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GENE EXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTORS IN THE TISSUES OF HUMAN GLIOMAS AND MENINGIOMAS

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Summary: Northern blot analysis showed transcripts of two types of the fibroblast growth factor (FGF) receptor genes, *flg* and *bek*, in almost all the tissues samples of 18 human gliomas and 22 human meningiomas, which produced abundant basic and/or acidic FGF. From immunohistochemistry, FGF receptors were expressed in the tumor cells of a glioma and a meningioma. RNA expression of these FGF receptors was also detectable in normal human brains and normal bovine meninges. The expression level of either FGF receptor gene was not significantly different between tumor tissues and normal tissues.

Basic and acidic fibroblast growth factors (FGFs) are mitogens and differentiation factors for neuroectoderm-derived cells or mesoderm-derived cells (1) as well as potent angiogenic factors (1,2). It has been suggested that FGFs are involved in neoplastic growth from the following evidences; a group of oncogenes, such as *hst/K-fgf*, *int-2*, *FGF-5* and *hst-2/FGF-6*, encodes FGF-related proteins (3-8);

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Abbreviations: FGF, fibroblast growth factor; SSC, 0.15M NaCl, 15mM sodium citrate; SDS, sodium dodecyl sulfate.

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certain cells can acquire transformed phenotypes after transfection of the basic FGF gene (9-12); many kinds of tumor cells produce FGFs as autocrine growth factors (2). We demonstrated using Northern hybridization that basic and/or acidic FGF were abundantly produced in more than 90% of human gliomas and meningiomas, which comprise about 50% of human brain tumors (13). Some glioma cells are also known to bear FGF receptors from the *in vitro* study by cross-linking with ^{125}I-FGFs (14). Recently, the cDNAs for two types of human FGF receptors, *flg* and *bek*, were isolated (15-17). In addition, these FGF receptors also demonstrate the capacity for binding with both basic and acidic FGFs with high affinity (16). In order to determine whether FGF receptor genes are expressed in brain tumor tissues *in vivo*, Northern blot analysis for *flg* and *bek* was performed using the same samples as were previously examined as to the expression of the FGF genes (13). Furthermore, immunohistochemical study showed the localization of FGF receptors in tumor tissues.

Materials and Methods

Tissue Samples. Tissue samples of brain tumors were obtained from 45 patients operated on at Kyoto University Hospital and affiliated hospitals. Tumor samples consisted of 18 gliomas, 22 meningiomas and five metastatic brain tumors (Table). Normal human brain tissues were obtained from two patients who required lobectomy. These samples were described previously (13).

Northern Blot Analysis. Northern blot analysis was performed as described (13). Briefly, Total RNA was isolated by the Guanidinium thiocianate/cesium chloride method. Twenty micrograms of total RNA were denatured in 1M glyoxal/50% dimethyl sulfoxide, fractionated by electrophoresis in 1% agarose gels and transferred to diazophenylthioether paper (Schleicher & Schuell, Inc., Keene, NH). The following cDNA probes were used for hybridization: *flg*, [a 2.8-kb EcoR I fragment (15)] and *bek*, [a 2.25-kb EcoRI fragment (16)]. These probes were labeled with [∂-^{32}P]dCTP by random priming and hybridization was carried out. The final washes were performed twice under stringent conditions using 0.1X SSC and 0.5% SDS at 65°C for 30 minutes each time (13). Filters were then autoradiographed for two days at -70°C. Densitometric measurement of band intensities was performed by a Zeineh soft laser scanning densitometer (Biomed).

Immunohistochemistry. Polyclonal antibodies against a synthetic peptide from human FLG [residues 782-805 (16)] were raised in rabbits according to the previous report (18). The antibodies crossreacted with a synthetic FLG fragment on a Western blot analysis. Frozen sections were prepared from tissue samples of a glioblastoma and a meningioma. Six-micron-thick sections were treated with the polyclonal anti-human FLG antibodies

(1:4000), biotynilated anti-rabbit IgG antibody (1:200)(Vector), followed by an avidin-biotin procedure (Vector) (13). The sections were counterstained with methylgreen. The staining specificity of antibody against FGF receptor was assessed by comparing the staining pattern after absorption by antigen.

Results and Discussion

Northern blot analysis. A 4.2-kb band for *flg* was detectable in all 18 gliomas and 22 meningiomas and three of five metastatic brain tumors (Fig. 1-A and Table 1). A transcript for *bek* was detected at 4.4 kb (Fig. 1-B) in all 18 gliomas, 19 of 22 meningiomas and three of five metastatic brain tumors (Table 1). Both types of the FGF receptor genes were also expressed in normal human brains and bovine meninges. There was no significant difference in the expression levels of the *flg* or *bek* gene between tumor tissues and normal tissues. In addition, there was no significant correlation of expression levels of the FGF receptor genes to those of the FGF genes (assessed by Spearman's rank correlation test, $P > 0.01$).

Immunohistochemistry. Dense staining with anti-human FLG antibody was observed in glioblastoma cells and meningioma cells (Fig. 2-A and C). In the control experiment, no

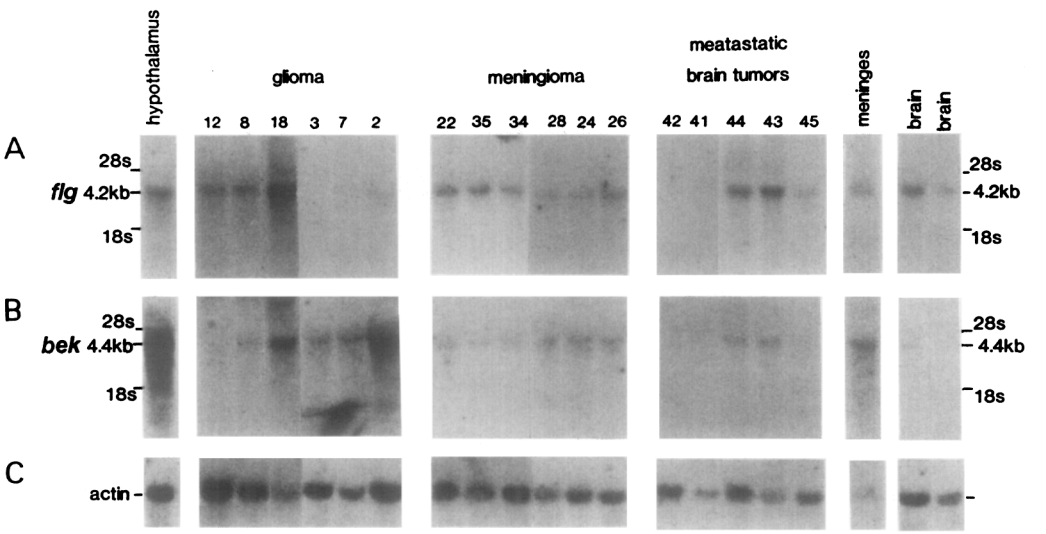

Fig. 1. Northern hybridization of FGF receptors. Twenty micrograms of total RNA were loaded in each slot except in the bovine hypothalamus (20μg of poly $(A)^+$ RNA). The number of each lane indicates the patient number in Table 1.

Table 1

Sample lists and relative amounts of mRNA measured by a densitometer

	Patient	WHO classification	basic FGF	acidic FGF	*flg*	*bek*
Gliomas	1	astrocytoma	91	128	24	14
	2	astrocytoma	83	0	35	60
	3	astrocytoma	74	>142	22	23
	4	astrocytoma	102	108	20	10
	5	astrocytoma	0	0	114	23
	6	anplastic astrocytoma	>274	85	60	19
	7	anplastic astrocytoma	154	>142	21	25
	8	anplastic astrocytoma	124	0	100	13
	9	glioblastoma	270	28	78	19
	10	glioblastoma	167	50	89	12
	11	glioblastoma	152	>142	29	12
	12	glioblastoma	150	87	100	11
	13	glioblastoma	120	25	68	12
	14	glioblastoma	61	137	20	4
	15	oligodendroglioma	52	0	31	13
	16	malignant oligodendroglioma	176	69	100	8
	17	oligodendroglioma	98	>142	33	11
	18	malignant ependymoma	191	0	120	40
Meningiomas	19	meningioma	46	0	89	32
	20	meningioma	167	>142	94	61
	21	meningioma	59	0	36	0
	22	meningioma	80	0	85	19
	23	meningioma	54	0	35	4
	24	meningioma	0	0	32	21
	25	meningioma	0	0	13	10
	26	meningioma	120	0	60	23
	27	meningioma	141	0	74	12
	28	meningioma	133	0	32	22
	29	meningioma	117	>142	28	14
	30	meningioma	85	0	63	6
	31	meningioma	96	0	34	5
	32	meningioma	48	0	30	0
	33	meningioma	57	0	21	0
	34	meningioma	85	0	73	13
	35	meningioma	37	0	79	14
	36	meningioma	59	0	26	17
	37	meningioma	178	0	57	19
	38	meningioma	170	0	81	14
	39	meningioma	41	0	37	12
	40	meningioma	87	130	78	19
Metastatic	41	malignant lymphoma	0	0	0	0
brain tumor	42	lung cancer	0	0	0	0
	43	sarcoma	0	0	96	10
	44	adenocarcinoma	0	0	79	10
	45	renal cell carcinoma	0	0	5	6
Control		bovine hypothalamus	100	100	100	100
		bovine meninges	0	0	35	24
		normal human brain	0	0	71	14
		normal human brain	0	0	15	7

All values of mRNA of growth factor and receptor 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 were used in all samples except in the bovine hypothalamus [20μg of poly$(A)^+$ RNA]. The values of FGFs were taken from reference 13.

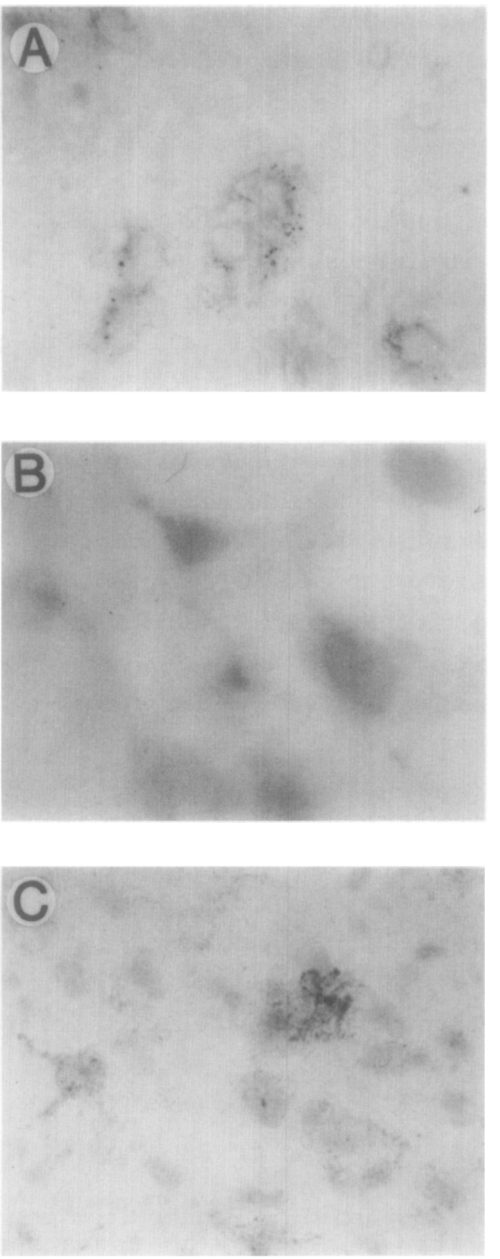

Fig. 2. **Immunohistochemistry.** Remarkable immunoreactivities toward anti-FLG antibodies were detected in tumor cells of glioblastoma tissues (Panel A, x160) while they were undetectable in a section of glioblastoma treated with the absorbed antibody (Panel B, x400). Dense staining of FLG protein was also detected in meningioma cells (Panel C, x160).

immunoreactivity was detected when the absorbed antibody was applied to a glioblastoma section (Fig.2-B).

Autonomous cell growth and tumorigenesis may result from the constitutive interaction of cellular growth factors with

their corresponding receptors (19). We have demonstrated that human gliomas and meningiomas produce FGFs *in vivo* while the expression of FGFs was not detected in normal brain tissues and meningeal tissues (13). Immunohistochemical study also showed immunoreactivities toward anti-basic FGF in most of gliomas and meningiomas *in vivo* (20,21). Moreover, the biological activity of basic or acidic FGF was reportedly demonstrated in human glioma cells *in vitro* (14,22). The present study showed that almost all of the gliomas and the meningiomas expressed either *flg* or *bek* gene *in vivo* as well as basic and acidic FGF genes. These suggest that tumor-derived FGFs play a crucial role in tumorigenesis of gliomas and meningiomas through an autocrine mechanism (13,14,20-22).

Some of the metastatic brain tumors, which showed no transcript of basic or acidic FGF, expressed both *flg* and *bek* genes, suggesting that FGFs are involved in these tumor growths as a paracrine factor.

The present study has shown that almost all of the gliomas and the meningiomas actually express the FGF receptor genes *in vivo*. However, while gene expressions of both FGFs were elevated in gliomas and meningiomas more than in normal brains and meninges, there was no significant difference of expression levels of the *flg* or *bek* gene between tumor tissues and normal tissues. It is suggested that a significant amount of tumor-derived FGFs is involved in tumorigenesis of gliomas and meningiomas through the constitutive interaction of with cellular FGF receptors.

Acknowledgments

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