Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3733–3737, June 1987
Cell Biology

The human transforming growth factor type α coding sequence is not a direct-acting oncogene when overexpressed in NIH 3T3 cells

(v-*erbB*/cell transformation/epidermal growth factor receptors/monoclonal antibody)

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Communicated by Peter Duesberg, February 5, 1987

ABSTRACT **A peptide secreted by some tumor cells *in vitro* imparts anchorage-independent growth to normal rat kidney (NRK) cells and has been termed transforming growth factor type α (TGF-α). To directly investigate the transforming properties of this factor, the human sequence coding for TGF-α was placed under the control of either a metallothionein promoter or a retroviral long terminal repeat. These constructs failed to induce morphological transformation upon transfection of NIH 3T3 cells, whereas viral oncogenes encoding a truncated form of its cognate receptor, the EGF receptor, or another growth factor, *sis*/platelet-derived growth factor 2, efficiently induced transformed foci. When NIH 3T3 clonal sublines were selected by transfection of TGF-α expression vectors in the presence of a dominant selectable marker, they were shown to secrete large amounts of TGF-α into the medium, to have downregulated EGF receptors, and to be inhibited in growth by TGF-α monoclonal antibody. These results indicated that secreted TGF-α interacts with its receptor at a cell surface location. Single cell-derived TGF-α-expressing sublines grew to high saturation density in culture. However, when plated as single cells on contact-inhibited monolayers of NIH 3T3 cells, they failed to form colonies, whereas v-*sis*- and v-*erbB*-transfected cells formed transformed colonies under the same conditions. Moreover, TGF-α-expressing sublines were not tumorigenic in nude mice. These and other results imply that TGF-α exerts a growth-promoting effect on the entire NIH 3T3 cell population after secretion into the medium but little, if any, effect on the individual cell synthesizing this factor. It is concluded that the normal coding sequence for TGF-α is not a direct-acting oncogene when overexpressed in NIH 3T3 cells.**

Transforming growth factor type α (TGF-α) is a 50-amino acid peptide in its fully processed form (1, 2). TGF-α is produced by rodent cells transformed by Moloney murine sarcoma virus or Kirsten murine sarcoma virus (3), by embryos in early fetal development (4, 5), and by many human tumor cells (6). This peptide has both structural and functional homology to the potent mitogen, epidermal growth factor (EGF) (1, 7–9) and binds to the same receptor as EGF (8). Reports that TGF-α confers anchorage-independent growth to NRK cells in culture (10) have led to the proposal that TGF-α causes malignant transformation *in vivo* of cells releasing this factor (11).

EGF receptor binding of TGF-α or EGF triggers a cascade of intracellular events, including stimulation of the tyrosine protein kinase activity intrinsic to this receptor (12). Stimulation of anchorage-independent growth of a specific target cell by addition of TGF-α can be blocked by an anti-EGF receptor antibody (8), further indicating that the EGF receptor plays an integral role in cell growth stimulation by TGF-α.

Analysis of the cDNA for TGF-α has revealed that the 50-amino acid TGF-α is encoded as an internal part of a 160-amino acid precursor (2, 13). Moreover, expression vectors for the complete TGF-α precursor protein have been developed, and constitutive expression of TGF-α has been reported to induce anchorage-independent growth and increase tumor formation by the rat-1 cell line in nude mice (14). In this report we have investigated the ability of TGF-α to transform the mouse NIH 3T3 cell line by expressing the human TGF-α-coding sequence at high levels in these cells. In addition, we compared the effects of TGF-α on NIH 3T3 cells to that of two viral oncogenes, in particular v-*erb*, which is a derivative of the cellular receptor for EGF, and v-*sis*, which is closely related to human platelet-derived growth factor 2 (PDGF-2). Our findings have implications concerning the mechanisms by which a growth factor synthesized by a cell possessing the cognate receptor may play a role in the neoplastic process.

MATERIALS AND METHODS

Plasmid Constructions. Plasmid pSVTGFα contains the entire coding region of human TGF-α cDNA (2) under the transcriptional control of the simian virus 40 early promoter. This vector is identical to pMTE4E (14) except that it lacks both the neomycin resistance and dihydrofolate reductase genes and contains an additional 185-base-pair (bp) pBR322-derived fragment that precedes the hepatitis B-derived fragment. A 595-bp fragment containing the coding region for TGF-α but lacking a potential polyadenylylation signal 43 bp 3′ to the stop codon was isolated from pSVTGFα by *Cla* I digestion and, subsequently, *Sau*3AI digestion. pZipTGFα was constructed by ligation of the *Bam*HI-linkered 595-bp fragment into the *Bam*HI site of the vector pZipNeoSV(X)1 (15). The 595-bp TGF-α fragment was also ligated into the *Bgl* II site of the parent vector p341-3 to generate pMTTGFα. p341-3 expresses cDNAs under the control of the mouse metallothionein-1 gene (P. Howley, personal communication).

Transfection and Isolation of Cell Lines. Transfections were carried out in 100-mm dishes by the calcium phosphate precipitation method (16, 17) using 40 μg of calf thymus DNA as carrier and the indicated amount of plasmid DNA. After 20 hr, the medium was changed to fresh serum-supplemented Dulbecco's modified Eagle's medium (DMEM). For selection of pZipTGFα transfectants, cells were exposed to 10 μg of DNA and selected in 750 μg of G418 (Geneticin, GIBCO)

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Abbreviations: LTR, long terminal repeat; DMEM, Dulbecco's modified Eagle's medium; TGF-α, transforming growth factor α; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; Mo-MuLV, Moloney murine leukemia virus.

per ml either as mass cultures or following cell transfer to isolate individual colonies. With pMTTGF plasmid DNA, TGF-α-expressing clones were selected in the presence of G418 after cotransfection of 10 μg of pMTTGF DNA with 0.2 μg of pSV2neo (18). The v-*sis* oncogene in simian sarcoma virus DNA, designated pSSV-11 (19), and a recombinant plasmid, pMuLV/*erbB*, containing the v-*erbB* gene under the transcriptional control of a Moloney murine leukemia virus (MuLV) long terminal repeat (LTR) (20) were also utilized. Cell lines were frozen soon after expansion, and all experiments were done with lines that had been in mass culture <2 weeks.

Growth in Serum-Free Medium. The complete protocol for growth in serum-free medium will be described elsewhere (W. G. Taylor, O. Segatto, and S. A. Aaronson, unpublished data). Serum-free medium consisted of a mixture of MCDB 401 medium (Irvine Scientific), Leibovitz's L-15 medium (GIBCO), and Ham's F-12 medium (GIBCO) containing 10 nM selenium (GIBCO) and 20 μg of transferrin per ml (GIBCO). Where indicated, 10 ng of EGF per ml (GIBCO) was added. Cell counts were performed in a Coulter counter.

Assay of EGF Receptors. Binding assays were performed as described (21) using [^{125}I]EGF (175–275 μCi/μg, Biomedical Technologies, Norwood, MA; 1 Ci = 37 GBq).

Assay of TGF-α. Samples were assayed by an EGF receptor competition kit (Biomedical Technologies). All determinations were confirmed by a sandwich ELISA for TGF-α using TGF-α monoclonal antibody as solid-phase antibody and polyclonal rabbit anti-TGF-α serum as detecting antibody (T. S. Bringman, P. B. Lindquist, and R. Derynck, unpublished data). Human and mouse EGF were not detectable in the ELISA assay. Recombinant human TGF-α and purified TGF-α monoclonal antibody TGF-α1 were prepared as described (2, 14).

RESULTS

Effects of TGF-α Expression Vectors on the Phenotype of NIH 3T3 Cells. To test the biological activity of human TGF-α, the coding region of the TGF-α cDNA was cloned into two different expression vectors and introduced into NIH 3T3 cells. Previous work has shown that retroviral LTRs provide efficient promoter/enhancer elements for gene transcription (22). Therefore, the TGF-α cDNA sequence encoding the human TGF-α precursor protein (2) was cloned into the *Bam*HI site of the Mo-MuLV-based vector pZip-NeoSV(X)1 (15), which contains the bacterial transposon Tn*5* neomycin resistance gene conferring G418 resistance in mammalian cells (18) (Fig. 1). In this vector (pZipTGFα), TGF-α should be translated from an mRNA transcribed under the control of the LTR. We also placed the TGF-α coding sequence under the control of the metallothionein promoter, which can be induced by heavy metals (pMT-TGFα, Fig. 1).

To assess the transforming potential of these human TGF-α expression vectors, we transfected the DNA of each onto NIH 3T3 cells. Neither induced detectable transformed foci with as much as 10 μg of DNA added per plate. Moreover, induction of the metallothionein promoter by inclusion of 80 μM $ZnCl_2$ in medium of cells transfected with pMTTGFα DNA did not result in the appearance of foci. In contrast, both the v-*sis* oncogene of simian sarcoma virus and the v-*erb* oncogene of avian erythroblastosis virus induced readily observable transformed foci at titers of more than 10^3 foci/μg of DNA under identical conditions (19, 20). A comparison of representative cultures transfected with pZipTGFα, v-*sis*/PDGF-2, and v-*erbB* oncogenes is shown in Fig. 2.

To ensure that TGF-α was expressed by our constructs, we cotransfected pMTTGFα with the pSV2neo plasmid that confers G418 resistance (18). Drug-resistant colonies were expanded and analyzed for their ability to secrete TGF-α into the culture medium (Table 1). Eighteen of 27 MTTGF cell lines secreted TGF-α at concentrations ranging from 1 to 20 ng/ml, as judged by an EGF receptor competition assay. Mass cultures of drug-resistant cell lines isolated from pZipTGFα-transfected cells expressed similar high levels of this protein (Table 1). These results were confirmed by an ELISA assay specific for TGF-α.

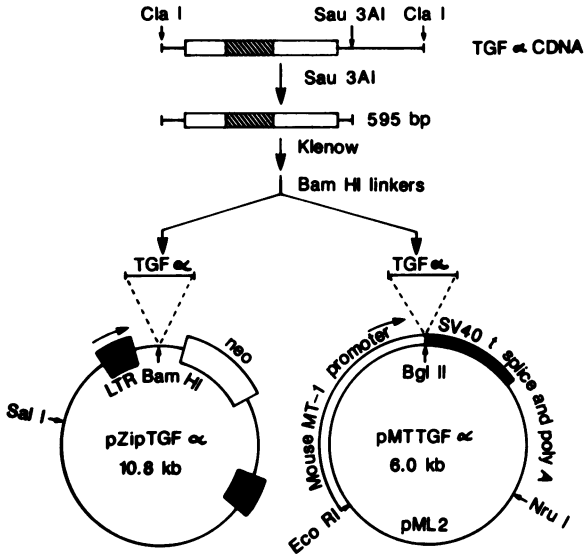

FIG. 1. Expression vectors for TGF-α. The rectangle contains the entire coding region for TGF-α. The dashed box within the rectangle represents the coding region for the mature 50-amino acid TGF-α. Arrows indicate the transcriptional orientation of the individual promoters.

The lines chosen for further study were passaged twice and rescreened for TGF-α production. As shown in Table 1, the levels of TGF-α secreted by $ZnCl_2$-treated MTTGF cell lines were 2- to 8-fold higher than observed with uninduced cultures. Moreover, the amounts of TGF-α released were at least 5- to 10-fold higher than secreted by viral-transformed or spontaneous tumor cell lines reported to be high producers of TGF-α (10, 23).

Although transfection of TGF-α recombinant plasmids onto NIH 3T3 cells did not produce any observable altered foci, each of the G418-resistant clonal lines expressing high levels of TGF-α exhibited a morphology (see Fig. 3*A Middle*)

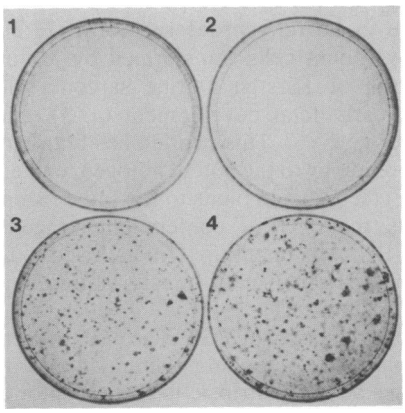

FIG. 2. Focus assay following transfection of NIH 3T3 by plasmid DNAs. NIH 3T3 cells were transfected as indicated with 0.4 μg of plasmid DNA and 40 μg of carrier calf thymus DNA by the calcium phosphate precipitation technique. Cells were fixed with formalin and stained with Harris hematoxylin 14 days post-transfection: 1, no plasmid; 2, pZipTGFα; 3, pSSV-11; 4, pMuLV/*erbB*.

Table 1. Properties of clonally derived TGF-α-expressing NIH 3T3 sublines

Cell line	Human TGF-α secreted,* ng/ml		Saturation density,† cells/cm² × 10⁵	[125I] EGF bound, fmol per 10⁶ cells‡
	−$ZnCl_2$	+$ZnCl_2$		
neo				
SV2neo-7-1	<0.2	<0.2	1.1	16
SV2neo-20-4	NT	NT	0.9	NT
SV2neo-21-2	NT	NT	1.4	NT
TGF-α				
MTTGF-15-2	2.1	16.5	4.4	<0.4
MTTGF-16-7	7.4	13.0	3.2	<0.4
MTTGF-16-14	1.4	4.7	4.5	<0.4
ZipTGF-11M	14.1	NT	4.2	NT
ZipTGF-12M	5.8	NT	3.8	NT
erbB				
ERB-18-2	NT	NT	4.7	NT
ERB-21-M	NT	NT	4.6	NT

*Confluent 10-cm plates were washed three times with DMEM and supernatants collected after a 24-hr incubation with 5 ml of DMEM with or without 30 μM $ZnCl_2$.

†The maximum cell number obtained when 5×10^3 cells per cm^2 were inoculated onto 20-cm^2 Petri dishes under conditions where the medium containing 10% calf serum was changed every 3 days. The saturation density was taken as that value where successive cell counts at 2-day intervals showed no increase in cell number.

‡[125I]EGF binding to confluent cultures was performed as described. All measurements were performed in triplicate, and results were subjected to Scatchard analysis. Nonspecific binding was <2% of total binding. NT, not tested. M, cell lines derived from mass cultures.

readily distinguishable from control NIH 3T3 lines (see Fig. 3*A Top*). These cells were refractile and grew in multiple layers without a regular growth pattern, whereas the control cells grew as a flat contact-inhibited monolayer. Moreover, the saturation density achieved in 10% calf serum by each of the TGF-α-expressing lines was at least 2- to 4-fold higher than that of control cell lines and comparable to that of a v-*erbB* transformant (Table 1).

Growth Alteration in TGF-α-Expressing Sublines Is Mediated by TGF-α Interaction with Cell Surface EGF Receptors. To determine the site of interaction of TGF-α with its receptor under conditions in which both were synthesized by the same cell, we initially examined the level of EGF receptors in TGF-α-producing NIH 3T3 sublines by measuring binding of [125I]EGF. As shown in Table 1, confluent cultures of each of the lines tested lacked detectable EGF receptors, whereas a representative pSV2neo NIH 3T3 transfectant similarly selected for G418 resistance bound 16 fmol of EGF per 10^6 cells.

Recent studies have indicated that a monoclonal antibody to TGF-α inhibits the anchorage-independent growth of rat-1 cells expressing TGF-α (14). To test whether such antibody could inhibit growth of TGF-α-transfected cells, we exposed a confluent culture of the TGF-α-expressing NIH 3T3 subline MTTGF-15-2 to the TGF-α monoclonal antibody. As shown in Table 2, the TGF-α antibody inhibited [3H]thymidine incorporation by 34% but did not diminish DNA synthesis by control NIH 3T3 cells. These findings implied that the antibody could specifically inhibit the growth stimulatory actions of TGF-α at a cell surface location. In contrast, EGF, which had no stimulatory effect on MTTGF-15-2 cells, increased DNA synthesis of the control cells 2-fold (Table 2). These results confirmed that EGF receptors associated with such TGF-α-expressing cells were fully occupied or downregulated. The reversibility of this downregulation was demonstrated by the ability of MTTGF-15-2 cells to respond to

Table 2. Effect of TGF-α monoclonal antibody on [3H]thymidine incorporation into control (SV2neo-7-1) and MTTGF subline

Additions to medium	SV2neo-7-1, cpm	MTTGF-15-12, cpm
None	14,214 ± 3,287	19,691 ± 1,515
TGF-α antibody	17,105 ± 3,450	12,954 ± 293
EGF	28,138 ± 2,227	17,975 ± 2,264
TGF-α antibody followed by EGF	26,060 ± 4,386	33,391 ± 2,963

Trypsinized cells (10^5) of the indicated cell line were plated into 24-well plates. After overnight incubation in DMEM containing 10% calf serum, the cells were washed two times with phosphate-buffered saline and then incubated with 1 ml of DMEM supplemented with 5 μg of transferrin per ml, 1 μM selenium, and, where indicated, 40 μg of Protein A-Sepharose purified TGF-α antibody. Two days later the medium was changed, and either 10 ng of EGF or 40 μg of TGF-α antibody was added or both EGF and TGF-α antibody were added. The cells were then processed as described (24).

EGF with a 2.6-fold stimulation in [3H]thymidine incorporation following TGF-α antibody exposure for 48 hr. These results suggest that prolonged antibody exposure bound sufficient TGF-α to unmask functional EGF receptors. Thus, it appeared that TGF-α works through cell surface EGF receptors for at least some of its growth-promoting activity.

Mechanism Accounting for the Overgrowth of TGF-α-Expressing NIH 3T3 Cells. There was an apparent paradox between the lack of focus formation induced by the TGF-α expression vectors upon primary transfection of NIH 3T3 and the densely growing nature of clonal lines selected following transfection as TGF-α-expressing cells. It was possible that introduction of the TGF-α coding sequence required more cell generations for expression of a genetically determined transformed phenotype than was required for the viral oncogenes v-*sis* or v-*erbB*. Alternatively, the growth-promoting effects of TGF-α might not be exerted specifically on the cell synthesizing this growth factor but instead act on the entire cell population after secretion into the medium. By the former hypothesis, stably TGF-α-transformed cells might be expected to form densely growing colonies when plated as single cells on monolayers of contact-inhibited NIH 3T3, an assay for transformed cells (25). If the latter were the case, the number of TGF-α-secreting cells plated might not secrete a sufficient quantity of TGF-α into the medium to cause the culture to grow in a transformed manner.

As shown in Fig. 3*B*, v-*sis* and v-*erbB* transformants efficiently formed readily detectable colonies when plated on NIH 3T3 monolayers. In striking contrast, as many as 10^4 cells of a representative TGF-α-producing subline, MTTGF-15-2, failed to yield visible colonies under these same conditions (Fig. 3*B*). This lack of growth did not represent random clonal variation because none of eight TGF-α sublines analyzed yielded detectable colonies in the monolayer assay (data not shown). These results strongly implied that the overgrowth potential of the TGF-α-expressing sublines was not an intrinsic genetic property.

To determine whether added TGF-α could induce the growth alterations observed in dense cultures of clonal TGF-α-expressing NIH 3T3 cells, we added TGF-α to control NIH 3T3 cells. At a TGF-α concentration of 5 ng/ml, comparable to that released by dense TGF-α sublines (Table 1), the cells grew to 2- to 4-fold increased saturation density and exhibited morphologic alterations (Fig. 3*A Bottom*) indistinguishable from those of clonally derived TGF-α-producing NIH 3T3 sublines.

As independent confirmation that TGF-α did not specifically stimulate growth of the individual cell synthesizing this factor, we utilized a chemically defined medium in which NIH 3T3 cells display a strict EGF requirement for growth

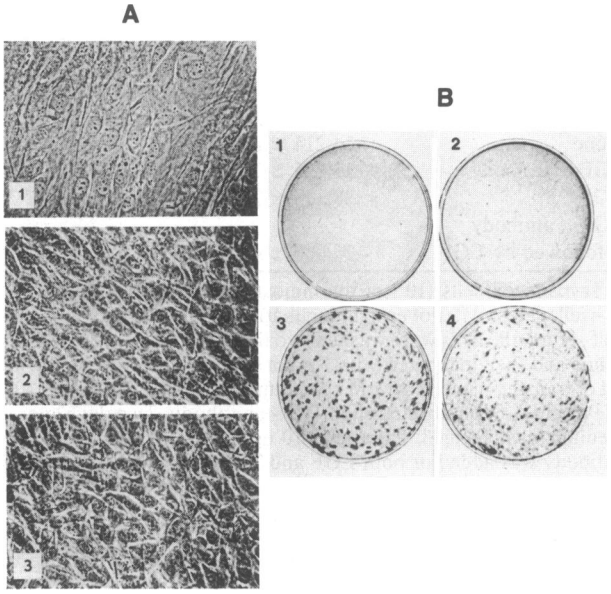

FIG. 3. Effect of TGF-α on cell morphology. (*A*) Phase-contrast photomicrographs of cell line SV2neo-7-1 (*Top*), cell line MTTGF-15-2 (*Middle*), and cell line SV2neo-7-1 (*Bottom*) grown for 6 days in medium containing 5 ng of recombinant human TGF-α per ml. (×200.) (*B*) Growth of isolated cell lines on monolayers of contact-inhibited NIH 3T3 cells. NIH 3T3 cells were grown to confluence in 60-mm plates in 5% calf serum. Monodispersed cells of each cell line were plated on the monolayer. Media containing 5% calf serum were changed the next day and subsequently every third day. Plates were stained with Giemsa 16 days later: 1, SV2neo-7-1, 10^3 cells; 2, MTTGF-15-2, 10^4 cells; 3, *sis*-1, 10^3 cells; 4, *erb*-18-2, 10^3 cells.

(O.S., W. Taylor, and S.A.A., unpublished data). As shown in Fig. 4, when a TGF-α-producing subline, MTTGF-15-2, was plated at relatively high density of 1.9×10^3 cells/cm^2, it grew well in the absence of EGF supplement. Under the same conditions, control NIH 3T3 cells failed to grow at 1.9×10^3 cells/cm^2 in the absence of EGF. However, when the plating density of the TGF-α subline was lowered 8-fold, there was no detectable growth of the cells in the absence of EGF addition to the medium (Fig. 4*B*).

To directly assess the effect of TGF-α expression on the *in vivo* growth properties of the cells, transfectants containing pZipTGFα or pMTTGFα, as well as v-*sis* or v-*erbB*, were inoculated subcutaneously in nude mice. As shown in Table 3, v-*sis* and v-*erb* transfectants rapidly formed tumors with as few as 10^4 cells inoculated. In contrast, several TGF-α-expressing NIH 3T3 lines, which secreted large quantities of TGF-α (Table 1), failed to induce tumors even when as many as 10^6 cells were inoculated. All of the above results strongly argue that TGF-α exerts little or no direct effect on the growth properties of the individual cell from which it is secreted.

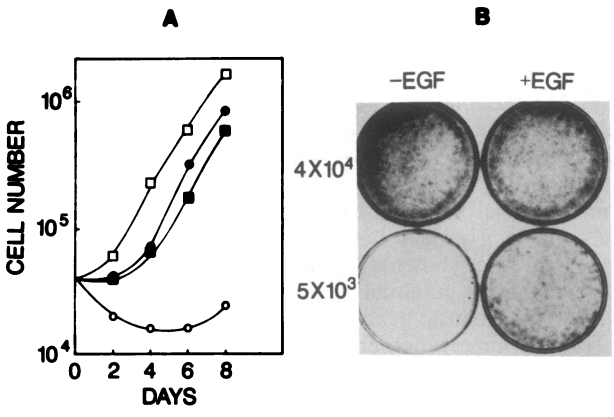

FIG. 4. Growth of cell lines in serum-free medium. (*A*) Cells were plated (4×10^4 cells) in 60-mm dishes in serum-free medium with or without 10 ng of EGF per ml. The cells were counted every 2 days. Each point represents the average of duplicates; variation between replicates was <10%. ●, MTTGF-15-2; □, MTTGF-15-2 + EGF; ■, SV2neo-7-1 + EGF; and ○, SV2neo-7-1. (*B*) Cells from the MTTGF-15-2 subline were plated in 60-mm dishes in serum-free medium at either 4×10^4 or 5×10^3 cells per plate. Where shown, 10 ng of EGF per ml was included in the medium. The plates were stained with Giemsa 12 days later.

DISCUSSION

Genes involved in pathways that lead to normal cell proliferation in response to growth factors appear to be frequent targets of genetic alterations associated with malignancy. As yet, the v-*sis*/PDGF-2 oncogene (24) is the only growth factor-encoding sequence that has been isolated from an acute transforming retrovirus (26). However, there is accumulating evidence that expression of coding sequences for other growth factors can convert cell lines to malignancy (27). Thus, growth factors expressed by cells possessing cognate receptors appear capable of playing important roles in the neoplastic process.

A growth factor, designated TGF-α, was initially detected in tissue culture fluids of a variety of virally and spontaneously transformed cells. The secretion of TGF-α by transformed but apparently not by normal cells (3), as well as the ability of this growth factor to induce anchorage-independent growth of NRK cells (10), led to the proposal that TGF-α causes malignant transformation *in vivo* of cells releasing this factor (11). The molecular cloning of TGF-α cDNA (2) provided the opportunity to directly test the biologic effects of expression of this growth factor by cells possessing its receptor.

In primary DNA transfection assays, TGF-α expression vectors failed to induce morphological transformation of NIH 3T3 cells, whereas v-*sis* and v-*erbB* oncogenes were efficient at transforming the same cells. We showed that TGF-α secretion by cultures selected using markers linked to the TGF-α coding sequence was several-fold higher than levels of TGF-α release reported for any viral or spontaneous tumor cells. Moreover, the lack of focus-forming activity of the TGF-α vectors was not due to the inability of NIH 3T3 cells to respond to TGF-α. Control NIH 3T3 cells possessed EGF receptors, demonstrated overgrowth in serum-containing medium supplemented with TGF-α, and grew in chemically

Table 3. *In vivo* growth properties of TGF-α-expressing NIH 3T3 lines

Cell line	No. of tumors/no. of animals*		
	10^6 cells	10^5 cells	10^4 cells
neo			
SV2 neo-7-1	0/4	0/4	0/4
SV2 neo-9M	0/4	0/4	0/4
TGF-α			
MTTGF-16-7	0/4	0/4	0/4
MTTGF-15-2	0/4	0/4	0/4
ZipTGF-11M	0/4	0/4	0/4
ZipTGF-12M	0/4	0/4	0/4
erbB			
ERB-21M	4/4	4/4	1/4
sis			
sis-13M	4/4	3/4	1/4

*NIH Swiss nude mice were inoculated subcutaneously with the indicated number of cells from individual cell lines. The animals were observed twice weekly for 6 weeks for the formation of tumors >2 mm in diameter.

defined medium in which EGF or TGF-α was the primary growth factor supplement.

By enriching the transfected cell population for only those cells containing the TGF-α expression vector, we were able to demonstrate a biological effect of TGF-α secretion. Such cultures grew to much higher saturation density than control NIH 3T3 cells. Our findings that monoclonal antibody directed against TGF-α inhibited the growth of such cells implies that the TGF-α synthesized must interact with its receptor at an antibody-accessible site at the cell surface. Moreover, we showed that TGF-α works through the EGF receptor: (*i*) densely growing TGF-α-expressing NIH 3T3 lines lacked detectable EGF receptors; (*ii*) TGF-α-expressing cell lines were not mitogenically stimulated by EGF; and (*iii*) prolonged TGF-α antibody exposure unmasked EGF receptors capable of responding to EGF by stimulated growth. These findings indicate that TGF-α secretion can provide a growth stimulus to cells possessing EGF receptors and that this interaction occurs at the cell surface.

The growth-stimulating effect of TGF-α secretion even on NIH 3T3 cell populations selected to contain the TGF-α vector was limited in that such cells failed to exhibit properties of malignant cells *in vitro* or *in vivo*. The cells did not form colonies on contact-inhibited monolayers, nor did they form tumors in nude mice. By contrast, v-*sis* and v-*erbB* transformants formed single-cell-derived foci when plated on NIH 3T3 monolayers and were tumorigenic under the same assay conditions.

Rosenthal *et al.* (14) reported that 10^6 rat-1 cells transfected with a TGF-α expression vector formed tumors in 9/9 animals, whereas 3/9 animals developed tumors following inoculation of the same number of untransfected control rat-1 cells. The growth of tumors in each case was similar and nonprogressive, in contrast to the rapid growth of tumors observed with *ras*-transfected rat-1 cells (14). In our present studies, v-*erbB*- and v-*sis*-transformed NIH 3T3 cells were at least 100-fold more tumorigenic, and *ras* transformants were at least 10^3-fold more malignant than NIH 3T3 cells containing TGF-α expression vectors and secreting large amounts of this growth factor. Thus, our results as well as those of Rosenthal *et al.* (14) indicate that unlike potent oncogenes including *sis*, which encodes a different growth factor, the TGF-α coding sequence exerts little if any effect on the malignancy of either NIH 3T3 or rat-1 cells. The small increase in tumorigenicity of already malignant control rat-1 cells may simply reflect an indirect enhancing role of TGF-α secretion on growth of already neoplastic cells through a feeding effect or by its recently reported angiogenesis-inducing activity (28).

In contrast to TGF-α, the coding sequence for one subunit of another growth factor, human *sis*/PDGF-2, has transforming activity if expressed in NIH 3T3 and other cells that contain PDGF receptors (19). Unlike the case with TGF-α, the *sis*/PDGF-2 gene product is not actively secreted but remains tightly cell associated (29). Whether *sis*/PDGF-2 interacts with its receptor within the cell or at a surface location, it is capable of triggering the autonomous growth of a single cell in which it is synthesized (19). Our present studies demonstrate that growth stimulation by secreted TGF-α must be mediated indirectly by its "feeding effect" on the entire culture with little, if any, direct action on the cell in which it is synthesized. Thus, the mode of growth factor synthesis and secretion may play critical roles in determining its actions. If, for example, the growth factor were processed through the cell as an inactive precursor or in a different cellular compartment from its receptor and/or were rapidly secreted and diffused, it may exert little or no direct effect on growth of that cell. Quantitative considerations such as the amount of growth factor synthesized and number of receptors may have important bearing as well. It will be of interest to determine whether alterations that lead to aberrant expression and/or processing of TGF-α, other than the conditions tested here, can cause this growth factor to acquire potent transforming properties.

We thank Paolo Di Fiore and Jacalyn H. Pierce for helpful discussions and their gift of v-*erbB*-transfected cell lines. We also thank Paul Arnstein for testing the tumorigenicity of cell lines, and M. Winkler for providing purified TGF-α. O.S. was supported by a fellowship from the Associazione Italiana per la Ricerca sul Cancro.

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