Two Distinct Tumor Cell Growth-inhibiting Factors from a Human Rhabdomyosarcoma Cell Line

Charlotte M. Fryling,1 Kenneth K. Iwata,2 Patricia A. Johnson, Walter B. Knott, and George J. Todaro3

ABSTRACT

Tumor cell growth-inhibiting factors (TIFs) have been shown to inhibit the growth of tumor cell lines in culture. TIF-1, the first TIF to be described, is a low-molecular-weight, acid- and heat-stable polypeptide with no antiviral activity. A second class of TIFs (TIF-2) has now been isolated from the conditioned media of a human rhabdomyosarcoma cell line and partially purified by polyacrylamide gel filtration, cation exchange, and reverse-phase high-pressure liquid chromatography. Partially purified preparations of TIF-2 inhibit the growth of a variety of human tumor cells in soft agar and monolayer cultures and are mitogenic for normal human and mouse cells. TIF-2 has no antiviral activity. The growth-inhibitory effects of TIF-2 are reversible when the affected cells are no longer exposed to the factor. Although both TIF-1 and TIF-2 are obtained from the same source, they can be distinguished by their molecular weight, heat lability, elution pattern from reverse-phase high-pressure liquid chromatography, and their effect on the growth of mink lung epithelial cells. The growth of a human tumor cell variant, selected for resistance to growth inhibition by TIF-1, is inhibited by TIF-2. TIFs may therefore be a family of related polypeptides which selectively inhibit the growth of tumor cells.

INTRODUCTION

Previous studies have shown that the human rhabdomyosarcoma cell line A673 produces TGFs (17) and TIFs (12). TGFs (17) and sarcoma growth factors (3) confer the transformed phenotype on certain normal indicator cells and promote the anchorage-independent growth of these cells. TGFs also enhance the growth of tumor cells in soft agar (12). The TGFs produced by this cell line compete for binding to the EGF receptor and are classified as TGFα (15). TIF-1, which does not bind to the EGF receptor, is an acid- and heat-stable, low-molecular-weight polypeptide (M, 10,000 to 16,000) that is inactivated by trypsin (12). Preparations of TIF-1 inhibit the growth of human tumor cells in soft agar and monolayer culture and stimulate the growth of normal human fibroblasts and a normal human kidney epithelial cell line. It blocks the stimulatory effect of both TGFα and EGF in a soft agar assay with human tumor cells.

A second class of TIFs (TIF-2) has been isolated and partially purified from the same rhabdomyosarcoma cell line. TIF-2 shares many properties in common with TIF-1 but can be distinguished by its molecular weight, chromatographic properties, and its effect on certain target cells, thus indicating that it is a distinct TIF. The growth of A673 cells, in particular, and of tumor cells, in general, may be regulated by the TGFs and TIFs which these tumor cells themselves produce.

MATERIALS AND METHODS

Cell Culture. Cell cultures were maintained at 37°C in 75-sq-cm Falcon No. 3024 tissue culture flasks with complete growth medium composed of Dulbecco's modification of Eagle's medium with 10% FBS (Grand Island Biological Co., Grand Island, NY). The cell lines A673, a human rhabdomyosarcoma, A549, a human lung adenocarcinoma, and A375, a human melanoma line, have been described previously (8). A375 Ag5 is a soft agar clone of A375. A375 Ag5-IR is a TIF-1-resistant variant derived by continually treating subconfluent A375 Ag5 cells with TIF-1. HuF is a normal human foreskin fibroblast strain received from J. Levy (Cancer Research Institute, University of California at San Francisco). NIH/3T3 clone 7 was established in this laboratory.

Conditioned Media. A673 cells were grown to confluence on 850 sq-cm roller bottles (Corning 25140) in 50 ml of complete growth medium. The monolayers were rinsed twice with serum-free Dulbecco's modified Eagle's medium and incubated in 50 ml of serum-free Waymouth's medium for 8 h. The medium was discarded and replaced with 50 ml of fresh, serum-free Waymouth's medium, and the cells were incubated for 48 h. The conditioned media were then collected, clarified by centrifugation, concentrated, dialyzed, and rechromatographed. The supernatant fluid was then lyophilized. The procedure has been described in more detail elsewhere (12).

Bio-Gel P-100 Chromatography of Concentrated Conditioned Media. Concentrates from 227 liters of conditioned media were chromatographed on several Bio-Gel P-100 columns. The lyophilized samples were resuspended in 10 ml of 1 M acetic acid, centrifuged at 175,000 × g for 30 min, and applied to a column (5 × 82.5 cm) of Bio-Gel P-100 (100 to 200 mesh; Bio-Rad), equilibrated with 1 M acetic acid, and dialyzed overnight against 5 HIM ammonium acetate (pH 4.5) at 4°C, collecting 12.4-ml fractions. Protein was determined for every other fraction by absorbance at 280 nm. Aliquots of fractions were collected, lyophilized, and utilized as described below.

CM-cellulose Chromatography. Fractions from several Bio-Gel P-100 columns (total protein, 85 mg) containing TGF activity (M, 18,000 to 22,000) were combined, lyophilized, resuspended in 5 ml of 1 M acetic acid, and dialyzed overnight against 5 mM ammonium acetate (pH 4.5) at 4°C. The dialyzed material was centrifuged at 175,000 × g for 30 min at 22°C, and the supernatant was placed on a column (1.5 × 3 cm) of CM-cellulose (Whatman, CM-23). The column was eluted with a 400-ml linear salt gradient from 5 mM ammonium acetate (pH 4.5) to 500 mM ammonium acetate (pH 8.8) with a flow rate of 80 ml/h at 22°C. Fractions of 10.3 ml were collected, and conductance was determined using a conductivity meter (Radiometer, Copenhagen, Denmark). Fraction aliquots were sterilized by adding 0.5 ml of 1 M acetic acid and were lyophilized.

rp-HPLC. Lyophilized samples from the CM-cellulose purification step were reconstituted in 1 M acetic acid and dialyzed overnight against water. The dialyzed material was concentrated by lyophilization, and the concentrated samples were redissolved in 1 M acetic acid and dialyzed overnight at 4°C. The dialyzed samples were chromatographed on a Bio-Rad HPX-87H column using a flow rate of 0.5 ml/min and a temperature of 80°C. Fractions of 0.5 ml were collected, lyophilized, and utilized as described below.

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3 Present address: Oncogene Science, Inc., 3005 First Avenue, Seattle, WA 98121.
4 The abbreviations used are: TGFs, transforming growth factors; TGFα, type α TGFs; TGFβ, type β TGFs; TIFs, tumor cell growth-inhibiting factors; EGF, epidermal growth factor; rp-HPLC, reverse-phase high-pressure liquid chromatography; FBS, fetal bovine serum; TFA, trifluoroacetic acid; [125I]IodUrd, 5-[125I]iodo-2'-deoxyuridine; CM, carboxymethyl.
were resuspended in 1 ml of 0.05% TFA and injected on a Waters C_{18}-
\muBondapak column (0.39 x 30 cm). A Waters 720 System Controller
was used for the gradient system. The column eluate was monitored
with a variable-wavelength UV detector (Waters, Model 481) set at 214
nm. Elution was achieved using a 3-step (15 min, 80 min, 15 min) linear
gradient of acetonitrile (0 to 25%, 25 to 45%, and 45 to 100%, respecti-
vely) in 0.05% TFA at a flow rate of 1.0 ml/min.

A fraction containing TIF-2 activity from a rp-HPLC as described above
was lyophilized, resuspended in 1 ml of 0.05% TFA, and rechromato-
graphed by injection on a Waters C_{18}-\muBondapak column (0.39 x 30
cm). Elution was achieved using a 3-step (10 min, 80 min, 10 min) linear
gradient of 2-propanol (0 to 29%, 29 to 37%, 37 to 100%, respectively)
in 0.05% TFA at a flow rate of 1.0 ml/min.

Soft Agar Assay. Lyophilized TIF was resuspended in complete
medium containing 10\(^4\) A375 Ag5 cells and 0.34% agar (Difco Noble
Agar). This suspension was immediately pipetted over a base layer of
0.5% agar in complete medium in 35-mm Falcon 3001 tissue culture
dishes. Cells were incubated at 37°C in a 5% CO\(_2\)-95% air atmosphere,
and photomicrographs were made after 8 days.

TGF Assay. TGF activity was determined by the ability to compete in a
\[^{125}\text{I}\]EGF radioreceptor binding assay (17) and to stimulate the
growth of normal rat kidney cells (NRK 49F) in soft agar.

TIF Assay. Test cells were subcultured (100 \muL/well) in 96-well Nunc
167008 tissue culture plates at a density that allowed continuous growth
throughout the time period of the assay. Most cells were seeded at a
density of 3 x 10\(^4\) cells/well, while A549 required a seeding density of
4.5 x 10\(^4\) cells/well. Cells were treated with TIF for 96 h and with [\(^{3}H\)]
IdUrd (Amersham IM.355V) for the last 24 h as described previously (12).
Inhibition-stimulation of growth was expressed as the percentage of
radioactivity as described (12).

RESULTS

Conditioned media from the human rhabdomyosarcoma cell
line A673, partially purified by Bio-Gel P-100 chromatography,
had been shown to contain both TGFs (17) and TIFs (12).
Fractions with TGF activity (M, 18,000 to 22,000) from the P-100
column showed little TIF activity as determined by the TIF
assay (12), using the human lung adenocarcinoma cell line A549.
When the fractions containing TGF activity were subjected to rp-
HPLC, another peak of TIF activity eluted between approximately
38 and 42% acetonitrile, while the majority of the protein eluted at a lower
acetonitrile concentration. A single fraction [Fraction 24 (Chart
2)] which eluted between approximately 38 and 39% acetonitrile
and which contained TIF-2 activity was lyophilized and further
purified by rp-HPLC using a 3-step linear 2-propanol gradient
(Chart 3). TIF-2 eluted between approximately 29 and 31% and
again was purified away from the majority of the protein.

The HPLC-purified TIF-2 was not inactivated by trypsin. It was
stable at 56°C but, after heating to 100°C for 3 min, the level of
inhibition of cell growth dropped 30% in a TIF assay. No inter-
feron activity was detected when TIF-2 was tested in an antiviral
interferon assay.

Material from each purification step was tested in the TIF
assay using the human lung adenocarcinoma cell line (A549) and
normal human foreskin fibroblasts (HuF) as the test cells. Growth
inhibition of A549 cells and growth stimulation of HuF cells were
observed at each purification step, as can be seen in Table 1.

The growth-inhibitory effects of TIF-2 seem to be reversible,
TUMOR CELL INHIBITORY FACTORS FROM A HUMAN CELL LINE

Figure 1. Comparison of CM-cellulose and HPLC-purified TIF-2 from A673-conditioned media.

Table 1

Comparison of CM-cellulose and HPLC-purified TIF-2 from A673-conditioned media.

Table 2

Mitogen assay with TIF-2.

Table 3

Effect of TIF-2 on normal and transformed cell lines.

since the replacement of TIF-2 with fresh media leads to renewed growth of A549 cells. Since preparations of TIF-2 stimulated the growth of normal fibroblasts, it was also tested in a mitogen assay (see "Materials and Methods") with serum-starved, quiescent HuF cells and normal mouse NIH 3T3 clone 7 cells (Table 2). Levels of [3H]l[3H]dUrd incorporation were as good as or better than when cells were treated with 10% FBS.

Tumorigenicity in vivo and anchorage-independent growth in vitro appear to be closely related characteristics of tumor cells (1, 13). The human melanoma cell line A375 was treated with TIF-2 in a soft agar assay, and photomicrographs were made at 8 days. As shown in Fig. 1, control cells typically grow to colony sizes measuring an average of 0.5 to 1.0 mm in diameter. The colony sizes of cells treated with TIF-2 were significantly reduced (averaging 0.1 to 0.2 mm in diameter), yet there was no evidence of cytotoxicity. The small colony growth that was seen occurred within 1–3 days after treatment, and there was no further increase in colony size after 3 weeks. The human lung adenocarcinoma cell line, A549, was also inhibited in a soft agar assay. TIF-2 thus inhibits anchorage-independent growth as well as monolayer cell growth.

TIF-2 (partially purified) was also tested for its effect on other normal and tumor cells. Table 3 shows representative examples of the effects of TIF-2 preparations on various cell lines. Although high concentrations of TIF-2 were used, similar results have been seen with TIF-2-purified material. The growth of human fibroblasts and epithelial embryonic kidney cells was stimulated. In contrast, human tumor cell growth was inhibited. When high tissue culture passages of A549 and A673 cells were tested in the same assay (data not shown), inhibition of cell growth was greatly reduced, suggesting that the closer the tumor cell line is to the primary explant the more sensitive it is to inhibition of growth by TIF-2. The effect of TIF-2 on the growth of SV40-transformed fibroblasts was examined. Whereas the growth of normal WI-38 cells (CCL 75) was stimulated by TIF-2, the growth of WI-38 cells transformed by SV40 (CCL 75.1) was inhibited. The growth of SV40-transformed mouse cell line was also inhibited. Normal rat cell growth is stimulated by TIF-2.

The continuous mink lung cell line (CCL 64) is very responsive to inhibition of growth by TIF-1 (12). As shown in Table 4, inhibition of the growth of CCL 64 cells was dose dependent and detected at very low concentrations of TIF-1. TIF-2, however, had no significant effect on the growth of these cells. Low concentrations of both TIF-1 and TIF-2 inhibited the growth of

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Amount tested (µg/100 µl)</th>
<th>% of inhibition of A549</th>
<th>% of stimulation of HuF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.26</td>
<td>15</td>
<td>37 ± 15.4</td>
<td>38 ± 14.2</td>
</tr>
<tr>
<td>2.6</td>
<td>26</td>
<td>3301 ± 313</td>
<td>194</td>
</tr>
<tr>
<td>13.0 µg</td>
<td>6777 ± 667</td>
<td>399</td>
<td></td>
</tr>
<tr>
<td>FBS 10% (v/v)</td>
<td>3875 ± 877</td>
<td>228</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD derived from triplicate wells assayed in one experiment.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Amount tested (µg/100 µl)</th>
<th>[3H]dUrd incorporation (cpm)</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH clone 7</td>
<td>Control</td>
<td>1699 ± 176</td>
<td>100</td>
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</tr>
<tr>
<td>TIF-2</td>
<td>0.10 µg</td>
<td>2396 ± 487</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>TIF-2</td>
<td>0.52 µg</td>
<td>2814 ± 965</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>TIF-2</td>
<td>2.6 µg</td>
<td>3 a 301 ± 313</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>TIF-2</td>
<td>13.0 µg</td>
<td>6777 ± 667</td>
<td>399</td>
<td></td>
</tr>
<tr>
<td>FBS 10% (v/v)</td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>[3H]dUrd incorporation</th>
<th>% of inhibition</th>
<th>% of stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human</td>
<td>HuF foreskin fibroblast</td>
<td>72 ± 6.7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TID adult skin fibroblast</td>
<td>121 ± 18.8</td>
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<td></td>
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<tr>
<td>WI-38 embryonic lung fibroblast</td>
<td>11 ± 7.6</td>
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<tr>
<td>HEK p3 embryonic kidney</td>
<td>21 ± 10.9</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human tumor A549 p17 lung carcinoma</td>
<td>53 ± 1.9</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>A673 p10 rhabdomyosarcoma</td>
<td>46 ± 11.4</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF-7 breast cancer</td>
<td>71 ± 8.3</td>
<td>0</td>
<td></td>
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<tr>
<td>A2058 p13 melanoma</td>
<td>55 ± 0.5</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Nonhuman CCL 64 normal mink lung</td>
<td>5 ± 18.2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRK-49 normal rat kidney</td>
<td>52 ± 7.4</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV40 transfectants SV40-W1-38 human</td>
<td>24 ± 6.9</td>
<td>0</td>
<td></td>
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</tr>
<tr>
<td>SV3T3 clone 5 (mouse)</td>
<td>36 ± 5.2</td>
<td>0</td>
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<td></td>
</tr>
</tbody>
</table>

a Mean ± SD derived from triplicate determinations from a representative experiment. b The number following the letter "p" denotes the passage number of the cell in tissue culture.

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eventually settled on the surface and grew as a monolayer. After growth of the parent line is inhibited by TIF-1, TIF-2, and inter-
were tested in the TIF assay and, as shown in Table 5, the 
cells (averaging 0.1 to 0.2 mm in diameter). Both of these lines 
grew to colony sizes averaging 0.5 to 1.0 mm in diameter. In 
4-5 passages, they grew as rapidly as did the parent line, A375 
was further purified by CM-cellulose chromatography. Partially purified human 
TIF-2, and leukocyte Interferon on the human melanoma, clonal 
soft agar, with only a few small colonies composed of 3 to 5 
Springfield, VA. 
leukocyte Interferon (Lot P-321) was obtained from Meloy Laboratories, Inc., 
plate. When transferred to a new tissue culture flask, the cells 
cells became enlarged, rounded, and no longer attached to the 
monolayers were treated with TIF-1 for 10 days. The treated 
formed A375 Ag5-IR. To derive this variant, A375 Ag5 cells (1 x 
line A375 Ag5, and a TIF-1-resistant variant of this line, desig 
the growth of normal human cells in monolayer cultures, and are 
TGFs and 2 classes of TIF designated TIF-1 (12) and TIF-2. 
The human rhabdomyosarcoma cell line, A673, produces 
molecular weight of TIF-2 suggests that perhaps it is a precursor 
the proliferation of which is inhibited by TIF-1. Although the larger 
molecules have similar biological activities. Both of these molecules 
can stimulate or inhibit growth depending on the experimental 
transformed phenotype may be necessary for the growth-inhibitory activity of the TIFs. This is demonstrated by the growth inhibition of virally transformed cells the normal 
counterpart of which is stimulated to grow (SV40-transformed 
both TIFs have no detectable antiviral activity. TIF-2 can be 
distinguished from TIF-1 by its apparent molecular weight, tryp-
insensitivity, heat lability at 100°C, elution pattern from rp-
HPLC, and inability to inhibit the growth of certain target cells, 
the TIF assay (see "Materials and Methods"). 
Expressed as percentage of decrease of control [125I]dUrd incorporation as in 
Mean ± SD determined from triplicate wells from a representative experiment.

Table 4 
Comparison of tumor inhibitors on A375 Ag5 and TIF-1-resistant A375 Ag5-IR 
cells 
TIF-1 and TIF-2 were partially purified by Bio-Gel P-100 chromatography. TIF-2 
was further purified by CM-cellulose chromatography. Partially purified human 
leukocyte interferon (Lot P-321) was obtained from Meloy Laboratories, Inc., 
Springfield, VA. 

| Sample | Concentration tested (μg/100 μl) | % of inhibitiona 
|--------|---------------------------------|----------------- 
| TIF-1  | 0.0057 0 ± 3.62 23 ± 24.0 
|       | 0.0057 37 ± 6.0 45 ± 6.3 
|       | 0.057 59 ± 6.0 78 ± 15.8 
|       | 0.57 75 ± 3.6 78 ± 0.7 
| TIF-2  | 0.0032 8 ± 9.4 6 ± 10.3 
|       | 0.032 33 ± 17.0 14 ± 13.2 
|       | 0.32 50 ± 6.5 8 ± 17.3 
|       | 3.2 65 ± 5.9 5 ± 18.2 

a As in the TIF assay (see "Materials and Methods"). 
b Expressed as percentage of decrease of control [125I]dUrd incorporation as in 

Table 5 
Comparison of tumor inhibitors on A375 Ag5 and TIF-1-resistant A375 Ag5-IR 
cells 
TIF-1 and TIF-2 were partially purified by Bio-Gel P-100 chromatography. TIF-2 
was further purified by CM-cellulose chromatography. Partially purified human 
leukocyte interferon (Lot P-321) was obtained from Meloy Laboratories, Inc., 
Springfield, VA. 

| Sample | Amount testedb | A375 Ag5 | A375 Ag5-IR |
|--------|----------------|----------|------------- 
| TIF-1  | 1.73 μg 44 ± 8.72 2 ± 6.7 
| TIF-2  | 9.75 μg 34 ± 10.6 25 ± 6.8 
| Interferon | 100 IU 55 ± 1.5 54 ± 6.8 

Table cells | Concentration per 100 μl in TIF assay. 

A549 cells. 
A comparison was made of the effects of exposure to TIF-1, 
TIF-2, and leukocyte interferon on the human melanoma, clonal 
line A375 Ag5, and a TIF-1-resistant variant of this line, designated 
A375 Ag5-IR. To derive this variant, A375 Ag5 cells (1 x 
10⁶) were seeded on 24-well Nunc 169590 plates, and the 
monolayers were treated with TIF-1 for 10 days. The treated 
cells became enlarged, rounded, and no longer attached to the 
plate. When transferred to a new tissue culture flask, the cells 
eventually settled on the surface and grew as a monolayer. After 
4-5 passages, they grew as rapidly as did the parent line, A375 
Ag5. When both cell types were plated in soft agar, A375 Ag5 
grew to colony sizes averaging 0.5 to 1.0 mm in diameter. In 
contrast, the A375 Ag5-IR variant showed almost no growth in 
soft agar, with only a few small colonies composed of 3 to 5 
cells (averaging 0.1 to 0.2 mm in diameter). Both of these lines 
were tested in the TIF assay and, as shown in Table 5, the 
growth of the parent line is inhibited by TIF-1, TIF-2, and inter-
feron. The variant remained sensitive to growth inhibition by TIF-
2 and interferon, but growth was not inhibited by TIF-1.

DISCUSSION

The human rhabdomyosarcoma cell line, A673, produces 
TGFs and 2 classes of TIF designated TIF-1 (12) and TIF-2. 
Partially purified preparations of both TIFs inhibit the growth of 
human tumor cells in soft agar and monolayer cultures, stimulate 
the growth of normal human cells in monolayer cultures, and are 
mitogenic to normal human and mouse fibroblasts. Although high 
concentrations of partially purified TIFs were used in these 
experiments, the inhibitory and stimulatory activities of both TIF-
1 and TIF-2 coelute through several purification procedures. 
However, until each factor is purified to homogeneity, it will not 
be known if the stimulation of normal cells is due to the action 
of TIFs or to the presence of other, unidentified, but perhaps 
new growth factors. Tucker et al. (18) have recently shown that 
purified growth inhibitor from BSC-1 cells and TGFβ from plate-
lets have similar biological activities. Both of these molecules 
can stimulate or inhibit growth depending on the experimental 
conditions (18). The transformed phenotype may be necessary 
for the growth-inhibitory activity of the TIFs. This is demonstrated by 
the growth inhibition of virally transformed cells the normal 
counterpart of which is stimulated to grow (SV40-transformed 
WI-38 cells and normal WI-38 cells).

Both TIFs have no detectable antiviral activity. TIF-2 can be 
distinguished from TIF-1 by its apparent molecular weight, tryp-
scinsensitivity, heat lability at 100°C, elution pattern from rp-
HPLC, and inability to inhibit the growth of certain target cells, 
the TGFβ inhibitor characterized by Holley et al. (9–11, 18). In contrast, TIF-2 has little or no effect 
on the proliferation of this cell line. This difference in sensitivity 
may be explained by the presence of specific receptors on the 
cell surface with which TIF-1 interacts but which do not bind TIF-
2.

Holley et al. (11) has shown that the African green monkey 
kidney BSC-1 inhibitor prevents the stimulation of DNA synthesis 
by EGF in BSC-1 cells (11). Similarly, TIF-1 and TIF-2 can 
antagonize the effects of TGFα in soft agar and monolayer 
cultures. Other growth factors which are produced by trans-
fected cells [e., platelet-derived growth factor (4, 5, 19) and 
insulin-like growth factor (2, 7, 14)] may also interact with TIFs 
to control tumor cell proliferation. TIFs may thus be another 
important class of autocrine growth regulators (16) which are 
secreted by tumor cells.

As shown by Downward et al. (6) the close similarity between 
the EGF receptor and the v-erb-B oncogene product suggests 
other possible mechanisms for triggering proliferative responses. 
Since TIFs do not bind to the EGF receptor but can counteract 
the stimulatory effects of EGF and TGFα (12), perhaps they can 
also block the effects of activated growth factor receptors.

The effects of the TIFs on the variant melanoma cell line 
demonstrate that tumor cells can become nonresponsive to one 
TIF and yet remain sensitive to another. If tumor cells are capable 
of producing 2 or more classes of TIFs, it may be possible that 
some aggressive primary or metastatic tumors may be lacking 
certain TIFs and/or their receptors or may have acquired resist-
ance to the TIFs which they are producing. Determination of 
levels of TIF production and identification of tumor cell surface 
receptors may thus indicate which class of TIF could have 
therapeutic potential against particular tumors.

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REFERENCES


Fig. 1. Effect of TIF-2 on the growth of human melanoma cells (A375 Ag5) in soft agar. A, no treatment; B, TIF-2 (64 μg/ml) after CM-cellulose chromatography. x84.
Two Distinct Tumor Cell Growth-inhibiting Factors from a Human Rhabdomyosarcoma Cell Line
