Isolation and partial purification of growth factors with TGF-like activity from human malignant gliomas

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The effect of concentrated conditioned medium from each of eight human malignant glioma cell lines on the growth of indicator cells (normal rat kidney fibroblasts (NRK), clone 14) was determined in monolayer and in soft agar assay systems. The conditioned medium from all cell lines was mitogenic in the monolayer assay, but only SF-210, U-343 MG-A, and U-251 MG produced soluble factors that caused NRK cells to grow in soft agar. The soluble growth-promoting factors from these three cell lines were acid- and heat-stable (60°C for 30 minutes) but were inactivated by trypsin (100 μM/ml) and dithiothreitol (50 μM). The growth factors from SF-210 and U-343 MG-A were further purified by molecular-sieve chromatography. The partially purified growth factor from U-343 MG-A retained transforming growth factor (TGF)-like activity, had a molecular weight of 9 kD, was potentiated by TGF-β in the soft agar assay, competed effectively with 125I-epidermal growth factor (EGF) radiolabeled for the EGF receptor on A 431 epidermoid carcinoma cells, and was completely inhibited by monoclonal antibodies to TGF-α. The partially purified growth factor from SF-210 had a molecular weight of 17 kD, was not inhibited by monoclonal antibodies to platelet-derived growth factor (PDGF) or TGF-α, and did not bind to a heparin-Sepharose column. These results imply that U-343 MG-A secretes a growth factor with TGF-α-like activity, and SF-210 secretes a TGF with neither TGF-α nor TGF-β activity.

KEY WORDS • growth factor • transforming growth factor • glioma • brain neoplasm

There is increasing evidence that growth factors may play a role in the pathogenesis of certain malignancies of the central nervous system. A number of human brain tumors have been reported to produce platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). Increased expression of the erb-B oncogene, which encodes for the EGF receptor, has been found in glioblastoma multiforme. Growth factors that can cause phenotypic transformation of normal cells are called “transforming growth factors” (TGF’s). Properties of the transformed phenotype induced by TGF’s include the loss of density-dependent inhibition of cell growth in monolayer culture, characteristic morphological alterations, and the capacity for anchorage-independent growth. Transforming growth factors can be found in almost all neoplastic and certain non-neoplastic tissues from all species thus far studied. These factors have been partially purified from several human tumor cell lines, from solid mouse, rat, and human tumors, from the conditioned medium of chemically transformed rat and mouse cells, and from non-neoplastic tissues, including human placenta, kidney, and lung, mouse embryos, and human urine, serum, and platelets. Only recently, however, have attempts been made to isolate TGF’s from human malignant gliomas. This report describes a series of experiments that demonstrated secretion of proteins with TGF-like activity by three of eight human malignant glioma cell lines.

Materials and Methods

Cell Cultures

Eight well-characterized cell lines derived from human malignant gliomas were studied: SF-126, SF-188, SF-210, SF-265, SF-539, SF-295, U-343 MG-A, and U-251 MG. Normal rat kidney fibroblast (NRK) cells,
clone 14, were used as the target cells for the glioma-derived TGF-3's and were the generous gift of Dr. Lewis T. Williams, University of California, San Francisco. The cultures were maintained at 37°C in 75-sq cm plastic tissue-culture flasks in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS).

Source of TGF's: Collection of Glioma-Conditioned Medium

Serum-free media conditioned by the malignant glioma cell lines were the source of TGF's. Glioma cells (1 x 10^7) were placed in 850-sq cm plastic roller bottles* with 100 to 150 ml of MEM containing 10% FCS and gentamicin (50 µg/ml) and incubated at 37°C and 0.2 rpm in a 95% air:5% CO₂ atmosphere. When the cell layer reached 80% confluency, the cultures were rinsed twice for 3 hours each in 50 ml of fresh serum-free MEM to eliminate serum proteins. The rinsing medium was discarded. Over the next 8 days, five 150-ml aliquots of conditioned serum-free MEM were collected from each roller bottle, sterilized by passage through a 45-µm mesh filter,† and stored at -70°C until analyzed for activity in the mitogenic and soft agar assays. The protein concentration of the conditioned medium was measured with a protein determination kit.‡

Mitogenic Assay

Aliquots of the glioma-conditioned medium with known amounts of protein were analyzed for their ability to stimulate the incorporation of ³H-thymidine by NRK indicator cells. Approximately 1 x 10^3 NRK cells were seeded into each well of a 96-well, flat-bottom microtiter plate§ and allowed to adhere overnight in MEM and 10% FCS. The medium was removed and replaced with serum-free medium, the attached cells were incubated for 24 hours, and the conditioned medium was added. Twenty-four hours later, the cells were pulsed with 0.01 µCi of ³H-thymidine¶ and incubated at 37°C for 18 hours. One hour before harvesting, 10 µl of an enzyme cocktail consisting of pronase (0.05% of Kaken proteinase (proteolytic) U/mg) and collagenase (0.02% of 125 U/mg) was added to each well.** The cells were harvested onto Fiberglas filter paper with an automated multiple-sample harvester. Each sample was dried, placed in scintillation vials along with 3 ml of ¹³H-thymidine manufactured by NEN Research Products, DuPont, Boston, Massachusetts.

Concentration of Glioma-Conditioned Medium

Conditioned medium that stimulated the growth of NRK cells in soft agar was concentrated approximately 100-fold with a hollow fiber concentrator (CH2, cartridge SLY1, molecular weight cut-off 3000) at room temperature. The medium was then lyophilized to dryness with a multi-port lyophilizing unit.††

Acid Extraction of Concentrated Glioma-Conditioned Medium

Concentrated conditioned medium was resuspended in 1 M acetic acid and dialyzed for 48 hours in dialysis tubing at 4°C against a total of 100 volumes of 1 M acetic acid. The dialysate was lyophilized to dryness, resuspended in 5 mM acetic acid and 50 mM NaCl, and tested in the mitogenic and soft agar assays. The protein concentration of the conditioned medium after acid extraction was measured with a Bio-Rad protein determination kit.

EGF-Radioreceptor Analysis

The binding of ¹²⁵I-EGF to malignant glioma cells in culture was performed as described previously. Clone A 431 of the human squamous cell carcinoma cell line (kindly provided by Dr. L. T. Williams), which carries a high number of EGF receptors/cell, was used

* Roller bottles manufactured by Becton Dickinson, Oxnard, California.
† Mesh filter manufactured by Amicon, Danvers, Massachusetts.
‡ Protein determination kit manufactured by Bio-Rad, Richmond, California.
§ Microtiter plate manufactured by Nunc, Kamstrup, Denmark.
¶ ³H-thymidine manufactured by NEN Research Products, DuPont, Boston, Massachusetts.
** Kaken proteinase obtained from Calbiochem, San Diego, California; collagenase obtained from U.S. Biochemical Corp., Cleveland, Ohio.
†† Betalfluor obtained from National Diagnostics, Somerville, New Jersey.
†† TGF-α obtained from Genentech, South San Francisco, California; TGF-β obtained from R & D Systems, Minneapolis, Minnesota.
§ Fiber concentrator manufactured by Amicon, Danvers, Massachusetts.
‖ Lyophilizing unit manufactured by VirTis, Gardiner, New York.
as an internal control. Approximately $1 \times 10^5$ malignant glioma cells were seeded into each well of a 12-well Costar plate and allowed to adhere for 24 hours in MEM containing 10% FCS. On the day of the binding analysis, the medium was aspirated and the wells were washed once with 2 ml of binding medium consisting of MEM, 1 mg/ml bovine serum albumin (BSA, type V), and 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), pH 7.4, at room temperature. The binding medium was then aspirated and 1 ml of binding medium containing $^{125}$I-EGF (10 $\mu$Ci, 100,000 cpm) or $^{125}$I-EGF and various concentrations of purified unlabeled EGF (receptor grade) were added, and the cells were incubated in the binding mixture for 2 hours at 37°C.

To demonstrate that TGF-α competes for the EGF receptor, A 431 cells were incubated with various concentrations of unlabeled, recombinant human TGF-α in conjunction with the radiolabeled $^{125}$I-EGF. All binding reactions were stopped by washing four times with Hanks' balanced salt solution containing 1 mg/ml BSA at 4°C. The cells in each well were then solubilized with 1 ml 2% sodium dodecyl sulfate for 10 minutes. The total amount of radioactivity bound to the cell monolayer was measured with a gamma counter. Nonspecific binding was determined by adding a 1000-fold excess (1 $\mu$M) of unlabeled EGF. Specific binding was calculated by subtracting the nonspecific binding from total binding.

**Molecular Sieve Chromatography**

The lyophilized, acid-extracted dialysates of SF-210 and U-343 MG-A were applied to columns (1.5 × 90 cm) of Bio-Gel P-60 200-400 mesh equilibrated with 1 M acetic acid. The columns had been previously calibrated with ovalbumin (molecular weight ratio (M₀) 44,000), myoglobin (M₀ 17,000), cytochrome C (M₀ 14,000), insulin (M₀ 5700), and vitamin B12 (M₀ 1350). The columns were eluted at 22°C with 1 M acetic acid at a constant flow of 12 ml/hr, and 2-ml fractions were collected. Fractions were monitored either by absorbance at A₂₈₀ or by individual protein determinations. Aliquots of 10 to 20 $\mu$l from alternate fractions were dried at room temperature in a Speed Vac concentrator* for mitogenic, soft agar, and EGF-radioreceptor studies.

**Physicochemical Treatment of Partially Purified Glioma-Derived TGF's**

After acid extraction, the growth factors (10 $\mu$g protein) from SF-210, U-251 MG, and U-343 MG-A were tested for stability in response to limited proteolysis, heat, reducing agents, and acid and alkaline milieus. After molecular sieve chromatography, the partially purified TGF's from U-343 MG-A and SF-210 were characterized by physicochemically treating 10-$\mu$l aliquots (1 $\mu$g protein dissolved in 5 mM acetic acid and 50 mM NaCl) of the active fractions before performing soft agar assays under the conditions listed below.

To test trypsin sensitivity, the glioma factors were incubated for 2 hours at 37°C with trypsin (100 $\mu$g/ml) alone or with trypsin (100 $\mu$g/ml) plus soybean trypsin inhibitor (200 $\mu$g/ml). Trypsin and soybean trypsin inhibitor were preincubated together in a small volume of MEM for 30 minutes at 37°C. Aliquots with trypsin only received soybean trypsin inhibitor (200 $\mu$g/ml) after the 2-hour incubation.

The heat stability of the growth factors was tested by heating in a 60°C water bath for 30 minutes or by boiling for 3 minutes. The sensitivity of the glioma factors to reducing agents was determined by exposing the samples to 50 mM dithiothreitol at 37°C for 16 hours. To remove the dithiothreitol before analysis, the samples were dialyzed in dialysis tubing against 5 mM acetic acid and 50 mM NaCl before testing in the soft agar system.

The acid stability of the glioma growth factors was assessed by dialyzing aliquots in dialysis tubing for 20 hours at 4°C against 0.1 M acetic acid (pH 3.0, two changes of 1000-fold volume). After filtration, these samples were dialyzed again against 5 mM acetic acid and 50 mM NaCl for 12 hours before testing by soft agar analysis. The stability of the glioma growth factors in a basic milieu was tested in a similar fashion, except that ammonium hydroxide (25 mM, pH 12.0) was used as the dialyzing solution.

Epidermal growth factor- and TGF-α-like activity within the glioma growth factors was assessed by analyzing aliquots in the EGF-radioreceptor studies, by adding TGF-β (100 ng/ml) to the samples before the soft agar assay, and by incubating the samples overnight with anti-TGF-α monoclonal antibody (100 $\mu$g/ml). In the latter case, control samples consisted of adding anti-TGF-α monoclonal antibody (100 $\mu$g/ml) alone.

Because of the high affinity of all angiogenic factors for heparin, fibroblast growth factor (FGF)-like activity within the glioma growth factors was determined by first passing aliquots of purified factor over a column (1.5 × 10 cm, 30 ml) of heparin-Sepharose† equilibrated with 0.6 M NaCl, 0.01 M Tris-HCl, pH 7.5. After a wash of about 5 column volumes, the glioma-derived samples were eluted with a gradient of 100 ml of 0.6 to 3.0 M NaCl in 0.01 M Tris-HCl, pH 7.5, at a flow rate of 20 ml/hour at 4°C. Wash and elution samples were then dialyzed exhaustively against 5 mM acetic acid and 50 mM NaCl and then tested in the soft agar assays.

Platelet-derived growth factor-like activity was analyzed by incubating the glioma-derived factor aliquots in serially diluted anti-PDGF monoclonal antibody

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* Speed Vac concentrator manufactured by Savant, Farmingdale, New York.

† Heparin-Sepharose manufactured by Pharmacia, Uppsala, Sweden.
and conditioned medium), despite seeding densities as high as $1 \times 10^4$ cells/dish. Conditioned medium from SF-210, U-343 MG-A, and U-251 MG stimulated the growth of NRK cells in soft agar (Figs. 3 and 4). Acid-extracted conditioned medium from these three cell lines stimulated growth to an even greater extent (Fig. 5).

By itself, TGF-α caused NRK indicator cells to grow in soft agar. The addition of TGF-β (1.0 ng/ml) to TGF-α significantly increased the number of colonies that formed in soft agar (data not shown). In contrast, TGF-β by itself did not stimulate the growth of NRK indicator cells in soft agar.

**EGF Radioreceptor Analysis**

The specific binding of $^{125}$I-EGF to malignant glioma cell lines is shown in Table 2. All malignant glioma cell lines had demonstrable specific binding of radiolabeled EGF to the cell surface; however, no cell line or culture bound $^{125}$I-EGF to the same degree as A 431. Representative saturation-binding curves for malignant glioma cell lines are shown in Fig. 6. The U-343 MG-A cell line demonstrated the greatest level of specific binding in addition to saturability of the EGF-receptor.

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**Results**

**Mitogenic Assay**

With the hollow-fiber filter concentrator, it was possible to reduce the volume of glioma-conditioned medium 100-fold. Conditioned medium from all eight glioma-derived cell lines had a mitogenic effect on NRK indicator cells (Table 1). In most cases, the increase in $^3$H-thymidine uptake was dose-dependent (Figs. 1 and 2). Acid-extracted conditioned medium from cell lines SF-210, U-343 MG-A, and U-251 MG also stimulated the growth of NRK cells, although to a lesser degree than conditioned medium (Fig. 2).

**Soft Agar Assay**

Normal rat kidney cells did not form colonies in soft agar under control conditions (absence of growth factors and conditioned medium), despite seeding densities as high as $1 \times 10^4$ cells/dish. Conditioned medium from SF-210, U-343 MG-A, and U-251 MG stimulated the growth of NRK cells in soft agar (Figs. 3 and 4). Acid-extracted conditioned medium from these three cell lines stimulated growth to an even greater extent (Fig. 5).

By itself, TGF-α caused NRK indicator cells to grow in soft agar. The addition of TGF-β (1.0 ng/ml) to TGF-α significantly increased the number of colonies that formed in soft agar (data not shown). In contrast, TGF-β by itself did not stimulate the growth of NRK indicator cells in soft agar.

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**FIG. 1.** Mitogenic effect of glioma-conditioned medium on normal rat kidney (NRK) cells. Medium conditioned by the four glioma cell lines shown here caused a dose-dependent increase in the uptake of $^3$H-thymidine ($^3$H-Tdr, in cpm). Conditioned medium from U-251 MG had the greatest effect on NRK cells and increased thymidine uptake by 775% compared to control NRK cells incubated in serum-free (S-F) medium.

**FIG. 2.** Mitogenic effect of acid-extracted conditioned medium from selected glioma cell lines on normal rat kidney (NRK) cells. A dose-dependent increase in the uptake of $^3$H-thymidine ($^3$H-Tdr, in cpm) is seen for all cell lines compared to control NRK cells incubated in serum-free (S-F) medium; however, the increases were smaller than those observed with conditioned medium (see Fig. 1).

**FIG. 3.** Effect of glioma-conditioned medium on growth of normal rat kidney (NRK) cells in soft agar. Of the eight glioma-derived cell lines tested, only three (SF-210, U-343 MG-A, and U-251 MG) produced soluble factors that stimulated anchorage-independent growth. The increase in colony formation was dependent on the protein concentration and was greatest with medium conditioned by SF-210.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Lowest Mitogenic Dose (μg protein/ml)</th>
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<tbody>
<tr>
<td>SF-126</td>
<td>10</td>
</tr>
<tr>
<td>SF-188</td>
<td>0.5</td>
</tr>
<tr>
<td>SF-210</td>
<td>5</td>
</tr>
<tr>
<td>SF-268</td>
<td>4</td>
</tr>
<tr>
<td>SF-295</td>
<td>9</td>
</tr>
<tr>
<td>SF-539</td>
<td>10</td>
</tr>
<tr>
<td>U-343 MG-A</td>
<td>7.5</td>
</tr>
<tr>
<td>U-251 MG</td>
<td>1</td>
</tr>
</tbody>
</table>

* An increase of at least 200% in the incorporation of $^3$H-thymidine compared with serum-free controls was considered a mitogenic response. NRK = normal rat kidney.
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FIG. 4. Photomicrograph of a large colony of normal rat kidney cells that grew in soft agar after incubation with medium conditioned by SF-210 (protein content, 10 μg/ml). Only colonies that contained more than 40 cells or were greater than 40 μm were scored as positive. Scale bar = 40 μm.

Molecular Sieve Chromatography

The majority of the protein in the acid-extracted conditioned medium from glioma cell lines SF-210 and U-343 MG-A was found in the void volume of the molecular sieve column chromatograms (Figs. 7 and 8). Bio-Gel P-60 column fractions from SF-210 (fractions 23 to 43) contained a peak of colony-stimulating activity with an apparent molecular weight of 17 kD. When the active fractions were pooled, lyophilized, and resuspended in 5 mM acetic acid and 25 mM NaCl, a dose-dependent stimulation of 3H-thymidine incorporation and colony formation among NRK cells was found (data not shown). The Bio-Gel P-60 fractions from U-343 MG-A with retained TGF-like activity had an apparent molecular weight of 9 kD (Fig. 8). Aliquots from the pooled active fractions were similarly mito-

FIG. 5. Effect of acid-extracted conditioned medium from cell lines SF-210, U-343 MG-A, and U-251 MG on the growth of normal rat kidney (NRK) cells in soft agar. The largest number of colonies (205) was formed by NRK cells incubated with medium conditioned by U-343 MG-A (protein content, 10 μg/ml).

FIG. 6. Representative 125I-labeled epidermal growth factor (EGF) saturation-binding curves for glioma cells in culture. The U-343 MG-A cell line demonstrated the highest degree of specific 125I-EGF binding, with saturability of the EGF-receptor.

FIG. 7. Bio-Gel P-60 gel filtration column for purification of SF-210 glioma-derived conditioned medium. Acid-extracted conditioned medium from SF-210 (4 liters) was passed over a column (1.5 × 90 cm) of Bio-Gel P-60 200-400 mesh pre-equilibrated with 1 M acetic acid. Fractions 22 to 42 contained a single peak of normal rat kidney (NRK) cell colony stimulating activity at an apparent molecular weight of 17 kD. The bulk of the proteins contained within the acid-extracted sample were found in the void volume (Vo). Molecular weight standards are ovalbumin (44 kD), myoglobin (17 kD), and insulin (6 kD). O.D. = optical density; Vt = terminal volume.

FIG. 8. Molecular sieve chromatography (Bio-Gel P-60) of acid-extracted conditioned medium from the U-343 MG-A cell line. A broad peak of activity (line with squares) is seen between fractions 60 and 100 with an apparent molecular weight of 9 kD. The bulk of the protein (plain line) was found in the void volume (Vo). Molecular weight standards are as shown. NRK = normal rat kidney; Vt = terminal volume.
**TABLE 2**

<table>
<thead>
<tr>
<th>Cell Culture</th>
<th>Derivation</th>
<th>125I-EGF Specific Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF-126</td>
<td>glioblastoma multiforme</td>
<td>3861</td>
</tr>
<tr>
<td>SF-188</td>
<td>glioblastoma multiforme</td>
<td>4674</td>
</tr>
<tr>
<td>SF-210</td>
<td>glioblastoma multiforme</td>
<td>3524</td>
</tr>
<tr>
<td>SF-539</td>
<td>gliosarcoma</td>
<td>2875</td>
</tr>
<tr>
<td>U-251 MG</td>
<td>glioblastoma multiforme</td>
<td>3644</td>
</tr>
<tr>
<td>U-343 MG-A</td>
<td>anaplastic astrocytoma</td>
<td>5800</td>
</tr>
<tr>
<td>A 431</td>
<td>squamous cell carcinoma†</td>
<td>40,000</td>
</tr>
</tbody>
</table>

* EGF = epidermoid growth factor.
† Used as an internal control.

**Physicochemical Characterization of Glioma TGF's**

After acid extraction, the growth factors from all three glioma cell lines were acid- and heat-stable but showed lability in response to limited proteolysis and reducing agents (Table 3). The growth factors from SF-210 and U-343 MG-A were further characterized after molecular sieve chromatography, as described below (Table 4). The potency of the growth factor isolated from U-343 MG-A was greatly increased by TGF-β (10 ng/ml) but not by EGF (10 ng/ml). Passage of the partially purified growth factor over a column of heparin-Sepharose did not significantly alter the effect of the growth factor on NRK cell colony formation. Monoclonal antibodies to TGF-α completely eliminated NRK cell colony formation. In contrast, the purified growth factor from SF-210 was not potentiated by the addition of TGF-β or EGF, and was not inhibited by anti-TGF-α or anti-PDGF monoclonal antibodies in the soft agar assay (Table 4). Prior treatment of the factor by heparin-Sepharose affinity chromatography had no effect on colony formation. Finally, the partially purified factor did not bind to the EGF receptor.

**Discussion**

Six major types of tumor-derived endogenous growth factors have now been well described: TGF-α, TGF-β, bombesin, monolayer mitogens such as PDGF and EGF, insulin-like growth factors (IGF-I and IGF-II), and heparin-binding angiogenic factors...
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such as FGF. Of these factors, the TGF's are the most likely mediators of the malignant transformation of normal cells.

The TGF's belong to a family of heat- and acid-stable peptides requiring intrachain disulfide bonds for activity. Malignant cells in culture produce two types of TGF's: those that bind to the EGF receptor (TGF-\(\alpha\)) and those that do not (TGF-\(\beta\)). Transforming growth factor-\(\alpha\) has significant sequence homology with mouse and human EGF; it is released into the culture medium of a variety of malignant cells of both ectodermal and mesodermal origin. Transforming growth factor-\(\beta\), a 25-kD polypeptide, has been isolated from acid/ethanol extractions of both malignant and nonmalignant whole cells; in combination with TGF-\(\alpha\) or EGF, it stimulates NRK indicator cells to form colonies in soft agar. Transforming growth factor-\(\beta\) has been sequenced, and complementary DNA clones encoding the protein have been purified.

The bioassay used to measure TGF-like activity in a tumor sample or cell line is based on the fact that anchorage-independent growth of cells in culture has a particularly high correlation with neoplastic growth in vivo. Untransformed non-neoplastic cells do not form viable colonies in soft agar. Therefore, the growth of normal cells in soft agar bioassay systems can be an important quantitative measure of the relative amounts of TGF's in a given sample. Several epithelial and fibroblastic cell lines have been used to study the effects of TGF's on colony formation in soft agar. Because of their exquisite sensitivity to TGF's, NRK cells (clone 14) and AKR-2B cells are most commonly used for such studies.

Although the utility of certain indicator cell lines in bioassays of TGF's is generally accepted, some caveats are needed. First, Salomon, et al., showed that normal mouse or rat mammary epithelial cells are better than fibroblasts as target cells for TGF's isolated from medium-conditioned medium by breast carcinoma cell lines. Thus, TGF's produced by one tumor cell line may be able to stimulate the anchorage-independent growth of one type of indicator cell but not that of another. Second, Rizzino, et al., demonstrated that the use of a single indicator cell line to analyze TGF's may be fraught with difficulties because of potential growth factor synergism. In their study, FGF and PDGF enhanced the growth of NRK cells in soft agar depending on whether plasma-supplemented, serum-supplemented, or serum-free medium was used. Finally, the effect of TGF on the growth of normal indicator cells, including NRK cells, has varied considerably in different laboratories. For example, some researchers contend that TGF-\(\alpha\) by itself stimulates the growth of NRK cells in soft agar, while others have found that TGF-\(\alpha\) (or EGF) stimulates anchorage-independent growth of these cells only in the presence of TGF-\(\beta\). Nevertheless, the ability of cells to grow in soft agar probably remains the best in vitro assay for neoplastic transformation.

Todaro, et al., were the first to show that growth-stimulating factors released by human melanoma, rhabdomyosarcoma, and bronchogenic carcinoma cell lines could induce the anchorage-independent growth of normal human fibroblasts. These normal human fibroblasts were capable of achieving a 4.3% cloning efficiency in soft agar when exposed to the tumor-derived TGF's. In our study, three of eight well-characterized human malignant glioma cell lines produced soluble factors that stimulated anchorage-independent growth of NRK cells in soft agar.

The glioma factor isolated from U-343 MG-A by Bio-Gel P-60 chromatography was acid- and heat-stable and had a molecular weight of 6 to 10 kD. Its transforming activity was completely destroyed under basic conditions (pH 12.0), by reducing agents, and by incubation with anti-TGF-\(\alpha\) monoclonal antibodies. In addition, NRK cell colony formation was potentiated when TGF-\(\beta\) (10 ng/ml) was added to the partially purified TGF from this U-343 MG-A. Finally, aliquots of partially purified TGF from the gel filtration column competed directly with the \(^{125}\)I-EGF receptor on A 431 epidermoid carcinoma cells. These physicochemical data imply that A 431 glioma cells secrete a TGF-\(\alpha\)-like growth factor.

Bringsman, et al., have recently shown that TGF-\(\alpha\) is synthesized as a much larger (160 residue long) precursor that is a glycosylated and palmitoylated transmembrane protein. Cleavage of this large precursor molecule at various domains results in the detection of tumor-derived conditioned medium of several molecular species (5 kD, 12.5 kD, 15 kD, 18 kD, and 20 kD), many of which retain TGF-\(\alpha\) activity. It is therefore conceivable that the proteins we recovered from molecular sieve chromatography represent heterogeneous forms of TGF-\(\alpha\).

It is unlikely that the partially purified U-343 MG-A TGF-\(\alpha\)-like molecular complex contained FGF or PDGF. Like all of the angiogenic factors, FGF has a high affinity for heparin. When the partially purified U-343 MG-A complex was passed through a column of heparin-Sepharose, the NRK cell transforming activity did not bind to the column. When the purified U-343 MG-A growth factor was incubated with aliquots of anti-PDGF monoclonal antibodies, its activity was not altered. Thus, it appears that when conditioned medium from the malignant glioma cell line U-343 MG-A is processed according to TGF-purification protocols, a conventional TGF-\(\alpha\)-like molecule can be isolated. Interestingly, only the TGF-like factor from U-343 MG-A demonstrated good saturability of the A 431 EGF receptor. Since U-343 MG-A also possessed the highest specific binding of \(^{125}\)I-EGF, it is possible that autocrine stimulation of U-343 MG-A by TGF-\(\alpha\) takes place.

The partially purified growth factor isolated from SF-210 is also acid- and heat-stable and shows lability toward base and reducing agents. In contrast to the TGF-\(\alpha\)-like factor isolated from U-343 MG-A, the SF-
210 glioma factor is not potentiated by addition of TGF-β to the NRK soft agar system, is not inhibited by monoclonal antibodies to TGF-α, and does not compete with radiolabeled EGF for the EGF receptor. Furthermore, the SF-210 TGF-activity does not bind to a heparin-Sepharose column and is not eliminated by incubation with monospecific antibodies to PDGF. By molecular sieve chromatography, the SF-210 glioma factor has an apparent molecular weight of 17 kD. These results imply that the purified SF-210 glioma factor is neither TGF-α nor TGF-β. Furthermore, this purified TGF does not appear to resemble other conventional growth factors by molecular weight or physicochemical criteria.

Recently, a third class of TGF’s (TGF-γ) that induce the clonogenic growth of NRK cells in the absence of EGF has been described. Compounds with TGF-γ-like activity neither bind to the EGF-receptor nor potentiate TGF-α. Interestingly, TGF-γ has been found in high concentration in a neuroblastoma. Tsushima, et al., have identified a 15-kD species with TGF-γ-like activity from human anterior pituitary glands. However, until the final purification of these TGF-γ factors is achieved, the term must be used to describe a heterogeneous collection of soluble polypeptides, the behavior of which differs considerably from that of conventional TGF’s.

In conclusion, the data presented here support the hypothesis that human malignant gliomas secrete growth factors with TGF-like activity. Further purification to homogeneity and identification of these factors are currently underway. Additional experiments are needed to determine if these glioma-derived TGF’s are important in the pathogenesis of malignant gliomas.

Acknowledgments

The authors thank Mary Ellen Kuhlmann for manuscript preparation and Stephen Ordway for editorial assistance.

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Manuscript received May 23, 1988. Accepted in final form May 24, 1989. This work was supported by Grants CA 13525 and CA 31882 from the National Cancer Institute, National Institute of Health, and by a grant from the Preuss Foundation. Dr. Rutka is a fellow of the Medical Research Council of Canada.

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