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Synthesis of Messenger RNAs for Transforming Growth Factors α and β and the Epidermal Growth Factor Receptor by Human Tumors

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ABSTRACT

Human tumors and tumor cell lines were analyzed for the presence of mRNA coding for transforming growth factors α and β (TGF-α and -β) and the epidermal growth factor receptor. TGF-α mRNA was not detectable in hematopoietic tumor cell lines but was found in a variety of solid tumor cells, particularly carcinomas. Many of the tumors that contained TGF-α mRNA also expressed high levels of epidermal growth factor receptor mRNA. The concentration of TGF-α in the media of several tumor cell lines did not necessarily correlate with TGF-α mRNA levels, as a substantial fraction of TGF-α can remain cell associated. The levels of TGF-β mRNA in tumor cell lines and tumor specimens were variable, but higher in tumors than in the adjacent normal tissues.

INTRODUCTION

TGF-α[3] is a polypeptide which is structurally related to epidermal growth factor (1, 2) and competes with it for binding to the same receptor (3, 4). The 50-amino acid-long human TGF-α is derived from a 160-amino acid precursor (2). TGF-α (or EGF) can induce the reversible phenotypic transformation of normal rat kidney cells in the presence of structurally unrelated growth factor TGF-β (5, 6). Several cell lines transformed by retroviruses as well as some chemically and "spontaneously" transformed cell lines have been shown to release TGF-α in the surrounding culture medium (7). There is substantial evidence for the synthesis of TGF-α during early fetal development (8, 9), yet no normal adult cells are known to secrete TGF-α. This suggests that TGF-α may function as an embryonic version of EGF. The production of TGF-α by various tumor cells suggests that it may play an important role in cellular transformation when expressed abnormally. It has been proposed that TGF-α or other growth factors may play a role in tumor formation via an autocrine mechanism whereby TGFs help sustain the transformed character of the same cell population from which they are secreted (10, 11). The binding of either TGF-α or EGF to receptors on the surface of the target cells initiates a sequence of cellular responses culminating in cell division. The EGF/TGF-α receptor has been shown to be related to members of the *src* family on oncogenic tyrosine kinases and could represent the product of the oncogene c-*erb*B (12, 13).

TGF-β is a dimeric protein consisting of two identical 112-amino acid polypeptides (14). The monomer of human TGF-β is initially synthesized as the C-terminal segment of a 390-amino acid precursor (15, 16). TGF-β stimulates proliferation of some cells (17) but inhibits the growth of others (18, 19). Its effect on the growth of a particular cell type seems to depend on many variables such as cell type, physiological state of the cell, and presence of other growth factors. TGF-β is synthesized by many normal and transformed cell lines (5, 15). However, it has been demonstrated that retroviral transformation can result in greatly elevated levels of TGF-β mRNA and TGF-β synthesis (16, 20).

We report here the results from an extensive screening of many human tumor cell lines and tumors as well as several normal tissues for the presence of TGF-α and -β mRNA. The amounts of TGF-α associated with the cells and secreted in the medium were also measured for several of the tumor cell lines using a TGF-α specific immunoassay. Since the action of TGF-α is mediated by the EGF receptor (3, 4, 21), we also assessed the level of human EGFR transcripts in all these RNA samples.

MATERIALS AND METHODS

Cells, Tissues, and RNA Extraction. All cell lines tested (Table 1) were grown in Dulbecco's minimal essential medium, supplemented with 10% fetal bovine serum. Cells grown in suspension were harvested in late logarithmic growth and cells grown in monolayer were harvested at early confluency. RNA was prepared by lysing the cells in a hypotonic buffer containing NP-40 followed by removal of the nuclei as described (22). Tumor tissues were surgically removed and immediately frozen at −80°C. Total RNA from tissues was prepared as described (23). The polyadenylated mRNA fraction was isolated by oligo dT-cellulose chromatography (24).

Northern Hybridization. Polyadenylated RNA (4 μg) was electrophoresed into a formaldehyde/1.2% agarose gel (25) and blotted onto nitrocellulose (26). The nitrocellulose filters were hybridized with ^{32}P-labeled (27) cDNA probes in 50% formamide, 5 × standard saline citrate, 0.1% sodium dodecyl sulfate, 0.1% sodium pyrophosphate, 50 mM sodium phosphate (pH 6.8), 2 × Denhardt's solution, 10% dextran sulfate at 42°C for 15–20 h. Extensive washings were done in 0.2 × standard saline citrate, 0.1% sodium dodecyl sulfate at 50°C. The levels of a specific mRNA were scored by eye on a relative basis from $^{+}$ to $^{++++}$ depending upon the intensity of the autoradiographic signal. The scoring was standardized by including samples of HT1080 and PC3 mRNA (Table 1) in all gels.

The TGF-α probe consists of the cDNA insert contained in pTGF-C1 (2) and comprises the complete coding sequence. The 1050 base pair TGF-β cDNA insert of plasmid pβC$_1$ (15) was used to probe for TGF-β mRNA. The 1.1 kilobase pair *Eco*RI fragment from plasmid pHER-A64-2, which corresponds to the distal segment of the coding region and part of the 3′ untranslated sequence (13), was used in Northern hybridization for the EGFR mRNA.

Measurement of TGF-α in Cell Lysates and Medium. Cells grown to confluence in 175 cm^2 flasks were cultured for 48 h in 50 ml Dulbecco's minimal essential medium with or without 10% fetal bovine serum. The medium was collected, clarified by centrifugation, dialyzed using Spectrapor 6 (2000 molecular weight cut-off) dialysis tubing against 100 volumes of 0.1 M acetic acid and lyophilized. The cells were washed twice with 50 ml phosphate buffered saline and scraped from the flask in 8 ml acid/ethanol solution [93% ethanol, 0.23 M HCl, 0.5 mM phenylmethylsulfonyl fluoride, pepstatin (5 μg/ml)]. 4 ml of water was

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[3] The abbreviations used are: TGF, transforming growth factor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; mRNA, messenger RNA; ELISA, enzyme-linked immunosorbent assay.

Table 1 *TGF-α, TGF-β, and EGFR mRNA in cell lines*

Summary of Northern hybridization results for the different human cell lines. The levels of mRNA for TGF-α, TGF-β, or EGF receptor were scored on a relative basis from + to ++++ as explained in the "Materials and Methods" section.

		TGF-α	EGFR	TGF-β
CH2	T-cell hybridoma	–[a]	ND[b]	+
CEM	T-lymphoblast	–	ND	+
Molt-4	T-lymphoblast	–	ND	+++
SUBC	T-lymphoblast	–	ND	+++
Daudi	Burkitt's lymphoma	–	ND	++
EB-3	Burkitt's lymphoma	–	ND	+
Raji	Burkitt's lymphoma	–	ND	+++
Jiyoye	Burkitt's lymphoma	–	ND	++
HL60	Promyelocytic leukemia	–	ND	+++
U937	Histiocytic lymphoma	–	ND	+++
no name	Myeloma	–	ND	+++
A431	Epidermoid carcinoma	++	[c]	++
HeLa	Cervical epitheloid carcinoma	+++	ND	+
SW13	Adenocarcinoma adrenal cortex	–	ND	+++
MCF-7	Mammary carcinoma	+	ND	+
MDA-MB436	Mammary carcinoma	++	++++	++
HBL-100	Breast epithelial (phenotypically transformed)	+	++++	+++
1072F	Renal carcinoma	+++	ND	++
7860-KEC39	Renal carcinoma	++++	++	+++
7860	Renal carcinoma	++++	+++	++++
7860-ECI	Renal carcinoma	+++	++	++++
769P	Renal carcinoma	+++	++	++
CAK-1	Renal carcinoma	+	++	++
A172	Glioblastoma	–	+++	+++
A2058	Melanoma	+	ND	ND
TuWi	Wilms tumor	–	ND	+
Detroit 562	Pharyngeal carcinoma	–	ND	+
T24	Bladder carcinoma	–	ND	+
253J-168	Transitional cell carcinoma (bladder)	++++	ND	++
KB	Oral epidermoid carcinoma	+++	ND	++++
HepG2	Hepatoma	++	ND	++
A549	Lung carcinoma	+	ND	ND
PC3	Prostate carcinoma	++	++++	++++
HT1080	Fibrosarcoma	++++	+	++++
HOS-2	Osteosarcoma	+++	++++	++++
A204	Rhabdomyosarcoma	–	ND	+
RD	Embryonal rhabdomyosarcoma	–	ND	++
IMR-90	Normal lung diploid fibroblast	–	+	+
FS-4	Normal foreskin diploid fibroblast	–	++	++

[a] –, absence of detectable mRNA.
[b] ND, not determined.
[c] 10–50-fold overexpression of EGR receptor mRNA.

added to the cell extract and the flask was rinsed with 5 ml of 73% ethanol, 0.18 M HCl, 0.5 mM phenylmethylsulfonyl fluoride solution. The combined cell extracts were homogenized with a polytron, incubated at 4°C for 16 h, clarified by centrifugation, dialyzed against 100 volumes of 0.1 M acetic acid, and lyophilized. Prior to assay, the extracts from the medium or the cells were dissolved in 0.15 M NaCl/0.1 M acetic acid, cleared by centrifugation, and adjusted to pH 7.0–7.8 with 1 M Tris. All samples were assayed in a sensitive sandwich ELISA for TGF-α using monoclonal anti-TGF-α as solid-phase antibodies and polyclonal rabbit anti-TGF-α as detecting antibodies.[4] No cross-reactivity with EGF was detectable in this assay.

RESULTS

TGF-α mRNA. We initially examined a variety of human cell lines derived from different tumor types for the presence of TGF-α mRNA. No TGF-α mRNA was detectable in any cell line of hematopoietic origin. However, TGF-α mRNA of about 4.5–4.8 kilobases was clearly present in various other tumor cell lines of carcinoma or sarcoma origin (Table 1; Fig. 1). Examination of all the hybridization data indicates that TGF-α mRNA is most likely to occur in carcinoma cell lines, although some sarcoma cell lines such as HT1080 also contain relatively high levels of this mRNA. TGF-α mRNA was not detectable in the two normal fibroblast lines examined.

The study of these cell lines reveals preferential expression of TGF-α mRNA in some tumor types. In order to determine the biological and clinical relevance of this finding, we examined solid tumors which had been surgically removed from patients. RNAs from many different types of carcinomas and sarcomas were screened by Northern hybridization (Table 2, Fig. 1). TGF-α mRNA was detected in many of the samples and its size was identical in all cases. Due to the limitations of the detection method, very low levels of TGF-α mRNA may have remained undetected. Furthermore, the concentrations of TGF-α mRNA may be diluted due to the presence of nontumor tissue in the tumor specimens. TGF-α mRNA could not be detected in the normal tissues examined (Table 2). The data obtained with cell lines and tissues lead to the conclusion that TGF-α is made by a variety of solid tumors, but may be absent in hematopoietic tumors. TGF-α mRNA levels were preferentially high in renal carcinomas and in squamous carcinomas independent of their localization. In addition, TGF-α mRNA can also be unambiguously detected in many tumors of neuronal origin and in many mammary carcinomas.

TGF-α Polypeptide Levels. Since a variety of cell lines contained clearly detectable levels of TGF-α transcripts, we wanted to determine if these cells synthesized TGF-α. Four cell lines with relatively high TGF-α mRNA levels were grown to confluency and then cultured for 48 h in the presence or absence of serum. The TGF-α present in either the medium or the cells was assayed using a TGF-α specific ELISA. In separate experiments the TGF-α concentrations obtained with the ELISA have been shown to agree with the results obtained with the commonly used EGF-receptor binding assay (4) (data not shown). Table 3 summarizes the TGF-α levels obtained for four different cell lines. These data show that the TGF-α expression levels are generally higher in the presence than in the absence of serum. Serum itself did not contain any TGF-α as measured by the ELISA. However, the presence of serum may stimulate TGF-α synthesis or enhance the efficiency of correct proteolytic cleavage of the TGF-α precursor. It is also striking that the partitioning of TGF-α between the media and cell fractions is very different for each of these cell lines. For the A2058

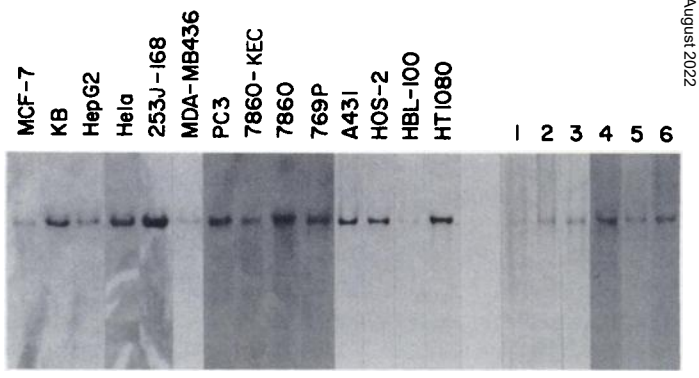

Fig. 1. Some examples of Northern hybridization for TGF-α mRNA using RNA derived from various tumor cell lines and tumor specimens. The tumor cell lines are designated with their name above each lane, while the tumor specimens are numbered 1 through 6 as follows: 1, adenocarcinoma in endometrium of uterus; 2, metastatic adenocarcinoma in stomach; 3, squamous carcinoma of head and neck; 4, large-cell carcinoma in lung; 5, nephroma; 6, apudoma. The intensity of the TGF-α mRNA band may not correspond to the levels designated in Tables 1 and 2 due to differences in autoradiograph exposure time. Patient code names: 1, Dix; 2, Glo; 3, Pit.

[4] T. S. Bringman, P. B. Lindquist, and R. Derynck. Submitted for publication.

Table 2 *TGF-α, TGF-β, and EGFR mRNA in tumor and normal tissues*

Summary of Northern hybridization results for a variety of surgically removed tumor specimens and several normal tissues. The levels of mRNA for TGF-α, TGF-β or the EGF receptor were scored on a relative basis, as explained in the legend to Table 1.

	TGF-α	EGFR	TGF-β
Metastatic squamous carcinoma, neck (Bio)[a]	+	++	+++
Metastatic squamous carcinoma, neck (Hix)	+	+++	+
Squamous carcinoma, head, and neck (Pit)	++	++++	++
Squamous carcinoma, lung (Cag)	++++	+++	++++
Squamous carcinoma (W-D)	−	++	++
Squamous carcinoma (W-M)	+	++	+
Squamous carcinoma (W-U)	+	+	+
Squamous carcinoma (W-2)	+	+++	++
Adenocarcinoma endometrium uterus (Dix)	+	+	+++
Metastatic adenocarcinoma stomach (Glo)	++	++++	+++
Ovarian carcinoma (Mil)	−	++	+++
Ovarian fibroma	+	+++	+
Mammary carcinoma (Mur)	+	++	++++
Seminoma	+	+	++
Adenocarcinoma, lung (W-F)	−	+	++
Adenocarcinoma (W-G)	−	+	+
Mammary carcinoma (W-L)	−	+	+
Bronchogenic adenocarcinoma (W-P)	−	++	++
Bronchogenic adenocarcinoma (W-GG)	−	+	+++
Adenocarcinoma, stomach (W-V)	−	++	++
Adenocarcinoma, colon (W-EE)	−	−	+
Papillary adenocarcinoma (W-II)	−	−	+
Renal carcinoma	++	++	++
Renal carcinoma	+++	+++	++
Metastatic melanoma lung (Alo)	+	+	++
Melanoma jejunum	+	+	+++
Melanoma	+++	+	+++
Apudoma	++	++++	−
VIP-oma	+	++++	++
Glioblastoma multiforma (W-BB)	−	+	++
Hepatoma	−	+++	+++
Embryonal carcinoma	−	−	+
Large cell carcinoma, lung	+++	*[b]	++
Metastatic serous carcinoma (W-H)	−	+	+
Leiomyosarcoma, stomach (Bow)	+	++	++++
Liposarcoma	−	+	+
Fibrous histiosarcoma (W-W)	−	+	+
Leiomyosarcoma (W-FF)	−	+	+
Pancreas islet tumor (W-HH)	−	−	+
Normal liver (A)	−	+++	+
Normal liver (B)	−	ND	ND
Normal pancreas	−	ND	ND
Normal lung (W-J)	−	−	+
Normal lung	−	++	+++
Normal stomach	−	+	+
Normal jejunum	−	+	+
Normal uterus (W-B)	−	−	+
Normal uterus (W-A)	−	+	+
Placenta, 18–20 wk	−	++++	++
Placenta term	−	*[b]	++
Peripheral blood lymphocytes (uninduced)	−	ND[c]	+
Peripheral blood lymphocytes (PHA-induced)	−	ND	+++

[a] Patient code name.
[b] *, extreme abundance of EGF receptor mRNA.
[c] ND, not determined.

Table 3 *Synthesis of TGF-α by several tumor cells*

Amounts of TGF-α expressed in a 50 ml culture by different human tumor cells grown and extracted as detailed in the "Materials and Methods" section.

	Cells	Medium
A549 (2.0×10^7 cells; 10% serum)	<0.1 ng	21.5 ng
A549 (2.0×10^7 cells; serum free)	>0.122 ng[a]	<0.5 ng
A2058 (5.8×10^6 cells; 10% serum)	<0.1 ng	24.9 ng
A2058 (5.8×10^6 cells; serum free)	<0.1 ng	6.21 ng
7860 (5.7×10^6 cells; 10% serum)	0.29 ng	<0.5 ng
7860 (5.7×10^6 cells; serum free)	0.51 ng	<0.5 ng
HT1080 (2.6×10^7 cells; 10% serum)	0.91 ng	2.1 ng
HT1080 (2.6×10^7 cells; serum free)	<0.1 ng	0.7 ng

[a] Level could not be accurately determined due to an anomalous dilution-response curve in the ELISA.

melanoma cell line, TGF-α can be found only in the medium, with no detectable TGF-α in the cellular fraction. In contrast, all the TGF-α activity in the 7860 renal carcinoma cell line is cell associated, with no activity in the medium.

EGF-receptor mRNA. To examine whether some of the TGF-α expressing tumors may represent examples for autocrine growth promotion, we compared the levels and distrubtion of EGFR mRNA with results obtained for the TGF-α mRNA in many of the RNA samples. EGFR mRNA was detected in the majority of solid tumors. The two previously described EGFR mRNA species of 10.5 kilobases and 5.8 kilobases (13) were present in most samples. However, in the case of the renal carcinoma cell line 7860, a single strong EGFR mRNA band with an apparently aberrant length of about 3.8 kilobases was visible (data not shown). In confirmation of previous findings (13), extremely high levels of EGFR mRNA were present in term placenta and in A431 cells. High levels of EGFR mRNA were found in most squamous carcinomas, also in agreement with previous data showing high numbers of membrane-bound EGF receptors on tumors of this type (28–30). High levels of EGFR mRNA were also detected in the renal carcinoma cells. These latter two tumor types were found to also synthesize relatively high levels of TGF-α mRNA. Thus there seems to be a correlation between the presence of TGF-α mRNA and elevated EGFR mRNA levels in the case of renal and squamous carcinoma. However, as illustrated with the fibrosarcoma cell line HT1080 and the glioblastoma line A172, no absolute relationship can be established. HT1080 cells contain relatively high levels of TGF-α mRNA and relatively low levels of EGFR mRNA, while A172 cells have very high levels of EGFR mRNA and no detectable TGF-α mRNA (Table 1).

TGF-β mRNA. A synergistic effect between TGF-α and -β has been shown to induce phenotypic transformation of normal rat kidney fibroblasts (6). We therefore measured the levels of TGF-β mRNA in the various tumor cell lines and tissues. We previously reported the presence of TGF-β mRNA in a variety of cell sources (15). In this study, TGF-β mRNA was also detected in all cell lines and in all tumor tissues tested. Low levels were observed in several normal tissues and TGF-β mRNA was not detectable in liver mRNA. The levels of TGF-β mRNA in the samples were highly variable and not dependent upon the transformed character of the cells. For example, the normal fibroblast FS-4 cells contain much higher TGF-β mRNA levels than many tumor cell lines. In order to examine TGF-β mRNA levels in matched samples of tumor and normal tissues, Northern hybridizations were performed using mRNA from surgically removed tumors and from clinically normal tissue located adjacent to the tumor site. The results indicate that in all six cases there was more TGF-β mRNA in the tumor tissue than in the adjacent clincially normal tissue (Fig. 2). The extent of increase was variable for the different pairs of tissue samples.

DISCUSSION

Examination of a variety of tumor cells by Northern hybridization has revealed that many carcinoma and sarcoma cell

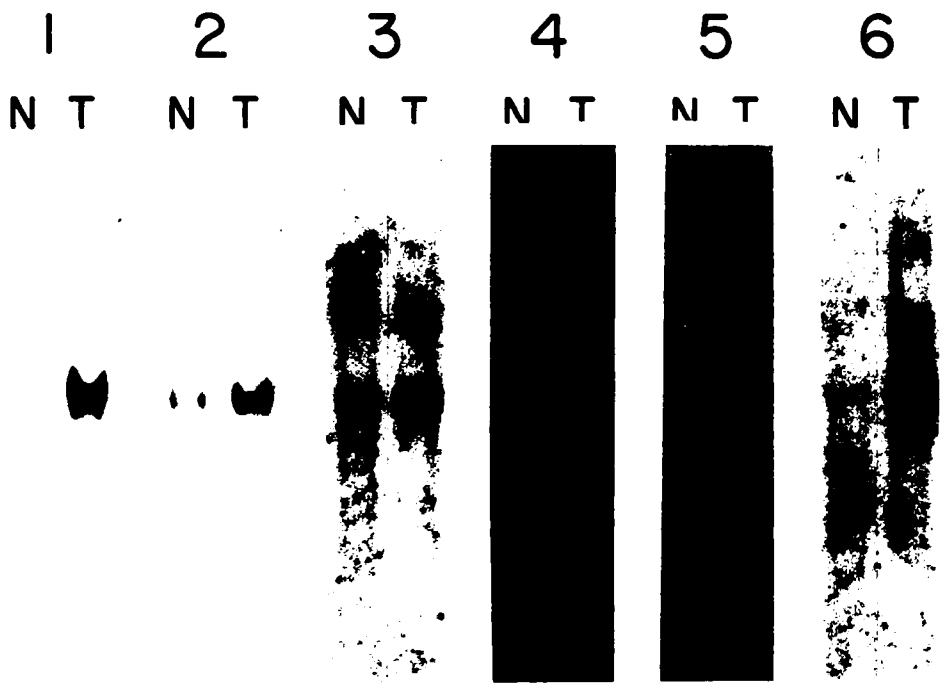

Fig. 2. TGF-β mRNA levels, determined by Northern hybridization in surgically removed tumor specimens (*T*) and the surrounding clinically normal tissue (*N*) derived from six patients. 1, leiomyosarcoma of stomach; 2, melanoma of jejunum; 3, squamous carcinoma; 4, bronchogenic adenocarcinoma; 5, metastatic serous carcinoma; 6, adenocarcinoma of lung.

lines synthesize TGF-α mRNA. The cell line A431, which expresses 10 to 50 times more EGF receptors than most other cell types (31), is particularly interesting since the effects in these cells of exogenously added EGF have been very well studied. From our results it is clear that the A431 cells synthesize TGF-α mRNA (Fig. 1) and also release low levels of TGF-α in the medium (data not shown). This suggests that the endogenous production of TGF-α and its interaction with EGF receptors in an autocrine fashion may have to be considered when evaluating the effects of exogenous EGF in this cell line.

Our hybridization data indicate that TGF-α mRNA is not synthesized by any of the hematopoietic tumor cell lines examined. Cells of the hematopoietic lineage are known to lack EGF receptors (32). In contrast, many tumor cells derived from solid tumors synthesize TGF-α and all of these cells also synthesize EGFR mRNA. This suggests that TGF-α synthesis could act in an autocrine fashion in these solid tumors since TGF-α has been shown to exert its action through the EGF receptor (3, 4, 21). While TGF-α mRNA can be found in tumors belonging to many types of carcinomas and sarcomas, the overall highest and most consistent synthesis can be found in four different tumor types. Squamous carcinomas and renal carcinomas synthesize relatively high levels of TGF-α mRNA, but TGF-α mRNA can also be found in mammary carcinomas and in tumors derived from neuroectodermal origin, such as melanomas. TGF-α synthesis may also be relatively common in hepatomas since it has been demonstrated in two hepatoma cell lines (33) and TGF-α mRNA is also present in the HepG2 hepatoma cell line. The lack of a sufficient number of tumor samples of particular tumor types precludes making other generalizations.

The synthesis of EGF receptors through which TGF-α exerts its action (3, 4, 21) by several mammary carcinomas (34) and their abundance in squamous carcinomas (27–29) have previously been reported. Our Northern hybridizations confirm these findings and also indicate relatively high levels of EGFR mRNA in renal carcinomas. We have previously shown that endogenous TGF-α expression can induce transformation of immortalized fibroblasts through an autocrine mechanism (35). It is therefore tempting to speculate in the context of an autocrine mechanism that expression of TGF-α by the tumor cells may contribute to tumor development. There may be a relationship between the levels of TGF-α mRNA and EGFR mRNA, since many TGF-α mRNA-containing tumors also have relatively high levels of EGFR mRNA. However, an absolute relationship does not exist since the HT1080 fibrosarcoma cell line, which contains relatively high TGF-α mRNA levels, has only low levels of EGFR mRNA. Also, we could not detect any TGF-α mRNA in the A172 glioblastoma cell line, which contains high levels of EGFR mRNA.

Malignancy-associated hypercalcemia occurs relatively frequently in patients with renal carcinoma, squamous carcinomas, breast carcinoma, and melanoma (36). Tumors of these types are most consistent for the presence of TGF-α mRNA. *In vitro* studies have indicated that TGF-α is a very potent inducer of Ca^{2+}-release in mouse calvariae and in rat fetal long bone (37, 38). It can thus be speculated that the TGF-α synthesized by these various tumor types triggers or contributes to malignancy-induced hypercalcemia. In this context, it may be relevant that the HOS-2 cell line derived from an osteosarcoma contains highly elevated TGF-α mRNA levels. It is important, however, to recognize that TGF-α induced Ca^{2+}-release may only be one mechanism, since parathyroid hormone-like molecules synthesized by the tumors also could play a role in this type of hypercalcemia. These latter factors could also contribute, perhaps in synergism with TGF-α, to the other types of malignancy-associated hypercalcemia.

Previous studies have indicated that TGF-α is a potent inducer of angiogenesis in an *in vivo* model (39). Hematopoietic tumors which do not require neovascularization for their development do not contain TGF-α mRNA. However, the endogenous synthesis of TGF-α in various types of solid tumors may not only contribute to an autocrine growth stimulation of these tumor cells, but may also play a paracrine role in the tumor-induced angiogenesis, probably in concert with other angiogenic factors.

Our analysis of TGF-α synthesized by several tumor cells using the antibody-based assay indicates that TGF-α can be

detected in the culture medium of some cells, such as HT1080, but that a significant amount may remain cell associated. The most striking example is the 7860 cell line, which does not have any detectable TGF-α in the medium, yet contains easily detectable levels in cell lysates. The nature of the cell-associated TGF-α is not clear. It could either represent fully processed TGF-α which is not yet secreted into the culture medium or TGF-α which has not yet been cleaved from the precursor. The sequence of the 160-amino acid-long TGF-α precursor contains an internal 22-amino acid hydrophobic region, which suggests that the precursor may be a transmembrane protein (2). A protease specific for the Ala-Val-Val sequences which flank mature TGF-α could cleave the external segment of the precursor and in this way release the 50-amino acid-long TGF-α. It is conceivable that this protease may either be absent or occur in only limited amounts in some cells, such as the 7860 renal carcinoma cells. This could result in the presence of the unprocessed TGF-α precursor in the membrane of these cells.

Previous studies have indicated that TGF-β expression is higher in some retrovirally transformed fibroblasts than in their nontransformed counterparts (6). The present study indicates that the TGF-β mRNA levels in tumor cells are generally not higher than in actively dividing normal cells. The fact that we are studying solid tumor tissue samples and the size limitations of the tumor biopsies make it impossible to accurately determine the levels of TGF-β secreted by the tumor cells. However, in all six cases in which surgically removed tumor specimens were compared with corresponding clinically normal surrounding tissue, the TGF-β mRNA level was highest in the tumor tissue. While it is possible that the higher levels of TGF-β mRNA are a direct result of the transformed character of the tumor cells, it may be that they correlate with the higher degree of mitotic activity of these cells. This latter interpretation is supported by the induction of TGF-β mRNA in lymphocytes upon mitogenic stimulation (15, 40) and could also explain the relatively high levels of TGF-β mRNA in the retrovirus-transformed fibroblasts (6). It should also be taken into consideration that tumors develop from the amplification of a specific cell type in tissues, or organs which may contain a heterogeneity of cell types. For this reason the surrounding clinically normal tissue may not be a matched control since the relative abundance of the different cell types will be different from the tumor tissue.

In conclusion, we have studied the expression of TGF-α, TGF-β, and the EGF receptor in a wide variety of tumor types. The high number of different tumor types and the poor availability of some types of tumor tissues imposes limitations on this type of study. However, the apparent synthesis of TGF-α by several types of solid tumors has potential diagnostic value, especially since low levels of TGF-α have been detected in the urine of several cancer patients (41, 42). Additional studies will be required to evaluate in detail the synthesis of TGF-α by many specific tumor types and to assess the endogenous synthesis of other growth factors in tumors.

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