Epidermal growth factor receptor in human glioma

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Distribution of the epidermal growth factor (EGF) receptor in the surgical specimen of the human glioma was studied by immunohistochemical techniques using a monoclonal anti-EGF receptor antibody. Of 11 gliomas examined, EGF receptors were detected in nine glioblastomas and in one fibrillary astrocytoma. In the majority of cells, staining was observed over the cell membrane. Nuclear and cytoplasmic staining was also seen. In four glioblastomas, EGF receptor-positive cells were diffusely distributed in the tumor tissue. In one glioblastoma and one fibrillary astrocytoma, only a few positive cells were observed. These results imply the possible role of EGF receptors in the cellular proliferation of the human glioma.

KEY WORDS • brain neoplasm • glioma • glioblastoma • epidermal growth factor receptor • oncogene • monoclonal antibody

Epidermal growth factor (EGF) is a polypeptide consisting of 53 amino acids and has been demonstrated to exhibit a wide variety of biological activities, such as stimulation of the growth and differentiation of the epithelial tissue. The effects of EGF on the cells are mediated by binding to the specific receptor on the membrane—the EGF receptor. The presence of EGF receptors has been confirmed in various tissues including liver, endometrium, bladder, skin, and placenta. Glial cells are reported to bind EGF. Recently, Libermann, et al., demonstrated increased levels of EGF receptors in glioblastomas and meningiomas by measuring their binding capacity with EGF. In the present study, the distribution of EGF receptors in human gliomas was investigated using an immunohistochemical method.

Materials and Methods
As soon as the tumor was removed surgically, the specimens were immediately frozen in Freon and stored at −80°C. The presence of EGF receptors was demonstrated by the biotin-streptavidin immunoperoxidase method. Tumor sections, 8 μ thick, were prepared with a cryostat at −18°C, air-dried for 60 minutes at room temperature, and fixed in chloroform:acetone (50:50) for 5 minutes at 4°C. The slides were rinsed three times in 0.05 M Tris buffer (pH 7.6, containing 0.08% sodium hydrochloride) and covered for 20 minutes with 3% normal sheep serum diluted by Tris buffer. The slides were then incubated with a murine monoclonal antibody to the EGF receptor diluted 1:10 by 1% normal sheep serum overnight at 4°C. This monoclonal antibody is raised against the EGF receptor of the epidermoid carcinoma cell line A431, which is known to have an unusually large number of EGF receptors. Immunoprecipitation and immunofluorescence studies concerning the biochemical character of this antibody demonstrated that it does not inhibit EGF binding to the receptor, which indicates that it recognizes the antigenic site of the receptor other than the EGF binding site.

After three washes in Tris buffer, the tumor sections were incubated with biotinylated anti-mouse sheep immunoglobulin (IgG), diluted 1:100 for 30 minutes at room temperature. The sections were rinsed three times in Tris buffer and incubated with streptavidin-biotinylated peroxidase complex diluted 1:300 for 20 minutes at room temperature. After three washes in Tris buffer, the sections were reacted with 0.05% diaminobenzidine dissolved in Tris buffer containing 0.01% hydrogen peroxide for 5 to 10 minutes. The sections were then rinsed in water and counterstained by hematoxylin. The sections that were incubated with normal mouse serum instead of the primary antibody served as control specimens. As positive control samples, full-term human placenta, which is reported to be rich in EGF receptors, was similarly stained.
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TABLE 1
Summary of immunohistochemical studies in 11 gliomas

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Location of Tumor</th>
<th>Histology</th>
<th>Grade</th>
<th>Cytoplasmic Membrane</th>
<th>Nucleus</th>
<th>Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15, F</td>
<td>rt frontal</td>
<td>glioblastoma</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>57, M</td>
<td>bifrontal</td>
<td>glioblastoma</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>25, M</td>
<td>rt frontoparietal</td>
<td>glioblastoma</td>
<td>weak</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>28, M</td>
<td>rt temporal</td>
<td>glioblastoma†</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>36, F</td>
<td>lt frontal</td>
<td>glioblastoma</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>62, M</td>
<td>rt occipital</td>
<td>glioblastoma</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>37, F</td>
<td>rt frontal</td>
<td>glioblastoma†</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>40, M</td>
<td>rt frontal</td>
<td>glioblastoma</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>40, M</td>
<td>lt frontal</td>
<td>glioblastoma</td>
<td>weak</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>17, F</td>
<td>cervical</td>
<td>fibrillary astrocytoma</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>67, F</td>
<td>rt frontal</td>
<td>glioblastoma</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Code: ++ = more than 50% of tumor cells are positively stained; + = positive cells are seen scattered in the tissue; weak = few cells are positively stained; - = negative.
† Tumor recurrence.

No immunoreactive products were demonstrated in any control section. In the human placenta, the outer surface of the syncytiotrophoblast was intensely stained.

The results of immunohistological studies in 11 tumors are summarized in Table 1. Histopathological diagnosis of the tumors was glioblastoma in 10 cases and fibrillary astrocytoma in one. Immunoreactive EGF receptor-positive tumor cells were demonstrated in 10 of the 11 tumors; in four of these the positive cells were diffusely distributed (Fig. 1). The intensity of the staining was different from cell to cell. In two tumors, only a few tumor cells were stained positively. The most intense staining was observed over the cytoplasmic membrane (Fig. 2 left), but the nucleus and cytoplasm were also frequently stained (Fig. 2 right). In some cells both membrane and nucleus were positively stained.

All of the patients except one (Case 5) were alive at the time of writing. No apparent correlations between the degree of staining and clinical parameters (such as age, sex, tumor location, or clinical course) were observed.

Discussion

It has been demonstrated that glial cells have a binding capacity to EGF and that the density of the receptor is higher in astrocytes and lower in oligodendrogliaomas. The evidence being accumulated indicates that EGF and its receptor system may play a role in carcinogenesis by affecting the cell regulatory system. It has been shown that growth of both epithelial and mesenchymal human tumors is significantly increased by EGF. Recently, Libermann, et al., demonstrated high levels of EGF receptors in glioblastomas and meningiomas as compared with the normal brain.
tissue by measuring the autophosphorylation reaction. The EGF receptors in these brain tumors were biochemically not distinguishable from those in the A-431 cell line which has been demonstrated to have increased levels of EGF receptors. These observations suggest that overexpression of the EGF receptor in human gliomas may play a role in the malignant transformation of the glial cell. However, information concerning the actual distribution of the EGF receptor in the tumor tissue cannot be obtained by biochemical analysis alone. Therefore, immunohistochemical investigations were undertaken to study the distribution of EGF receptors in human gliomas. Our results demonstrated that EGF receptors are expressed in the majority of glioblastomas, although the density of the receptor on each tissue or cell is variable.

Studies have shown that EGF receptors are located on the cell surface. The biological properties have been examined in the isolated cell membrane. Immunohistochemical study of EGF receptors in normal human tissue showed that most of the immunoreactive products are present on the cytoplasmic membrane, but positive immunostaining was also found in the nucleus and cytoplasm of the normal tissue. In our study, nuclear or cytoplasmic staining was not an infrequent finding. One cannot ignore the possibility that some cross-reactive substances were present; however, these results may be explained by investigations reported in the literature. It has been shown that EGF receptors are internalized into the cytoplasm after they bind EGF, and transmit signals to the cell. Furthermore, down-regulation of the receptor is observed. Structural changes of the receptor may occur during these processes, but it is not unexpected that immunoreactive products were detected in the cytoplasm and perinuclear region. In fact, Waterfield, et al., demonstrated that in A-431 cells, the antibody used in this study was considered to be internalized with the EGF receptor molecule in the presence of EGF, and that the antibody-receptor complex could be visualized as intracellular vesicles. Another explanation is that amplification and rearrangement of the EGF receptor gene was demonstrated in some glioblastomas as in the epidermoid carcinoma cell line A-431, and that EGF receptors expressed in the glial tumor may be truncated. The product of v-erbB (the oncogene of the avian erythroblastosis virus) has a marked homology with the cytoplasmic portion of the human EGF receptor. Transformation of the cell by this virus may be caused by an improper signal for the cell division by the truncated receptor. The product of v-erbB is demonstrated to be present in the cytoplasm as well as over the plasma membrane. Furthermore, it is speculated that a site for the interaction between a growth factor and its receptor in the transformed cell may be intracellular: that is, the endoplasmic reticulum or Golgi body.

In human bladder tumors, the presence of EGF receptors was shown to be associated with invasive and poorly differentiated tumors. It is not possible to obtain any significant correlation between the expression of EGF receptors and the histopathological finding or clinical course of our patients at present. The mechanism of abnormal regulation of cell growth in glial tumors cannot be explained only by overexpression of EGF receptors. However, we believe that understanding the molecular and genetic process occurring in these tumors may give valuable information as to the development of more efficacious treatment.

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