# MINIREVIEW

# Role of Transforming Growth Factor–β in Tissue Injury and Repair

## (44066)

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*Abstract.* **Transforming growth factor-β (TGFβ) belongs to a family of multifunctional polypeptides which regulate normal cell growth, development, and tissue remodeling following injury. The ability of cells to produce TGFβ or to respond to this growth factor *via* cell surface receptors is highly conserved throughout the animal kingdom. TGFβ is a potent growth inhibitor of many normal and transformed cell lines; abnormalities in TGFβ signaling have been linked to tumorigenicity. Disruption of the TGFβ1 gene *in utero* produces a wasting syndrome characterized by systemic inflammation, suggesting that this growth factor plays an important role in limiting the inflammatory response. TGFβ is a dominant mediator of the pathologic extracellular matrix accumulation that characterizes progression of tissue injury to end-stage organ failure. Recent studies directed towards characterization of the TGFβ genes, dissection of the mechanisms by which TGFβs are produced and activated, and identification of TGFβ signaling pathways have established the important roles that these family members play in cell and tissue homeostasis. In this overview, TGFβ structure-function relationships and their relevance to a few select models of tissue injury/wound repair are highlighted.** [P.S.E.B.M. 1997, Vol 214]

Transforming growth factor–β's (TGFβ) are members of a superfamily of polypeptide factors that control development and tissue homeostasis in organisms from Drosophila to humans. Homologous peptides of the TGFβ superfamily can be grouped into four major families (1): (i) the MIS, or Müllerian inhibitory substance family, which includes MIS, a protein that regulates Müllerian duct regression in male embryos (2); (ii) the inhibin/activin family, which includes the inhibins, proteins that block follicle stimulating hormone release by pituitary cells (3), and activins, which stimulate FSH release by pituitary cells (4, 5); (iii) the Vg-related family, which contains a variety of members that regulate bone development (bone morphogenic proteins, or BMPs) (6), neural tube differentiation (dorsalin-1) (7), growth and differentiation (growth and differentiation factor 1, or GDF-1) (8), dorsal-ventral patterning in Drosophila (decapentaplegic transcript, DPP) (9), induction of mesoderm from ectoderm in Xenopus (the Vgl gene [10] and the murine Vg-1 homolog Vgr-1 [11]); and (iv) the TGFβ family, which contains the five known isoforms of TGFβ and is the subject of this review. Family members, thought to be derived from a common ancestral gene, share homologous positioning of at least seven cysteine residues (12). Each of these proteins is encoded as a larger precursor and, with the exception of MIS, is processed to a C-terminal monomeric unit of 100–134 amino acids (2).

The multifunctional peptide now called transforming growth factor–β1 was initially characterized as a growth-stimulating peptide capable of inducing anchorage independent growth in normal fibroblast cell lines (13). This activity

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was subsequently found to be a combined effect of two distinct peptides—TGFα, which has homology to epidermal growth factor, and TGFβ (14, 15). TGFβ was purified from human platelets (16), placenta (17), and bovine kidney (18), and characterized as a disulfide-linked homodimeric peptide with a molecular mass of 25 kDa. Five distinct TGFβ genes have been identified in vertebrates, and three of these (TGFβ1, -2, and -3) are expressed in mammals (12). The TGFβ isoforms are between 60% and 80% homologous to each other (12).

The human TGFβ1 gene maps to chromosome 19q13.1–q13.3 (19) and contains 7 exons (20). The cDNA sequence indicates that the monomer is synthesized as the carboxy-terminal 112 amino acids of a 390–amino acid precursor (21). TGFβ1 sequence is highly conserved: the amino acid sequence is identical in humans (21), monkeys (22), cows (23), pigs (24), and chickens (25), whereas there is a single amino acid substitution in the mouse peptide (26). TGFβ1 mRNA is approximately 2.4 kb, although there is evidence for alternate splicing in the porcine gene (27). Platelets are the most abundant human source of TGFβ1 (16).

The human TGFβ2 gene is found on chromosome 1q41 (28); the sequence of this gene product is also highly conserved (29, 30). Porcine, but not human, platelets containing TGFβ2 (31). One of two bone-derived factors capable of inducing cartilage production and a glioblastoma-multiforme–derived factor with potent immunosuppressive activity were subsequently identified as TGFβ2 (32, 33). Like TGFβ1, TGFβ2 is encoded as a precursor molecule (414 amino acids) which is cleaved to a 112–amino acid fragment with 71% homology to TGFβ1 (29, 34). TGFβ2 differs from TGFβ's 1, 3, and 4 in that it lacks the TGD matrix recognition sequence (arg-gly-asp) (29).

There is considerable overlap in the activity of TGFβ1, 2, and 3 in cultured cells (31). However, TGFβ1 has been reported to be more potent than TGFβ2 in inhibiting proliferation of hematopoietic progenitor cells (35, 36) and aortic endothelial cells (37, 38) and in deactivating macrophages (39), whereas TGFβ2 is more potent than TGFβ1 at inducing mesodermal differentiation markers in Xenopus embryo cells (40).

The gene encoding the third member of the TGFβ family, TGFβ3, is found on human chromosome 14q23–24 (28). Like the other TGFβ's, the amino acid sequence of TGFβ3 is highly conserved in many animal species, including humans (41), mice (42), chickens (43, 44), and pigs (41). The human TGFβ3 gene contains seven exons; the protein is encoded by a single mRNA species of 3.5 kb (43). The TGFβ3 precursor is 412 amino acids in length (45) and is cleaved to a 112–amino acid active peptide. High levels of TGFβ3 transcripts are observed during embryonic development (42, 46, 47). The intact human and porcine epidermis constitutively expresses TGFβ3; this isoform may play an important role in wound repair (48–50).

The other members of the TGFβ family, TGFβ4 and -5, have not yet been identified in mammalian tissues. TGFβ4 was isolated from a chick embryo chondrocyte library (46). The TGFβ4 precursor is only 304 amino acids in length and lacks a signal peptide. The active peptide contains a 2–amino acid insertion in the coding region, resulting in an active peptide of 114 amino acids rather than 112 amino acids (46). TGFβ5, isolated and characterized from a Xenopus laevis oocyte library (51), encodes a putative protein of 382 amino acids which is cleaved to a 112–amino acid peptide, typical of other members of the TGFβ family.

The crystal structure of TGFβ2 has recently been established (52, 53). Of the nine cystine residues in each monomer, eight residues form four intrachain disulfide bonds and one forms an interchain bond. Three of the four intrachain disulfide bonds are grouped to define a topological "cystine knot." This motif defines a new superfamily of homo- or heterodimeric growth factors, which include members of the nerve growth factor, platelet-derived growth factor, and human chorionic gonadotropin families (reviewed in Ref. 54). Despite the structural similarity, there is minimal amino acid sequence homology among these families.

## TGFβ: Structure-Function Relationships

Since many cells express mRNA for one or more forms of TGFβ (41) and virtually all cells possess receptors for TGFβ (55), production and activation of TGFβ must be tightly regulated. Proposed mechanisms for TGFβ regulation, outlined in Figure 1, include transcriptional regulation of the TGFβ1 genes, stability of TGFβ mRNA, translation of mRNA, storage of TGFβ within platelet α-granules, activation of latent TGFβ, and inactivation of TGFβ by circulating proteins and extracellular matrix macromolecules

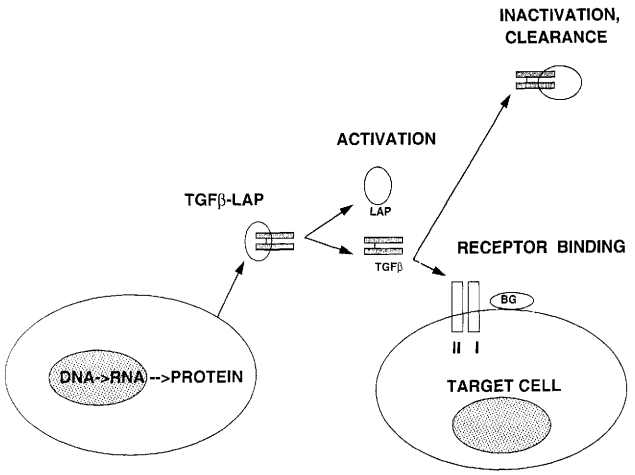


**Figure 1.** Potential mechanisms for TGFβ regulation. The promoter regions of the various TGFβ isoforms interact with distinct nuclear binding proteins to activate transcription of the TGFβ genes. TGFβ production may also be regulated by postranscriptional mechanisms. TGFβ is secreted as a latent complex (TGFβ-LAP), which must be activated prior to receptor binding. I and II refer to TGFβ receptor subtypes. Accessory molecules such as betaglycan (BG) facilitate binding of active TGFβ to its receptor. TGFβ may be inactivated and cleared after binding to serum proteins, including $\alpha_2$-macroglobulin.

(12). Since transcriptional activation of the TGFβ genes, activation of latent TGFβ, and receptor-ligand interactions have been shown to play an important role in pathophysiologic states, these processes will be emphasized.

**Transcriptional Regulation.** Abnormal expression of TGFβ1 is a characteristic finding in a variety of fibrotic disorders involving multiple organ systems, including the kidney, liver, lung, skin, and vessels (reviewed in Ref. 56). Transcriptional activation of the TGFβ genes is an important tissue response to injury. TGFβ isoforms appear to have unique promoter elements that are selectively activated by particular developmental and/or environmental signals (42, 57, 58).

In several normal and transformed cell lines, TGFβ1 is capable of positively regulating its own expression (59). Characterization of the TGFβ1 promoter (60) has led to identification of sequences that confer response to TGFβ (61). There are two transcriptional start sites for human TGFβ1 mRNA, 271 nucleotides apart (60). Two distinct regions of the TGFβ1 promoter have been identified, which are responsive to phorbol esters and to autoinduction by TGFβ1 (60, 62). The TGFβ1 promoter contains no TATA box or CAAT box, is extremely G-C rich, and contains numerous CCGCCC repeats, two of which bind the transcription factor Sp1 (62). Gel mobility shift studies have demonstrated that major transcription factor–binding sites responsible for TGFβ1 induction (by phorbol ester or by TGFβ1) include several regions that bind the AP-1 complex (63). Enhanced *jun* expression in an early genomic response to TGFβ1 stimulation (64); both TGFβ and *jun* expression are mediated by an AP-1 binding site (64, 65).

The TGFβ2 and TGFβ3 promoters have little sequence homology to the TGFβ1 promoter (58). The TGFβ2 promoter differs from the TGFβ1 promoter in that it contains three TATA-like sequences but lacks a functional AP-1 site (66). The TGFβ2 gene contains multiple transcript initiation sites; a cyclic AMP-responsive element (CRE) binds nuclear proteins and increases transcriptional activity of the promoter (67, 68).

The TGFβ3 promoter contains a TATA box 21 base pairs upstream from the transcription site. A cAMP-responsive element (CRE) and an AP-2 binding site are found near the TATA box. The cAMP-responsive element is important for both basal and forskolin-induced expression of the TGFβ3 gene (57). An additional sequence containing three consecutive TCCC repeats may, in some cells, regulate the developmental and tissue-specific expression of TGFβ3 (69). The transcription factor Sp1 stimulates TGFβ1 and TGFβ3 transcription, but not TGFβ2 (70).

**Latency.** TGFβ is secreted as an inactive, high–molecular weight precursor complex (71–74). Since the receptors for TGFβ are almost universally expressed by cells (75), local activation of latent TGFβ may be important in regulating cellular responses to this molecule.

The TGFβ1 gene encodes a 390-amino acid precursor molecule, which contains a signal peptide, the active TGFβ molecule, and the latency associated peptide (LAP) (76) (Fig. 2). Following removal of the signal peptide (amino acid residues 1–29), the gene product undergoes proteolytic cleavage between two arginine residues at positions 278 and 279 (77) to produce mature TGFβ1 (residues 279–390) and the LAP (residues 30–278) (78). Prior to secretion, disulfide-linked homodimers of TGFβ1 noncovalently associates with homodimers of LAP to produce the small latent TGFβ complex, which is inactive (74, 79). The LAP is apparently necessary for correct folding of TGFβ1 during synthesis (80). Purified LAP is capable of associating with and inactivating mature TGFβ (76), presumably by rendering the resulting complex incapable of binding the TGFβ receptor (75).

The TGFβ latent complex isolated from platelet α granules or from several cultured cell lines consists of a large latent TGFβ complex, which contains the small latent complex and an additional 125- to 160-kDa protein, the latent TGFβ–binding protein (LTBP) (81–83). The LTBP contains multiple epidermal growth factor–like repeats (83) and binds the small latent complex by disulfide bonds. Although LTBP is not required for latency, this additional protein may facilitate secretion of the large latent complex (82).

TGFβ can be released from latent complexes and thereby activated by transient acidification (pH < 3.5), alkanization (pH > 12.5), exposure to 0.02% sodium dodecyl sulfate or 8 *M* urea, but not by exposure to high concentrations of NaCl (5 *M*) (82, 84). Osteoclasts are capable of activating bone-derived latent TGFβ *in vitro* (85). Latent TGFβ produced by fibroblasts may be activated by proteases such as plasmin and cathepsin D (84).

Carbohydrate structures may also play a role in TGFβ

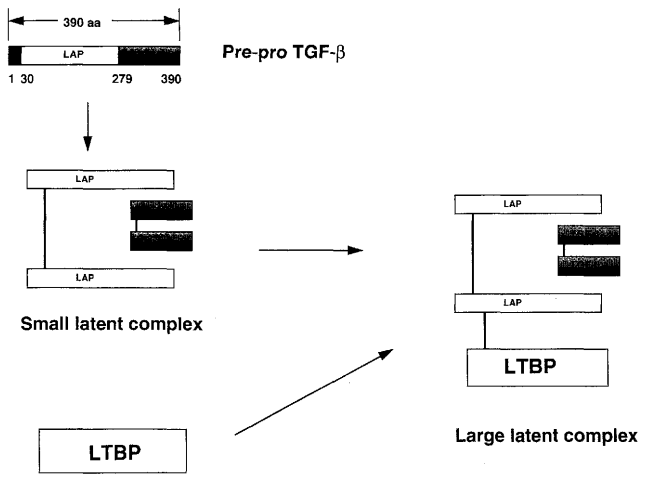


**Figure 2.** Latent TGFβ1 complexes. TGFβ1 is synthesized as a 390–amino acid (aa) precursor peptide (top), which contains the signal peptide (amino acid residues 1–29), the LAP (residues 30–278), and TGFβ (residues 279–390). The small latent complex consists of TGFβ homodimer, noncovalently associated with the homodimeric form of LAP (middle). Thick lines indicate the presence of disulfide bonds (number and position of disulfide bonds not drawn to scale). In some cells, the small latent complex associates with an additional 125- to 160-kDa protein, the latent TGFβ-binding protein (LTBP), to form the large latent complex.

activation. Latent TGFβ contains mannose 6-phosphate (86) and can bind cell surface mannose 6-phosphate receptors (87). Glycosidase treatment leads to deglycosylation of the LAP and activates latent TGFβ, without affecting the TGFβ1 homodimer or the LTBP (88). Sialic acid and mannose 6-phosphate activate latent TGFβ1, presumably by displacing active TGFβ1 from the carbohydrate structures present in the latent complex (88). Similar effects are observed following sialidase treatment (88).

Once activated, TGFβ1 binds other serum or matrix proteins. In plasma, essentially all TGFβ is complexed to $\alpha_2$-macroglobulin (88, 90). When injected into rats, clearance of circulating active TGFβ1 is rapid, with a half-life of 2.2 min (91). However, it is not clear whether (92) or not (93) the $\alpha_2$-macroglobulin–TGFβ complex is cleared *via* the hepatic $\alpha_2$-macroglobulin receptor. Free TGFβ can associate with fibronectin; this complex is active (94). TGFβ1 binds soluble and cell-associated forms of betaglycan (95); this interaction may protect TGFβ from degradation or may facilitate TGFβ clearance.

**TGFβ Receptors.** A large number of distinct high-affinity cell-surface binding receptors for TGFβ have been identified, in both normal and in transformed cell lines (55, 75). Characteristics of these binding proteins are summarized in Table I. Although these receptors possess binding constants in the low nanomolar range (1), many of the members of this group are not involved in signal transduction and may serve to prevent degradation of active TGFβ or to clear active TGFβ.

An overview of TGFβ receptor binding and activation is provided in Figure 3. Both TGFβ receptor type I and type II are required for signal transduction (101, 102). Chemically mutagenized mink lung epithelial cells no longer responsive to the growth-suppressive effects of TGFβ have defects of the TGFβ type I and/or type II receptor. Using somatic cell hybridization, TGFβ response can be restored by using appropriate complementation groups, leading to expression of both type I and type II receptors on hybrid cells (1). Homo-oligomeric complexes of TGFβ receptor type I or type II are not capable of signal transduction (103).

The TGFβ type I and type II receptors are unique in that they function as membrane-bound serine/threonine protein kinases (1). Recent studies have led to the hypothesis that TGFβ signaling begins with ligand binding to the TGFβ type II receptor. The type II receptor then associates with and phosphorylates the cytoplasmic domain of the type I receptor (104). Phosphorylation of the type I receptor by the type II receptor is necessary for signaling (105). The activated type I receptor then phosphorylates cytoplasmic proteins, which transduce the signal into the nucleus (106). Although, in many cell systems, the relative effects of TGFβ1, -2, and -3 are similar, the type I/type II receptor complex binds TGFβ1 and TGFβ3 with higher affinity than TGFβ2 (1).

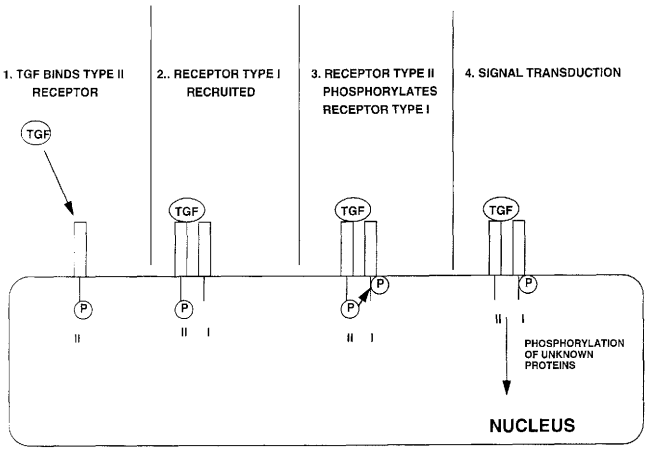


**Figure 3.** Overview of TGFβ receptor binding and signaling. Binding of active TGFβ to the type II receptor (1) leads to recruitment of the type I receptor (2). The type II receptor then phosphorylates the type I receptor (3), leading to phosphorylation of unknown cytoplasmic proteins (4).

In many cell lines, the most abundant cell surface receptor for TGFβ is betaglycan (107–109). Betaglycan is a polymorphic proteoglycan that binds TGFβ1, -2, and -3 with equal affinity (95, 110). Betaglycan presents TGFβ to the kinase subunit of the signaling receptor, enhancing cellular responses to TGFβ (111). Non–membrane-bound forms of betaglycan are also released by cultured cells into the medium (95) and may play a role in sequestration of active TGFβ (112).

**Table I.** TGFβ Binding Proteins

| Receptor type | Characteristics | Reference |
|---|---|---|
| Type I | 65 kDa; dimerizes with type II receptor; signal transduction | 1 |
| Type II | 85–110 kDa; dimerizes with type I receptor; binds TGF-β | 1 |
| Betaglycan (formerly called type III receptor) | 280–320 kDa<br>120–140 kDa (core protein) | 96 |
| GH3 (formerly called type IV receptor) | 64 kDa; binds TGFβ1, TGFβ2, activin, inhibin | 97 |
| Endoglin | Dimer, 95-kDa monomers | 98 |
| Phosphatidylinositol-linked binding proteins | 100, 180 kDa | 99 |
| TGFβ1 binding | | |
| TGFβ2 binding | 60, 140 kDa | |
| G1-260 | 260, 170, 85 kDa; isolated from rat glomeruli | 100 |

The remaining TGFβ receptors described in Table I have a more limited tissue distribution than TGFβ receptor types I, II, or betaglycan. For example, the type IV receptor is found only on GH3 pituitary cells (97). None of these receptors are thought to function as a signaling receptor (1). Like betaglycan, they may be involved in the presentation of TGFβ to the type I/type II receptor complex or the sequestration/clearance of active TGFβ. The inhibitory effect of heparin on vascular smooth muscle proliferation may be due to the ability of heparin to bind TGFβ1 (113).

**TGFβ Signal Transduction.** Since TGFβ inhibits proliferation of many normal and transformed cell lines, there has been great interest in dissecting the TGFβ signaling pathway, with the goal of identifying intermediates that may act as tumor suppressor gene products (114). Recent studies have utilized the yeast two-hybrid screen system to identify cytoplasmic proteins that interact with the TGFβ receptors. The TGFβ type I receptor interacts with at least two proteins, including the immunophilin FKBP12 (115) and farnesyl transferase-α (116, 117), whereas the TGFβ type II receptor interacts with TRIP-1 (118). However, the functional significance of these interactions in mammalian systems is not yet clear.

In growth inhibited epithelial cells, TGFβ induces $p44^{mapk}$, a component of the mitogen activated protein kinase (MAPK) pathway. It is hypothesized that autocrine production of TGFβ following $p44^{mapk}$ activation is responsible for suppression, rather than induction, of mitogenesis in responsive cells (199). Genetic selection studies based on the yeast MAPK pathway have led to the identification and characterization of a murine protein kinase, TAK1 (TGFβ-activated kinase 1), which is a distinct member of the MAP kinase kinase kinase (MAPKKK) family (120). More recent studies have led to the identification of human TAB1 (TAK1-binding protein), a gene product that may function as an activator of the TAK1 MAPKKK in TGFβ signal transduction (121). The general role of these recently identified proteins in TGFβ signal transduction and the mechanism by which cellular signals resulting from TGFβ-ligand interactions at the cell surface are conveyed to the nucleus clearly awaits further investigation.

## Effects of TGFβ

TGFβ has a wide variety of effects on cell and tissue differentiation, growth, and response to injury. An incomplete summary of the many effects of TGFβ is shown in Table II. Isoforms of TGFβ are widely expressed early in embryogenesis and exhibit both cell- and stage-specific patterns in all three germ layers (126). *In vitro,* TGFβ both promotes and inhibits the differentiation of pluripotent cells into mesenchymal cells (122–125).

**Immunosuppression.** TGFβ has potent immunosuppressive properties. An agent capable of suppressing T cells, isolated from human glioblastoma multiforme cells, was identified as TGFβ2 (33). TGFβ inhibits the activation and proliferation of immune cells (128, 129) and can inhibit the effector function of differentiated immune cells (129–131). The immunosuppressive actions of TGFβ1 are demonstrated in murine models of autoimmune disease, including collagen-induced arthritis (157), and chronic relapsing experimental allergic encephalomyelitis (158). In experimental allergic encephalomyelitis models, both TGFβ1 and TGFβ2 are protective (158, 159), and administration of an anti-TGFβ antibody exacerbates the disease process (160). The anti-inflammatory role of TGFβ is dramatically illustrated in "knock-out" models, in which the TGFβ1 gene is disrupted and the animals rapidly die with systemic inflammation (161, 162).

**Table II.** Effects of TGFβ

| Effect | Reference |
|---|---|
| Differentiation | |
| Myocyte: inhibits | 77, 122, 123 |
| Adipocytes: inhibits | 124 |
| Chondrocyte: stimulates | 125 |
| Embryogenesis: mouse | 126, 127 |
| Immune system | |
| Immunosuppression (TGFβ2) | 33 |
| T-cell proliferation: inhibits | 128 |
| B-cell proliferation: inhibits | 129 |
| Antibody production: inhibits | 129 |
| Natural killer cells: inhibits | 130 |
| Lymphokine-activated killer cells: inhibits | 131 |
| Eosinophils: decreases survival | 132 |
| Directs heavy chain class switch to IgA, IgG2B | 133 |
| Proliferation | |
| Epithelial cells: inhibits | 134, 135 |
| Osteoblast: inhibits | 136 |
| Hepatocytes: inhibits | 137 |
| Mesangial cells: biphasic | 138 |
| Fibroblasts: biphasic | 139 |
| Matrix synthesis | |
| Fibrillar (type I) collagen | 140, 141 |
| Basement membrane (type IV) collagen | 142 |
| Fibronectin | 143 |
| Integrins: stimulates | 144–146 |
| Matrix remodeling | |
| Plasminogen activator: inhibits | 147 |
| Plasminogen activator inhibitor: stimulates | 147–149 |
| Stromelysin and collagenase: inhibits | 150, 151 |
| Tissue inhibitor of metalloproteinase: stimulates | 151 |
| Elastase: inhibits (?) | 152 |
| Transin: stimulates | 153 |
| Inflammation | |
| Chemotactic for monocytes | 154 |
| Monocyte TNF, IL-1 production: stimulates | 155 |
| Chemotactic for fibroblasts | 156 |

**Proliferation.** The role of TGFβ in regulation of cell proliferation has been intensely studied. TGFβ has complex effects on cell division, with both stimulatory and inhibitory effects depending upon cell type, TGFβ concentration, cell

density, and the cellular microenvironment, including the presence of other growth factors and of extracellular matrix macromolecules. In general, TGFβ inhibits proliferation of epithelial cells (134, 135) and endothelial cells (163–165). TGFβ is capable of antagonizing the effect of several mitogens that act through a tyrosine kinase receptor such as EGF, PDGF, FGF, and IGF-1 (166). The potent immunosuppressive activity of TGFβ is, in part, mediated by inhibition of lymphocyte proliferation (128, 129).

The effects of TGFβ on the cell cycle are highlighted in Figure 4. TGFβ inhibits proliferation by activating at least two distinct inhibitors of cyclin-dependent kinases, which are necessary for phosphorylation of the retinoblastoma (Rb) gene and for cell cycle progression from G1 to S phase (167, 168). Mammalian cells contain several cyclin-cyclin–dependent kinases which are activated during G1 phase of the cell cycle, including cyclin E-Cdk2, cyclin D-Cdk4, and cyclin D-Cdk6 (169, 170). TGFβ stimulates the production of p15Ink4B, a nuclear protein which binds to and inhibits the activity of the cyclin D-Cdk4,6 complex (171). The promoter of the p15Ink4B gene contains several Sp1 consensus sites, which appear important in transcriptional activation of this gene by TGFβ (172).

The nature of the second TGFβ-induced cyclin-dependent kinase inhibitor varies by cell type, but the target is Cdk2 (167). In mink lung epithelial cells, p15Ink4B (induced by TGFβ) promotes the redistribution of the universal Cdk inhibitor, p27Kipl, from Cdk4 and Cdk6 to Cdk2, leading to inactivation of the cyclin E-Cdk2 complex (167, 173). *In vitro*, p27Kip1 inhibits Rb phosphorylation by cyclin E-cdk2, cyclin A-cdk2, and cyclin D2-Cdk4 (174). In epithelial cells, TGFβ stimulates the production of p21Cip1, which binds and inactivates Cdk2 (167, 175). The role of cyclin-dependent kinase inhibitors in specific cell systems awaits further definition. For example, three groups have disrupted the p27 gene in "gene knock-out" mice. Although organ hyperplasia, pituitary tumors, and infertility in females were noted (176–178), TGFβ was capable of inducing cell arrest in T cells and embryonic fibroblasts from p27 –/– animals, suggesting that p27kip1 is not essential for the inhibitory effect of TGFβ on the cell cycle (176).

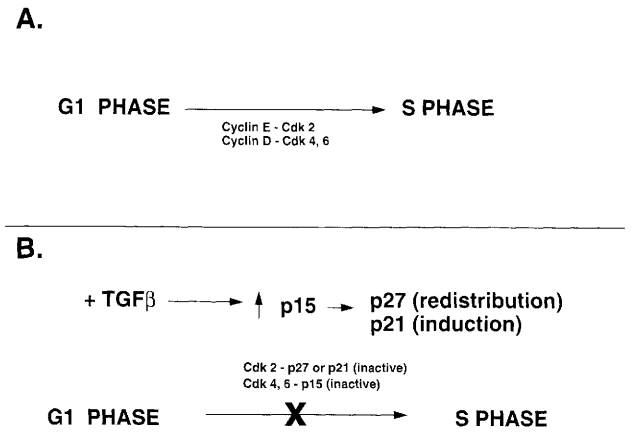


**Figure 4.** Role of TGFβ in cell cycle inhibition. In the cell cycle, G1 → S phase progression is associated with activation of Cyclin D-Cdk4,6 and Cyclin E-Cdk2, leading to phosphorylation of the retinoblastoma (Rb) gene product. TGFβ stimulates the production of p15, which directly inactivates Cdk4 and Cdk6. Depending on cell type, p15 induces p21 or facilitates redistribution of p27 from Cdk4 and Cdk6 to Cdk2. Both p21-Cdk2 and p27-Cdk2 are inactive.

In some situations, the effects of TGFβ on proliferation may not be inhibitory. In mesenchymal cells, more complex, biphasic responses to TGFβ have been described (138, 139, 179). In general, these complex responses are secondary to induction of other cellular responses by TGFβ. Factors contributing to these responses include cell density (138), age of donor from which fibroblast cultures are obtained (139), TGFβ-mediated stimulation of PDGF production in fibroblasts (180), and TGFβ-mediated stimulation of extracellular matrix production (181).

The observation that TGFβ induces anchorage-independent growth of rat kidney fibroblasts has led to speculation that this growth factor may play a role in carcinogenesis. Further studies showed that most tumor cells express TGFβ mRNA (182) and many secrete TGFβ. Many tumor cells and cell lines are growth inhibited by TGFβ (183–185). During carcinogenesis, cells may lose their sensitivity to negative growth regulation by TGFβ; mechanisms may include failure to synthesize, process, or release TGFβ; loss of receptors for TGFβ; loss of ability to activate latent TGFβ; or a failure in the intracellular TGFβ signaling pathway (134, 186). Deletion of p15Ink4B, a TGFβ-regulated cyclin-dependent kinase inhibitor, is a common finding in human bladder tumors (187). As the TGFβ signal transduction cascade is further characterized, it is likely that additional tumor suppressor genes will be identified.

**Apoptosis.** The role of TGFβ in cell-cycle regulation is not limited to proliferation. In several growth-inhibited cell systems, including endothelial cells (188), endocervical epithelial cells (189), hepatocyes (190), and B lymphocytes (191), TGFβ is capable of triggering apoptosis. The precise mechanism for this effect has not yet been determined. The observation that TGFβ induces apoptosis in tumor cell lines, including gastrointestinal (192), ovarian (193), and breast (194), may prove to have therapeutic implications. Tamoxifen, an anti-estrogenic compound widely used as adjunctive therapy for patients with breast cancer, induces apoptosis in breast cancer cells, perhaps through stimulation of TGFβ production (194).

**Extracellular Matrix Production.** TGFβ promotes extracellular matrix deposition through several distinct mechanisms: increased production of matrix macromolecules, increased synthesis of protease inhibitors, and inhibition of extracellular matrix proteases. TGFβ1 stimulates fibrillar collagen (collagen type I) mRNA expression and protein synthesis by a variety of fibroblast cell lines (141, 143, 195, 196) and by tissue fibroblasts *in vivo* (140). Steady-state collagen I mRNA in TGFβ1-treated cells is increased by transcriptional and, in some cell systems, by post-transcriptional mechanisms (197, 198). Cloning of the promoters for the α1 and α2 collagen type I genes has led

to the identification of several elements that confer response to TGFβ, including an NF-1 site upstream of the mouse α2 (I) collagen I promoter (195) and a TGFβ response element (TbRE) containing an Sp-1–binding site within the human α2 (I) collagen I promoter (199). The α1 (I) collagen I gene also contains a TGFβ responsive element with an Sp-1–binding site (196).

TGFβ promotes the production and deposition of type IV collagen, the predominant collagen type found in basement membranes (142, 200). TGFβ increases collagen IV mRNA levels by stimulating transcription of the collagen IV genes (142). Although the collagen IV promoter has been isolated from human (201), mouse (202), and rat (203), sequence elements responsive to TGFβ have yet to be defined. In addition to collagen types I and IV, TGFβ stimulates the production of collagen type II, the predominant collagen type found in cartilage (204), collagen type III, a fibrillar collagen that frequently associates with collagen type I (205), collagen type V (206), and collagen type VII, a collagen type found in anchoring fibrils of the skin (207). TGFβ stimulates the production of a wide variety of other extracellular matrix macromolecules, including fibronectin (143, 197, 205), thrombospondin (197), osteopontin (208), tenascin (209), elastin (210), and proteoglycans (211), including decorin (212). The proteoglycans betaglycan (12) and decorin (212) may play a role in the binding and inactivation of TGFβ.

TGFβ inhibits degradation of the extracellular matrix by inhibiting production of several different proteases and by stimulating the production of specific protease inhibitors. For example, TGFβ stimulates the production of plasminogen activator inhibitor (147) and tissue inhibitor of metalloproteinase (151) but decreases the synthesis of plasminogen activator (147), stromelysin, and collagenase (150, 151). These effects are brought about both by changes in gene transcription and changes in mRNA stability.

In addition to the well-established role of TGFβ in promoting extracellular matrix deposition, TGFβ modulates cell-matrix interactions by regulating expression of cell-matrix adhesion protein receptors. TGFβ-treated cells have an increased affinity for fibronectin and collagen (141); this effect is due to induction of integrin expression by TGFβ (146). Integrin subunits induced by TGFβ include those that mediate cell-matrix adhesion and those that mediate cell-cell adhesion (144, 145). These interactions play an important role in tissue remodeling following injury.

## Role of TGFβ in Select Models

**TGFβ Null Mutations.** Insight regarding the important role of TGFβ1 in growth and development has been obtained from gene knock-out studies (161, 162). Approximately 50% of TGFβ1-null mice die before birth. The primary cause of prenatal lethality is defective endothelial and hematopoietic development, particularly within the yolk sac (213, 214). The other 50% of TGFβ1-null mice are born without any apparent developmental abnormalities. These mice grow normally for approximately 2 weeks, after which they develop a wasting syndrome characterized by an extensive systemic inflammatory response and death by 3–4 weeks of age (161, 162). The inflammatory infiltrate consists of lymphocytes and macrophages and is found in many organs, with heart (endocarditis, myocarditis) and lungs (endothelialitis, interstitial pneumonia) most severely affected. Lymph nodes and spleen contain proliferative lesions, with immunoblasts and lymphocytes in both B- and T-cell zones. The lesions are similar to those described in a variety of autoimmune disorders, graft-versus-host disease, or some viral diseases. A number of autoantibodies have been identified in TGFβ knock-out mice (215), including those directed against DNA and ribonucleoproteins. These studies suggest that TGFβ plays a critical role in homeostatic regulation of immune cell proliferation and extravasation into tissues.

TGFβ1 (–/–) mice have a 3- to 5-fold increase in epidermal labeling index compared with normal mice (162), indicating that TGFβ1 plays an important role in negative regulation of keratinocyte growth.

Although skeletal architecture is grossly normal in TGFβ1 (–/–) mice, histologic analysis revealed modest reduction in the thickness of proximal tibial growth plates, with irregular chondrocyte columns (162). Other abnormalities in extracellular matrix structure or function have not been observed in TGFβ1-null mutants. However, recent studies have demonstrated that TGFβ1-null mice can receive maternal TGFβ1 *via* the placenta, which may affect embryogenesis and early development (216).

**Transgenic Models.** The role of persistent overexpression of TGFβ has been studied with the use of transgenic animals, in which the TGFβ gene coupled to a tissue-specific gene is introduced into mouse embryos. For example, pancreatic islet cell overexpression of TGFβ coupled to an insulin promoter leads to chronic pancreatitis and fibrosis of the pancrease (217). When the TGFβ gene is linked to the glial fibrillary acidic protein gene to target the gene product to astrocytes, central nervous system expression of laminin and fibronectin is increased, and the animals develop hydrocephalus (218). Osteoblast-specific overexpression of TGFβ linked to an osteocalcin promoter is associated with widespread bone loss with osteoblastic matrix deposition and osteoclastic bone resorption (219). Hepatic overexpression of a TGFβ transgene leads to the development of multiple tissue lesions, including hepatic fibrosis, glomerulonephritis, arteritis, myocarditis, and atrophy of the pancreas and testis (220).

**Wound Repair Models.** Wound repair is a tightly regulated process which involves the inflammatory response, proliferation of fibroblasts and transformation of fibroblasts into myofibroblasts, and extracellular matrix synthesis. TGFβ plays an important role in all of these processes. There are abundant sources of TGFβ in injured tissue; platelets store TGFβ in α-granules (221), and activated macrophages (155) and lymphocytes (128) can pro-

duce TGFβ. Since the TGFβ1 promoter is responsive to autoinduction by TGFβ, platelets and inflammatory cells may stimulate transcription of this gene in regenerating epidermal and dermal cells (222). TGFβ1 is a potent chemoattractant for monocytes (154) and fibroblasts (156), and may promote influx of these cells to the site of injury.

Through the use of isoform-specific antibodies, TGFβ1, -2, and -3 are identified in epidermal and dermal cells during excisional wound repair; TGFβ2 and -3 expression in migrating epithelium is greater than that of TGFβ1 (49). Exogenous administration of TGFβ1 to wounded animals stimulates collagen fibrillogenesis at the site of injury (223–225) and thereby promotes wound healing. However, persistent expression of TGFβ1 induces tissue fibrosis; examples of this phenomenon have been well described in several models of glomerulonephritis.

**Renal Disease Models.** Abnormal deposition of extracellular matrix macromolecules is a predominant morphologic manifestation of progressive renal disease. Glomerulosclerosis, characterized by expansion of the mesangial matrix, by the development of a thickened and irregular glomerular basement membrane, and by obliteration of glomerular capillaries with loss of filtration surface area (226), is the hallmark of irreversible renal damage. When TGFβ was initially isolated and characterized, bovine kidney was found to be an abundant source of this peptide (18). Therefore, the relationship between abnormal TGFβ production and extracellular matrix deposition in the kidney has been the focus of many studies. Compared with whole kidney, glomeruli express high levels of TGFβ1, with lower levels of TGFβ2 (227). Glomerular mesangial cells are a major source of renal TGFβ, but over 96% of total TGFβ is released in a latent form (228). As in other tissue systems, additional sources of TGFβ during renal inflammation include platelets (221), macrophages (155), and lymphocytes (128). Glomerular endothelial, mesangial, and epithelial cells possess high-affinity receptors for TGFβ (138). TGFβ inhibits the proliferation of glomerular epithelial cells, whereas more complex, biphasic effects of this growth factor are observed on mesangial cells (138). As in other cell systems, TGFβ stimulates glomerular cell production of extracellular matrix macromolecules, including collagen IV (138), fibronectin (138), and proteoglycans (229). TGFβ also inhibits glomerular plasminogen activator synthesis and stimulates the production of plasminogen activator inhibitor (230).

Several *in vivo* glomerulonephritis models have been utilized to firmly establish the role of TGFβ in progression of renal injury. In one widely studied model, an antibody directed to the Thy-1 antigen, expressed on mesangial cells, leads to a complement-dependent mesangiolysis, followed by cytokine-mediated mesangial cell proliferation and matrix production (231, 232), which resolves within several weeks. In this model, elevated expression of TGFβ was temporally associated with the increased matrix deposition (233); an anti-TGFβ antibody blocked the matrix deposition (234). Decorin, a proteoglycan capable of binding and inactivating TGFβ, also was able to block extracellular matrix deposition following induction of Thy 1.1 glomerulonephritis (235). Animals given a second dose of antibody directed against the Thy-1 antigen 1 week after the first dose develop progressive kidney fibrosis (236). This progressive glomerular lesion is associated with persistent expression of TGFβ1 mRNA and protein (236).

Increased production of active, but not total, TGFβ is also observed in anti-glomerular basement membrane disease in rabbits (237), and is associated with extracellular matrix deposition during disease progression (238, 239). *In vivo* transfection of the TGFβ1 gene into normal rat kidneys leads to increased production of TGFβ1 in glomeruli and to the rapid development of glomerulosclerosis (240). TGFβ produces similar lesions when administered to animals *via* an osmotic minipump (224). High glucose levels, characteristic of diabetes mellitus, induce transcription of the collagen genes (241); glucose also stimulates TGFβ gene expression (242). Other renal diseases associated with increased TGFβ expression include obstructive uropathy (243, 244) and IgA nephropathy (245, 246). The number of progressive inflammatory and noninflammatory renal diseases associated with abnormal TGFβ production and/or activation continues to increase, suggesting that persistent expression of TGFβ represents the "final common pathway" leading to end-stage renal disease (Fig. 5).

## Summary and Future Directions

TGFβ plays a critical role in cell and tissue growth, development, and differentiation. In adult tissues, TGFβ promotes homeostasis by regulating cell proliferation and in balancing extracellular matrix production and degradation. During wound repair, the inhibitory effects of TGFβ on inflammatory and immune effector cells may be important in limiting the magnitude of response to tissue injury. Transient TGFβ production may therefore promote wound healing and restitution of tissue architecture. Persistent expression and/or activation of TGFβ, due to repeated tissue injury or to a failure of effector cell clearance, may lead to fibrosis and end-stage organ failure. To intervene in this process,

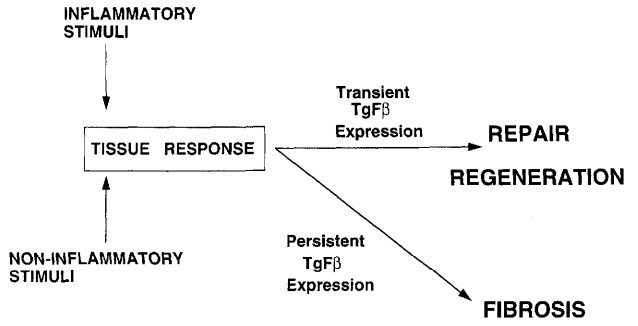


**Figure 5.** TGFβ expression following tissue injury. Transient TGFβ expression may promote tissue repair, whereas persistent TGFβ expression due to ongoing or unresolved tissue injury may lead to organ failure and fibrosis.

mechanisms regulating TGFβ gene expression, activation of latent TGFβ, and TGFβ clearance need to be more clearly defined. The wide range of biologic actions of TGFβ suggests many potential therapeutic applications of TGFβ agonist or antagonist therapy.


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