Multiplying Transforming Growth Factors in Human Malignant Effusions

Misuzu Kurokawa Seo, Kathy E. Lynch, and Daniel K. Podolsky

Departments of Medicine, Harvard Medical School and Massachusetts General Hospital (Gastrointestinal Unit), Boston, Massachusetts 02114

ABSTRACT

Human malignant effusions were found to contain transforming growth factor (TGF) activity capable of stimulating anchorage independent growth of nontransformed rodent fibroblasts. Bio-Gel P-60 chromatography of acid-ethanol extracts demonstrated the presence of three populations of TGF activities in 57% of malignant effusions. Two activities were similar to those of TGFα and TGFβ as judged by their size (Mr ≈ 6,000 and ≈ 25,000, respectively), biological activity (ability to stimulate anchorage independent growth of NRK fibroblasts in the absence or presence of epidermal growth factor, respectively), and capacity to competitively inhibit binding of 125I-labeled epidermal growth factor to A-431 membranes and 125I-TGFβ to baby hamster kidney fibroblasts, respectively. In addition a third factor which stimulated anchorage independent growth of nontransformed rodent fibroblast and human colonic epithelial cells was also recovered following Bio-Gel P-60 chromatography of extracts from several cytology positive human malignant effusions of patients with colonic and breast carcinoma as well as other malignancies. The latter malignant effusion related transforming growth factor was not present in benign or cytology negative effusions. Malignant effusion related TGF factor was inactivated by sulfhydryl reducing agents, heat, and trypsin treatment but was stable in 1% acetic acid and ethanol. Partial purification was accomplished by chromatography of an acid-ethanol extract on Bio-Gel P-60 followed by high performance liquid chromatography with C18-Bondapak to yield a nearly pure protein with apparent molecular weights of 64,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis when run in nonreducing conditions and 32,000 when run in reducing conditions. Malignant effusion related TGF was able to stimulate anchorage independent growth of nontransformed fibroblasts in the absence of other growth factors. It did not competitively inhibit binding of 125I-labeled epidermal growth factor, 125I-TGFβ, or 125I-labeled platelet derived growth factor. Therefore, this factor isolated from human malignant effusions may be distinct from previously described transforming growth factors. Collectively these observations indicate that human malignant effusions contains a multiplicity of transforming growth factors. It is possible that the malignant effusion related transforming growth factors play a role or reflect the metastatic growth properties of various tumors.

INTRODUCTION

Over the past several years a number of proteins have been identified which stimulate anchorage independent growth by nontransformed fibroblasts (1–4) leading to their designation as TGF. These proteins were initially isolated as products of transformed cells in culture and solid tumor tissue but have also been isolated from normal tissues (e.g., placenta) and biological fluids (5–8). Characterization of TGFs from a variety of sources led to the recognition of two TGF classes. The first, TGFα, competitively inhibited EGF binding and was found to cause anchorage independent growth of nontransformed fibroblasts without the need for other supplementary peptides (9–12). Although it is recognized that high and low molecular weight forms may exist, subsequent work has proven that TGFα is structurally homologous to EGF and interacts with the EGF receptor to effect its activity. The second class of TGF, designated TGFβ, was found to bear no homology to EGF but required its presence to stimulate colony formation by the nontransformed NRK fibroblasts in soft agar (13–18).

Further study has suggested that the biological activities of these TGFs as well as other growth factors are situational. The situational variability in the functional activities of TGFs is illustrated by TGFβ which may stimulate proliferation of fibroblasts but may inhibit epithelial cell proliferation and/or promote epithelial differentiation (15–21). Furthermore the effects of one growth factor may be modulated by the presence of a second factor. Thus the presence of EGF may fundamentally alter response of some fibroblasts to TGFβ (21, 22).

Since the identification of TGFα and TGFβ, a number of other tissue products with growth factor activity have been recognized. Colon tumor derived cell lines in particular have been found to produce a variety of growth factors (23–26). Although several of these colon tumor derived factors appear to be related to TGFα or TGFβ, others have been less well characterized. Some may be related to other growth factors recognized as products of nontransformed cells such as platelet derived growth factor or fibroblast growth factor. In the studies described here the presence of several TGF activities in human malignant effusion is demonstrated. In addition to activities seemingly similar to TGFα and TGFβ, another TGF which appears categorically distinct from TGFα and TGFβ activities has been found to be common in malignant effusions of patients with a variety of carcinomas.

MATERIALS AND METHODS

Specimens. Human effusions (0.7–3.7 liters) collected in a sterile manner for diagnostic and/or therapeutic indications were obtained from the Cytology Laboratory of the Massachusetts General Hospital following cytological assessment. Effusions were centrifuged at 10,000 g for 10 min to remove cellular debris and stored at 4°C or −20°C. Prior to use, specimens were again sterilized by passing them through a 0.45-μm filter. Diagnostic designations were confirmed by review of medical charts. These studies were approved by the Human Studies Committee of the Massachusetts General Hospital.

Cell Culture and Soft Agar Colony Stimulation Assay. BHK and NRK fibroblasts used as indicator cells were grown in DMEM supplemented with 10% FCS containing penicillin and streptomycin as described (27). HCMC were maintained as described previously (28). Soft agar colony stimulation assays were performed in 60-mm plastic dishes using a 3.0-ml bottom layer of 0.5% agar in 10% FCS-DMEM overlaid with 3.0 ml of 0.4% agar in 10% FCS-DMEM containing 1 × 104 indicator cells and test or control samples (up to 100 μl) (29). In some assays, purified EGF (4 ng/ml) (BRL) was added with TGF test samples. Control wells containing EGF only, purified TGFβ (100 pm), or a combination of EGF and TGFβ were incubated in parallel with test samples. TGFβ was the generous gift of Dr. Michael Sporn. Dishes were incubated at 37°C in a humidified atmosphere of 5% CO2 for 10–14 days. Fibroblast colony formation was quantified as the number of colonies containing >50 cells in each of 5 1 cm2 sections counted under a dissecting microscope. The specific activity of colony formation was calculated as described (27).
MULTICIPICITY OF TGF IN HUMAN MALIGNANT EFFUSIONS

Identification and Purification of Human TGF. For initial detection of TGF activity in effusions, samples (10 ml) were dialyzed against either PBS or water using dialysis tubing with a molecular weight cutoff of <3500. Samples dialyzed against water were lyophilized and then resuspended in an equal volume of PBS. No loss of TGF activity was observed following lyophilization.

For purification of TGF activity, malignant effusions (200–1400 ml) were extracted overnight (4°C) with stirring in 2 volumes absolute ethanol and 0.04 volume concentrated HCl. The supernatant was collected and saved following centrifugation (10,000 x g for 15 min) and the pellet was again extracted overnight by addition of 0.5 volume of ethanol:water:concentrated HCl (2:1:0.04, v/v/v) followed by repeat centrifugation as before to obtain a second supernatant. The two supernatants were combined and then adjusted to pH 5.3 by addition of concentrated NH₄OH followed by addition of 2 m ammonium acetate (pH 5.3) to achieve a final concentration of 25 mM. Subsequently, the centrifugation as before to obtain a second supernatant. The two collected and saved following centrifugation (10,000 x g for 15 min) and extracted with 2 volumes ethanol and 4 volumes anhydrous ether at −20°C for 48 h. The precipitate was collected by centrifugation (15,000 x g for 25 min), resuspended in a minimal volume of 1.0 m acetic acid, and dialyzed (4°C) against 1% acetic acid (v/v) before being lyophilized.

Crude TGF extract was resuspended in 1.0–2.5 ml 1% acetic acid and applied to a Bio-Gel P-60 column (2.5 x 100 cm) equilibrated in the same solution, which was then developed at 15.0 ml/h collecting 2.5-ml fractions with constant A₂₈₀ monitoring. Every third to fifth fraction was either analyzed for TGF activity directly after dialysis against PBS or first lyophilized after dialysis against water. Fractions with demonstrated TGF activity were pooled, dialyzed against water, and lyophilized. Semipurified TGF(s) was dissolved in 0.1% trifluoroacetic acid and chromatographed at room temperature on a preparative C₁₈-RP Bondapak reverse phase column (12 x 300 mm). The column was developed at a flow rate of 0.8 ml/min with an ascending gradient of acetonitrile in 0.1% trifluoroacetic acid reaching 20% (v/v) in 10 min followed by isocratic elution for 5 min before further ascending gradient to 70% acetonitrile over 115 min, collecting 1.0-ml fractions. The column eluant was monitored as before. Later samples were chromatographed over 120 min at 0.8 ml/min on a C₁₈-RP Bondapak using a linear 30–60% acetonitrile gradient in 0.5% trifluoroacetic acid. Identification of TGF activity within positive fractions recovered from HPLC was made following SDS-PAGE in nonreducing conditions by elution of 2.5-mm slices in 1.0 ml PBS, followed by dialysis against water, lyophilization, and determination of TGF activity as before.

Physical and Chemical Treatments. Semipurified preparations (0.5–1.0 mg) of human effusion TGFs (pre-HPLC) were dissolved in PBS or 1% acetic acid. Samples were alternatively placed at 65°C in a water bath for 30 min or in a boiling water bath for 3 min. Additional samples were treated with 10 mM dithiothreitol, or with L-1-lysylamido-2-phenylethyl chloromethyl ketone-trypsin (100 μg) with or without soybean trypsin inhibitor (200 μg) at room temperature for 6 h. After the various treatments, TGF activity was again assayed as before using NRK and BHK indicator cells in soft agar colony formation assays. Prior to assessment of TGF activity, samples and appropriate controls were dialyzed against 1% acetic acid and lyophilized.

Inhibition of Growth Factor Binding. 125I-EGF and 125I-PDGF were purchased from Collaborative Research. TGF/β was radiolabeled as described (30). Membranes from A-431 cells were prepared as described (31). Binding of growth factor in the presence or absence of human TGF containing samples was assessed using A-431 membranes for EGF and NRK or BHK cells for PDGF and TGF/β by methods described previously (30–33). The lower limits of sensitivity of the radioreceptor assays were 0.01 pm for EGF and TGF/β and 0.05 pm for PDGF.

General Methods. Protein was measured by the method of Lowry et al. (34). SDS-PAGE was performed in reducing and nonreducing conditions using 7.5–12.5% polyacrylamide gels as described by Laemmli (35).

RESULTS

As demonstrated in Table 1, a large proportion of human malignant effusions (57%) effectively stimulated anchorage independent growth of nontransformed fibroblasts. Effusions were obtained from patients with a wide variety of malignancies (see legend to Table 1). Although the presence of TGF activity was not strictly associated with specific primary cell types, the rate of positivity of effusion from patients with primary adenocarcinoma of the breast and digestive tract was higher than that of effusions from patients with squamous or nonepithelial primary lesions (78% vs. 24%). Comparison of two different fibroblast lines, NRK and BHK, indicates that the latter was a more sensitive indicator of TGF activity in human malignant effusion. Cytology negative patients with known malignancy also contained TGF activity although the level of activity in these samples was significantly less than that found in cytology positive effusions. Similarly effusions from patients with benign disease demonstrated TGF activity but at a significantly lower level than effusion from patients with cancer.

TGF activity in malignant effusions was stable to extraction with acid-ethanol and ether (Table 2) facilitating purification of TGF activities from large volumes of malignant effusion. Extracted material was chromatographed on Bio-Gel P-60 and the eluant was monitored for its ability to stimulate colony growth of NRK or BHK cells in agar with and without supplementation with EGF. As demonstrated in the representative chromatographic profile depicted in Fig. 1, most malignant

### Table 1 TGF activity in human effusions

<table>
<thead>
<tr>
<th>Effusion</th>
<th>Total no.</th>
<th>BHK (% positive)</th>
<th>NRK (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant cytology</td>
<td>79</td>
<td>707 ± 350 (57)</td>
<td>515 ± 275 (39)</td>
</tr>
<tr>
<td>Malignant cytology</td>
<td>14</td>
<td>310 ± 215 (21)</td>
<td>385 ± 185 (29)</td>
</tr>
<tr>
<td>Benign</td>
<td>11</td>
<td>140 ± 70 (18)</td>
<td>190 ± 110 (27)</td>
</tr>
</tbody>
</table>

* Mean ± SD colonies (>50 cells/each) of positive effusions after seeding 10⁶ indicator cells (either BHK or NRK fibroblasts)/well in the presence of 50 μg EGF diffusion derived protein prepared as described in the text after subtraction of buffer controls (mean <40).

† Positive effusions defined as >3-fold increase in colonies (>50 cells/colony)/soft agar.

‡ Effusions from patients with colonic (n = 21), breast (n = 19), unknown primary carcinoma (n = 18), pancreatic carcinoma (n = 8), squamous cell carcinoma (n = 7), melanoma (n = 1), lymphoma (n = 2), and sarcoma (n = 3).

§ Effusions from patients with lung (n = 7), colonic (n = 3), renal cell carcinoma (n = 2), and lymphoma (n = 2).

∥ Effusions from patients with congestive heart failure and pneumonia.

### Table 2 Partial purification of human malignant effusion TGF activities

<table>
<thead>
<tr>
<th>Step</th>
<th>TGF activity (colonies/10 μg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHK</td>
<td>NRK</td>
</tr>
<tr>
<td>Crude effusion</td>
<td>310</td>
</tr>
<tr>
<td>Acid-ethanol extract</td>
<td>430</td>
</tr>
<tr>
<td>Bio-Gel P-60*</td>
<td>1700</td>
</tr>
<tr>
<td>II</td>
<td>&lt;50</td>
</tr>
<tr>
<td>III</td>
<td>310</td>
</tr>
<tr>
<td>C₁₈ reverse phase HPLC*</td>
<td>6340</td>
</tr>
</tbody>
</table>

* Number of colonies (>50 cells each) in soft agar at 14 days after seeding 10⁶ indicator cells/6 ml agar/60-mm dish in the absence or presence of 4 ng/ml EGF.

† Representative isolation of TGF from malignant effusion (colonic adenocarcinoma primary with peritoneal metastasis) dialyzed against 1% acetic acid prior to initial evaluation.

‡ Recovery calculated relative to number of colonies formed using BHK indicator cells in the presence of EGF (4 ng/ml).

§ Peaks of TGF activity recovered following Bio-Gel P-60 chromatography of acid-ethanol extract as described in the text and Fig. 1.
The effusion contained three chromatographically separable TGF activities. The earliest eluting activity (Fig. 1, Peak I) eluted just after the void volume and was able to stimulate colony growth of BHK without the need for EGF supplementation. TGF Peak I did not stimulate colony growth of NRK without EGF as actively and overall appeared to be less potent when this fibroblast was used as the indicator line. A second TGF activity (Fig. 1, Peak II) with an apparent molecular weight of about 25,000–27,000 was detected when colony growth was measured in the presence of EGF. In this instance NRK appeared to be a more sensitive indicator of TGF activity. No TGF activity was apparent at this elution volume when EGF was omitted from the colony growth assay. A third TGF activity (Fig. 1, Peak III) was present in the late eluting fraction. The estimated molecular weight of this activity is 5500–8000. The Peak III TGF activity, like that of Peak I, was demonstrable in the absence of EGF. In contrast to Peak I, Peak III transforming growth factor activity was detectable when either NRK or BHK fibroblasts were used as indicator cells.

The relative recoveries of the different TGF activities (Peaks I, II, and III) varied widely among those cytology positive malignant effusions which demonstrated TGF activity in the unfractionated extract. The Peak II TGF activity was consistently present in those malignant effusions containing TGF activities (Table 3). TGF Peak III was also consistently found in malignant effusions. However, the absolute amounts of these activities varied substantially and appeared to be independent of each other. As a result the relative contributions of Peaks II and III to total TGF activity assessed with the BHK indicator cell line were highly variable. In contrast to TGF Peaks II and III, Peak I was not always present in malignant effusions. Several effusions were found to contain TGF activity eluting in the middle and late migrating positions but lacking any of the Peak I activity. The colony growth promoting characteristics of the two peaks recovered from those effusions lacking Peak I were comparable to those of Peaks II and III found in effusions containing all three activities.

As noted above some cytology negative effusions from patients with malignant or benign disease also demonstrated TGF activity (Tables 1 and 3). Nonetheless the percentage of cytology negative effusions exhibiting TGF activity was significantly lower than that observed for cytology positive malignant effusions. Assessment of the TGF activities recovered following Bio-Gel P-60 chromatography of these effusions demonstrated that Peak III activity accounted for the majority of this activity. In addition lesser amounts of Peak II TGF activity were also found, particularly in those cytology negative effusions from patients with malignancy. However, most of these cytology negative or benign effusions lacked Peak I activity, while the remainder of these effusions contained very little of this activity.

The semipurified TGF activities, Peaks I, II and III, recovered from Bio-Gel P-60 were characterized more fully (Table 4). All three activities were inactivated by treatment with trypsin and heat (boiling). Treatment with a sulphydryl reducing agent, dithiothreitol also resulted in loss of activity indicating a requirement for disulfide bonds for activity.

Subsequently the ability of the material recovered from Bio-Gel P-60 to inhibit binding of radiolabeled EGF to A-431 cell derived membranes and TGFß and PDGF to BHK and NRK cells was assessed (Table 5). TGF Peak I did not appear to inhibit binding of any of the labeled growth factor factor ligands in radioreceptor assays with a sensitivity below 0.01 pM. In contrast, Peak II inhibited binding of TGFß to BHK but did not affect binding of PDGF and minimally inhibited EGF binding. Finally Peak III effectively inhibited binding of the labeled EGF to A-431 membranes but did not alter binding of PDGF or TGFß. It should be noted that this radioreceptor assay does not distinguish between EGF and TGFα insofar as both ligands bind to the EGF receptor. These data indicate that TGF Peak II in malignant effusions is similar to TGFß in its biological activities as assessed by pattern of colony growth stimulation, cellular binding properties, and molecular size. Collectively, these data suggest that the human effusion Peak II TGF activity is related to or identical to TGFß with calculated concentration of 1.4 pmol TGFß-like activity/10 µg protein. By the same criteria, TGF Peak III in malignant effusions appears

### Table 3 Representation of TGF activities in human effusions

<table>
<thead>
<tr>
<th>Type*</th>
<th>Mean % of total TGF (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I*</td>
</tr>
<tr>
<td>Malignant cytology (n = 31)</td>
<td>43 (0–61)</td>
</tr>
<tr>
<td>Malignant cytology (n = 8)</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benign (n = 7)</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

* Effusions with demonstrated TGF activity.
* Total TGF activity assessed by stimulation of colonies (>50 cells each) using BHK indicator line.
* Peaks of TGF activity recovered after Bio-Gel P-60 chromatography as described in the text and Fig. 1.

### Table 4 Effects of chemical and physical treatment on human malignant effusion TGF activities

<table>
<thead>
<tr>
<th>Treatment</th>
<th>I*</th>
<th>II*</th>
<th>III*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Trypsin</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5 mM dithiothreitol</td>
<td>18</td>
<td>15</td>
<td>61</td>
</tr>
<tr>
<td>65°C, 20 min</td>
<td>&lt;10</td>
<td>90</td>
<td>85</td>
</tr>
<tr>
<td>100°C, 3 min</td>
<td>&lt;10</td>
<td>45</td>
<td>15</td>
</tr>
</tbody>
</table>

* TGF activity determined by number of colonies (>50 cells/colony) using BHK indicator cell line as indicator line (I and II) and NRK cells in the presence of 4 ng/ml EGF (II); untreated control range, 450–620/10⁴ cells.
* TGF activities recovered from Bio-Gel P-60 as described in the text and Fig. 1.
### Table 5 Properties of human effusion TGF activities

<table>
<thead>
<tr>
<th>Peak</th>
<th>BHK</th>
<th>HCMC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-EGF</td>
<td>+EGF</td>
</tr>
<tr>
<td>I</td>
<td>1520 ± 210</td>
<td>1250 ± 390</td>
</tr>
<tr>
<td>II</td>
<td>&lt;50</td>
<td>190 ± 80</td>
</tr>
<tr>
<td>III</td>
<td>230 ± 105</td>
<td>210 ± 90</td>
</tr>
</tbody>
</table>

**Notes:**
- Peak recovered following Bio-Gel P-60 chromatography of human malignant effusion as detailed in "Materials and Methods" and Fig. 1.
- TGF activity expressed as number of colonies (>50 cells each for fibroblasts; >5 cells each for HCMC) ± SD/1.0 µg protein/10⁶ indicator in the absence or presence of 1 ng/ml EGF. Buffer controls subtracted (<40).
- Binding of radioiodinated EGF, TGFβ, and PDGF to A-431 cell membrane and BHK cells, respectively, performed as described previously (30-33). Expressed as percentage inhibition of binding in the presence of test peak (10.0 µg) relative to binding in the absence after subtraction of nonspecific binding. Labeled molar label persisting in the presence of 100-fold excess of unlabeled ligand. Sensitivities of assays equal 0.01 pmol for EGF and TGFβ and 0.05 pmol for PDGF. Nonspecific binding, 8-14% total binding.

### DISCUSSION

Transforming growth factors have been identified in a wide variety of tissues and biological fluids (5-12). While they were initially identified as products of tumors and transformed cells and their names connote ability to stimulate acquisition of some growth properties normally associated with malignant transformation by nontransformed cells, it is clear that such concepts are too narrow. The most well characterized factors, TGFα and TGFβ or closely homologous analogues, appear to be produced in nontransformed cells. Moreover these peptide growth factors appear to be part of a larger group of peptides including some hormones, which can effect alteration in the rate and pattern of normal cellular growth through interaction with specific cell surface receptors. It has also been recognized that these factors do not uniformly stimulate cellular growth. Instead the effects of these growth factors appear to be conditional and each exhibits a range of growth effects which depends upon the target cells, the presence or absence of additional growth factors, and perhaps other conditions. TGFβ for example stimulates proliferation and anchorage independent growth of fibroblast lines yet leads to growth inhibition and promotion of differentiation in many epithelial cell lines (10, 36).

The studies detailed in this report demonstrate several TGF activities in human malignant effusions. Separation and characterization of TGF activities indicate that a substantial proportion of this activity may be attributed to factors similar or identical to TGFα (EGF) and TGFβ (24-26). These observations are consistent with previous studies which have identified TGFα and TGFβ-like activities in solid tumors (23, 24). To a lesser extent, similar activities were also present in cytology negative effusions from patients with malignant and benign disorders. These findings too are consistent with previous demonstrations of TGF activities in other diverse biological fluids and tumors from patients with malignancy as well as normal controls (24, 25, 27). Indeed, in past studies TGF activities found in both malignant tissue and benign sources have appeared to be related or identical to TGFα or TGFβ or both. In some instances, these previously described factors have been...
MULTIPlicITY OF TGF IN HUMAN MALIGNANT EFFUSIONS

Fig. 3. SDS-PAGE of human MER-TGF. MER-TGF (2.0 ng) recovered from C18-Bondapak HPLC as described in text and Fig. 2 were electrophoresed in SDS gradient gel in reducing conditions using previously described methods (35), and protein was visualized through silver staining procedure. Lane a, molecular weight standard; Lane b, acid-ethanol extract of malignant effusion prior to Bio-Gel P-60; Lane c, MER-TGF from a single malignant effusion; Lane d, MER-TGF isolated from pooled malignant effusion (n = 4).

found to be larger than the prototype TGFs (24-26).

While TGFα- and TGFβ-like activities appear to be present in human malignant effusions and contribute to overall TGF activity in these fluids, an additional TGF activity was found to be present in many samples. This additional TGF activity is distinguishable from TGFα and TGFβ-like factors in both its spectrum of biological activity as well as its physical characteristics. It appears that this additional TGF, designated human malignant effusion related TGF (=MER-TGF), can stimulate a wide range of cells to grow in an anchorage independent manner including most notably at least one nontransformed colonie epithelial line (HCMC). In contrast to TGFβ, MER-TGF does not require EGF or TGFα to stimulate anchorage independent growth. Furthermore MER-TGF does not seem to compete for the same binding sites as EGF, TGFβ, or PDGF. While MER-TGF is a sulphydryl dependent peptide, it is somewhat larger than TGFβ or PDGF and substantially larger than EGF. However, size and subunit structure are not necessarily inherently distinguishing features, with the recognition that higher molecular weight forms of both TGFα and TGFβ have been found (24-26). Further structural characterization of human MER-TGF will permit more detailed comparison with previously described TGFs.

The presence of MER-TGF in association with the presence of malignant cells in effusions suggests that this peptide may be a tumor cell product. Insofar as such effusions are in a sense suspension cultures of malignant cells, it is possible that this factor contributes to the growth characteristics of these cells, and the activity of this substance in the soft agar colony assay could mirror its intrinsic biological activity. However, the mechanism of these effects as well as the range of its functional properties requires further study. Within the limits of these initial studies, MER-TGF appears to be consistent in its growth stimulating effects and without signs of the bifunctional potential which have been noted for TGFβ (22, 23). Further characterization of this tumor cell product, with development of appropriate structural or immunological probes will facilitate study of the functional properties of MER-TGF in tumor cell growth. Previously recognized TGFs have been found to have counterparts in normal cells; therefore, it will be important to define nontransformed sources of MER-TGF or more TGF-like proteins.

MER-TGF was initially found in effusions containing adenocarcinoma cells deriving from several types of primary tumors, including most notably colonic, breast, and ovarian cancers. We have not yet observed this activity in a limited number of samples from patients with non-epithelial cell derived malignancies (e.g., lymphoma, sarcomas). It will be of interest to determine whether MER-TGF production is particularly associated with epithelial derived tumor sources suggesting a role in regulation of epithelial cell growth. If MER-TGF does participate in control of epithelial cell growth or differentiation, it exerts these effects through receptors and mechanisms unrelated to previously described growth factors.

REFERENCES


Fig. 4. SDS-PAGE of human MER-TGF and recovery of biological activity. MER-TGF (2 μg) recovered from C18-Bondapak HPLC as described in text and Fig. 2 was electrophoresed in SDS-PAGE in nonreducing conditions. Lane A, silver staining; Lane B, TGF activity fractions (2.5 mm) were eluted in 1.0 ml PBS containing 0.5% SDS overnight, dialyzed against water, and lyophilized, and TGF activity was determined as described in text using BHK indicator line.


Multiplicity of Transforming Growth Factors in Human Malignant Effusions

Misuzu Kurokawa Seo, Kathy E. Lynch and Daniel K. Podolsky


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/7/1792

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.