Expression of Epidermal Growth Factor Receptors in Human Brain Tumors

T. A. Libermann, N. Razon, A. D. Bartal, Y. Yarden, J. Schlessinger,1 and H. Soreq2

ABSTRACT

The expression of receptors for epidermal growth factor (EGF-R) was determined in 29 samples of brain tumors from 22 patients. Primary gliogenous tumors, of various degrees of cancer, five meningiomas, and two neuroblastomas were examined. Tissue samples were frozen in liquid nitrogen immediately after the operation and stored at -70° until use. Cerebral tissue samples from 11 patients who died from diseases not related to the central nervous system served as controls.

Immunoprecipitation of functional EGF-R-kinase complexes revealed high levels of EGF-R in all of the brain tumors of nonneuronal origin that were examined. The level of EGF-R varied between tumors from different patients and also between specimens prelevated from different areas of the same tumor. In contrast, the levels of EGF-R from control specimens were invariably low.

The biochemical properties of EGF-R in brain tumor specimens were found to be indistinguishable from those of the well-characterized EGF-R from the A-431 cell line, derived from human epidermoid carcinomas. Human brain EGF-R displays a molecular weight of 170,000 by polyacrylamide-sodium dodecyl sulfate gel electrophoresis. It is phosphorylated mainly in tyrosine residues and shows a 2-dimensional phosphopeptide map similar to that obtained with the phosphorylated EGF-R from membranes of A-431 cells.

Our observations suggest that induction of EGF-R expression may accompany the malignant transformation of human brain cells of nonneuronal origin.

INTRODUCTION

The binding of EGF3 to its specific membrane receptors (EGF-R) on target cells initiates a complex array of cellular responses involved with regulation of cellular growth in vitro and in vivo (for review, see Ref. 5). Specific binding of EGF has been demonstrated in many established cell lines derived from various origins (2, 4, 12). A general early response, elicited by binding of EGF, is the rapid stimulation of phosphorylation of several endogenous membrane proteins (6, 15, 18, 27). Of these, the major protein was identified as EGF-R. It displays a molecular weight of 170,000 by polyacrylamide-sodium dodecyl sulfate gel electrophoresis upon denaturing conditions (6). The EGF-stimulated phosphorylation has been observed in several cultured cell lines (6, 15, 30), in certain tissue homogenates (16), in enriched membrane prepar-

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The abbreviations used are: EGF, epidermal growth factor; EGF-R, epidermal growth factor receptor(s); PBS, phosphate-buffered saline (0.13 mM NaCl-3 mM KCl-1 mM KH₂PO₄-18 mM NaHCO₃-0.9 mM CaCl₂-5 mM MgCl₂); CNS, central nervous system; SDS, sodium dodecyl sulfate.

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1 T. A. Libermann, Y. Yarden, and J. Schlessinger, unpublished results.
**MATERIALS AND METHODS**

**Brain Tissues.** Specimens from brain tumors were invariably prelevated at the time of surgery. Control specimens, from the brain of patients who died from diseases not related to the CNS, were generally prelevated 24 hr postmortem (after about 24 hr at 4°C). Whenever possible, prelevation at surgery was used for control samples as well (see Table 2). All tissues were frozen in liquid nitrogen following prelevation and were stored at -70°C until use.

**Histological and Pathological Preparations.** Tissue excised at the time of prelevation was fixed in 4% buffered formaldehyde for 16 hr at room temperature, washed twice with PBS, and embedded in paraffin. Five-μm sections were prepared, mounted on glass slides, and stained with hematoxylin-eosine. Histological characteristics such as hypercellularity, extreme nuclear pleomorphism, numerous mitoses, pseudo palisading around areas of tumor necrosis, and marked endothelial hyperplasia and proliferation of capillaries were detected in sections derived from glioblastoma multiforme specimens, defined according to the recent classification accepted by the WHO (32). Astrocytomas, Grades I and II, were characterized by predominance of astrocytes in the tissue sections, with moderate nuclear pleomorphism, a variable degree of endothelial hyperplasia of capillaries, and mitoses in at least some portions of the tumors. Rosettes of meningeal cells, surrounded by whorls of cell layers and occasional invading vascular channels, were detected in sections of meningiomas. In neuroblastomas, small poorly differentiated cells with a tendency to form rosettes and pseudo rosettes were observed. The summary of the pathological findings for each of the tumor specimens used is presented in Table 1, as confirmed independently by a senior pathologist.

**Immunoprecipitation of EGF-R-Kinase Complexes.** Samples of frozen brain tissue (net weight, about 25 mg) were homogenized in 4 volumes per weight of ice-cold solubilization buffer containing 20 mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, pH 7.4, 150 mm NaCl, 1% Triton X-100, 10% glycerol, 0.03% NaN3, and 1% Trasylol (aprotinin; Sigma Chemical Co., St. Louis, MO). Incubation was at 0°C (in ice) for 60 min. Insoluble material was removed by 15-min centrifugation in an Eppendorf centrifuge at 4°C.

Antibodies against membranes of A-431 cells were elicited in rabbits and IgG purified from the antiserum by ion-exchange chromatography on DEAE-52 columns (14). For each incubation mixture, 10 μg of anti-A-431 membrane IgG were incubated with 2 mg of protein A-Sepharose 4B (Pharmacia, Sweden) for 30 min at room temperature. The protein A-Sepharose-bound antibodies were centrifuged 20 sec at room temperature, in an Eppendorf centrifuge, and unbound antibodies were washed away with PBS. Immunoprecipitation of EGF was carried out with 100-μl aliquots of solubilized brain tissue in the presence of 100 μl of PBS, 10% glycerol (final concentration), and the centrifuged pellet of protein A-Sepharose-bound antibodies. Incubation was for 2 hr at 4°C. Immunoprecipitates were centrifuged 20 sec at room temperature in an Eppendorf centrifuge and washed 4 times with 1 ml of solubilization buffer.

**EGF-R Kinase Autophosphorylation Analysis.** Autophosphorylation of immunoprecipitated EGF-R was carried out using the above-described pellets in the presence of 30 μl of solubilization buffer containing 2 mm MnCl2 and 2 μCi of γ-[32P]ATP (New England Nuclear, Boston, MA; NEN-002A; 2000 to 3000 Ci/mmol). Incubation was for 10 min on ice. Phosphorylation was stopped by adding 30 μl of sample buffer [30% glycerol-15% β-mercaptoethanol-10% sodium dodecyl sulfate-0.5 mm Tris HCl (pH 6.8)-0.09% bromphenol blue]. Samples were then boiled and electrophoretically separated on 5 to 15% gradient SDS-polyacrylamide gels (20). Gels were dried in vacuum and autoradiographed for 12 hr, using Agfa Curix film and Cawo intensifying screen. Labeled bands were aligned on the dried gels using the autoradiograms, cut out, and counted according to the method of Ceronvec.

**Biochemical Characterization of Phosphorylated EGF-R.** Gel-eluted bands with a molecular weight of 170,000 were used for biochemical characterization. Immunoprecipitation with the monoclonal anti-EGF-R antibody TLS-IgG (23) and in vitro phosphorylation of the well-characterized EGF-R from membranes of A-431 cells (9) were carried out in parallel, as a reference. Two-dimensional phosphopeptide analysis was performed according to the method of Elder et al. (11). Phosphoamino acid analysis was performed according to the method of Hunter and Sefton (19).

**RESULTS**

Homogenates from primary human brain tumors and from nonmalignant brain tissue were tested for the presence of specific EGF-R. Receptors were immunoprecipitated with polyclonal rabbit antibodies, which were generated against membranes enriched with EGF-R from the human cell line A-431, derived from epidermoid carcinoma (14). Functional EGF-R-kinase complexes were detected by phosphorylation in vitro of the immunoprecipitates, using γ-[32P]ATP. Fig. 1 reveals the results of a representative experiment, in which the phosphorylated receptors from a variety of such tumors were separated by SDS gel electrophoresis followed by autoradiography of the dried gels.

Of the various types of tumors which were examined, high levels of EGF-R-kinase activity were determined in samples derived from tumors of nonneuronal origin, such as glioblastomas (Fig. 1, Lanes d, f, h, and n) and meningiomas (Fig. 1, Lanes b, g, and l). In contrast, tissue samples derived from neuroblastomas (Fig. 1, Lanes c and e) or from the brains of patients who died from diseases not related to the CNS (Fig. 1, Lanes k and m) revealed EGF-R-kinase activities that were much lower than those observed in the samples prelevated from tumors of nonneuronal origin. The major phosphorylated proteins, in all tissues tested, displayed molecular weights of about 170,000 and 150,000. These comigrate with the phosphorylated EGF-R from membranes of A-431 cells and with its major breakdown product (Fig. 1, Lane a).

The gel-separated, 170,000 band, representing phosphorylated EGF-R from primary glioblastoma (Fig. 1, Lane d), was extracted from the dried gel and subjected to 2-dimensional phosphopeptide analysis. A similar separation was carried out, in parallel, with the phosphorylated EGF-R from membranes of A-431 cells, precipitated with the monoclonal anti-EGF-R antibody TLS-IgG (29). The 2 phosphopeptide maps obtained were very similar (Fig. 2). Moreover, in both cases, we could show by phosphoamino acid analysis of the in vitro-phosphorylated receptors that tyrosine residue is the main amino acid phosphorylated (Fig. 3; Ref. 31). These observations suggest that the phosphorylation sites on EGF-R receptors of human glioblastoma are similar to those of the well-characterized receptors from A-431 cells.

Several control experiments were carried out to verify the attribution of high levels of EGF-R-kinase complexes to the malignant cells of nonneuronal origin in human brain tumors. (a) In order to exclude that the variation in the level of receptors could reflect different numbers of cells in the tissue samples, the amount of DNA was measured in most of the samples tested. The total amount of DNA varied between 0.4 and 1.0 mg/g of wet weight tissue, and there was no correlation to the level of receptor. (b) Determination of the content of protein revealed that, in 80% of the samples tested, the concentration of protein ranged between 70 and 140 mg/g tissue. Moreover, the general size distribution of the total tissue proteins appeared by SDS-polyacrylamide gel analysis to be rather similar in all of the samples that were tested (not shown). This observation indicates...
that the variation in EGF-R level cannot be explained by selective enhancement of in situ proteolysis in part of the samples. (c) To exclude the possibility that different mode of preleval of samples was the major cause for the 50- to 200-fold differences in EGF-R level between tumor and control specimens, we incubated samples from glioblastoma tumors for 24 hr or more at 4°. This incubation, which mimics the conditions under which postmortem samples are preleval, reduces the level of EGF-R by 2- to 4-fold only (not shown). Thus, all of these control experiments confirm that the high levels of functional EGF-R-kinase complexes in human brain tumors reflect an intrinsic property of the malignant cells of nonneuronal origin within these tumors.

Table 1 summarizes the results of experiments in which tissue homogenates from various tumors, and from different areas within specific tumors, were tested for the level of gel-extracted phosphorylated EGF-R. In Table 2, the parallel observations for control samples are presented. In 11 of 11 samples from patients who died from diseases not related to the CNS, the level of EGF-R appeared to be lower by 50-fold than in other regions of these particular tumors. An example of the morphological variability between different areas of a single tumor is demonstrated in Fig. 5, presenting sections from the periphery, the intermediate zone, and the necrotic core of a single tumor of the glioblastoma multiforme type. Hyperplasia of endothelial cells, rosettes of undifferentiated glial cells, and pseudopalisades are most conspicuous in the periphery of the tumor. The intermediate zone is of an older age and includes monstrous (giant) cells with hyper-

<table>
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<tr>
<th>Sample</th>
<th>Age Sex</th>
<th>Histology</th>
<th>Lobe Side</th>
<th>Site of sample within tumor</th>
<th>No. of operation</th>
<th>Further therapy</th>
<th>Pathological condition</th>
<th>³²P-labeled EGF-R (cpm/band)</th>
</tr>
</thead>
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<td>1</td>
<td>57 M</td>
<td>GM (monstrocellular)</td>
<td>T Rt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Died 6 mo after operation</td>
<td>100</td>
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<tr>
<td>2</td>
<td>40 M</td>
<td>Astrocytoma Grade II</td>
<td>Fr Rt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 1 yr</td>
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<tr>
<td>3</td>
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<td>Astrocytoma Grade II</td>
<td>Fr Rt</td>
<td>Periphery</td>
<td>1st</td>
<td>None</td>
<td>Alive 1 yr</td>
<td>6,195</td>
</tr>
<tr>
<td>4</td>
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<td>GM (Astrocytoma Grade IV)</td>
<td>Fr Lt</td>
<td>Core</td>
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<td>None</td>
<td>Died 3 mo after operation</td>
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</tr>
<tr>
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<td>GM (Astrocytoma Grade IV)</td>
<td>Fr Lt</td>
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<tr>
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</tr>
<tr>
<td>8</td>
<td>71 F</td>
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<td>Fr Lt</td>
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<td>Fr Lt</td>
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<td>1st</td>
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<td>Died (perioperative death)</td>
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<td>10</td>
<td>21 M</td>
<td>GM (Astrocytoma Grade III)</td>
<td>Cerebellum + brain stem</td>
<td>Core</td>
<td>3rd</td>
<td>—</td>
<td>Died 22 mo after 2nd operation</td>
<td>400</td>
</tr>
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<td>GM (Astrocytoma Grade III)</td>
<td>Cerebellum + brain stem</td>
<td>Periphery</td>
<td>3rd</td>
<td>—</td>
<td>Died 22 mo after 2nd operation</td>
<td>1,225</td>
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<td>12</td>
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<td>GM (Grade IV)</td>
<td>Cerebellum + brain stem</td>
<td>Core</td>
<td>4th</td>
<td>—</td>
<td>Died 22 mo after 2nd operation</td>
<td>945</td>
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<tr>
<td>13</td>
<td>57 M</td>
<td>GM</td>
<td>Fr Rt</td>
<td>Core</td>
<td>2nd</td>
<td>—</td>
<td>Died 7 mo after 1st operation</td>
<td>1,680</td>
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<tr>
<td>14</td>
<td>64 F</td>
<td>GM (malignant glioma)</td>
<td>T Lt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Died (perioperative death)</td>
<td>1,800</td>
</tr>
<tr>
<td>15</td>
<td>43 M</td>
<td>GM (Astrocytoma Grade III)</td>
<td>T Rt</td>
<td>Core</td>
<td>2nd</td>
<td>—</td>
<td>Died 3 mo after 2nd operation</td>
<td>25,235</td>
</tr>
<tr>
<td>16</td>
<td>37 M</td>
<td>GM</td>
<td>Fr Rt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 7 mo</td>
<td>6,130</td>
</tr>
<tr>
<td>17</td>
<td>62 M</td>
<td>GM</td>
<td>Fr Lt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Died (perioperative death)</td>
<td>83,573</td>
</tr>
<tr>
<td>18</td>
<td>50 F</td>
<td>GM</td>
<td>Fr Rt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 4 mo</td>
<td>4,444</td>
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<tr>
<td>19</td>
<td>50 F</td>
<td>GM</td>
<td>Fr Rt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 4 mo</td>
<td>18,000</td>
</tr>
<tr>
<td>20</td>
<td>71 M</td>
<td>GM</td>
<td>P Rt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Died 5 mo after 1st operation</td>
<td>1,140</td>
</tr>
<tr>
<td>21</td>
<td>54 M</td>
<td>GM</td>
<td>T Lt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 3 mo after operation</td>
<td>2,465</td>
</tr>
<tr>
<td>22</td>
<td>68 F</td>
<td>Metastasis of lung cancer</td>
<td>T Rt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 7 mo after operation</td>
<td>11,856</td>
</tr>
<tr>
<td>23</td>
<td>5 M</td>
<td>Neuroblastoma</td>
<td>Brain stem</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 6 mo after operation</td>
<td>1,500</td>
</tr>
<tr>
<td>24</td>
<td>5, 12 M</td>
<td>Neuroblastoma</td>
<td>Spinal</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Died (perioperative sudden death)</td>
<td>300</td>
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<tr>
<td>25</td>
<td>48 F</td>
<td>Meningioma (angio-blastic)</td>
<td>Fr Rt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 1 yr</td>
<td>17,900</td>
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<td>26</td>
<td>67 M</td>
<td>Meningioma</td>
<td>T Lt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 10 mo</td>
<td>2,800</td>
</tr>
<tr>
<td>27</td>
<td>17 M</td>
<td>Meningioma</td>
<td>P Lt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 2 mo</td>
<td>9,444</td>
</tr>
<tr>
<td>28</td>
<td>54 M</td>
<td>Meningioma</td>
<td>T Lt</td>
<td>Core</td>
<td>1st</td>
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<td>Died (perioperative death)</td>
<td>8,196</td>
</tr>
<tr>
<td>29</td>
<td>64 F</td>
<td>Meningioma</td>
<td>Fr Lt</td>
<td>Core</td>
<td>1st</td>
<td>None</td>
<td>Alive 10 mo</td>
<td>4,020</td>
</tr>
</tbody>
</table>

* T, temporal; Rt, right; Fr, frontal; Lt, left.

² Irradiated with 5,000 R 18 months before second operation; first operation, Astrocytoma Grade I.

³ Irradiated with 5,000 R 21 months before.

⁴ Partial irradiation with 5,000 R after first operation 2 months previously.

⁵ Irradiated with 5,000 R 5 years before at first diagnosis, Astrocytoma Grade II.
another case (Sample 15), the patient was operated on and the effect of the radiotherapy applied after the first operation. In all of the samples taken from this patient, perhaps reflecting recurrent tumor, of the glioblastoma multiforme type (Sample 12). However, the EGF-R level was generally low (<2000 cpm)

In 2 cases where patients were X-irradiated prior to the tissue prelevation on the second or third operation (Table 1, Samples 10 to 13), EGF-R levels appeared to be relatively low (lower than 2000 cpm/sample). A wider selection of samples will be necessary to reveal whether tumors of neuronal origin consistently display low levels of EGF-R. The levels of functional EGF-R-kinase complexes in the different tumor samples are presented in Chart 1 as a function of survival of the patients, in months from the time of operation, and in comparison with the levels of receptors in samples from control patients. No correlation of EGF-R level with survival time could be observed, although the possibility that such a correlation exists cannot be excluded at present.

**DISCUSSION**

Human brain tumors of nonneuronal origin appear by our findings to differ from control brain specimens with regard to their apparent very high content of EGF-R. The evaluation of the amount of EGF-R is based on the autophosphorylation enzymatic reaction. Since we have found that the level of [32P]phosphate incorporated into EGF-R is proportional to the amount of EGF-R, this method can be used as a reliable mean for monitoring the amount of EGF-R.

The measurement of EGF-R levels in normal and malignant brain tissue can be affected by several factors. Differences in the sensitivity of detection of functional EGF-R-kinase complexes may induce an apparent change in the estimation of EGF-R levels. However, it is unlikely that such changes could cause the striking differences of up to 300-fold between tumor and control samples. Also, the concentration of DNA and of protein appears to be rather consistent in all of the samples tested, indicating that the variations in EGF-R levels are not due to major differences in number of cells/sample. In addition, the high levels of EGF-R in gliomas and meninigiomas are unlikely to be due to invasion by blood vessels, since neuroblastomas, which display an equally high distribution of vascular formations, showed relatively low elevation of EGF-R levels relative to nonmalignant irradiated 5 years prior to prelevation of the sample, and the EGF-R level was very high in the recurrent tumor (25,235 cpm). Further experiments will be required to determine whether brain irradiation reduces the level of the EGF-R-kinases and whether reappearance of EGF-R-kinase can be correlated with the recurrence of the tumor.

In the 2 samples of neuroblastoma that were quantitated, labeling was below 2000 cpm/sample. A wider selection of samples will be necessary to reveal whether tumors of neuronal origin consistently display low levels of EGF-R. The levels of functional EGF-R-kinase complexes in the different tumor samples are presented in Chart 1 as a function of survival of the patients, in months from the time of operation, and in comparison with the levels of receptors in samples from control patients. No correlation of EGF-R level with survival time could be observed, although the possibility that such a correlation exists cannot be excluded at present.

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**Chart 1. Levels of functional EGF-R-kinase complexes in different samples of human brain tissue.** EGF-R was immunoprecipitated and autophosphorylated as described in the text. The M, 170,000 band was excised from gels and counted. Values represent [32P] incorporated into the EGF-R-kinase complexes from 25-mg tissue samples. Classification is after Tables 1 and 2. Different samples from a single tumor are aligned together. Samples derived from the peripheral region of tumors (P); samples prelevated after X-irradiation (*); postoperative death (†).
control forebrain samples. It should be noted that the high cell type complexity within tumor specimens certainly contributes to the variations observed in EGF-R levels between different tumors and between samples derived from a single tumor.

The heterogeneity of cell types within primary human brain tumors does not allow a simple interpretation for the large difference in the level of EGF-R determined in these tumors. One possible interpretation is that the reduced level of EGF-R in nonmalignant brain could be due to the fact that most of the cells in the normal brain are in a quiescent phase of the cell cycle, whereas in malignant brain tumors, a large population of mitotic or polydictoid cells can be observed. This interpretation is in line with the recent observation of Robinson et al. (21), who reported that certain cultured cells possess, when metabolically deprived, 20-fold more receptors for EGF in the S phase of the cell cycle compared to the G1 phase (21). It also agrees with the observation of a high level of EGF-R in developing cultures of primary rat astrocytes (25). An alternate explanation is that a certain cell population, possessing EGF-R, appears to be more abundant in the brain gliogenous tumor cells. This explanation is also consistent with the fact that some tumors express more receptors than do others.

Several retroviruses and growth factors were found to increase a tyrosine-specific kinase activity in established cell lines. This activity was demonstrated to be associated with either the protein product of the viral transforming gene (19) or receptors for the growth factors (10, 27). The expression of EGF-R in several established cell lines has been shown to be transformation sensitive, and many cell lines exist in which transformation by viruses or other transforming agents results in a loss of requirement for EGF for growth and in a marked reduction in binding of EGF (7, 17, 26). Thus, the effect of transformation on EGF-R expression may vary with the cell type in question. Further experiments, using immunofluorescence analysis on tumor sections with monoclonal antibodies against EGF-R, will be necessary in order to clarify certain points which we raised in this study. Immunofluorescence of morphologically defined tumor sections can also reveal whether the quantitative level of EGF-R expression in individual cells within these sections is related to the state of dedifferentiation of cells.

Glioblastoma multiforme tumors consist of up to 45% of the total number of intracranial tumors and end invariably in death of the patients, and both radiotherapy and chemotherapy cannot prevent the recurrence of the tumor (1, 24). Better understanding of the molecular processes underlying the transformation of these gliogenous cells and their proliferation may yield new insights to evaluate the efficacy of various treatments aimed to abolish these malignant tumors.

REFERENCES

Fig. 1. Gel electrophoresis of autophosphorylated EGF-R immunoprecipitated from human brain tissue. Preparation of samples, immunoprecipitation of EGF-R-kinase complexes, autophosphorylation, and gel analysis were carried out as described in Materials and Methods. Each immunoprecipitation mixture contained 25 mg of tissue. Membranes from A-431 cells (a) were used as a standard source for immunoprecipitable EGF-R-kinase complexes (9). Exposure was for 12 hr at —70°, with a CAWO intensifying screen and Agfa Curix film. The tissue samples examined were of the following sources: b, meningioma (see Table 1, Sample 27); c, neuroblastoma (Table 1, Sample 23); d, metastatic lung cancer (Table 1, Sample 22); e, neuroblastoma (Table 1, Sample 24); f, glioblastoma multiforme (Table 1, Sample 19); g, meningioma (Table 1, Sample 28); h, glioblastoma multiforme (Table 1, Sample 21); i, recurrent glioblastoma multiforme (Table 1, Sample 20); j, astroblastoma; k, normal brain (see Table 2, Sample 10); l, meningioma (Table 1, Sample 28); m, fetal brain (Table 2, Sample 11); and n, glioblastoma multiforme (Table 1, Sample 21).

Fig. 2. Two-dimensional tryptic analysis of phosphopeptides derived from the M, 170,000 band of EGF-R from membranes of A-431 cells and from a glioblastoma multiforme tumor. Immunoprecipitation of EGF-R from membranes of A-431 cells (a) was performed with the monoclonal anti-EGF-R antibody TLS-lgG. Polyclonal, rabbit anti-A-431 membrane IgG was used to immunoprecipitate the EGF-R from a tissue sample derived from human glioblastoma tumor (b; see Table 1, Sample 6). Phosphopeptide thin-layer chromatography was according to the method of Elder et al. (11). Exposure was for 48 hr.
Fig. 3. Two-dimensional phosphoamino acid analysis of EGF-R immunoprecipitated from human glioblastoma multiforme. Immunoprecipitation was as in the legend to Fig. 2. Phosphoamino acid analysis was performed by thin-layer chromatography according to the method of Hunter and Sefton (19). Migration coordinates of phosphoserine (P-SER), phosphothreonine (P-THR), and phosphotyrosine (P-TYR) are noted.

Fig. 4. Histological appearance of representative brain tissue samples. Preparation of specimens, fixation, and staining were as described in "Materials and Methods." Note the different histological appearance of tissues from trauma, various glioblastoma multiforme tumors, hemorrhagic infarction, meningioma, and astrocytoma specimens.
Fig. 5. Histological differences between various areas in a single tumor. Tissue samples were prelevated from the periphery, intermediate zone, and core regions of a single glioblastoma multiforme tumor. Histological analysis was as described in "Materials and Methods." Note the increased necrosis in the core region.
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