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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

(acidic fibroblast growth factor/transforming growth factor type β1/brain neoplasm/Northern blot analysis/*in situ* hybridization)

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ABSTRACT **The growth autonomy of human tumor cells is considered due to the endogenous production of growth factors. Transcriptional expression of candidates for autocrine stimulatory factors such as basic fibroblast growth factor (FGF), acidic FGF, and transforming growth factor type β were determined in human brain tumors. Basic FGF was expressed abundantly in 17 of 18 gliomas, 20 of 22 meningiomas, and 0 of 5 metastatic brain tumors. The level of mRNA expression of acidic FGF in gliomas was significant. In contrast, transforming growth factor type β1 was expressed in all the samples investigated. The mRNA for basic FGF and its peptide were localized in tumor cells *in vivo* by *in situ* hybridization and immunohistochemistry, showing that basic FGF is actually produced in tumor cells. Our results suggest that tumor-derived basic FGF is involved in the progression of gliomas and meningiomas *in vivo*, whereas acidic FGF is expressed in a tumor origin-specific manner, suggesting that acidic FGF works in tandem with basic FGF in glioma tumorigenesis.**

Gliomas, which comprise 35% or more of human intracranial tumors (1), are derived from various types of glial cells of neuroectodermal origin in the brain. They are frequently rich in vascularity and the abundance of their vascularity on cerebral angiograms and/or histology is usually associated with malignant progression (2). Meningiomas comprise 15% or more of intracranial tumors (1). They are derived from meningeal tissues of mesodermal origin. Most meningiomas are clinically and histologically benign, but they often grow into a large mass and show increased vascularity (3).

Basic and acidic fibroblast growth factors (FGFs) have been recently shown to be mitogens and differentiation factors both for neuroectoderm- and mesoderm-derived cells (4, 5). In addition, both FGFs could also be responsible for capillary ingrowth into various tissues (4). Glioma cells are reported to have FGF receptors (6), and mesodermal cells probably also bear FGF receptors because they respond to basic FGF (4). We supposed that glioma- or meningioma-derived FGF might be involved both in their progression as an autocrine growth factor and in tumor angiogenesis.

It is essential to examine tumor tissue specimens for the presence of mRNAs that encode growth factors, since the presence of mRNAs indicates the production by tumor itself rather than the passive absorption or the binding of growth factor peptides synthesized elsewhere. Therefore, we performed Northern blot analysis for basic and acidic FGFs on tissue specimens from 45 human intracranial tumors. In addition, *in situ* hybridization and immunohistochemical studies were performed to determine the cellular source of mRNA for basic FGF and its peptide in tumor tissues. This report describes the localization of mRNA and peptide of basic FGF in glioma cells and meningioma cells *in vivo*.

MATERIALS AND METHODS

Tissue Samples. Tissue samples were obtained from 45 patients operated on at the Kyoto University Hospital and affiliated hospitals. The samples consisted of 18 gliomas, 22 meningiomas, and 5 metastatic brain tumors (Table 1). The resected samples were frozen in liquid nitrogen and stored at −70°C. Samples of normal human brain, bovine hypothalamus, bovine meninges, and normal human monocytes were used as control specimens. Normal human brain tissues were obtained from two patients who required lobectomy in tumor operations. The histology of each tumor specimen was evaluated upon hematoxylin and eosin staining of the tissue adjacent to that used for RNA extraction.

Northern Blot Analysis. Total RNA was isolated by the guanidinium thiocyanate/cesium chloride method (7). Poly(A)$^+$ RNA was selected by oligo(dT)-cellulose affinity chromatography for bovine hypothalamus and human monocytes. Twenty micrograms of RNA was denatured in 1 M glyoxal/50% dimethyl sulfoxide, fractionated by electrophoresis in 1% agarose gels, and transferred to diazophenylthioether paper (Schleicher & Schuell). Before transfer, the gels were stained with ethidium bromide to verify the quality and quantity of RNA loaded. The following cDNA probes were used for hybridization; human basic FGF [a 0.4-kilobase (kb) *Bam*HI fragment from pTB627 (8)], human acidic FGF [a 0.8-kb *Eco*RI/*Bgl* II fragment from pMJ23b (9)], and a chemically synthesized 51-mer oligonucleotide, corresponding to nucleotides 1826–1876 of human transforming growth factor type β1 (TGF-β1) nucleotide sequence (10). Densitometric measurement of autoradiograms was performed by a Zeineh soft laser scanning densitometer (Biomed).

***In Situ* Hybridization.** Frozen sections were prepared from one piece of a glioblastoma sample, which was the same tumor used in immunohistochemistry. *In situ* hybridization was performed as described (11). Briefly, 6-μm-thick tissue sections were air dried on glass slides, which were treated with 0.05% poly(L-lysine) (Sigma), and fixed in 4% paraformaldehyde. Slides were pretreated with 0.2 M HCl (10 min), 0.01% Triton X-100 (5 min), and 0.5 μg of proteinase K per

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Abbreviations: FGF, fibroblast growth factor; TGF-β, transforming growth factor type β.

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Table 1. Sample lists and relative amounts of mRNA measured by a densitometer

Patient	WHO classification	Malignant grading	Basic FGF	Acidic FGF	TGF-β1	Patient	WHO classification	Malignant grading	Basic FGF	Acidic FGF	TGF-β1
		Gliomas									
1	Astrocytoma	Low grade	91	128	65	24	Meningioma		0	0	35
2	Astrocytoma	Low grade	83	0	76	25	Meningioma		0	0	14
3	Astrocytoma	Low grade	74	>142	92	26	Meningioma		120	0	49
4	Astrocytoma	Low grade	102	108	47	27	Meningioma		141	0	46
5	Astrocytoma	Low grade	0	0	138	28	Meningioma		133	0	53
6	Anaplastic astrocytoma	High grade	>274	85	77	29	Meningioma		117	>142	41
						30	Meningioma		85	0	66
7	Anaplastic astrocytoma	High grade	154	>142	61	31	Meningioma		96	0	49
						32	Meningioma		48	0	38
8	Anaplastic astrocytoma	High grade	124	0	122	33	Meningioma		57	0	43
						34	Meningioma		85	0	122
9	Glioblastoma	High grade	270	28	53	35	Meningioma		37	0	40
10	Glioblastoma	High grade	167	50	63	36	Meningioma		59	0	68
11	Glioblastoma	High grade	152	>142	35	37	Meningioma		178	0	72
12	Glioblastoma	High grade	150	87	147	38	Meningioma		170	0	127
13	Glioblastoma	High grade	120	25	25	39	Meningioma		41	0	38
14	Glioblastoma	High grade	61	137	27	40	Meningioma		87	130	148
15	Oligodendroglioma	Low grade	52	0	26		Metastatic brain tumors				
16	Malignant oligodendroglioma	High grade	176	69	47	41	Malignant lymphoma		0	0	48
						42	Lung cancer		0	0	135
17	Oligodendroglioma	Low grade	98	>142	33	43	Sarcoma		0	0	>153
18	Malignant ependymoma	High grade	191	0	28	44	Adenocarcinoma		0	0	>153
						45	Renal cell carcinoma		0	0	>153
		Meningiomas					Control specimens				
19	Meningioma		46	0	37		Bovine hypothalamus		100	100	100
20	Meningioma		167	>142	29		Bovine meninges		0	0	34
21	Meningioma		59	0	20		Human monocytes		0	0	61
22	Meningioma		80	0	126		Normal human brain		0	0	
23	Meningioma		54	0	34		Normal human brain		0	0	

Lung cancer, large cell carcinoma of lung; sarcoma, sarcoma from synovial membrane; adenocarcinoma, adenocarcinoma in orbit. Malignant grading is according to the classification determined by the World Health Organization (WHO). Low grade, glioma grade I or II; high grade, glioma grade III or IV. All values of growth factor mRNA are expressed relative to the level of mRNA in bovine hypothalamus poly(A)$^+$ RNA, which was arbitrarily set at 100. Twenty micrograms of total RNA was used in all samples except in bovine hypothalamus and human monocytes [20 μg of poly(A)$^+$ RNA].

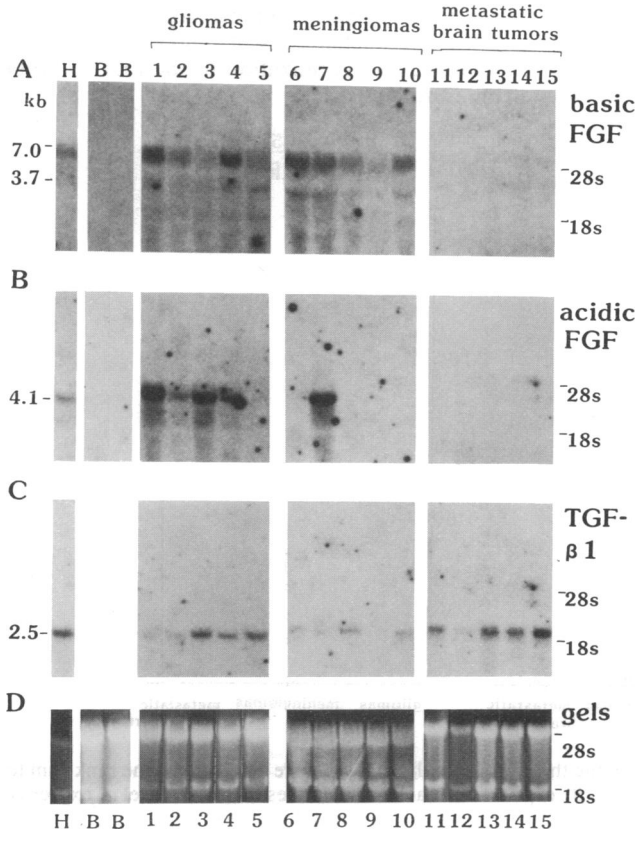

FIG. 1. Northern blot analysis of basic and acidic FGFs and TGF-β1. (*A* and *B*) Hybridization profiles with the basic FGF and the acidic FGF cDNA probes, respectively, which were labeled with [α-^{32}P]dCTP by random priming. Hybridization was carried out in a solution of 50% formamide/5× SSC at 42°C. The final washes were done twice under stringent conditions with 0.1× SSC/0.5% SDS at 65°C for 1 hr each time (20). (*C*) Hybridization profile with the synthesized TGF-β1 probe, which was phosphorylated with [γ-^{32}P]ATP by T4 polynucleotide kinase. Hybridization was done in a solution of 30% formamide at 42°C and washes were performed twice under less stringent conditions with 0.5× SSC/0.5% SDS at 50°C for 1 hr each time (20). (*D*) rRNA stained with ethidium bromide in agarose gels before transfer. Lanes: H, bovine hypothalamus; B, normal human brains; 1–5, gliomas (nos. 11, 17, 3, 7, and 2, respectively); 6–10, meningiomas (nos. 27, 29, 30, 21, and 31, respectively); 11–15, metastatic brain tumors (nos. 42, 41, 44, 43, and 45, respectively). The numbers in parentheses indicate the sample numbers of tumor sample tissues shown in Table 1. Twenty micrograms of total RNA was loaded in each slot except in lane H [20 μg of poly(A)$^+$ RNA].

Table 2. Differences in frequency of detected mRNA expression between the three groups

	Gliomas	Meningiomas	Metastatic tumors
Basic FGF	17/18	20/22	0/5
Acidic FGF	13/18	3/22	0/5
TGF-β1	18/18	22/22	5/5

The differences in frequency of each growth factor expression between three groups were assessed by the χ^2 test. They were significant in basic FGF ($\chi^2 = 26.01$; $P < 0.01$) and acidic FGF ($\chi^2 = 17.93$; $P < 0.01$).

ml (10 min; 37°C). They were postfixed in 4% paraformaldehyde and thereafter washed with 2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0). Prehybridization was carried out at 37°C for 2 hr in the hybridization buffer. Hybridization was performed at 42°C for 36 hr in the hybridization buffer containing the probe of human basic FGF (a 1.9-kb *Pst* I fragment from pTB 627), labeled with biotin-7-dATP by a nick-translation kit (Bethesda Research Laboratories), at a concentration of 20 μg/ml. Thereafter, slides were washed serially in 50% formamide/2× SSC at 37°C, 2× SSC, and 1× SSC at room temperature. The BluGENE kit (Bethesda Research Laboratories) was used for the detection of biotinylated hybridized probes. As a negative control for probes, the labeled plasmid pUC18 was used.

Immunohistochemistry. Two samples of one glioblastoma and one meningioma, other than the 45 samples described above used in Northern blot analysis, were fixed in Bouin's fixative, embedded in paraffin, and sectioned.

The mouse monoclonal antibody mAb 78 against human recombinant basic FGF (IgG2) (12) was applied to 6-μm-thick tissue sections followed by the avidin-biotin procedure (13) with a Vecstatin kit (Vector Laboratories). The sections incubated with normal mouse serum instead of mAb 78 served as negative controls.

RESULTS

Northern Blot Analysis. Fig. 1*A* shows that two major bands were detected at 7.0 and 3.7 kb by Northern blot analysis of basic FGF in gliomas and meningiomas. Those correspond to the previously reported two basic FGF messages (4). Four bands for basic FGF mRNA have been detected in a human tumor cell line such as SK-HEP 1 (15). Two minor bands were shown in some of the samples positive for 7.0- and 3.7-kb bands. However, only one 7.0-kb band for basic FGF mRNA was detectable in bovine hypothalamus as described (16). A transcript of 4.1 kb for acidic FGF was detected in most of the gliomas and in some of the meningiomas (Fig. 1*B*). The five metastatic brain tumors and normal human brains showed no transcript for either basic FGF or acidic FGF, while the bovine hypothalamus [poly(A)$^+$ RNA] showed both signals. A transcript of 2.5 kb for TGF-β1 was found in all the samples (Fig. 1*C*).

Table 1 shows the expression levels of basic FGF, acidic FGF, and TGF-β1 in each sample. The five metastatic brain tumors and other normal controls showed no signals of either basic FGF or acidic FGF. TGF-β1 transcript was found in all the tumor samples and in all the normal tissues. In gliomas, the expression levels of basic FGF were greater in high grade than in low grade gliomas ($P < 0.01$).

The transcript for basic FGF was found in 17 of 18 gliomas and in 20 of 22 meningiomas (Table 2). The basic FGF transcript was far more frequently expressed in both gliomas and meningiomas than in metastatic brain tumors. Acidic FGF mRNA was detected in 13 of 18 gliomas and in 3 of 22 meningiomas. Acidic FGF transcript was more frequently expressed in gliomas than in meningiomas and metastatic brain tumors.

Fig. 2*A* shows that the expression level of basic FGF in both gliomas [129.95 ± 68.93 (mean ± SD)] and meningiomas (84.47 ± 50.09) was significantly higher than that in metastatic brain tumors. The expression level of acidic FGF was significantly higher in gliomas (71.47 ± 57.48) than in meningiomas (18.84 ± 47.43) or metastatic brain tumors (Fig. 2*B*). The expression level of TGF-β1 was higher in metastatic brain tumors (128.14 ± 40.72) than in gliomas (64.53 ± 37.22) or meningiomas (57.13 ± 38.47) (Fig. 2*C*).

***In Situ* Hybridization.** The biotinylated cDNA probes of basic FGF hybridized strongly to almost all glioblastoma cells as well as to the endothelial cells of blood vessels (Fig. 3 *Upper*). In contrast, no hybridization to the pUC18 probes was found (Fig. 3 *Lower*).

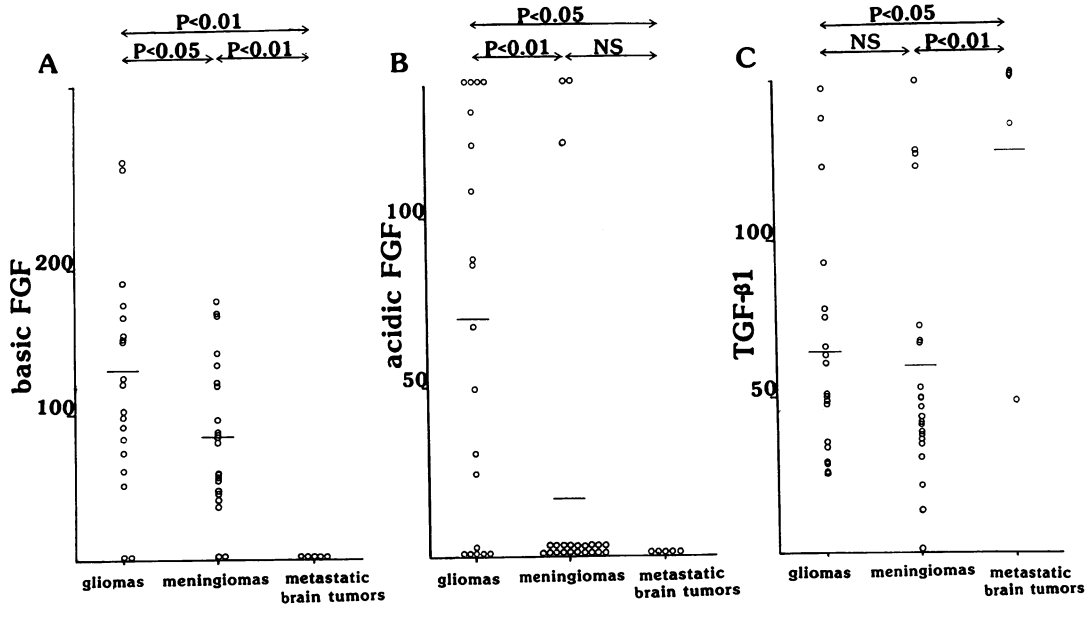

FIG. 2. Relative amounts of mRNA for individual growth factors between the three groups. All analyses were assessed by the rank sum test of Wilcoxon. (*A*) Basic FGF expression. (*B*) Acidic FGF expression. (*C*) TGF-β1 expression. Each bar indicates the mean level of expression in each group. NS, not significant.

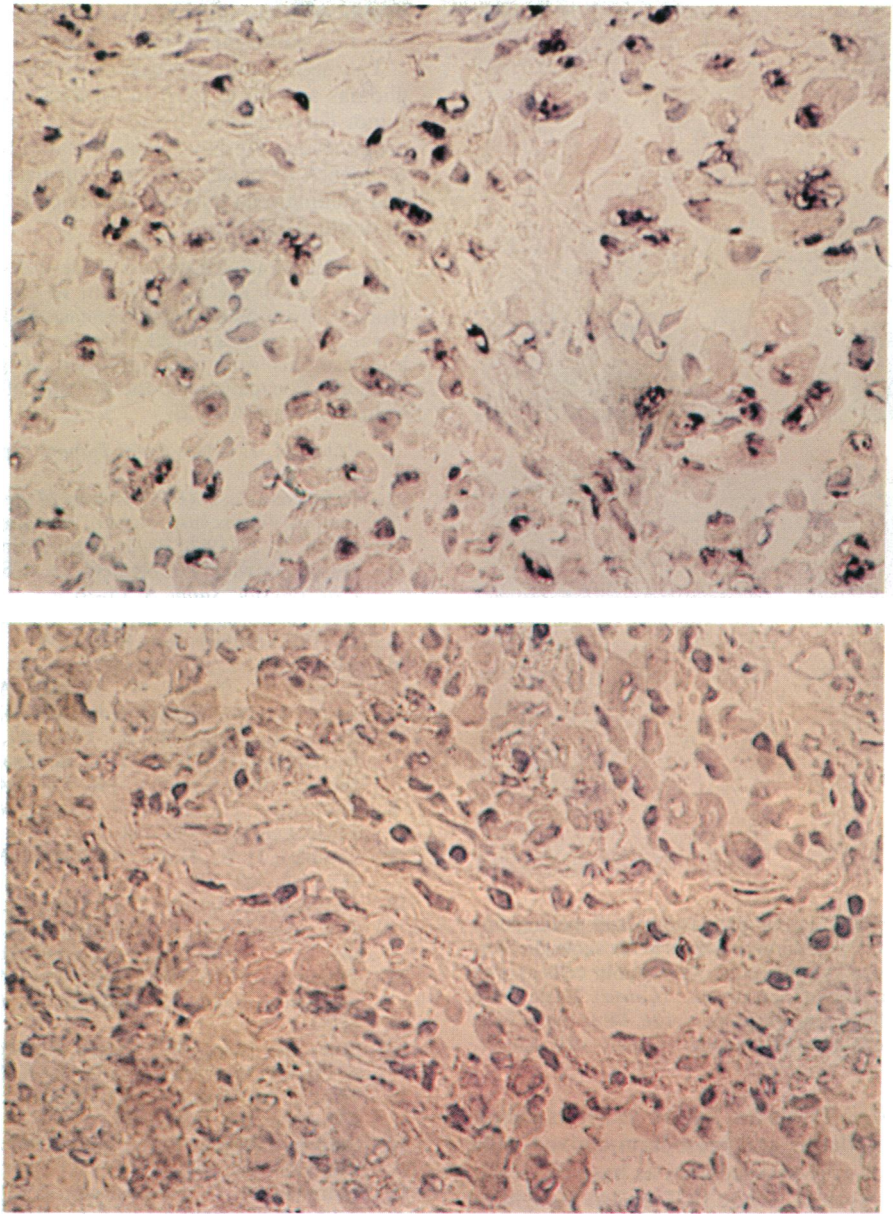

FIG. 3. Basic FGF mRNA expression in glioblastoma tissue. (*Upper*) *In situ* hybridization with biotinylated basic FGF cDNA probe. Strong hybridization to the basic FGF probes is indicated by dark purple precipitates in the cytoplasm of cells and they are defined as positive cells. Sections were counterstained with hematoxylin. (*Lower*) No hybridization signals of biotinylated plasmid pUC18. (×180.)

Immunohistochemistry. Many glioblastoma cells were densely stained with the anti-basic FGF antibody (Fig. 4 *Upper*). Almost all meningioma cells were also immunoreactive toward anti-basic FGF antibody (Fig. 4 *Lower*). In both tissues, endothelial cells were also basic FGF positive.

DISCUSSION

Significant amounts of mRNA for FGFs were detected in human gliomas and/or meningiomas; the expressions of basic FGF and acidic FGF in gliomas and basic FGF in meningiomas were significant, while that of TGF-β1 was expressed in all samples, including normal samples. This biased distribution of mRNA expression of FGFs specifically to the histological classifications suggests that each factor plays an important role in tumorigenesis in a tumor origin-specific manner.

Basic FGF has been widely purified from neuroectoderm- and mesoderm-derived cells and is known to act as a potent mitogen for these cells, which bear specific FGF cell-surface receptors (4). It was reported that primary astrocyte cultures produce basic and acidic FGFs (17) and glioma cells *in vitro* produce basic or acidic FGF and have FGF receptors (6, 14). However, mRNA for basic FGF was not previously identified in mammalian tissues except in bovine hypothalamus, human neurinomas, and human prostate (18–20). In fact, the expression level of basic FGF mRNA is reportedly too low to be detected in human tissues, such as in the placenta, kidney, fetal liver, fetal heart, or in breast cancers, even in the analysis with poly(A)$^+$ RNA (5, 18). In our analysis with total RNA, abundant mRNA expression of basic FGF was detected in tissues of human gliomas and meningiomas, while it was undetectable in any samples of metastatic brain tumors, normal human brain, normal bovine meninges, and other normal controls. In addition, *in situ* hybridization showed that the main source of basic FGF mRNA was tumor cells, and immunohistochemistry clearly showed that basic FGF peptide was actually produced in glioma cells and meningioma cells.

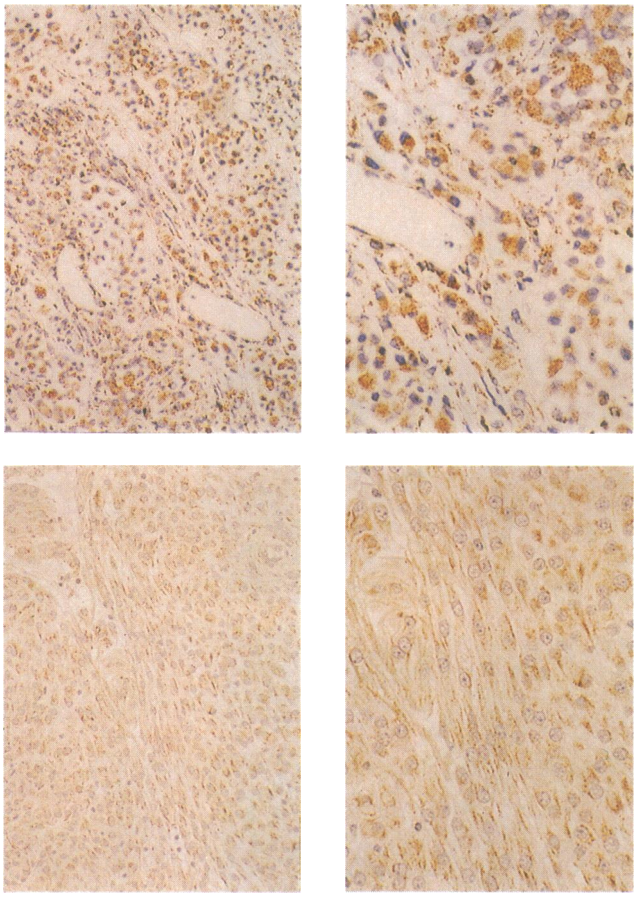

FIG. 4. Basic FGF production in glioblastoma and meningioma tissues. (*Upper*) Basic FGF immunoreactivities in the same glioblastoma tissue used for *in situ* hybridization. Most tumor cells as well as endothelial cells are stained positively. (*Lower*) Basic FGF immunoreactivities in a human meningioma tissue. Most tumor cells as well as endothelial cells showed staining. (*Upper*, ×70; *Lower*, ×140.)

Acidic as well as basic FGFs are known to act as potent mitogens for neuroectoderm- and mesoderm-derived cells and share the same cell-surface receptors as basic FGF (4). In the central nervous system, acidic FGF is also thought to be an autocrine growth factor for glioma cell cultures (6). We demonstrated that acidic FGF mRNA was expressed significantly in gliomas also *in vivo*, suggesting that tumor-derived acidic FGF as well as basic FGF are involved in the progression of gliomas.

Our result that mRNA expression of basic FGF increases in proportion to malignant gradings of gliomas is consistent with the following findings: FGFs confer tumorigenesis and autonomous cell growth when introduced via expression vectors into normal cells, such as Swiss 3T3 cells and hamster kidney-derived cells (21, 22). Normal adrenal cortical cells and myoblasts require exogenous basic FGF (5); however, mouse Y-1 adrenal cortical tumor cells and A 204 rhabdomyosarcoma cells can proliferate without exogenous basic FGF because of their ability to produce and to respond to their own basic FGF (23, 24).

Basic and acidic FGFs act as potent angiogenic factors *in vivo* (25). It has been established that the tumor angiogenesis factor originally isolated from chondrosarcoma is identical with basic FGF (26) and that glioma-derived acidic FGF acts as a mitogen for capillary endothelial cells (6). Our results suggest that tumor-derived basic and/or acidic FGFs contribute to tumor angiogenesis in gliomas and meningiomas *in vivo* in a paracrine fashion.

The significant expression of basic and acidic FGFs in gliomas and basic FGF in meningiomas suggests that these tumor-derived growth factors play a crucial role in tumor progression. We have demonstrated that abundant amounts of mRNA of basic FGF were transcribed in human glioma and meningioma tissues, and its cellular source is attributed to tumor cells.

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