEGF levels in cyst fluids were 2 to more than 2,000 times higher than in corresponding serum. Notably, maximum VEGF levels were found in cyst fluids from patients with recurrent glioblastoma. The median VEGF concentration in matched serum samples was 283 pg/ml (range 125–716 pg/ml), which was not significantly different from the VEGF values in sera taken from 145 healthy blood donors (median 369; range 30–1,722 pg/ml).

VEGF protein concentrations in serum and cyst fluid of a patient with a progressing metastatic brain tumor

In patient 15 (Table 1) the VEGF concentration in matched samples of sera and cyst fluids was assessed repeatedly during disease progression. In parallel, the patient was evaluated clinically and by MRI (Fig. 1). The VEGF concentration in cyst fluid specimens increased dramatically from 882 to 574,460 pg/ml during disease progression. Despite this approximately 650-fold increase in the VEGF concentration at the metastatic site, the serum VEGF level remained almost stable within the normal range (Fig. 2).

Discussion

Solid brain tumors of various histologies were found to secrete significant amounts of VEGF into cyst fluid, with maximum VEGF levels in recurrent glioblastoma and brain

metastases. These data confirm and extend previous findings of high VEGF levels in cyst fluid of glioblastomas [2, 3, 20, 22] and suggest a role of VEGF in local tumor progression and metastasis.

In 1983, Senger et al. [16] showed that VEGF promotes the accumulation of ascites fluid, and elevated VEGF levels were recently also reported in ascites fluid of patients with advanced ovarian cancer [7]. First attempts to quantify VEGF protein in CNS malignancies used a radioreceptor assay to measure VEGF levels [22]. Elevated VEGF concentrations were found not only in glioma tissue but also in tumor cyst fluid, indicating that VEGF is secreted from tumor cells into the cystic compartment of gliomas. These findings were recently confirmed using a more sensitive ELISA technique showing up to 300-fold higher VEGF concentrations in glioblastoma cyst fluid than in serum [20]. Additional evidence that VEGF might be involved in cyst formation stems from in situ hybridization studies in pilocytic astrocytoma demonstrating high levels of VEGF mRNA transcripts in the tumor cyst wall [8]. Immunohistochemical studies in gliomas demonstrated a correlation of VEGF expression in tumor tissue with the presence of peritumoral edema and cyst formation [19]. All cystic gliomas expressed VEGF irrespective of tumor grade [19]. In our study, all patients with tumors of astrocytic origin showed high VEGF concentrations in cyst fluid. In addition, patients with other brain tumor entities known to invariably express VEGF, such as ependymomas [13] and meningiomas [13, 15], showed high VEGF levels in cyst fluid. These findings further substantiate the theory that VEGF expression in tumor tissue is more strongly associated with cyst formation than with tumor grade and histology [19].

VEGF may play a key role in brain tumor biology for both angiogenesis and microvascular extravasation, leading to vasogenic edema [20] and cyst formation [19]. Recent data indicate that VEGF is involved in the induction or maintenance of endothelial fenestrations in tumor capillaries expressing receptors for VEGF. Fenestrated endothelia are found in endocrine organs, in specific regions of the brain, such as the choroid plexus, and in the tumor vasculature [4]. In a rat melanoma tumor model VEGF has recently been shown to play an important role in facilitating the growth of tumor lesions in the brain, not only by stimulating tumor neovascularization but also by altering vascular permeability in tumor and adjacent tissue [11]. Results from this experimental work provide strong evidence that an increase in microvascular permeability is the first step in a cascade of pathophysiological events involved in cyst formation. The continuous stimulation of endothelial cells by VEGF was shown to result in vasodilatation of tumor vessels along with a blood-tumor-barrier disruption. Tumor cells transfected with VEGF antisense constructs not only grew substantially slower following intracerebral inoculation, but also revealed major differences in vascular permeability in comparison to control xenografts constitutively secreting VEGF [11]. Others have demonstrated that VEGF induces the synthesis of plasminogen activators [12] and collagenase [21] by endothelial cells in vitro. These proteolytic enzymes are known to effectively degrade extracellular matrix proteins and thus might be important for the local disruption of tumor tissue giving rise to cyst formation. The hypoxic environment within the tumor cyst could further stimulate VEGF expression in tumor cells adjacent to the tumor cyst, similar to the VEGF expression predominantly found in glioblastoma cells surrounding perinecrotic zones [14]. Experiments with glioma cells have impressively shown that hypoxia is a strong stimulus for VEGF expression [17].

We noted a massive increase in the VEGF concentration in tumor cyst fluid during disease progression in a patient with a cystic brain stem metastasis from an adenocarcinoma of the lung. Eventually, the VEGF level reached 500,000 pg/ml. This VEGF level and the exceedingly high VEGF concentrations in cyst fluids from patients with recurrent glioblastoma are among the highest VEGF concentrations reported in vivo. For comparison, tumor cyst fluids from two glioblastoma patients reported elsewhere contained 9,044.6 and 7,892.6 pg/ml [19], which were similar to VEGF concentrations found in cyst fluid samples from two of our patients with de novo glioblastoma [19]. In addition, we recently measured elevated VEGF concentrations in cerebrospinal fluid (CSF) from patients with carcinomatous meningitis, also indicating the high local production of VEGF by tumor cells within the CSF compartment [18]. Following intrathecal chemotherapy VEGF levels in CSF decreased and correlated well to treatment response [18]. Maximum amounts of VEGF in cyst fluid from recurrent glioblastomas and brain metastases suggest that local VEGF production is related to tumor progression in this series.

Interestingly, none of the 14 patients investigated in this series had elevated VEGF serum levels as compared to 145 healthy blood donors. Even the sera of patients with maximal VEGF levels in cystic fluid did not contain abnormally high levels of VEGF. These findings are in keeping with previous results from studies of glioblastomas, where highly elevated VEGF concentrations in the tumor tissue were not accompanied by increased serum VEGF [20]. These findings may be explained by the relatively small compartment of the tumor cyst leaking into the large serum compartment. VEGF leaking from the cysts into the bloodstream may also be rapidly bound by vascular endothelium, blood cells and soluble VEGF receptors such as flt-1 [1].

Local production of VEGF in brain tumors might be crucial for tumor neovascularization, local tumor growth and cyst formation. Thus, therapeutic strategies targeting VEGF or its receptors could be envisaged to inhibit tumor-induced neovascularization. For the selection and monitoring of tumor patients undergoing such anti-angiogenic therapies surrogate markers of angiogenesis in blood, urine or malignant effusions are urgently needed. Therefore, the local VEGF levels in the tumor tissue or tumor-associated fluids should be evaluated in this respect.

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