Urinary epidermal growth factor in patients with gliomas: significance of the factor as a glial tumor marker

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Epidermal growth factor (EGF) content in urine from patients with glial tumors was examined by radioimmunoassay techniques with labeled human EGF and its rabbit EGF polyclonal antibody. There was no cross-reaction with transforming growth factor-α, which has a common receptor with EGF. Forty glial tumors were divided into three groups according to the clinical stage: Samples from Group A patients were obtained before therapy and/or biopsy; in these patients a large volume of tumor was apparent on computerized tomography (CT). Group B samples were obtained after gross total removal of the tumor and/or chemo- and radiation therapy; these patients showed a small volume of residual tumor on CT. Samples from Group C patients were obtained after gross tumor total removal and/or chemo- and radiation therapy; no tumor was detected on CT scans in these patients. Urinary EGF levels in Group A samples were statistically significantly higher than in samples from healthy individuals (p < 0.001), Group B patients (p < 0.10), and Group C patients (p < 0.02). In addition, high-grade glial tumors in Group A cases showed a significantly higher level of urinary EGF than low-grade tumors in Group A patients (p < 0.05), or patients with meningioma (p < 0.02), metastatic brain tumor (p < 0.05), and cerebral infarction (p < 0.001). Longitudinal changes of urinary EGF levels in glioma patients mostly synchronized with the clinical course and therapeutic interventions. Therefore, urinary EGF, as a glial tumor marker, may be of practical value for diagnosing a malignant glioma and evaluating for the efficacy of chemo- and radiation therapy.

KEY WORDS · urinary epidermal growth factor · glioma · urine · tumor marker

Epidermal growth factor (EGF) is a 53-amino-acid polypeptide (6045 D) growth factor, originally discovered in extracts of the male mouse submandibular gland. It has been shown that EGF stimulates deoxyribonucleic acid synthesis and cell division in various tissues including normal glia. Epidermal growth factor binds to a specific cell growth receptor (EGF receptor), the gene of which is the proto-oncogene of v-erbB, and is overexpressed in nearly one-half of malignant gliomas and neontal rat astrocytes. Human EGF (hEGF) has been identified with β-u-urogastrone, large amounts of which are extracted from human urine. Urinary excretion of hEGF occasionally increases in patients with malignant neoplasms other than bladder tumors. Stromberg, et al., demonstrated a high-molecular-weight form of EGF in urine from a patient with glioblastoma. In the present study, we measured urinary hEGF excretion in patients with neuroepithelial tumors. We report our results and discuss the role of EGF in the growth of glioma and the significance of urinary EGF as a tumor marker.

Materials and Methods

Patient Population

Spot urine samples were collected from 40 patients with glial tumors. There were 19 females and 21 males, ranging in age from 15 to 80 years. Glial tumors included 28 high-grade astrocytomas, seven low-grade astrocytomas, one ependymoma, one oligodendroglioma, one medulloblastoma, one mixed anaplastic oligoastrocytoma, and one ganglioglioma. Control groups, examined for their urinary EGF, were as follows: 12 patients with meningiomas, 14 patients with other primary intracranial tumors (three neurovascula aromas, two pituitary adenomas, four germinomas, two craniopharyngiomas, two malignant lymphomas, and one hemangioblastoma), nine patients with metastatic brain tumors, one case of radiation necrosis, 14 cases of cerebral infarction, and 12 healthy individuals. Urine samples voided in the morning were kept frozen at −20°C until assayed. The urine samples obtained from the patients with glial tumors were divided into three groups according to the clinical stage of the patient:
Group A samples were obtained before therapy and/or biopsy; these patients exhibited a large volume of tumor on computerized tomography (CT). Samples from Group B patients were obtained after gross total tumor removal and/or chemo- and radiation therapy; a small volume of residual tumor was demonstrated on CT scans in these patients. Group C samples were obtained after gross total removal of tumor and/or chemo- and radiation therapy; no tumor was detected on CT. The 24 Group A neoplasms consisted of 17 high-grade glial tumors (Grades 3 and 4) and seven low-grade tumors (Grades 1 and 2), according to the classification of Daumas-Dupont, et al.\cite*{1} There were eight cases in Group B and eight in Group C. In addition, longitudinal changes of urinary EGF levels in six glioma-bearing patients were examined in order to evaluate surgical intervention, chemo- and radiation therapy, and growth rate of tumors.

Radiomunoassay Procedure

The radiomunoassay (RIA) procedure of hEGF was similar to the method described by Hirata and Orth,\cite*{2} with minor modifications. Highly purified hEGF was used as the standard and labeled tracer. The RIA standard diluent was 10 mM Tris-HCl (pH 7.4), containing 0.1 mg/ml Merthiolate (thimerosal), and 5 mg/ml bovine serum albumin. The volume of the incubation mixture was 0.3 ml, and the mixture consisted of 0.1 ml of sample or standard and 0.1 ml of rabbit anti-hEGF (final dilution 1:20,000), all diluted in 10 mM Tris-HCl (pH 7.4). After incubation for 20 hours at room temperature, 0.1 ml Tris-HCl containing 125I-hEGF tracer (10,000 cpm), labeled by the method previously reported by Starkey and Orth,\cite*{3} was added, followed by incubation for 20 hours at room temperature. Then 0.1 ml of 25% polyethylene glycol was also added. After incubation for 15 minutes, the tubes were centrifuged and the supernatants were decanted. Finally, the precipitates were counted with a gamma scintillation spectrometer.

Since urine EGF levels exactly parallel those of urine creatinine,\cite*{4,5,6} the urine collections were monitored by creatinine determinations. The concentration of urinary creatinine was determined with the aid of an autoanalyzer. Student’s t-test was applied to assess the significance of the differences in mean values between groups.

Epidermal growth factor levels in the cerebrospinal fluid (CSF) of two Group A patients with glioma (Cases 5 and 6, Table 1) was also examined in this RIA system.

**Competitive Binding of Transforming Growth Factor-α**

Since the EGF receptor is also the receptor for transforming growth factor-α (TGF-α),\cite*{7,8} the cross-reaction to TGFα in this assay system was examined. Various volumes of recombinant human TGF-α were added into this assay system, and the immunoprecipitates were then counted with a gamma scintillation spectrometer.

**Results**

Urinary EGF levels in this study are expressed in terms of urine creatinine and displayed in Fig. 1. Levels in healthy individuals ranged from 3.9 to 15.8 ng/mg creatinine, with an average of 8.7 ng/mg creatinine. Urinary EGF levels in Group A patients ranged from 7.7 to 63.6 ng/mg creatinine, the average being 23.4 ng/mg creatinine. When Group A cases were divided into high- and low-grade groups, the average of the
TABLE 2
Urinary EGF from eight Group B patients with glial tumors*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Pathological Diagnosis</th>
<th>Tumor Grade</th>
<th>Location of Lesion</th>
<th>Urinary EGF†</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>19</td>
<td>F</td>
<td>anaplastic astrocytoma</td>
<td>3</td>
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<td>26</td>
<td>63</td>
<td>F</td>
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<td>3</td>
<td>thalamus</td>
<td>19.3</td>
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<tr>
<td>27</td>
<td>62</td>
<td>F</td>
<td>anaplastic astrocytoma</td>
<td>3</td>
<td>parietal lobe</td>
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<tr>
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<td>37</td>
<td>M</td>
<td>oligodendroglioma</td>
<td>2</td>
<td>frontal lobe</td>
<td>12.1</td>
</tr>
<tr>
<td>29</td>
<td>36</td>
<td>M</td>
<td>glioblastoma</td>
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<td>occipital lobe</td>
<td>9.7</td>
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<td>30</td>
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<td>F</td>
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<td>4</td>
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<tr>
<td>31</td>
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<td>3</td>
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<tr>
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<td>3</td>
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</table>

* Group B patients were examined after removal and/or chemoradiation therapy. These patients exhibited a small volume of residual tumor on computerized tomography. EGF = epidermal growth factor. Tumors graded according to the classification of Damas-Dupont, et al.*
† Expressed as ng/mg creatinine.

The urinary EGF level of Group B patients was significantly higher than that of healthy individuals (p < 0.005) and the 14 patients in the cerebral infarction group (p < 0.005), and slightly statistically higher than that of Group C patients (p < 0.10). However, there was no significant difference between levels in Group C patients and the healthy individuals (Fig. 1).

Surgical intervention and irradiation seemed to influence longitudinal fluctuations of urinary EGF in six glioma-bearing patients in Group A. Postoperative urinary EGF levels decreased in all except Case 4. In addition, the urinary EGF content in Cases 1, 2, and 4 decreased after irradiation. At the terminal stage of the disease, the urinary EGF level was very high (Fig. 2).

The urinary EGF content of the 12 patients in the meningioma group was statistically significantly lower than that of Group A patients (p < 0.02), but there was no statistically significant difference between the meningioma group and healthy individuals. Except for malignant lymphomas and hemangioblastomas, primary intracranial tumors were associated with a low level of urinary EGF. The nine patients with metastatic brain tumors showed a statistically significantly lower level of urinary EGF than did the Group A patients (p < 0.05) (Fig. 3). The one patient with radiation necrosis had a low urinary EGF level. Cerebrospinal fluid levels of EGF in patients with malignant gliomas were below 0.05 ng/ml.

No cross-reaction to TGF-α was observed in this assay system, indicating no interference from this factor in this system (Fig. 4).

Discussion

Distribution of EGF

Human EGF in adult human tissues is below 5.5 ng/gm wet tissue if extracted by affinity chromatography. The kidney, thyroid gland, and pancreas contain a high level of hEGF, however, brain tissue contains a very low level of hEGF (below 0.5 ng/gm wet tissue).
Urinary epidural growth factor as glial tumor marker

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Pathological Diagnosis</th>
<th>Tumor Grade of Tumor</th>
<th>Location of Tumor</th>
<th>Urinary EGF†</th>
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<tbody>
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<td>49, M</td>
<td>glioblastoma</td>
<td>4</td>
<td>temporal lobe</td>
<td>3.5</td>
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</tr>
</tbody>
</table>

* Group C patients were examined after removal and/or chemoradiation therapy, in these patients no tumor was shown on computerized tomography. EGF = epidermal growth factor. Tumors graded according to the classification of Daumas-Duport, et al.†

† Expressed as ng/mg creatinine.

Although the submandibular gland in mice has an exceptionally large amount of EGF ( 1.6 mg/gm wet tissue), the human submandibular gland contains only 1.3 ng/gm wet tissue. Human plasma contains hEGF at a mean of 16.3 ng/ml in males and 13.9 ng/ml in females. Hirata, et al. reported that EGF concentrations in human CSF were 171 pg/ml in normal individuals, 235 pg/ml in patients with brain tumors (including two medulloblastomas, one glioblastoma, two germinomas, one craniopharyngioma, one meningioma, and one metastatic pulmonary carcinoma), and 271 pg/ml in patients with pituitary tumors (including three nonfunctioning adenomas, two growth hormone-secreting adenomas, and two prolactin-secreting adenomas).

**EGF/TGF-α and EGF Receptors in Glioma**

The growth effects of EGF on gliomas are various. Werner, et al., reported that D-263MG and D-37MG glioma lines were stimulated by EGF, while D-247MG and U-343MGa exhibited no growth response, although all cell lines had close to 10³ EGF receptors per cell. Pollack, et al., demonstrated that three human glioma cell lines (T98G, U87, and U138) with high-affinity EGF receptors responded in a dose-dependent fashion to physiological concentrations of EGF.

The EGF receptors in glial tumors have been examined in various ways. Amplification and rearrangement of the EGF receptor gene and enhanced expression of EGF receptor messenger ribonucleic acid (mRNA) have been demonstrated in cultured cell lines. Enhanced EGF receptor immunoreactivity has been observed in tumor specimens. These studies on EGF receptors of gliomas suggested that the number of EGF receptors increased in high-grade glial tumors.

Epidermal growth factor competes with TGF-α for binding to its receptor and shows moderate sequence homology to TGF-α. The majority of biological activities of EGF are consonant with those of TGF-α, but TGF-α has more potent activity in angiogenesis and bone absorption. Low-molecular-weight (6 kD) α-type TGF activity was detected in urine from all subjects, but high-molecular-weight (30 to 35 kD) α-type TGF activity was detected in urine only from a majority of cancer patients. These α-type TGF activities of high molecular weight were detected in conditioned medium of a human glioma-derived cell line (YKG1). A strongly positive correlation between glial tumor grade and extent of TGF-α expression was found. Recently, TGF-α has been demonstrated immunohistochemically in high-grade gliomas. Since amplification of the EGF receptor gene has also been demonstrated in gliomas, TGF-α secreted by gliomas may provide autocrine stimulation through binding to amplified EGF receptors.

**Excretion of EGF Into Urine**

As hEGF passes into the glomerular filtrate, 50,000 to 60,000 ng of hEGF is finally excreted in the urine each day. Therefore, an amount of hEGF equal to that excreted in the urine must be synthesized and secreted into the blood to maintain the proper blood level of the factor; however, most of the urinary EGF may be produced in the kidney. Normal older individuals
exhibit a lower level of urinary EGF.37 Urinary EGF was found at a statistically significantly higher level in some patients with many malignant neoplasms (leukemia, malignant lymphoma, multiple myeloma, small cell and epidermoid carcinoma of the lung, maxillary cancer, esophageal cancer, gastric cancer, and medul-

ular carcinoma of the thyroid) than in controls.38 On the other hand, urinary EGF from patients with bladder tumors was statistically significantly lower, probably due to an excessive expression of EGF receptors.19

We have previously reported that α-type TGF activities of high molecular weight (20 to 50 kD) were detected at high levels in urine from all patients with high-grade astrocytoma, and at intermediate and low levels in urine from all patients with low-grade astrocytoma.20 It could not be distinguished whether α-type TGF activities were derived from TGF-α, EGF, or both, because both TGF-α and EGF had the ability to stimulate the formation of colonies and had common receptors. Stromberg, et al.,35 stated that glioma-associated urinary high-molecular-weight TGF was indistinguishable from a high-molecular-weight form of EGF. In our RIA system, however, only EGF could be detected (Fig. 4).

A high level of urinary EGF was detected in urine from patients with high-grade glial tumors in this assay (Table 1 and Fig. 3). This suggests that the above activities of α-type TGF of high molecular weight may have been due to an increased amount of urinary EGF in those high-grade glial tumors.

Stromberg, et al.,35 demonstrated that urinary high-
molecular-weight EGF/TGF-α detected in urine before removal of a glioblastoma disappeared after tumor removal. Our results revealed that urinary EGF levels in Group A patients were higher than in Group B or Group C patients. Comparing high-grade with low-grade gliomas in Group A patients, the urinary EGF level was higher in the high-grade than in the low-grade gliomas. Differential diagnosis between glioma and infarction, and between recurrent glioma and radiation necrosis are at times necessary. The patient with cerebral infarction showed a significantly lower level of urinary EGF than those with glioma.

Epidermal growth factor is distributed in various tissues, but it is suggested that urinary EGF may be derived from the kidney.30 Large amounts of the EGF detected in the urine of patients with high-grade gliomas may result from oversecretion of EGF stimulated by unknown factors released by high-grade gliomas. Since urinary EGF reflects the clinical stage and pathological grade, it is of practical value for diagnosis before surgery and for evaluation of therapies.

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References


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