Neurol Med Chir (Tokyo) 36, 865~869, 1996

Elevated Plasma Basic Fibroblast Growth Factor in Brain Tumor Patients

Masanori KURIMOTO, Shunro ENDO, Yutaka HIRASHIMA, Michiharu NISHIJIMA, and Akira TAKAKU

Department of Neurosurgery, Toyama Medical and Pharmaceutical University, Toyama

Abstract

We attempted to determine whether the plasma concentration of basic fibroblast growth factor (bFGF) was elevated in brain tumor patients. The plasma concentration of bFGF was measured in 55 brain tumor patients and 17 normal subjects by enzyme immunoassay. Upper limit of plasma bFGF in the normal subjects was 1.6 pg/ml. Elevated plasma bFGF levels were observed in 28 patients including 13 of 17 glioma patients, eight of 19 benign tumor patients, and seven of 19 malignant tumor patients. Twelve of 14 malignant glioma patients had elevated plasma bFGF levels. There was a good correlation ($r = 0.42$, $p < 0.05$) between the elevated plasma bFGF level and the tumor volume. Further studies are needed to determine whether the plasma bFGF level is a clinically useful diagnostic and prognostic marker for patients with brain tumors.

Key words: basic fibroblast growth factor, brain tumor, immunoassay, tumor marker

Introduction

Basic fibroblast growth factor (bFGF), originally isolated from the pituitary gland and brain, is a potent mitogen for both neuroectoderm- and mesoderm-derived cells.[5,6,14] This peptide has been identified in a wide variety of normal and malignant tissues including brain tumors such as gliomas and meningiomas.[13,16,17] Recently, evidence has accumulated linking bFGF expression with abnormal growth and progression in human gliomas. Glioma cells express messenger ribonucleic acid (mRNA) and peptide for bFGF[10,13,16] and bFGF receptors.[10] Exogenously added bFGF stimulates glioma proliferation in culture,[10] and neutralizing anti-bFGF antibody suppressed glioma growth *in vitro*.[8] In addition, bFGF is a potent inducer of new blood vessel formation which may be essential to the growth of solid tumors.[3] These findings support the autocrine and paracrine roles of bFGF in human gliomas. The level of bFGF in body fluid has been measured in patients with various cancers using enzyme or radioimmunoassay,[4,7,9,11] but not in patients with brain tumors.

This study measured the plasma bFGF level in patients with various types of brain tumors using a commercially available enzyme immunoassay and examined the relationship between the plasma bFGF level and the size of brain tumors.

Materials and Methods

The patients were 26 males and 29 females between 8 and 77 years of age. The diagnosis in all 55 patients was based on histological examination of surgical specimens. There were 17 gliomas, 19 non-glioma benign tumors, and 19 non-glioma malignant tumors.

Blood was collected by venipuncture and was immediately mixed with 1/10 volume of 3.8% sodium citrate. This citrated anticoagulated blood was centrifuged at 2500*g* for 15 minutes. The supernatant was stored as platelet-poor plasma. Platelet-poor plasma was also obtained from 17 healthy individuals (ranging in age from 18 to 58 years) to establish normal control levels.

The plasma levels of bFGF were determined using a commercially available enzyme immunoassay kit

Received April 15, 1996; Accepted August 29, 1996

Author's present address: M. Kurimoto, M.D., Brain Tumor Research Laboratory, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

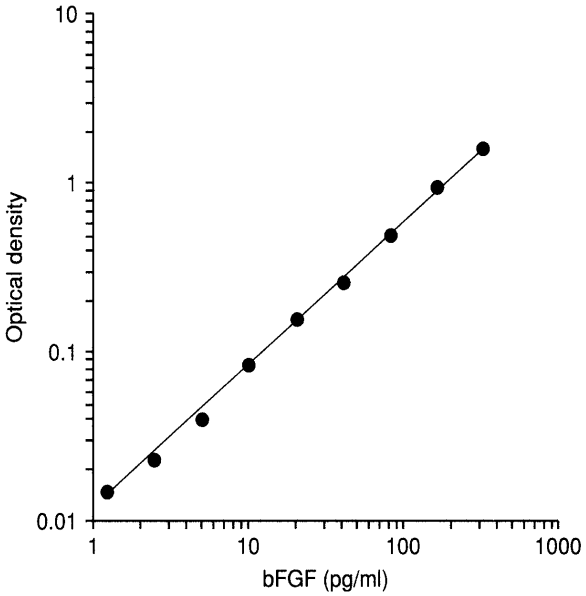

Fig. 1 Relationship between the logarithm of the concentration of standard bFGF and the logarithm of optical density at 450 nm.

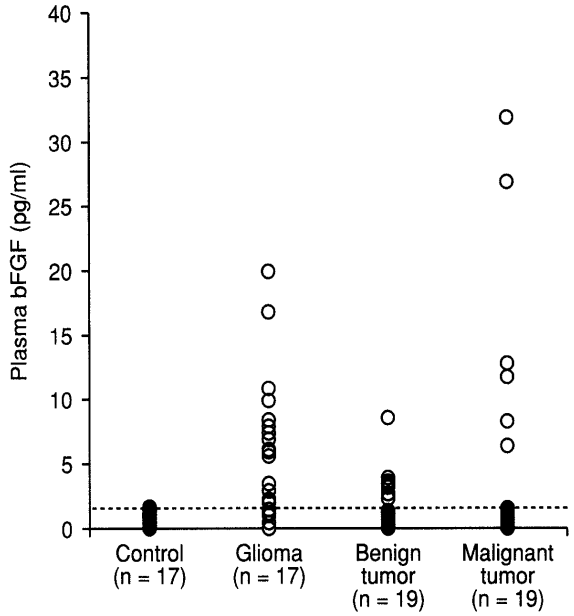

Fig. 2 Distribution of plasma bFGF levels in control individuals and patients with various brain tumors. The *broken line* indicates the upper limit of plasma bFGF in the controls.

(Amersham, Tokyo, Japan). This quantitative "sandwich" enzyme immunoassay uses a highly specific monoclonal antibody for bFGF bound to the wells of a microtiter plate, together with a polyclonal antibody to bFGF conjugated to horseradish peroxidase. Recombinant human bFGF was used as a standard. The enzyme immunoassay system could reliably detect recombinant human bFGF at levels as low as 1.25 pg/ml (Fig. 1).

The tumor volume was calculated based on preoperative magnetic resonance images. The maximal perpendicular dimensions were measured on 1-cm thick slices of axial T_2-weighted images to obtain the area on each slice. The approximate tumor volume was calculated by summing up these areas multiplied by the height (1 cm) of each slice. The volume of the cystic tumor was obtained by subtracting the cyst volume.

Results

The plasma bFGF levels in 13 of the 17 normal subjects were lower than the detection limit (1.25 pg/ml). The upper limit of plasma bFGF in normal individuals was 1.6 pg/ml (Fig. 2). We defined elevated plasma bFGF levels to be greater than 1.6 pg/ml. On the basis of this cut off value, elevated plasma bFGF levels were observed in 28 of 55 patients (Table 1). These were 13 of 17 glioma patients, eight of 19 benign tumor patents, and seven of 19 malignant tumor patients (Fig. 2). One of three patients with

Table 1 Glioma patients with elevated plasma bFGF

Case No.	Age/ Sex	Tumor type	Plasma bFGF (ng/ml)	Tumor volume (cm^3)
1	68/F	glioblastoma	7.2	125
2	30/M	glioblastoma	7.9	115
3	50/F	glioblastoma	2.0	100
4	41/F	glioblastoma	8.4	159
5	77/M	glioblastoma	17.1	172
6	64/F	glioblastoma	20.0	118
7	40/M	glioblastoma	3.3	69
8	36/F	astrocytoma (grade 3)	11.2	140
9	49/M	astrocytoma (grade 3)	10.0	144
10	54/M	astrocytoma (grade 3)	6.4	150
11	67/M	astrocytoma (grade 3)	9.3	131
12	12/M	astrocytoma (grade 3)	3.5	75
13	75/F	astrocytoma (grade 2)	3.1	59
14	48/F	meningioma	8.6	45
15	62/M	meningioma	2.8	60
16	32/M	meningioma	1.8	57
17	47/F	meningioma	3.1	120
18	76/F	malignant meningioma	3.6	88
19	45/M	pituitary adenoma	2.7	29
20	20/F	craniopharyngioma	2.5	33
21	68/F	craniopharyngioma	3.9	92
22	65/M	metastatic BT (L)	27.1	141
23	73/M	metastatic BT (L)	6.5	79
24	73/F	metastatic BT (M)	8.4	90
25	68/M	metastatic BT (L)	11.6	73
26	69/M	malignant lymphoma	12.8	79
27	63/F	malignant lymphoma	1.7	42
28	60/F	fibrosarcoma	32.0	192

BT: brain tumor, L: lung cancer, M: breast cancer.

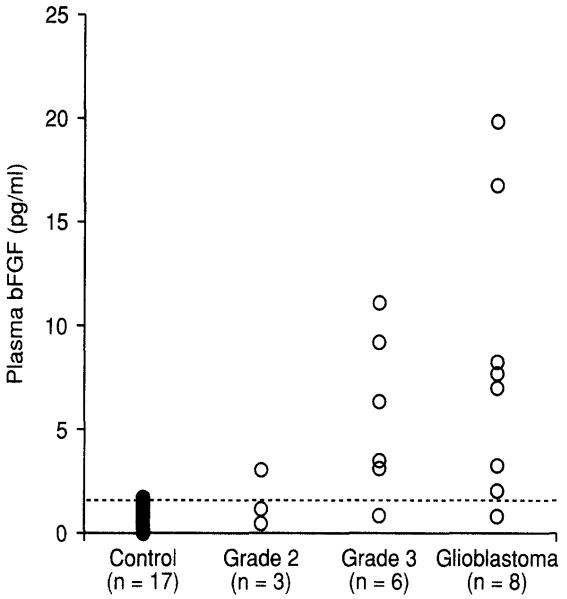

Fig. 3 Distribution of plasma bFGF levels in controls and glioma patients with various grades. The *broken line* indicates the upper limit of plasma bFGF in the controls.

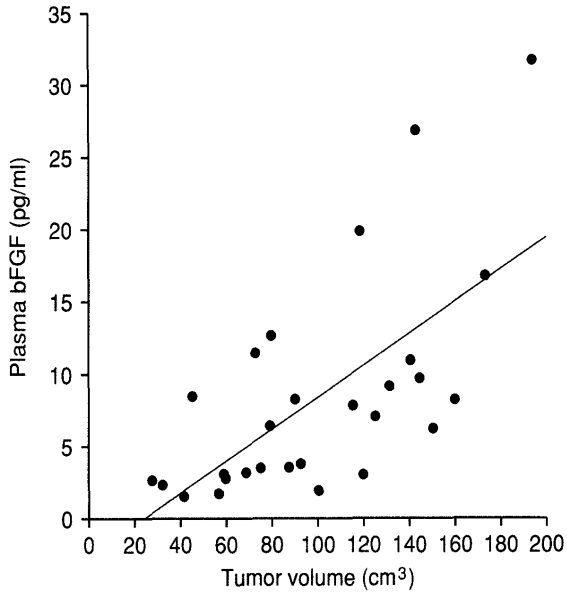

Fig. 4 Relationship between the size of brain tumors and the elevated levels of plasma bFGF.

grade 2 glioma, five of six patients with grade 3 glioma, and seven of eight patients with glioblastoma had elevated plasma bFGF (Fig. 3). In addition, the plasma levels of bFGF were higher in the patients with malignant glioma than those in the control group or the patients with low grade glioma (Fig. 3).

Pearson correlation analysis of the elevated levels of plasma bFGF and the tumor volumes in the 28 patients showed a good correlation ($r = 0.42$, $p < 0.05$) with a regression line of $Y = 0.11X - 2.7$ (when Y is plasma basic FGF level, X is tumor volume) (Fig. 4).

Discussion

Measurement of the plasma levels of bFGF in 55 brain tumor patients and comparison with 17 normal subjects showed that 28 patients had an increased level of plasma bFGF. The elevated plasma bFGF was quite often seen in patients with gliomas, especially malignant gliomas. This is consistent with the earlier observation that the expression level of bFGF increases proportionally with the degree of malignancy.[15,17] In addition, there was a good correlation between the elevated plasma levels and the tumor volume. These observations suggest that the plasma bFGF level is a potentially useful blood tumor marker for malignant gliomas or large brain tumors.

The main source of elevated plasma bFGF in glioma patients may be the tumor tissue. However, other sources of elevated plasma bFGF should be considered, because an elevated level of plasma bFGF was observed in patients with non-glioma malignant tumors such as metastatic carcinomas, in which expression of bFGF has not been observed.[16] The brain is one of the most abundant sources of bFGF.[5] In the injured brain, immunoreactive peptides and mRNA expression for bFGF are markedly increased in the reactive astroglial cells around the lesions.[2] Patterson *et al.*[12] observed that FGF-like activity appears in the cerebrospinal fluid of patients with acute brain injury. In addition, both mRNA and immunoreactive peptide of bFGF are expressed in the proliferating endothelial cells in malignant gliomas.[16,17] These facts support the hypothesis that bFGF is not only liberated from tumor cells but also from proliferating endothelial cells and reactive astroglial cells. In addition, bFGF has no signal peptide[1] and cell death may be the main mode by which bFGF is liberated to the systemic circulation.[14]

Recent developments in various assay systems using monoclonal antibodies have enabled the detection of small amounts of bFGF in body fluids. However, there has been a considerable inter-assay variability for serum or plasma bFGF levels in normal subjects. Kurobe *et al.*[9] reported that the mean level of bFGF in the sera of normal subjects is 190 pg/ml. This value is much higher than the values of a few to several pg/ml found in this study and by others.[7] Nonspecific binding and interference by se-

rum proteins might cause false positive results.[7] In addition, serum obtained from clotted blood contains large amounts of cytokines and growth factors. Therefore, we selected platelet-poor plasma as samples. Setting of the normal range is also a crucial factor for this kind of study. Since the upper limit of the normal range was 1.6 pg/ml, we defined elevated plasma bFGF levels to be greater than 1.6 pg/ml. This resulted in inclusion of 58% of benign tumor patients and 63% of malignant tumor patients. However, the majority of the malignant glioma patients (86%) were outside from normal range. If we set the normal range higher, most benign and malignant tumor patients would be included within the normal range.

This study is preliminary and the precise source of the elevated plasma bFGF in the brain tumor patients was obscure. However, our results indicate at least two points: 1) some patients with brain tumors, especially malignant gliomas, have elevated plasma bFGF levels, and 2) the level of bFGF correlates with tumor size. Since there are no suitable blood tumor markers for most types of brain tumors, we are now working to examine whether the plasma bFGF level is a clinically useful diagnostic and prognostic marker for patients with brain tumors.

References

1) Abraham JA, Margia A, Whang JL, Tumolo A, Friedman J, Hjerrild KA, Gospodarowics D, Fiddes JC: Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. *Science* 233: 545-548, 1986
2) Finklestein SP, Apostolides PJ, Caday CG, Prosser J, Philips MF, Klagsbrum M: Increased basic fibroblast growth factor (bFGF) immunoreactivity at the site of focal brain wounds. *Brain Res* 460: 253-259, 1988
3) Folkman J, Klagsbrun M: Angiogenic factors. *Science* 235: 442-447, 1987
4) Fujimoto K, Ichimori Y, Kakizoe T, Okajima E, Sakamoto H, Sugimura T, Terada M: Increased serum levels of basic fibroblast growth factor in patients with renal cell carcinoma. *Biochem Biophys Res Commun* 180: 386-392, 1991
5) Gospodarowicz D, Bialecki H, Greenburg G: Purification of the fibroblast growth factor activity from bovine brain. *J Biol Chem* 253: 3736-3743, 1978
6) Gospodarowicz D, Massoglia S, Cheng J, Fujii DF: Effect of fibroblast growth factor and lipoproteins on the proliferation of endothelial cells derived from bovine adrenal cortex, brain cortex, and corpus luteum capillaries. *J Cell Physiol* 127: 121-136, 1986
7) Ii M, Yoshida H, Aramaki Y, Masuya H, Hada T, Terada M, Hatanaka M, Ichimori Y: Improved enzyme immunoassay for human basic fibroblast growth factor using a new enhanced chemiluminescence system. *Biochem Biophys Res Commun* 193: 540-545, 1993
8) Kurimoto M, Nogami K, Ogiichi T, Nishijima M, Takaku A: Antiproliferative effect of the multiple autocrine loop blockade in human malignant glioma cell lines. *Neurol Med Chir (Tokyo)* 35: 723-727, 1995
9) Kurobe M, Kato A, Takei Y, Hayashi K: Fluorometric enzyme immunoassay of basic fibroblast growth factor with monoclonal antibodies. *Clin Chem* 38: 2121-2123, 1992
10) Morrison RS, Gross JL, Herblin WF, Reilly TM, LaSala PA, Alterman RL, Moskal JR, Kornblith PL, Dexter DL: Basic fibroblast growth factor-like activity and receptor are expressed in a human glioma cell line. *Cancer Res* 50: 2524-2529, 1990
11) Nguyen M, Watanabe H, Budson AE, Richie JP, Hayes DF, Folkman J: Elevated levels of an angiogenic peptide, basic fibroblast growth factor, in the urine of patients with a wide spectrum of cancers. *J Natl Cancer Inst* 86: 356-361, 1994
12) Patterson SL, Grady MS, Bothwell M: Nerve growth factor and a fibroblast growth factor-like neurotrophic activity in cerebrospinal fluid of brain injured human patients. *Brain Res* 605: 43-49, 1993
13) Paulus W, Grothe C, Sensenbrenner M, Janet T, Baur I, Graf M, Roggendorf W: Localization of basic fibroblast growth factor, a mitogen and angiogenic factor, in human brain tumors. *Acta Neuropathol (Berl)* 79: 418-423, 1990
14) Rifkin DB, Moscatelli D: Recent developments in the cell biology of basic fibroblast growth factor. *J Cell Biol* 109: 1-6, 1989
15) Takahashi JA, Fukumoto M, Igarashi K, Oda Y, Kikuchi H, Hatanaka M: Correlation of basic fibroblast growth factor expression levels with the degree of malignancy and vascularity in human gliomas. *J Neurosurg* 76: 792-798, 1992
16) Takahashi JA, Mori H, Fukumoto M, Igarashi K, Jaye M, Oda Y, Kikuchi H, 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 U S A* 87: 5710-5714, 1990
17) Zagzag D, Miller DC, Sato Y, Rifkin DB, Burstein DE: Immunohistochemical localization of basic fibroblast growth factor in astrocytomas. *Cancer Res* 50: 7393-7398, 1990

Address reprint requests to: M. Kurimoto, M.D., Department of Neurosurgery, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan.

Commentary

In this study by Kurimoto *et al.*, the authors have studied levels of basic fibroblast growth factor (bFGF) in the plasma of patients with brain tumors. There were 55 patients with glioblastoma multiforme, astrocytomas of lower grades, meningiomas, pituitary tumors, and metastatic brain tumors. There were 17 healthy patients whose plasma served as controls. By enzyme immunoassay, the authors determined that elevated bFGF levels were found in about half of all tumor-bearing patients. The most interesting observation was a direct correlation between bFGF levels and tumor size. The main source of the elevated plasma bFGF was thought to be the tumor tissue itself, although this was not conclusively proven by the authors. As there has recently been a lot of interest in the study of angiogenic factors in human brain tumors, especially vascular endothelial growth factor, another important parameter which the authors might wish to examine in future studies is the degree of vascularity of the brain tumors relative to bFGF levels. I commend the authors for their insightful and careful study.

James T. RUTKA, M.D., Ph.D., F.R.C.S.C.
Neurosurgery
The Hospital for Sick Children
Brain Tumor Research Laboratory
University of Toronto
Ontario, Canada

The authors have reported interesting data on the plasma basic fibroblast growth factor (bFGF) level of brain tumor patients. They found that the majority (12/14) of malignant glioma patients showed elevated plasma bFGF level. They postulated that the plasma bFGF was secreted mainly from brain tumors, and some part originated from the destroyed brain tissues adjacent to the tumor.

Because of the absence of signal peptide in the bFGF gene, cell death might be the major mechanism of FGF liberation to the systemic circulation, although tumor tissue may have an alternative secretion mechanism. The source of bFGF can be elucidated by further studies such as 1) matching the plasma bFGF level to the immunohistochemistry of surgical specimen for bFGF, 2) analysis of plasma bFGF changes by the treatment, and 3) measurement of bFGF in the cerebrospinal fluid and its changes by the treatment.

There are several previous reports of this kind, in which enzyme immunoassay could detect elevated plasma bFGF level in renal, lung, and brain tumor patients (see ref. 7 of this article). The present report along with this reference suggests that plasma bFGF can be a good marker for systemic neoplasm and good indicator of tumor regression or growth in response to treatment.

Kazuo YAMADA, M.D.
Department of Neurosurgery
Nagoya City University Medical School
Aichi, Japan