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Transforming Growth Factors and Control of Neoplastic Cell Growth

Jorma Keski-Oja, Edward B. Leof, Russette M. Lyons, Robert J. Coffey, Jr., and Harold L. Moses

Departments of Cell Biology, Pathology and Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Transforming growth factors (TGFs) are peptides that affect the growth and phenotype of cultured cells and bring about in nonmalignant fibroblastic cells phenotypic properties that resemble those of malignant cells. Two types of TGFs have been well characterized. One of these, TGFα, is related to epidermal growth factor (EGF) and binds to the EGF receptor, whereas the other, TGFβ, is not structurally or functionally related to TGFα or EGF and mediates its effects via distinct receptors.

TGFβ is produced by a variety of normal and malignant cells. Depending upon the assay system employed, TGFβ has both growth-inhibitory and growth-stimulating properties. Many of the mitogenic effects of TGFβ are probably an indirect result of the activation of certain growth factor genes in the target cell. The ubiquitous nature of the TGFβ receptor and the production of TGFβ in a latent form by most cultured cells suggests that the differing cellular responses to TGFβ are regulated either by events involved in the activation of the factor or by postreceptor mechanisms. The combined effects of TGFβ with other growth factors or inhibitors evidently play a central role in the control of normal and malignant cellular growth as well as in cell differentiation and morphogenesis. Since transforming growth factor as a concept has partially proven misleading and insufficient, there is a need to find a new nomenclature for these regulators of cellular growth and differentiation.

Key words: transforming growth factors, TGFβ, oncogene activation, growth stimulation, growth inhibition, neoplastic growth, cancer cell

Transforming growth factor-β (TGFβ) was discovered as a growth-stimulatory molecule in two laboratories concurrently [1,2]. Its major effect was the ability to induced soft agar growth of nontumorigenic fibroblastic cells. It was originally thought that TGFs would be found only in malignant cells since similar effects had earlier been ascribed to sarcoma growth factors (SGFs) that were found in medium conditioned by murine sarcoma virus-transformed fibroblasts [3]. It was later found

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that SGF is composed of an epidermal growth factor (EGF)-like growth factor (TGFα, see below) and TGFβ [4].

Recent observations, however, have demonstrated that TGFs are found in normal cells and tissues, and that other growth factors such as platelet-derived growth factor (PDGF) can stimulate anchorage-independent growth of nonmalignant cells in the presence of serum [5,6]. The ability of cells to grow in soft agar probably results from the synergistic action of intracellular factors such as activated oncogenes [5,7] and external ones such as polypeptide growth factors including PDGF, EGF, TGFα, and TGFβ [8] and their extracellular matrices [9].

Recent studies have demonstrated that TGFβ can function as a growth stimulator only for fibroblastic cells. A model has been proposed that suggests that its effect is indirect and is mediated via the induction of the *sis*-proto-oncogene that codes for the B chain of PDGF [7]. On the other hand, TGFβ is a potent growth inhibitor of several types of normal epithelial cells and several types of malignant cells [10]. TGFβ, or a very similar factor, had been described earlier as a growth inhibitor (GI) for certain epithelial cell types [11,12] before its growth stimulatory effects were discovered. TGFβ can thus function both as a stimulator and as an inhibitor of growth. These properties, when modified by other growth factors, may be essential in the regulation of both physiological and malignant growth. In addition, novel anchorage-independent growth modifying peptides have been purified and characterized from several different sources during the past few years [13–18]. Elucidation of their molecular properties will add to our understanding of the regulation of the malignant phenotype.

TGFα—RELATIONSHIPS TO EGF AND TGFβ

TGFα was originally observed in the conditioned medium of murine sarcoma virus-transformed cells as EGF-competing activity that was associated with anchorage-independent growth of fibroblastic rat kidney NRK cells [3]. The growth factor was termed sarcoma growth factor (SGF), and later it was shown that SGF was composed of TGFα and TGFβ molecules [4]. Examination of a variety of cell lines indicated that TGFα was produced by different malignant cells including those that were transformed by viruses but not by normal adult cells [19–21]. TGFα is possibly an embryonic form of EGF, which is inappropriately expressed in various malignancies.

The addition of TGFα to NRK cells resulted in the formation of small colonies of cells without exogenous TGFβ; however, the simultaneous presence of both TGFα and TGFβ resulted in enhanced soft agar colony formation [2]. Which TGF(s) is (are) required for growth in soft agar depends upon the cell assay system employed. AKR-2B, Balb/c-3T3, human foreskin fibroblasts, or EGF receptorless NR6 cells only require the addition of TGFβ to the serum supplemented medium for induction of soft agar growth [10]; there is no EGF or TGFα requirement.

Radioreceptor assays have shown that EGF and TGFα compete similarly for binding to cell surface EGF receptors [22–24]. Amino acid sequencing of rat, mouse, and human TGFαs demonstrated that the protein is highly conserved [25,26, cf.27], which indicates that the protein is phylogenetically important [28–30].

The human TGFα gene has been mapped to chromosome 2 (2p13) close to the breakage point in Burkitt's lymphoma [31,32], which suggests that a similar activation mechanism may operate in certain malignancies. Molecular cloning of TGFα indi-

cated that human TGFα is encoded by a 4.5–4.8-kb mRNA. Analyses of the cDNA clones indicated that the 50-amino acid TGFα is translated as a part of a precursor molecule of 160 amino acids. A very hydrophobic region of the TGFα precursor is located nine residues downstream of the COOH terminus of the 50-amino acid TGFα molecule. The structure of this region is characteristic of transmembrane regions of membrane proteins, and it is possible that TGFα is secreted from cells followed by a proteolytic cleavage at the cell membrane [cf. 27].

In addition to the TGFα molecule, several larger proteins with TGFα activity can be detected in the conditioned medium of transformed cells [cf.27]. These larger peptides are evidently derived from the same gene and are presumably from the same precursor [23]. Variations in the proteolytic processing may explain the different molecular sizes of TGFα activity.

Other molecules that are structurally related to EGF and TGFα and that contain EGF-like sequences include the EGF precursor molecule [33,34], the vaccinia virus growth factor (VVGF) [35–37], and the low density lipoprotein (LDL)-receptor [38,39]. Several serine proteinases including the plasminogen activators and blood coagulation factors contain EGF-like domains whose functions have not as yet been elucidated [40].

PURIFICATION AND MOLECULAR CHARACTERIZATION OF TGFβ

TGFβ (see Table I) is widely distributed in different tissues [cf 41–43] and has been purified from placenta [44,45], kidneys [46], and platelets [47,48] as well as from cultured cells [1,2,11; see 41–43]. Platelets have been the most common source for purification because of their high TGFβ content [48]. It has been estimated that platelets contain threefold greater amounts of TGFβ than PDGF. The intact molecule from all sources has a molecular weight of 25,000 and is composed of two apparently identical subunits of 12,500. The reduced, dissociated subunit is biologically inactive [48].

Derynck and coworkers [49] have cloned the gene for TGFβ from a human genomic library and from cDNA libraries derived from human term placenta and from the human fibrosarcoma line HT-1080. The amino acid sequence deduced from

TABLE I. Properties of TGFβ*

Molecule	25,000-dalton disulfide-linked homodimer (112 amino acids each chain) [48]
	Cleaved from a larger precursor protein [49]
Chromosomal location	19q (subbands q13.1–q13.3) [51]
Sources	Cultured cells [1–3], platelets [47,48], placenta [44,45], kidney [46]
Activation	Dissociation from a binding protein or cleavage from a precursor protein [43,80,81]
Receptor	High molecular weight dimeric glycoprotein (M_r 560,000) [61,62]
	Abundant in all cells [59]
	No enzymatic functions known
Homologies	Inhibin [52]; Activins [53,54]
	Müllerian-inhibiting substance [55]
Identities	Growth inhibitor (GI) [12]
	Cartilage-inducing factor-A (CIF-A) [57]
	Differentiation inhibitor of BRL cells [58]

*The reference nos. of some representative publications are included.

sequencing of overlapping cDNA fragments suggests a subunit of 112 amino acids. Amino acid sequence analysis of reduced human platelet-derived TGFβ further confirmed that the two chains were identical [49]. These studies furthermore suggested a precursor encoded in the 391-residue open reading frame where each subunit is encoded by residues 280–391. The murine TGFβ gene has recently been cloned, and the cDNA sequence has been determined [50]. The COOH-terminal precursor cDNA sequence representing the TGFβ coding region is identical in murine and human clones except for one amino acid at position 354 (serine in murine, alanine in human TGFβ). This high degree of evolutionary conservation suggests that most regions of the TGFβ molecule are necessary for biological activity and that TGFβ probably plays an essential role in normal growth and development. The human TGFβ gene has been localized to the long arm of chromosome 19 and to chromosome 7 in the mouse, which share four homologous loci [51].

Analysis of the TGFβ sequence indicated homology with the β-chains (β_A and β_B of inhibin [52]. Inhibin is a potent inhibitor of follicle-stimulating hormone (FSH) secretion, and is produced by both the ovaries and the testis. It is composed of two different polypeptide chains (α and β_A or β_B) and it is structurally related to certain glycoprotein hormones of the pituitary and placenta. Interestingly, the β-subunits can form homodimers and heterodimers, which have effects opposite to those of inhibin ("activins" [53,54]). In addition, a structural homology between Müllerian-inhibiting substance (M_r 150,000) and TGFβ has also been found [55]. These molecules appear to form a group of regulators of growth and differentiation with multiple functions [see 56]. In addition, biological and polypeptide analyses have shown that cartilage-inducing factor-A (CIF-A) [57] and the differentiation inhibitor of Buffalo rat liver (BRL)-cells [58] are actually TGFβ.

TGFβ RECEPTOR

TGFβ, unlike TGFα, has its own specific cell membrane receptors that like the TGFβ molecule itself, are ubiquitous. Specific binding of ^{125}I-TGFβ to various mesenchymal and epithelial cells in primary and secondary cultures and continuous cell lines, both normal and neoplastic, has been reported [59]. The development of radioreceptor assays for TGFβ has allowed the quantitation of dissociation constants (25–140 pM) and receptor number per cell (10,000–40,000) [59,60]. The receptors bind TGFβ from different species equally well (see Fig. 1) suggesting that these growth factor-receptor systems are highly conserved. The TGFβ receptor is different from either the EGF or platelet-derived growth factor (PDGF) receptors, which function as tyrosine-specific protein kinases. Affinity labeling of the receptor in mouse cells has identified both multimeric complexes, and a M_r 565,000 complex that is apparently a dimeric glycoprotein. The receptor dissociates in the presence of disulfide reducing agents into two subunits of M_r 280,000–290,000 [61]. Two types of TGFβ-receptors have recently been proposed on the basis of cross-linking studies [62]. Thus far no kinase or other enzymatic activity has been reported for the TGFβ receptor.

TGFβ enhances the affinity of the EGF-receptors of NRK cells [63,64], and the activation of the EGF-receptor appears to be essential for the action of TGFβ in these cells [65]. The need for insulin-like growth factors in TGFβ-induced cell transformation has also been proposed [66].

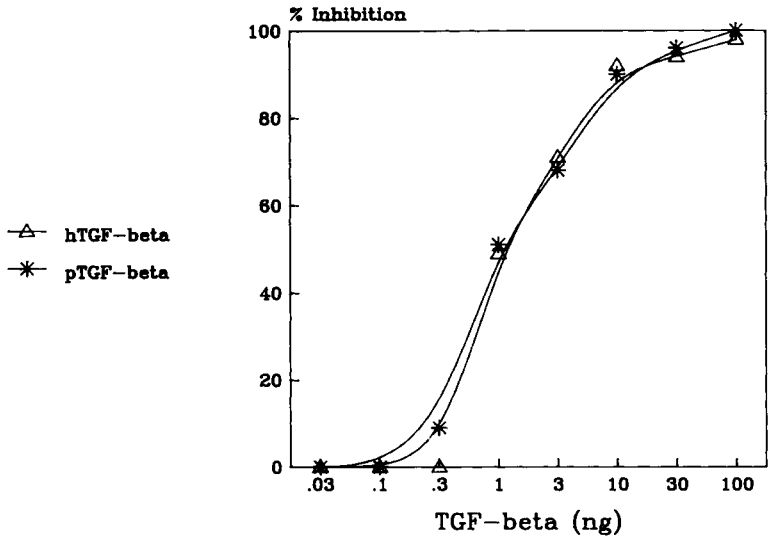

Fig. 1. Inhibition of the binding of human TGFβ (hTGFβ) to cellular receptors by human and porcine TGFβ (pTGFβ). The radioreceptor assays were carried out using ^{125}I-labeled hTGFβ and 84A cells [59]. The inhibition of binding with pTGFβ occurs at nanogram concentrations as with hTGFβ [59] which indicates that their affinities for the cellular receptors are of the same magnitude.

MECHANISM OF GROWTH STIMULATION BY TGFβ

Cell stimulation by TGFβ brings about alterations in the functions of the cell membrane. The uptake of glucose and amino acids into the cells is enhanced [65,66]. However, the relationships of these membrane changes to growth stimulation have not been elucidated.

Anchorage-Independent Growth

TGFβ stimulates soft agar growth of murine AKR-2B and 3T3 cells, rat NRK cells, and secondary cultures of human foreskin fibroblasts [1,2,10]. All of the cells stimulated to proliferate by TGFβ are mesenchymal in origin and are either fibroblasts or fibroblast-like cells. The requirement of additional growth factors in medium already containing serum for soft agar growth has led to significant confusion in the interpretation of the results [see 10]. Studies of growth factor requirements for growth in soft agar have, in general, demonstrated that the same growth factors that are required for cell growth as substratum-attached layers, in addition to TGFβ, are required for the support of proliferation of anchorage-independent growth [67,68].

Recently it has been shown that fibronectin plays a role in the soft agar growth of NRK (49F) cells [9]. Both fibronectin and procollagen production were enhanced by TGFβ, and fibronectin, at microgram concentrations, enhanced the soft agar growth of the indicator cells. Synthetic peptide inhibitors of fibronectin binding to its cell surface receptors inhibited the colony-forming effect of TGFβ, suggesting that the formation of pericellular matrix structures may have been important in the actions of TGFβ [9]. Recently it has also been reported that retinoic acid, in combination with insulin and EGF or PDGF, can induce soft agar growth of NRK cells [69]. Whether this takes place via the stimulation of extracellular matrix formation is not known. The multiple known biological effects of TGFβ (Table II) may take place via

TABLE II. Biological Effects of TGFβ*

Inhibits the growth of most normal, especially epithelial cells, and several malignant cells [10,72,74]
Acts as a mitogen for mesenchymal cells (activates the c-*sis* oncogene) [1,2,7,70]
Stimulates anchorage-independent growth of nonmalignant fibroblastic cells (frequently in concert with other growth factors) [1,2]
Inhibits adipogenic differentiation of 3T3 cells [105,106] and stimulates terminal differentiation of bronchial epithelial cells [108]
Enhances wound healing [87,88]
Induces fibroblast chemotaxis [97]
Regulates the plasminogen activator activity of cultured cells; induces endothelial type plasminogen activator inhibitors [91,92]
Enhances the production of connective tissue components [9,87]
Acts as a modifier of different immunological responses [98]

*The reference nos. of some representative publications are included.

the induction of multiple genes that act synergistically to support the cell's ability to grow in soft agar.

Mitogenicity of TGFβ

TGFβ stimulates DNA synthesis in quiescent substratum-attached cultures of mouse AKR-2B cells without other added growth factors in a completely defined medium, but with delayed kinetics relative to stimulation with other growth factors [70]. Stimulation with EGF and insulin, PDGF, fibroblast growth factor (FGF) or serum resulted in a 12–14-hr lag phase before the onset of DNA synthesis, which then peaked at 20–25 hr following stimulation. Cultures stimulated with TGFβ exhibited a lag phase that was prolonged to 24 hr with a peak of DNA synthesis between 30 and 35 hr. In an effort to determine why TGFβ stimulated DNA synthesis with such unusual, delayed kinetics relative to stimulation with other growth factors, the possibility was examined that TGFβ was acting as an indirect mitogen through induction of synthesis of endogenous growth factors. It was found that TGFβ stimulation of quiescent cultures of AKR-2B cells resulted in an early induction of c-*sis* mRNA [7] (see Fig. 2). The rise in c-*sis* mRNA was followed by a corresponding increase of a PDGF-like protein in the culture medium. In addition, PDGF-regulated genes (c-*fos* and c-*myc*) were stimulated by TGFβ with delayed kinetics relative to that seen with direct PDGF stimulation. The data suggest that the mitogenicity of TGFβ for adherent cells is mediated by the induction of c-*sis* (PDGF) with the subsequent autocrine stimulation of c-*fos*, c-*myc*, other PDGF inducible genes, and DNA synthesis. Although this model of indirect mitogenicity might explain the results of TGFβ stimulation of adherent mesenchymal cells, it does not account for the growth inhibitory actions of TGFβ (see below).

INHIBITION OF CELL PROLIFERATION BY TGFβ

Recently, Tucker et al [12] have shown that TGFβ and the growth inhibitor (GI) that Holley and coworkers [11] isolated from BSC-1 cell conditioned media are similar, if not identical, molecules. Under conditions in which TGFβ is stimulatory for fibroblastic AKR-2B cells, there is an inhibition of the early S phase induced by EGF and insulin or PDGF [12,70]. In addition, GI purified from medium conditioned by BSC-1 cells [71] and TGFβ purified from human platelets have almost identical

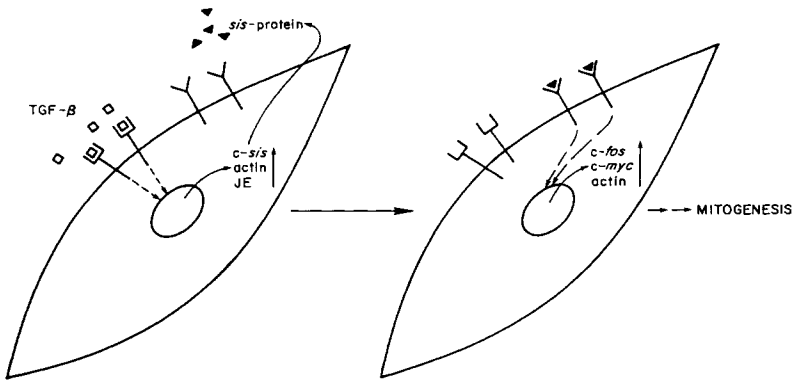

Fig. 2. Proto-oncogene activation by TGFβ. The binding of TGFβ to its cell surface receptors is followed by the activation of certain cellular proto-oncogenes [7]. Among the first activated genes are c-*sis*, actin, and JE genes [96]. The *sis*-protein presumably activates the c-*fos* and c-*myc* genes which then leads to DNA synthesis.

biological activities in stimulating growth of AKR-2B cells in soft agar and inhibiting DNA synthesis in BSC-1 and CCL-64 epithelial mink lung cells. GI was also able to compete for binding with ^{125}I-labeled TGFβ to membrane receptors nearly as effectively as the native platelet-derived TGFβ [59]. Both the GI and TGFβ have apparent molecular weights of 25,000 and migrate as a single polypeptide of 12,500 in sodium dodecyl sulfate (SDS)-polyacrylamide gels under reducing conditions [48,71]. In addition, our recent immunological analyses have indicated antigenic cross-reactivity (unpublished).

TGFβ is inhibitory for spontaneous soft agar growth of several human carcinoma cell lines [10,72]. No epithelial cell type, either neoplastic or non-neoplastic, has been demonstrated to be stimulated to proliferate by TGFβ. The epithelial cells or carcinoma cell lines that have been tested so far were either inhibited or showed no response to TGFβ under usual cell culture conditions [10,73]. TGFβ is a potent inhibitor of growth of secondary cultures of human foreskin keratinocytes [10,74]. The keratinocytes were reversibly inhibited in the G1 phase of the cell cycle by TGFβ. TGFβ is also a potent inhibitor of EGF-induced stimulation of DNA synthesis in primary cultures of rat hepatocytes [75,76], and it inhibits the growth of primary cultures of human megakaryocytic and erythroid precursors [77]. TGFβ inhibits effectively also fibroblast growth factor (FGF)-stimulated endothelial cell proliferation [78,79].

The mechanisms by which TGFβ inhibits cell proliferation are largely unknown. It is possible that TGFβ is primarily an inhibitor for all cell types and that stimulation of fibroblastic cells is fortuitous through the induction of c-*sis* and autocrine activity by the PDGF-like *sis*-protein, which is the direct mitogen.

POTENTIAL ROLES OF TGFβ IN NEOPLASIA AND OTHER DISEASE STATES

We used mouse embryo-derived cell culture model systems for neoplastic transformation (AKR-2B and C3H/10T½ cell lines), to demonstrate that the chemically transformed derivatives of these cell lines both produced and responded to

TGFβ [1,10]. Although the parent cell lines released as much TGFβ into serum-free conditioned medium as their chemically transformed derivatives, the TGFβ released by both the parent cells and the transformed derivatives was in an inactive form that was irreversibly activated by acid treatment.

These studies are in agreement with those of Lawrence et al [80] who demonstrated that many cell types release TGFβ in an inactive form. The inactive form of TGFβ released by cells in culture appears to be in a higher molecular weight form than the active molecule, perhaps reflecting an association with a binding protein [43,81]. The physiological mechanism of TGFβ activation is not known. Proteolytic cleavage from a larger precursor or dissociation from a binding protein appear to be the most plausible explanations (see Fig. 3).

Cellular Responsiveness to TGFβ

The major change observed in the chemically transformed cells relative to their parent cell lines was the development of a markedly increased sensitivity to TGFβ stimulation of growth in soft agar [10]. The possible mechanisms of the enhanced responsiveness of these cells to TGFβ were examined. Studies on the TGFβ receptor revealed very slightly reduced numbers of receptors on the chemically transformed cells relative to the parent cell lines with no detectable change in affinity. This suggested that a postreceptor mechanism was responsible for the increased TGFβ sensitivity observed in the chemically transformed AKR-2B cells. Using the C3H/10T½ cells, which are completely unresponsive to TGFβ with respect to stimulation of growth in soft agar, transfection was carried out with a mouse c-*myc* gene linked to an SV40 promotor and/or with an activated H-*ras* gene, both of which were cotransfected with the dominant neomycin-resistance marker (E.B. Leof and H.L. Moses, unpublished observations). The c-*myc* gene-transfected cells became highly responsive to stimulation of growth in soft agar by TGFβ, which suggested that c-*myc* expression, at least in part, controlled cellular responsiveness to TGFβ. The H-*ras* gene-transfected cells demonstrated marked morphologic transformation in

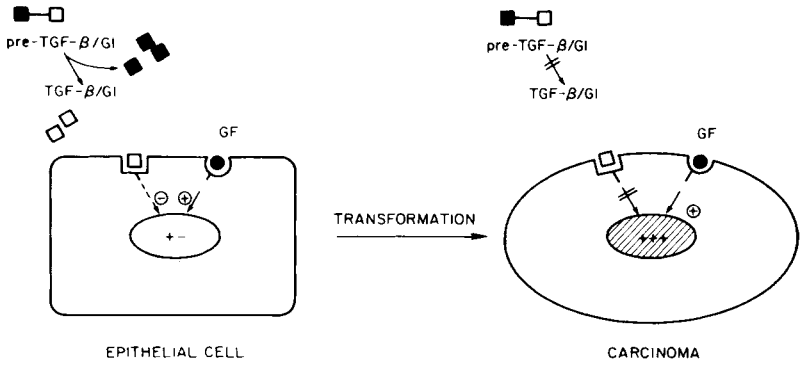

Fig. 3. Escape from TGFβ growth inhibition in carcinoma cells. During the growth of epithelial cells they receive both stimulatory and inhibitory signals from their cell membrane growth factor receptors. An alteration in the cell's ability to activate latent TGFβ or an alteration in the function of the receptor or postreceptor mechanisms may sensitize the cells in a pathological way to normal growth stimulatory signals. This may result in malignant growth [74]. Abbreviations: pre-TGFβ, the inactive precursor of TGFβ (possibly TGFβ bound to a binding protein); GI, growth inhibitor (structurally and immunologically identical with TGFβ).

culture and grew spontaneously in soft agar in the absence of TGFβ. The growth of the H-*ras* gene-transfected cells was only slightly enhanced by TGFβ. These data demonstrated that transfection of C3H/10T½ cells with an activated H-*ras* gene induced a similar phenotype to that induced by TGFβ, but without the requirement for added TGFβ. This suggests that $p21^{H\text{-}ras}$ may enhance autocrine stimulation by endogenous TGFβ or may be involved in the transduction of the TGFβ signal. However, divergent results of similar transfection experiments and TGFβ responsiveness using Fisher rat 3T3 cells have recently been reported [82].

Epithelial Cell Transformation

The potential role of TGFβ in neoplastic transformation of epithelial and other nonfibroblastic cell types may be entirely different from that involved in fibroblastic cells. We have observed that a squamous carcinoma cell line has lost the inhibitory response to TGFβ characteristically exhibited by normal keratinocytes [74]. Loss of TGFβ-effected growth inhibition in epithelial cells could result in an enhanced proliferative potential, which would produce the same effect as the activation of a stimulatory response (see Fig. 3).

The growth of many normal epithelial cells in primary or secondary culture, including human keratinocytes [83] and mammary [84] and bronchial [85] epithelial cells, is inhibited by serum or by the addition of pure platelet-derived TGFβ [10,74]. Although serum contains relatively large quantities of platelet-derived TGFβ [47], most of the TGFβ in serum is in an inactive form [43,80,81]. It is probable that the TGFβ in serum is responsible for the inhibitory effect of serum on epithelial cells [86]. It is of interest to note that many carcinoma cell lines grow well in serum, but are inhibited in their growth by active TGFβ [10].

These observations suggest that certain epithelial cells can activate the TGFβ present in serum and that at least some carcinoma cells have lost that capability. Since many cells, including epithelial cells [74], produce TGFβ in an inactive form, have receptors for TGFβ, and are capable of responding to active TGFβ, a major regulatory step in TGFβ action is probably at the level of activation of the inactive TGFβ precursor. If this is the case, the loss of the ability to activate TGFβ in cells that are normally inhibited by this molecule could lead to a growth advantage (Fig. 3).

Effects on Connective Tissue and Wound Healing

Recent studies by Roberts and coworkers [87] demonstrated that TGFβ induced a marked desmoplastic reaction (induction of angiogenesis and connective tissue formation) when injected into mice. Since TGFβ is abundant in platelets, its role in wound healing was soon appreciated [88]. Another growth factor of platelets, PDGF, enhances wound healing by increasing the proliferation of fibroblasts and the formation of granulation tissue [89]. These and other studies indicated that TGFβ might be involved in wound healing [90] and further suggested that the TGFβ released by carcinoma cells could contribute to the stromal cell proliferation necessary for the formation of large tumors. The data further suggested that TGFβ could play a major role in many disease states involving fibroblastic proliferation and collagen deposition. Potential mechanisms for augmented connective tissue formation are that TGFβ both enhances the expression of fibronectin and procollagen in cells [9] and regulates pericellular proteolysis by inducing the production of endothelial-type plasminogen activator inhibitors [91,92]. TGFβ-like factors that enhance the production of protein-

ase inhibitors are produced by 8387 human fibrosarcoma cells [93]. These kinds of factors may operate in the regulation of both normal and pathological proteolysis.

An interesting field of research with connections to wound healing is atherosclerosis. According to the response-to-injury hypotheses of Ross and coworkers [cf 94] an injury to the endothelium exposes the subendothelium at sites of turbulent blood flow; platelets become attached at these sites, aggregate and release PDGF. Given that TGFβ is also released from platelets [90] it may act directly or indirectly (via the induction of c-*sis*) in the pathogenesis of atherosclerosis. PDGF and TGFβ may participate in the formation of the atheroma plaques at least by stimulating the growth of smooth muscle cells [see 94]. PDGF enhances the transcription of the actin genes [95], and similar direct effects have recently been described for TGFβ [96]. The activation of the actin genes is probably an indication of the enhanced motility of cells and may explain in part the chemotactic effects of these growth factors [see 89,97].

Immunological Aspects

A potential role for TGFβ in the modulation of the immune response has recently been suggested. Mitogenic treatment of human T lymphocytes results in accumulation of TGFβ mRNA [98], and TGFβ appears to act as an antagonist of interleukin-2. In addition, Mizel et al [99] have shown the production of immunosuppressive factors by murine sarcoma virus (MSV)-transformed cells, and TGFβ inhibits the production of IgG and IgM [see 98]. These results suggest that TGFβ is capable of modifying immunological responses and might participate in the pathogenesis of different immunological diseases.

Effects on Bone Resorption

It has been suggested that growth factors such as EGF, TGFα, TGFβ and PDGF regulate bone resorption in animals [100,101]. This is possibly a cause of the hypercalcemia associated with many cancers [102]. The most important growth factor regulating bone resorption appears to be TGF∝, and it has been recently reported that it is more effective in this regard that EGF, indicating for the first time a difference in action for these closely related growth factors [103]. Interestingly, the tumor necrosis factors (TNFs) also have bone-resorbing activity, and may act in concert with TGF∝ in vivo [104].

TGFβ IN THE REGULATION OF DIFFERENTIATION

TGFβ appears to play a role in certain steps of cell differentiation. TGFβ inhibits insulin- and dexamethasone-induced differentiation of mouse 3T3 cells to adipocytes [105,106], and it also controls myogenesis [107]. On the other hand, it has been reported that it can enhance terminal differentiation of bronchial epithelial cells to squamous cells [108]. One of the functions of TGFβ appears to be to control the differentiation of BRL cells [58]. When the effects of SGFs (TGFα + TGFβ) on the developing tooth germ were studied in organ culture, it was found that they prevented morphogenesis and stimulated connective tissue formation and neovascularization [109]. The inhibition of tooth germ morphogenesis was probably due to the TGFβ inhibition of epithelial cell growth.

Available evidence thus suggests that TGFβ may, in concert with other growth factors, participate in various events of cell differentiation and morphogenesis and thus also have a function as a developmental protein.

CONCLUDING REMARKS

It is obvious that TGFβ has a multifaceted role in growth regulation. Both stimulatory and inhibitory properties have been reported. The ubiquitous nature and extreme evolutionary conservation of this molecule suggest that TGFβ plays a central role in mediating the cellular response to a variety of environmental stimuli. Characterization of the subsequent molecular response will undoubtedly expand our understanding of normal growth control as well as of cellular transformation.

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