Acidic and Basic Fibroblast Growth Factors Are Present in Glioblastoma Multiforme

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ABSTRACT

Immunohistochemistry was performed on paraffin sections from human glioblastoma multiforme and normal brain tissue. Acidic fibroblast growth factor (FGF) was abundantly present in astrocytes from all glioblastomas studied. Basic FGF was found in the matrix surrounding proliferating blood vessels in most of the glioblastomas. In contrast, astrocytes from normal brain did not contain acidic FGF, and perivascular matrix staining was not demonstrated for basic FGF in the normal brain. Both growth factors could be demonstrated in neurons, Purkinje cells, capillary endothelium, and arterial walls in the normal brain. This study implicates both growth factors in the pathogenesis of malignant glioma. Both may be significant mediators of angiogenesis in glioblastoma.

INTRODUCTION

Glioblastoma multiforme is a uniformly fatal brain tumor. Approximately 10,000 cases occur yearly in the United States (1). The tumor arises from the astrocytic series of neuroglial cells. It contains both poorly differentiated and pleomorphic cellular elements; hence, the name glioblastoma multiforme. Microscopically, these tumors are characterized by bizarre appearing cells with hyperchromatic nuclei, giant cells, and frequent mitotic figures. Various descriptive terms have been given to the pleomorphic cells. Plump distended astrocytes are gemistocytes; elongated fibril-producing cells are fibrillary astrocytes; thin hair-like cells are pilocytic astrocytes. Protoplasmic astrocytes are normal constituents of gray matter. They have short processes which terminate on blood vessels. Most glioblastomas are pleomorphic. They are mixtures of the various cell types. Abundant neovascularization is a hallmark of these tumors. Necrotic segments are common. Tumors of astrocytic origin are assigned grades I–IV with grade IV being the most anaplastic and clinically aggressive (2). Grade III and IV gliomas are classified as "malignant" because of their rapid progressive course. In this study we evaluated 12 high grade (III and IV) gliomas using the general descriptive term, glioblastoma multiforme.

Acidic and basic FGFs are members of the class of angiogenic factors with affinity for heparin. Both are capable of stimulating proliferation and motility of endothelial cells. They are the prototype molecules within each class of heparin affinity growth factors and are believed to play an essential role in angiogenesis (3). They are distinct but structurally related molecules sharing sequence homology through 53% of their primary structure (4). The genes for acidic FGF are found on chromosome 5, while those for basic FGF reside on chromosome 2 (5). Endothelial cell growth factor is the precursor of acidic FGF (6). Both acidic and basic fibroblast growth factors have been isolated from neural tissues (7).

MATERIALS AND METHODS

To determine whether the fibroblast growth factors are present in glioblastoma multiforme, we performed immunohistochemistry on paraffin-embedded tissue. Neutralizing polyclonal antibodies to acidic and basic fibroblast growth factors were used (F.G.F. Co., La Jolla, CA). The antibodies were prepared in rabbits against bovine antigen and purified by the manufacturer using protein A Sepharose chromatography. As a control, slides were probed with purified polyclonal rabbit immunoglobulin from nonimmunized animals (Dako Corp., Carpenteria, CA). To demonstrate the specificity of each antibody, additional slides were processed after the antibody was absorbed with antigen. Twelve tumors were probed. Brain tissues from five victims of trauma served as a normal tissue control. The Vectastain avidin-biotin complex peroxidase kit was used for detection (Vector Labs, Inc., Burlingame, CA). Ten µg of each polyclonal antibody was applied per slide. Five-µm slides were prepared on a microtome. They were deparaffinized in xylene, dehydrated in ethanol, rinsed for 5 min in PBS, incubated for 30 min in 0.3% H2O2 in methanol, washed in PBS for 10 min, preincubated in 1.5% goat serum for 30 min, incubated for 30 min with the primary antibody in PBS, 1.5% bovine serum albumin, washed for 10 min in PBS, incubated for 30 min with biotinylated sheep antibodies to rabbit IgG, washed for 10 min in PBS, incubated for 30 min with avidin-biotin complex, washed for 10 min in PBS, incubated for 4 min with the chromagen, diaminobenzidine, washed for 5 min in tap water, and counterstained with Mayer's hematoxylin. For those slides preabsorbed with antigen, the primary antibody was prepared by adding 5 µg of antigen to 10 µg of antibody in 50 µl of PBS, pH 7.2. The solution was shaken gently for 4 h, incubated overnight at room temperature, and centrifuged at 7000 rpm for 10 min in a microfuge. The supernatant was utilized as the primary antibody.

RESULTS

The distribution of acidic and basic fibroblast growth factors in normal brain tissue is demonstrated in Fig. 1. Acidic fibroblast growth factor was detected in capillary endothelial cells and within the walls of leptomeningeal arteries where it could be seen strikingly in the adventitial, as well as the muscular, layer. It was also detected in the cerebellar Purkinje cells and cortical neurons. Astrocytes failed to stain for acidic FGF. Basic FGF was found in fewer subpopulations of cells than acidic FGF in the normal brain. Like acidic FGF, basic FGF could be seen in the muscular layer of large blood vessels. However, the adventitia was spared. Purkinje cells also demonstrated staining for basic FGF. Sporadic neurons and capillaries demonstrated staining which was less dramatic in comparison to acidic FGF. These findings are summarized in Table 1.

Twelve high grade gliomas were evaluated (Fig. 2). The pattern of staining was consistent. All 12 contained numerous astrocytes whose cytoplasm was stained for acidic FGF. The intensity of the stain was considerably greater in the malignant as...
of gemistocytic or protoplasmic astrocytes were unstained, while focal stretches of interstitial matrix stained positively. The inhomogeneity of distribution may account for the lack of staining in three of the tumors.

The specificity of each antibody for the antigen in question is demonstrated in Fig. 4. Absorption with antigen resulted in inhibition of cytoplasmic staining for anti-acidic FGF and perivascular matrix staining for anti-basic FGF.

**DISCUSSION**

This study adds to a growing list of reports which implicate the fibroblast growth factors in the pathogenesis of human glioma. The presence of RNA coding acidic and basic FGFs was demonstrated by Takahashi et al. (10) using Northern blot analysis. In their study, 17 of 18 gliomas expressed basic FGF, while 13 of 18 expressed acidic FGF. One tumor was probed for basic FGF using in situ hybridization and immunohistochemistry. Hybridization signal was detected in malignant cells as well as within endothelial cells of blood vessels. Immunohistochemistry confirmed the presence of the basic FGF protein in malignant cells. Other studies have demonstrated the presence of acidic FGF, basic FGF, and receptors for basic FGF in cultured human glioma cells (11–13).

Staining for acidic FGF was exclusively intracellular. Basic FGF was demonstrated in the cytoplasm of fibrillary astrocytes. It also appeared to be present in the matrix surrounding proliferating vessels. Conclusions cannot be reached from this study regarding the mechanisms of extracellular deposition. The fibroblast growth factors lack classic secretory signal sequences (4). Folkman and Klagsbrun (3) have speculated that release from cells might occur after cellular damage or under tightly regulated conditions.

Basic FGF has been demonstrated in bovine corneal basement membrane and in developing retinal capillaries (14, 15). It has been demonstrated in the subependymal extracellular matrix of cultured bovine corneal and endothelial cells (16). Folkman et al. postulated that these structures may serve as storage depots which regulate the accessibility of the molecule to the vascular endothelium. They hypothesized that tissue injury could release basic FGF, thereby triggering angiogenesis. In this study we demonstrated the presence of both acidic and basic FGF in the cytoplasm of neurons, endothelium, and Purkinje cells. They may also serve as reservoirs for the vascular mitogens.

There was a considerable difference in the staining of malignant as opposed to normal brain tissue for both acidic and basic FGF. This is summarized in Table 1. In normal brain tissue acidic FGF was not demonstrated in astrocytes. In contrast, glioblastoma contains a large population of intensely staining opposed to normal tissue. The cytoplasm of gemistocytic and protoplasmic astrocytes stained most intensely. In nine of the 12 tumors, staining was uniform throughout the tumor. The vast majority of malignant astrocytes contained acidic FGF. However, in three tumors the staining was heterogeneous with areas of solid unstained cells. Tumors contain multiple subpopulations of cells. Heterogenous staining has been reported in the immunohistochemical analysis of other tumors (8). These findings concur with a report that glioma cells in culture produce acidic FGF (9).

Basic FGF was demonstrated in nine of the 12 tumors studied (Fig. 2). Staining was intense in the matrix surrounding proliferating blood vessels. In some sections basic FGF could be seen within the walls of the tumor vasculature (Fig. 3). Fibrillary astrocytes stained for basic FGF. The distribution of basic FGF was inhomogenous throughout the tumors. Typically, segments

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**Table 1 Summary of findings**

<table>
<thead>
<tr>
<th>FGF</th>
<th>Glioblastoma</th>
<th>Normal brain</th>
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<tbody>
<tr>
<td>Acidic</td>
<td>Abundantly present in gemistocytic astrocytes throughout tumor</td>
<td>Not present in astrocytes</td>
</tr>
<tr>
<td></td>
<td>Present in Purkinje cells, capillary endothelium, neurons, and muscular and adventitial layers of large arteries</td>
<td>Not present in perivascular matrix</td>
</tr>
<tr>
<td>Basic</td>
<td>Present in perivascular matrix</td>
<td>Present in Purkinje cells, muscular layer of large arteries and faintly in sporadic neurons and capillaries</td>
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Fig. 1. Immunohistochemistry of normal brain tissue of a trauma victim. Affinity-purified rabbit polyclonal antibodies to acidic FGF (A–C) and basic FGF (D–F) were used as the primary antibodies. The purified IgG fraction of rabbit serum from nonimmunized animals (G–I) was used as a control. A, acidic FGF is demonstrated in capillary endothelial cells and cortical neurons. Astrocytes failed to stain (× 400). B, acidic FGF is demonstrated in cerebellar Purkinje cells and in the cytoplasm of adjacent neurons (× 400). C, acidic FGF is demonstrated in the muscular layer and adventitia of a leptomeningeal artery (× 400). D, cortical neurons, capillary endothelial cells, and astrocytes fail to stain for basic FGF (× 400). E, basic FGF is demonstrated in the cytoplasm of a cerebellar Purkinje cell. Weak staining is also seen in a nearby capillary and neurons (× 400). F, the muscular layer of a leptomeningeal artery stains for basic FGF. The adventitial layer is relatively spared of the stain (× 200). G–I, purified nonimmune rabbit serum demonstrates no staining (G and H, × 400; I, × 200).

Fig. 2. Distribution of acidic and basic fibroblast growth factors in glioblastoma multiforme. The primary antibody is anti-acidic FGF (A, D, and G), anti-basic FGF (B, E, and H), and nonimmune polyclonal rabbit immunoglobulin (C, F, and I). A–C, glioblastoma 1: acidic FGF is demonstrated in the cytoplasm of astrocytes clustered about a blood vessel. Basic FGF is seen outlining the lumina of numerous blood vessels. The control antibody demonstrates no staining (A, × 100; B and C, × 200). D–F, glioblastoma 2: astrocytes stain deeply for acidic FGF. Basic FGF is demonstrated along fibrillary astrocytes and the perivascular matrix. The gemistocytic astrocytes which stain for acidic FGF are unstained for basic FGF. Control polyclonal antibodies show no staining (D, × 200; E and F, × 100). G–I, glioblastoma 3: heterogeneous staining for acidic FGF is demonstrated. Basic FGF is demonstrated intensely in the matrix surrounding numerous proliferating blood vessels. The control slide demonstrates no staining (G–I, × 200).
ACIDIC AND BASIC FGF IN Glioblastoma

Fig. 4. Immune staining is inhibited by absorption of the antibody with antigen. A and B, a glioblastoma is immunohistochemically probed with anti-acidic FGF (A) and anti-basic FGF (B). Note presence of staining for both antibodies. C and D, adjacent sections from the same tumor are probed with anti-acidic FGF absorbed with acidic FGF (C) and anti-basic FGF absorbed with basic FGF (D). Inhibition of staining in comparison with A and B demonstrates that both antibodies recognize their respective antigens.

gemistocytic astrocytes. Often they were clustered about blood vessels. In glioblastoma basic FGF was abundantly present in the matrix surrounding proliferating blood vessels. This was not seen in the perivascular matrix from any section of normal brain examined.

Neovascularization is a necessary phenomenon for both normal and neoplastic growth. Tumor growth is dependent upon the ability of the tumor to recruit a vasculature. Glioblastoma multiforme is a lethal brain tumor characterized by aberrant neovascularization. Thick walled proliferative vascular channels are common. Both acidic and basic fibroblast growth factors are mitogenic for endothelial, as well as glial, cells. Both are expressed abundantly in glioblastoma. Each has a unique pattern of distribution. The gemistocytic and protoplasmic astrocytes which stained for acidic FGF failed to stain for basic FGF. Fibrillary astrocytes produced basic but not acidic FGF. This indicates that each growth factor is produced by a separate subpopulation of cells. Acidic FGF was seen in the cytoplasm of astrocytes, often in proximity to blood vessels (Fig. 2). Basic FGF was consistently demonstrated in the matrix around proliferating blood vessels (Fig. 2). It could also be seen within some vessel walls (Fig. 3). Close proximity to the vasculature suggests a role for both fibroblast growth factors in the angiogenic process. The abundant presence of both growth factors suggests that each has a role in the pathogenesis of glioblastoma multiforme. In particular, the presence of basic FGF in perivascular matrix and within tumor vasculature suggests that this molecule plays a role in the vascular proliferation which accompanies glioblastoma multiforme.

ACKNOWLEDGMENTS

The authors thank Brad Goldblatt for technical assistance, Drs. Paul Monteleone and John Yerger for critical review, and Cindy Miller, Karen Ryan, and Diane McLachlan for assistance in preparing the manuscript.

REFERENCES

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