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Expression of Vascular Endothelial Growth Factor and Its Possible Relation with Neovascularization in Human Brain Tumors[1]

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ABSTRACT

To examine which growth factors correlate with neovascularization in human brain tumors, the mRNA levels of transforming growth factor α, transforming growth factor β, basic fibroblast growth factor, and vascular endothelial growth factor (VEGF) genes were determined by a Northern blot analysis in surgically obtained human gliomas and meningiomas. The vascular development was determined by counting the number of microvessels which were immunostained with von Willebrand factor. We normalized the growth factor mRNA levels *versus* the glyceraldehyde phosphate dehydrogenase mRNA level. In the 17 gliomas and 16 meningiomas examined, the mRNA of transforming growth factors α and β, basic fibroblast growth factor, and VEGF were expressed at various levels. Among those 4 growth factors, the mRNA levels of VEGF, but not those of transforming growth factors α and β and basic fibroblast growth factor, correlated significantly with vascularity in both gliomas (correlation coefficient $r = 0.499$; $P < 0.05$) and meningiomas (correlation coefficient $r = 0.779$; $P < 0.001$). These findings thus suggest that VEGF may be a positive factor in tumor angiogenesis in both human gliomas and meningiomas.

INTRODUCTION

Tumor angiogenesis supports tumor enlargement. The transition from limited to rapid tumor growth often accompanies the transition from the prevascular to the vascular phase (1, 2). Various growth factors which are produced from malignantly transformed cells (3, 4) are considered to develop neovascularization-tumor angiogenesis (5–7). Recent study has indicated that induction of angiogenesis may be due to the down-regulation of an inhibitor of angiogenesis under the control of wild type *p53* and may further be enhanced by additional expression of angiogenic factors such as VEGF[3] (8).

bFGF has no conventional signal sequence (9), but it shows a potent angiogenic activity in various model systems (6). bFGF acts as an angiogenic factor in the chick chorioallantoic membrane and corneal bioassays *in vivo* (10), and the expression of bFGF is associated with a switch to the angiogenic phase during the development of dermal fibrosarcoma in transgenic mice (11). Angiogenesis model systems *in vitro*, proliferation, cell migration, and plasminogen activator production as well as the formation of tube-like structures of bovine vascular endothelial cells are all enhanced in response to the exogenous addition of bFGF (12–16). In contrast, the TGF-β family antagonizes the effects of bFGF (17, 18). EGF and its related family, TGF-α, stimulates the angiogenesis in the hamster cheek pouch bioassay (19) or in the rabbit cornea (20). EGF/TGF-α is angiogenic in human microvascular endothelial cells which develop the capillary networks on Matrigel (21). EGF/TGF-α stimulates proliferation, migration, and plasminogen activator production as well as tubulogenesis (22–28). The exogenous addition of TGF-β inhibits the formation of tube-like structures in type I collagen gel (24, 29).

VEGF is another angiogenic factor which can induce neovascularization of the chick chorioallantoic membrane and corneal bioassays *in vivo* (30, 31). In an *in vitro* angiogenesis model, VEGF stimulates plasminogen activator synthesis and the formation of tube-like structures (32, 33). Furthermore, VEGF also appears to play an important role in both hypoxia-induced and embryonic angiogenesis (34, 35).

Human brain tumors such as gliomas and meningiomas often produce high levels of bFGF (36–38). Enhanced expression of bFGF mRNA is observed in many human glioma cell lines (16). On the other hand, TGF-α is also produced at high levels in various tumor cells (4), while TGF-β is also found in various human tumors (39, 40). VEGF is abundantly expressed in human gliomas (34, 41). These results indicate that bFGF, TGF-α, VEGF, and TGF-β which modulate angiogenesis are produced in human tumors, particularly, in human brain tumors.

We investigated which growth factors are closely involved in the angiogenesis of human brain tumors, and in our present study we thus examined whether the expression of 4 growth factors, TGF-α, TGF-β, bFGF, and VEGF, might be responsible for tumor angiogenesis in human gliomas and meningiomas.

MATERIALS AND METHODS

Samples. Thirty-three patients with brain tumors (17 gliomas and 16 meningiomas) form the basis of this study. In all patients, both a tumor resection and histological confirmation of the tumor were performed. All surgical specimens were snap-frozen immediately after removal and stored −80°C until processing.

Northern Blot Analysis. A human VEGF cDNA probe was kindly provided from H. A. Weich (Biotechnologische Forschung, Department of Gene Expression, Braunschweig, Germany) and bFGF cDNA was from D. B. Rifkin (New York University Medical Center). Human TGF-α and TGF-β cDNAs were donated from R. Derynk (Genentech, Inc., South San Francisco, CA). GAPDH cDNAs was donated from JCRB (Foundation for Promotion of Cancer Research, Tokyo, Japan). The Northern blot analysis was performed as described previously (16). The tumor tissue specimens were homogenized in liquid nitrogen and suspended in 4 M guanidine thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% Sarkosyl and 0.1 M β-mercaptoethanol. We added 2 M sodium acetate (pH 4.0), water-saturated phenol, and chloroform successively to samples. After vigorous mixing, the samples were left on ice for 20 min and then centrifuged at 10,000 × *g* for 20 min. The aqueous phase was aliquoted, mixed with isopropyl alcohol, and kept at −20°C for 20 min. The samples were then centrifuged at 10,000 × *g* for 20 min to obtain the RNA pellet, which was washed in 75% ethanol and then dissolved in sterile RNase-free water. The resulting RNA was fractionated through a 1% agarose gel containing 2.2 M formaldehyde, transferred onto a nylon membrane (Hybond N$^+$; Amersham, United Kingdom), and UV cross-linked with 0.25 J/cm^2 using Fluo-Link (Viler Lourmat, Marne-La-Vallee, France). The filter was hybridized to ^{32}P-labeled cDNA probes in Hybrisol (Oncor, Inc., Gaithersburg, MD) for 24 h at 40°C and then washed at room temperature in 2 × SSC (1 × SSC is 15 mM trisodium citrate dihydrate-150 mM sodium chloride) and 0.1% SDS, after which it was washed in 0.2 × SSC and 0.1% SDS. The mRNA levels were quantified by a densitometric analysis using a Fujix BAS 2000 bioimage

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[3] The abbreviations used are: VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; TGF-β, transforming growth factor β; EGF, epidermal growth factor; TGF-α, transforming growth factor α; GAPDH, glyceraldehyde phosphate dehydrogenase.

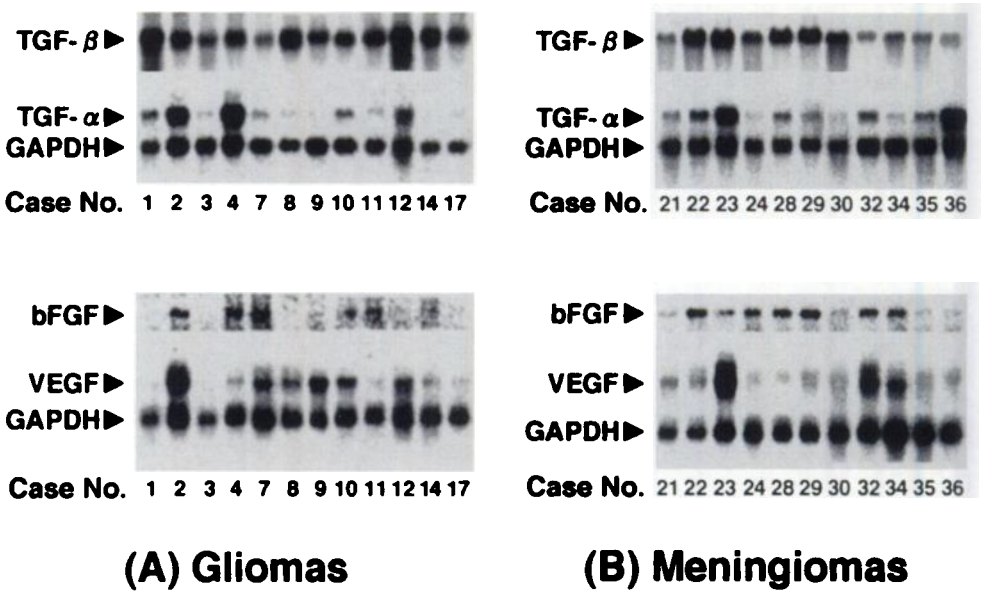

Fig. 1. Northern blot analysis of TGF-α, TGF-β, bFGF, and VEGF in 12 glioma samples (*A*) and 11 meningioma samples (*B*). Fifteen μg of total RNA were electrophoresed on a 1% agarose-formaldehyde gel which was then transferred to a nylon membrane and hybridized with various radiolabeled cDNA probes. As a control, we also used a GAPDH cDNA probe. Various mRNA levels of 4 growth factor genes were observed.

analyzer (Fuji Photo Film Co., Tokyo, Japan). The expression indices of VEGF, bFGF, TGF-α, or TGF-β were presented when normalized by the GAPDH mRNA level in each sample.

Vascular Counting. As a parameter to express tumor angiogenesis, the number of microvessels within the tumor tissue was counted under light microscopy using immunohistochemically stained sections. All specimens were cut into 6-μm slices in a cryostat and mounted on gelatin-coated slides. For the immunohistochemical study, von Willebrand factor (factor VIII) was stained to detect endothelial cells as described previously (42). After fixation with cooled acetone for 5 min, frozen sections were immersed in 3% hydrogen peroxide in methanol for 30 min for the endogenous peroxidase block. The murine monoclonal anti-factor VIII antibody was purchased from Dako (Glostrup, Denmark) as a primary antibody. It was diluted 200-fold with PBS and the specimens were incubated with primary antibody at 4°C overnight. The streptavidin-biotin method was used with the Histofine SAB (M) kit (Nichirei Co., Tokyo, Japan). After a washing in PBS, the sections were visualized by incubation with 300 μg/ml of diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) in 0.05 M Tris buffer (pH 7.6) containing 0.003% hydrogen peroxide for 5 min. The vascular count was expressed as the number of microvessels observed in an area of 1.035 mm^2 using light microscopy. In all samples, the number of microvessels was counted in five different areas and then we calculated their mean.

Statistics. The data were analyzed statistically by checking Pearson's correlation coefficient, and the criterion for statistical significance was $P = 0.05$.

RESULTS

Expression of TGF-α, TGF-β, bFGF, and VEGF mRNA in Gliomas and Meningiomas. We carried out a Northern blot analysis to examine whether any angiogenic growth factor was expressed in brain tumors. An example of a Northern blot analysis of TGF-α, TGF-β, bFGF, VEGF, and GAPDH (internal control, 1.3 kilobases) mRNA in the glioma and meningioma samples was presented (Fig. 1). The tumor mRNA level of the 4 growth factors in all gliomas and meningiomas were determined when normalized by those of the GAPDH gene. The expression indices were obtained to compare which growth factor correlates with vascularity within the tumor tissue among the 4 different growth factors and are summarized in Tables 1 and 2. Although the samples were limited in number, there appeared to be no close correlation between the histological tissue type and the level of each growth factor mRNA expression. The bFGF and VEGF genes were expressed in various degrees in both gliomas

Table 1 *Expression of growth factors and vascular counts in gliomas*

Case	Expression index[a]				Vascular count[b]	Histology
	VEGF	bFGF	TGF-α	TGF-β		
1	0.312	0.092	0.724	2.144	15.8	Astrocytoma
2	1.001	0.119	1.112	1.281	55.6	Astrocytoma
3	0.311	0.099	0.346	0.973	45.0	Astrocytoma
4	0.275	0.155	1.698	1.271	26.6	Astrocytoma
5	0.094	0.092	0.095	0.391	40.2	Astrocytoma
6	0.133	0.110	0.133	0.630	24.2	Astrocytoma
7	0.439	0.096	0.419	0.985	40.6	PNET[c]
8	0.564	0.067	0.388	1.415	132.2	Ependymoma
9	1.105	0.058	0.240	1.039	94.4	Ependymoma
10	0.516	0.063	0.443	1.108	60.8	Glioblastoma
11	0.372	0.127	0.381	1.709	13.4	Glioblastoma
12	0.641	0.075	0.709	1.695	41.8	Glioblastoma
13	0.389	0.069	0.495	0.990	42.2	Glioblastoma
14	0.468	0.118	0.273	1.568	65.0	Oligodendroglioma
15	0.592	0.162	1.234	1.728	20.2	Oligodendroglioma
16	0.451	0.091	0.654	2.205	11.6	Medulloblastoma
17	0.357	0.038	0.344	1.360	11.6	Medulloblastoma

[a] The expression indices of growth factors were presented when normalized by the GAPDH mRNA level.
[b] The vascular count was expressed as the number of microvessels observed in an area of 1.035 mm^2 using light microscopy.
[c] PNET, primitive neuroectodermal tumor.

Table 2 *Expression of growth factors and vascular counts in meningiomas*

Case	Expression index[a]				Vascular count[b]	Histology
	VEGF	bFGF	TGF-α	TGF-β		
21	0.607	0.062	0.309	0.902	49.2	Meningotheliomatous
22	0.576	0.172	0.475	1.875	60.6	Meningotheliomatous
23	1.237	0.063	0.946	1.909	286.0	Meningotheliomatous
24	0.266	0.088	0.212	1.306	10.4	Meningotheliomatous
25	0.491	0.063	4.636	3.735	16.8	Meningotheliomatous
26	0.469	0.095	0.643	1.859	125.0	Transitional
27	0.359	0.134	0.597	2.300	27.0	Transitional
28	0.280	0.131	0.373	1.987	22.8	Transitional
29	0.367	0.158	0.335	2.146	18.8	Fibrous
30	0.318	0.052	0.268	1.296	10.6	Fibrous
31	0.460	0.093	0.701	1.916	89.8	Fibrous
32	0.557	0.060	0.272	0.654	95.4	Angiomatous
33	0.039	0.095	0.019	0.574	16.4	Psammomatous
34	0.280	0.038	0.152	0.734	40.8	Anaplastic
35	0.260	0.028	0.368	0.748	17.2	Anaplastic
36	0.260	0.032	1.961	0.689	145.0	Anaplastic

[a] The expression indices of growth factors were presented when normalized by the GAPDH mRNA level.
[b] The vascular count was expressed as the number of microvessels observed in an area of 1.035 mm^2 using light microscopy.

and meningiomas. Glioma cases 2, 8, 9, 10, 12, and 15 and meningioma cases 21, 22, 23, and 32 expressed a high level of 3.9-kilobase VEGF mRNA with mRNA expression indices over 0.5. There appeared to be an expression of bFGF 7.0-kilobase mRNA in gliomas cases 2, 4, 11, and 15 and in almost all cases of meningiomas. TGF-α and TGF-β mRNA, with sizes of 4.5 and 2.5 kilobases, respectively, were expressed in most of the gliomas and meningiomas at varying levels.

Determination of Vascular Development. Fig. 2 demonstrated examples of immunohistochemical staining for factor VIII. Prominent tumor vessels were observed in case 9 (glioma; Fig. 2*A*) and case 23 (meningioma; Fig. 2*B*), and both showed relatively higher VEGF mRNA levels (Fig. 1). By contrast, there appeared to be a poor development of tumor vessels in case 1 (glioma; Fig. 2*C*) and case 29 (meningioma; Fig. 2*D*), and both showed relatively lower VEGF mRNA levels (Fig. 1).

The vascular counts in gliomas and meningiomas are summarized in Tables 1 and 2, respectively. They were 43.6 ± 31.0 (SD) in the gliomas and 61.2 ± 69.6 in the meningiomas.

Correlation of Growth Factor mRNA Level and Vascularity. We then examined which growth factor was the most closely correlated with vascularity in human brain tumors. On the basis of the data in Tables 1 and 2, we made a statistical analysis using Pearson's correlation coefficient. Scattered charts with regression lines are shown in Fig. 3. The correlation coefficients between the vascular counts and the expression indices of the four growth factors were 0.499 (VEGF), −0.342 (bFGF), −0.247 (TGF-α), and −0.244 (TGF-β) in gliomas (n = 17) and 0.779 (VEGF), −0.211 (bFGF), 0.084 (TGF-α), and −0.030 (TGF-β) in meningiomas (n = 16), respectively. The VEGF mRNA levels were then statistically correlated with tumor vascularity in gliomas ($P < 0.05$) and meningiomas ($P < 0.001$). VEGF was thus considered to be a candidate factor to induce angiogenesis in both the gliomas and meningiomas, while the other 3 growth factors were determined not likely to possess such an ability.

DISCUSSION

Our present study demonstrated that the expression of the VEGF gene, but not that of bFGF, TGF-α, and TGF-β genes, appeared to be closely correlated with vascularity in both human gliomas and meningiomas. Human gliomas produce a high level of bFGF (16, 36–38), which suggests the existence of a paracrine control of tumor angiogenesis. The *in vitro* model system for angiogenesis, tubular morphogenesis in bovine aortic endothelial cells, is enhanced by a co-culture human glioma cells expressing high bFGF mRNA levels, and this phenomenon is inhibited by the coadministration of neutralizing bFGF antibody (16). Furthermore, human glioma cell lines, which have a high level of bFGF mRNA, efficiently develop capillary networks in mice (16). Although the expression of bFGF in tumor cell lines or vascular endothelial cells is also considered to be responsible for angiogenesis in various model systems *in vitro* as well as *in vivo* (5, 6, 12–16), our data using clinical samples suggest that tumor angiogenesis in human gliomas and meningiomas is not likely due to the enhanced expression of bFGF in tumors.

In human microvascular endothelial cells, EGF and its related TGF-α induce cell migration and tubular morphogenesis in an *in vitro* model system (22–28). Human microvascular endothelial cells induce tube-like structures in type I collagen gel when cocultured with TGF-α producing esophageal cancer cells or keratinocytes, while the administration of neutralizing antibody against TGF-α blocks tubular morphogenesis by microvascular endothelial cells (24, 28). However, our present study suggested that it is unlikely that TGF-α was involved in the angiogenesis of brain tumors. On the other hand, TGF-β often blocks bFGF- or EGF/TGF-α-induced angiogenesis in the *in vitro* model systems (24, 29). The expression of TGF-β was thus expected to control angiogenesis as a negative factor *in vivo*. Our present study demonstrated that there appeared to be no negative correlation with the vascular counts in either gliomas or meningiomas.

VEGF is apparently up-regulated in central nervous system tumors in comparison with normal brain (43). There have been several reports on the localization of VEGF mRNA in tumor cells within human brain tumor tissue specimens using the technique of *in situ* hybridization (34, 41). It is also considered that the expression of VEGF mRNA within the tumor tissue in the present study is mainly derived from tumor cells and not from either endothelial cells or other cells. We have also examined the expression of VEGF mRNA in clinical samples of hemangioblastomas, neurinomas and metastatic brain tumors from lung cancer, and it was concluded that VEGF might also play an important role in the angiogenesis of those tumors because of the up-regulation of VEGF mRNA in these tumors.[4] The expression of VEGF is often increased in many human glioma cell lines.[4]

VEGF induces its signaling through interaction with its receptor, *flt*, carrying *fms*-like tyrosine kinase, which shows a 60% homology to the platelet-derived growth factor receptor family (44–46). The high-affinity tyrosine kinase receptor for VEGF, *flt*, is not expressed in the

[4] Unpublished data.

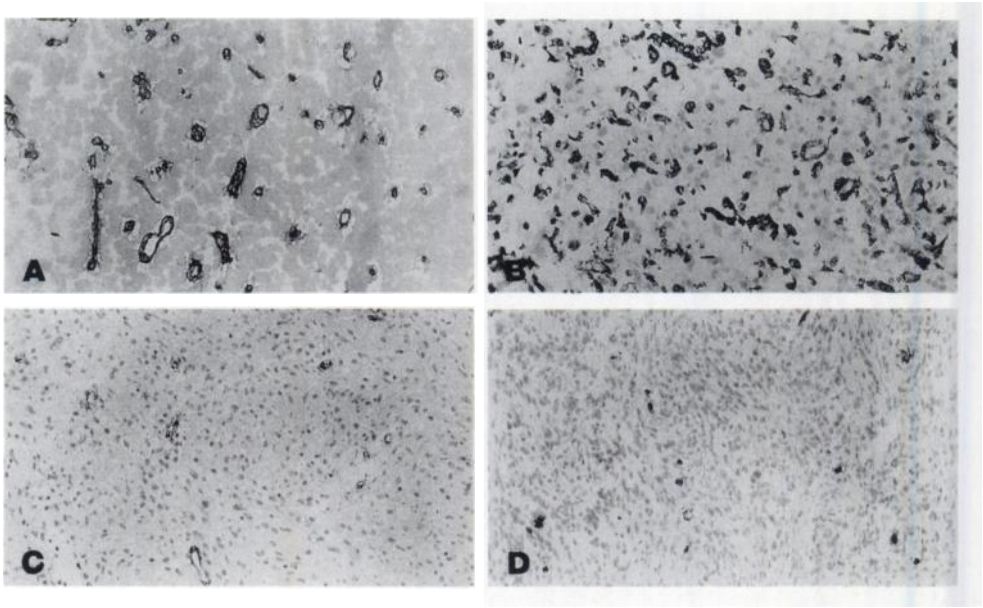

Fig. 2. Immunohistochemical staining of Factor VIII in human brain tumors. Frozen sections (6 μm) were incubated with anti-factor VIII antibody and the streptavidin-biotin method was performed. All vascular endothelial cells of frozen sections were stained immunohistochemically with anti-factor VIII antigen. Prominent tumor vascularizations were observed in *A* (glioma, case 9) and *B* (meningioma, case 23). In contrast, the tumor vessels were poorly developed in *C* (glioma, case 1) and *D* (meningioma, case 29). × 100.

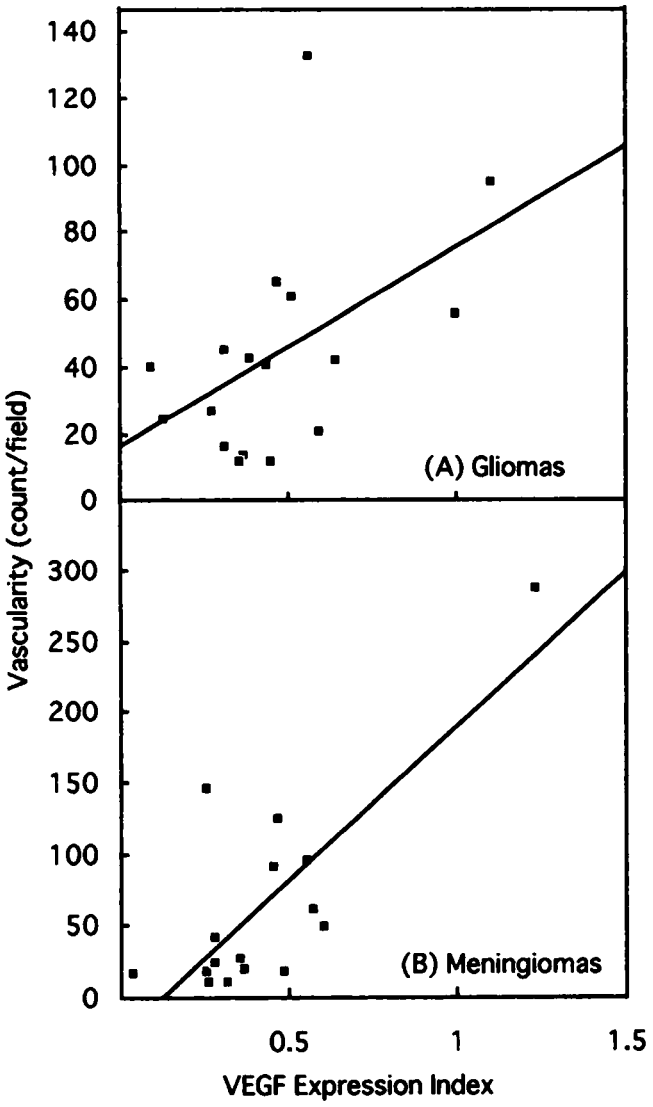

Fig. 3. Correlation between the VEGF expression and vascularity in the glioma (*A*) and meningioma (*B*) samples. The VEGF expression index and the vascular count were deduced from Tables 1 and 2. In *A*, the regression line $Y = 60.375X + 15.13$ and the correlation coefficient (r) = 0.499 (n = 17). In *B*, the regression line $Y = 216.453X - 27.85$ and the correlation coefficient (r) = 0.779 (n = 16).

normal brain endothelium but is up-regulated in the endothelium of the human gliomas, which thus suggests a paracrine control by VEGF of angiogenesis in brain tumors (41). Millauer *et al.* (47) have further demonstrated that human glioma growth in nude mice is inhibited by a dominant-negative *flk*-1 mutant (47). A relevant study by Kim *et al.* (48) also demonstrated that anti-VEGF mAb administration can inhibit the tumor growth of human rhabdomyosarcoma, glioblastoma multiforme, or leiomyosarcoma cell lines in nude mice. In the rat glioma model of tumor angiogenesis, the VEGF receptor family, *flt*-1 or *flk*-1, is expressed in endothelial cells within the tumor, while VEGF itself is expressed in the rat glioma cells (49). In this model, VEGF, but not bFGF or PDGF-B, is specifically induced in response to hypoxia (49). On the other hand, VEGF, which is highly hypoxia inducible in glioma cells, is considered to mediate hypoxia-initiated angiogenesis in brain tumors (34). These recent findings suggest the common notion that the *flt*/VEGF system may play a role in the angiogenesis of solid tumors, in particular brain tumors. In line with this theory, our present study with clinical samples may also indicate that the up-regulation of VEGF may contribute to the neovascularization of human gliomas and meningiomas.

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REFERENCES

1. Folkman, J., and Klagsburn, M. Angiogenic factors. Science (Washington DC), *235:* 442–447, 1987.
2. Folkman, J., Watson, K., Ingber, D., and Hanahan, D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature (Lond.), *339:* 58–61, 1989.
3. Todaro, G. J., Fryling, C., and De, L. J. Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. Proc. Natl. Acad. Sci. USA, *77:* 5258–5262, 1980.
4. Roberts, A. B., and Sporn, M. B. Transforming growth factors. Cancer Surv., *4:* 683–705, 1985.
5. Klagsbrun, M., and D'Amore, P. A. Regulators of angiogenesis. Annu. Rev. Physiol., *53:* 217–239, 1991.
6. Rifkin, D. B., and Moscatelli, D. Recent developments in the cell biology of basic fibroblast growth factor. J. Cell Biol., *109:* 1–6, 1989.
7. Kuwano, M., Ushiro, S., Ryuto, M., Samoto, K., Izumi, H., Ito, K., Abe, T., Nakamura, T., Ono, M., and Kohno, K. Regulation of angiogenesis by growth factors. Gann Monogr., *42:* 113–125, 1994.

8. Van Meir, E. G., Polverini, P. J., Chazin, V. R., Su Huang, H-J., de Tribolet, N., and Cavenee, W. K. Release of an inhibitor of angiogenesis upon induction of wild type *p53* expression in glioblastoma cells. Nat. Genet., *8:* 171–176, 1994.
9. D'Amore, P. A. Modes of FGF release *in vivo* and *in vitro*. Cancer Metastasis Rev., *9:* 227–238, 1990.
10. Lobb, R. R., Alderman, E. M., and Fett, J. W. Induction of angiogenesis by bovine brain derived class 1 heparin-binding growth factor. Biochemistry, *24:* 4969–4973, 1985.
11. Kandel, J., Bossy, W. E., Radvanyi, F., Klagsbrun, M., Folkman, J., and Hanahan, D. Neovascularization is associated with a switch to the export of bFGF in the multistep development of fibrosarcoma. Cell, *66:* 1095–1104, 1991.
12. Montesano, R., Vassalli, J. D., Baird, A., Guillemin, R., and Orci, L. Basic fibroblast growth factor induces angiogenesis *in vitro*. Proc. Natl. Acad. Sci. USA, *83:* 7297–7301, 1986.
13. Moscatelli, D. High and low affinity binding sites for basic fibroblast growth factor on cultured cells: absence of a role for low affinity binding in the stimulation of plasminogen activator production by bovine capillary endothelial cells. J. Cell. Physiol., *131:* 123–130, 1987.
14. Sato, Y., and Rifkin, D. B. Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. J. Cell Biol., *107:* 1199–1205, 1988.
15. Schweigerer, L., Neufeld, G., Friedman, J., Abraham, J. A., Fiddes, J. C., and Gospodarowicz, D. Capillary endothelial cells express basic fibroblast growth factor, a mitogen that promotes their own growth. Nature (Lond.), *325:* 257–259, 1987.
16. Abe, T., Okamura, K., Ono, M., Kohno, K., Mori, T., Hori, S., and Kuwano, M. Induction of vascular endothelial tubular morphogenesis by human glioma cells. A model system for tumor angiogenesis. J. Clin. Invest., *92:* 54–61, 1993.
17. Sato, Y., and Rifkin, D. B. Inhibition of endothelial cell movement by pericytes and smooth muscle cells: activation of a latent transforming growth factor-β 1-like molecule by plasmin during coculture. J. Cell Biol., *109:* 309–315, 1989.
18. Pepper, M. S., Belin, D., Montesano, R., Orci, L., and Vassalli, J. D. Transforming growth factor-β 1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells *in vitro*. J. Cell Biol., *111:* 743–755, 1990.
19. Schreiber, A. B., Winkler, M. E., and Derynck, R. Transforming growth factor-α: a more potent angiogenic mediator than epidermal growth factor. Science (Washington DC), *232:* 1250–1253, 1986.
20. Gospodarowicz, D., Bialecki, H., and Thakral, T. K. The angiogenic activity of the fibroblast and epidermal growth factor. Exp. Eye Res., *28:* 501–514, 1979.
21. Ito, K., Ryuto, M., Ushiro, S., Ono, M., Sugenoya, A., Kuraoka, A., Shibata, Y., and Kuwano, M. Expression of tissue-type plasminogen activator and its inhibitor couples with development of capillary network by human microvascular endothelial cells on Matrigel. J. Cell. Physiol., in press, 1995.
22. Mawatari, M., Kohno, K., Mizoguchi, H., Matsuda, T., Asoh, K., Van, D. J., Welgus, H. G., and Kuwano, M. Effects of tumor necrosis factor and epidermal growth factor on cell morphology, cell surface receptors, and the production of tissue inhibitor of metalloproteinases and IL-6 in human microvascular endothelial cells. J. Immunol., *143:* 1619–1627, 1989.
23. Mawatari, M., Okamura, K., Matsuda, T., Hamanaka, R., Mizoguchi, H., Higashio, K., Kohno, K., and Kuwano, M. Tumor necrosis factor and epidermal growth factor modulate migration of human microvascular endothelial cells and production of tissue-type plasminogen activator and its inhibitor. Exp. Cell Res., *192:* 574–580, 1991.
24. Okamura, K., Morimoto, A., Hamanaka, R., Ono, M., Kohno, K., Uchida, Y., and Kuwano, M. A model system for tumor angiogenesis: involvement of transforming growth factor-α in tube formation of human microvascular endothelial cells induced by esophageal cancer cells. Biochem. Biophys. Res. Commun., *186:* 1471–1479, 1992.
25. Matsuda, T., Okamura, K., Sato, Y., Morimoto, A., Ono, M., Kohno, K., and Kuwano, M. Decreased response to epidermal growth factor during cellular senescence in cultured human microvascular endothelial cells. J. Cell. Physiol., *150:* 510–516, 1992.
26. Sato, Y., Okamura, K., Morimoto, A., Hamanaka, R., Hamaguchi, K., Shimada, T., Ono, M., Kohno, K., Sakata, T., and Kuwano, M. Indispensable role of tissue-type plasminogen activator in growth factor-dependent tube formation of human microvascular endothelial cells *in vitro*. Exp. Cell Res., *204:* 223–229, 1993.
27. Morimoto, A., Tada, K., Nakayama, Y., Kohno, K., Naito, S., Ono, M., and Kuwano, M. Cooperative roles of hepatocyte growth factor and plasminogen activator in tubular morphogenesis by human microvascular endothelial cells. Jpn. J. Cancer Res., *85:* 53–62, 1994.
28. Ono, M., Okamura, K., Nakayama, Y., Tomita, M., Sato, Y., Komatsu, Y., and Kuwano, M. Induction of human microvascular endothelial tubular morphogenesis by human keratinocytes: involvement of transforming growth factor-α. Biochem. Biophys. Res. Commun., *189:* 601–609, 1992.
29. Tada, K., Fukunaga, T., Wakabayashi, Y., Masumi, S., Izumi, H., Kohno, K., and Kuwano, M. Inhibition of tubular morphogenesis in human microvascular endothelial cells by co-culture with chondrocytes and involvement of transforming growth factor β: a model for avascularity in human cartilage. Biochim. Biophys. Acta, *1201:* 135–142, 1994.
30. Wilting, J., Christ, B., Bokeloh, M., and Weich, H. A. *In vivo* effects of vascular endothelial growth factor on the chicken chorioallantoic membrane. Cell Tissue Res., *274:* 163–172, 1993.
31. Connolly, D. T., Heuvelman, D. M., Nelson, R., Olander, J. V., Eppley, B. L., Delfino, J. J., Siegel, N. R., Leimgruber, R. M., and Feder, J. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J. Clin. Invest., *84:* 1470–1478, 1989.
32. Bikfalvi, A., Sauzeau, C., Moukadiri, H., Maclouf, J., Busso, N., Bryckaert, M., Plouet, J., and Tobelem, G. Interaction of vasculotropin/vascular endothelial cell growth factor with human umbilical vein endothelial cells: binding, internalization, degradation, and biological effects. J. Cell. Physiol., *149:* 50–59, 1991.
33. Pepper, M. S., Ferrara, N., Orci, L., and Montesano, R. Vascular endothelial growth factor (VEGF) induces plasminogen activators and plasminogen activator inhibitor-1 in microvascular endothelial cells. Biochem. Biophys. Res. Commun., *181:* 902–906, 1991.
34. Shweiki, D., Itin, A., Soffer, D., and Keshet, E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature (Lond.), *359:* 843–845, 1992.
35. Breier, G., Albrecht, U., Sterrer, S., and Risau, W. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development, *114:* 521–532, 1992.
36. Zagzag, D., Miller, D. C., Sato, Y., Rifkin, D. B., and Burstein, D. E. Immunohistochemical localization of basic fibroblast growth factor in astrocytomas. Cancer Res., *50:* 7393–7398, 1990.
37. Takahashi, J. A., Mori, H., Fukumoto, M., Igarashi, K., Jaye, M., Oda, Y., Kikuchi, H., and Hatanaka, M. Gene expression of fibroblast growth factors in human gliomas and meningiomas: demonstration of cellular source of basic fibroblast growth factor mRNA and peptide in tumor tissues. Proc. Natl. Acad. Sci. USA, *87:* 5710–5714, 1990.
38. Stefanik, D. F., Rizkalla, L. R., Soi, A., Goldblatt, S. A., and Rizkalla, W. M. Acidic and basic fibroblast growth factors are present in glioblastoma multiforme. Cancer Res., *51:* 5760–5765, 1991.
39. Massague, J. The transforming growth factor-β family. Annu. Rev. Cell Biol., *6:* 597–641, 1990.
40. Moses, H. L., Yang, E. Y., and Pietenpol, J. A. TGF-β stimulation and inhibition of cell proliferation: new mechanistic insights. Cell, *63:* 245–247, 1990.
41. Plate, K. H., Breier, G., Weich, H. A., and Risau, W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas *in vivo*. Nature (Lond.), *359:* 845–848, 1992.
42. Samoto, K., Ikezaki, K., Yokoyama, N., and Fukui, M. P-glycoprotein expression in brain capillary endothelial cells after focal ischaemia in the rat. Neurol. Res., *16:* 217–223, 1994.
43. Berkman, R. A., Merrill, M. J., Reinhold, W. C., Monacci, W. T., Saxena, A., Clark, W. C., Robertson, J. T., Ali, I. U., and Oldfield, E. H. Expression of the vascular permeability factor/vascular endothelial growth factor gene in central nervous system neoplasms. J. Clin. Invest., *91:* 153–159, 1993.
44. Shibuya, M., Yamaguchi, S., Yamane, A., Ikeda, T., Tojo, A., Matsushime, H., and Sato, M. Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (*flt*) closely related to the *fms* family. Oncogene, *5:* 519–524, 1990.
45. Vries, C., Escobedo, J. A., Ueno, H., Houck, K., Ferrara, N., and Williams, L. T. The *fms*-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science (Washington DC), *255:* 989–991, 1992.
46. Vaisman, N., Gospodarowicz, D., and Neufeld, G. Characterization of the receptors for vascular endothelial growth factor. J. Biol. Chem., *265:* 19461–19466, 1990.
47. Millauer, B., Shawver, L. K., Plate, K. H., Risau, W., and Ullrich, A. Glioblastoma growth inhibited *in vivo* by a dominant-negative *Flk-1* mutant. Nature (Lond.), *367:* 576–579, 1994.
48. Kim, K. J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H. S., and Ferrara, N. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. Nature (Lond.), *362:* 841–844, 1993.
49. Plate, K. H., Breier, G., Millauer, B., Ullrich, A., and Risau, W. Up-regulation of vascular endothelial growth factor and its cognate receptors in a rat glioma model of tumor angiogenesis. Cancer Res., *53:* 5822–5827, 1993.