



Transforming Growth Factor- β (TGF- β) - Mini Review

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The transforming growth factor- β (TGF- β) gene family expresses a set of structurally and functionally related polypeptides (1-7). TGF- β s have been shown to regulate many cellular processes in a wide variety of cells and tissues, including fibroblasts, epithelium, bone and extracellular matrix (ECM) (2). TGF- β has been implicated in cell growth and division, migration, tissue repair, inflammation, angiogenesis, metastasis and tumorigenesis (1-7).

Three TGF- β highly homologous isoforms regulated by specific genes have been identified in mammals – TGF- β 1, TGF- β 2 and TGF- β 3 – and the three proteins share ~70% identical amino acid sequence (38). There are nine strictly conserved cysteine residues in each monomer (14). Eight of these form four intra-chain disulphide bonds which are clustered in a core region to form a 'cysteine knot' folding motif (14). The ninth cysteine forms an inter-chain disulphide bond, which along with two identical hydrophobic interfaces, stabilizes the TGF- β dimer.

While TGF- β 1 appears to play the major role, all three isoforms are observed during development and appear to display overlapping patterns of expression (2). Each isoform plays a distinct role, which appears to depend on the cell type, its state of differentiation and growth conditions, and especially on which other growth factors are present (2).

TGF- β 1 is also the prototype for a superfamily of over 40 different related bone morphogenic proteins (BMPs), presumed to derive from a common ancestral gene (23,34).

TGF- β Proteins

Each of the three human isoform genes encodes a dimeric pro-protein. Mature dimeric TGF- β is proteolytically cleaved from the C terminus of the precursor protein (15), but remains non-covalently bound (and therefore unable to react with TGF- β receptors) to its pro-protein, also known as the latency associated peptide (LAP), to form the small latent complex (SLC). Thus mature TGF- β , a 24 kDa homodimer, is non-covalently associated in an SLC with its 80 kDa LAP. LAP is required for the efficient secretion of TGF- β from cells, and along with the TGF- β binding proteins, it maintains TGF- β availability in a large extracellular reservoir that is readily accessible by activation, while preventing it from attaching to ubiquitous cell surface receptors.

TGF- β Binding Proteins (LTBPs)

Like other growth factors, TGF- β has binding proteins that regulate its distribution, function and extracellular activation in various tissues and body fluids, thereby modulating its metabolic effects (5).

There appear to be 4 isoforms of latent TGF- β binding proteins (LTBP-1 to -4), a family of extracellular glycoproteins that also includes the ECM proteins fibrillin-1 and -2.

The LTBPs are multi-domain proteins with a common, highly repetitive structural organization. They all contain three 8-cys repeats (37), a hybrid domain and a hinge domain which is proteolytically sensitive. The three 8-cys repeats are separated by calcium and non-calcium binding EGF-like repeats. These EGF-like repeats are thought to form a rigid sequence to separate the C-terminal (SLC-binding) region away from the matrix binding N-terminal domain, thus facilitating the interaction of the SLC with its activators (5).

Three of the LTBPs (LTBP-1,-3,-4) are involved in the sequestration of the SLC to form a large latent complex (LLC) (5) and thus target TGF- β availability to various extracellular structures. For example, LTBP-1 is involved in the sequestration of TGF- β and its storage as LTGF β (MWt 170 or 230 kDa) (36) in the ECM. LTBP-1, -3 and -4 are thought to bind covalently to the TGF- β pro-peptide via di-sulphide bonds between cysteine residues in the third 8-Cys repeat of LTBP-1 and Cys 33 in the pro-peptide (14,17,22,29).

Recently, purified recombinant LTBP-2 has been shown not to bind SLC (40). LTBP-2 is thought to be involved in cell adhesion and migration, as LTBP-2 has an RGD (putative integrin binding site) sequence in the N-terminal region. Melanoma cell lines have been shown to bind very efficiently to LTBP-2 in a concentration-dependent manner (40). This attachment can be prevented by β 1, α 3 and α 6 integrin antagonists and heparin (40). LTBP-2 has also been shown to be an integral component of elastin-containing microfibrils, and a possible role in embryo implantation has been suggested as LTBP-2 KO mice die between E3.5 (blastocysts) and E6.5 (embryo) (39). LTBP-2 appears to be involved in the epithelial-mesenchymal transformation (EMT), important in fetal development (24), but which also occurs during metastasis (13,32).

Regulation by LTBP-1 of the Activation of TGF- β in the extracellular environment

LTBP-1 is known to be involved in the regulation of TGF- β activation by integrin α V β 6. Although integrin α V β 6 recognises the RGD sequence on the TGF- β pro-peptide, the TGF- β binding domain and a basic amino acid sequence (a protease-sensitive hinge domain) with ECM targeting properties on LTBP-1 are also required to release the active peptide in the extracellular environment (9).

Activation of TGF- β by proteolytic events

Annexin II, a co-receptor for tissue plasminogen activator (tPA) and plasminogen promote cell-surface generation of the serine protease plasmin (22). Plasmin-mediated proteolysis has been shown to release active TGF- β s from the LLC (24).

The mannose 6-phosphate (M6P) / IGF-II receptor in human monocytes has been shown to bind a TGF- β 1 SLC. A complex forms between the SLC and the receptor for urokinase plasminogen activator, resulting in plasminogen activation of TGF- β by proteolysis (18).

The integrin RGD sequence on the pro-peptide of TGF- β 3 also binds to other ligands such as fibronectin and vitronectin through this RGD sequence (27) (via integrin α 8 β 1). This binding of SLC to integrin α 8 β 1 may be important in the development of fibrosis (27).

TGF- β Receptors

Three different high affinity cell surface TGF- β receptors have been identified – TGF- β Type I (T β RI), TGF- β Type II (T β RII) and TGF- β Type III (T β RIII).

T β RI has a highly conserved repetitive glycine-serine motif (known as the 'GS' domain) between the transmembrane and kinase domains. The kinase domains of TGF β RI are relatively well conserved, while those associated with TGF β RII are more distantly related.

T β RII has a C-terminal extension rich in serine and threonine that can be quite large (26,31,35). It appears to be the predominant signalling receptor (43) although it has greater affinity for binding TGF- β 1 and TGF- β 3 than it has for TGF- β 2 (38).

Analysis of the crystal structure of TGF- β 3 bound to the extracellular ligand-binding domain of T β RII shows this receptor interacts with homodimeric TGF- β 3 by binding identical finger segments at opposite ends of the growth factor (20) opening up the normally closed form of the monomeric subunits. It is further proposed that the dimerization of the ectodomains of T β RI and T β RII occurs by their binding at adjacent positions on the TGF- β ligand surface and directly contact each other via protein – protein interactions (20). The picture is further complicated as individual Type I and Type II receptors can form both ligand-dependent and ligand-independent homo- and hetero-meric complexes (30).

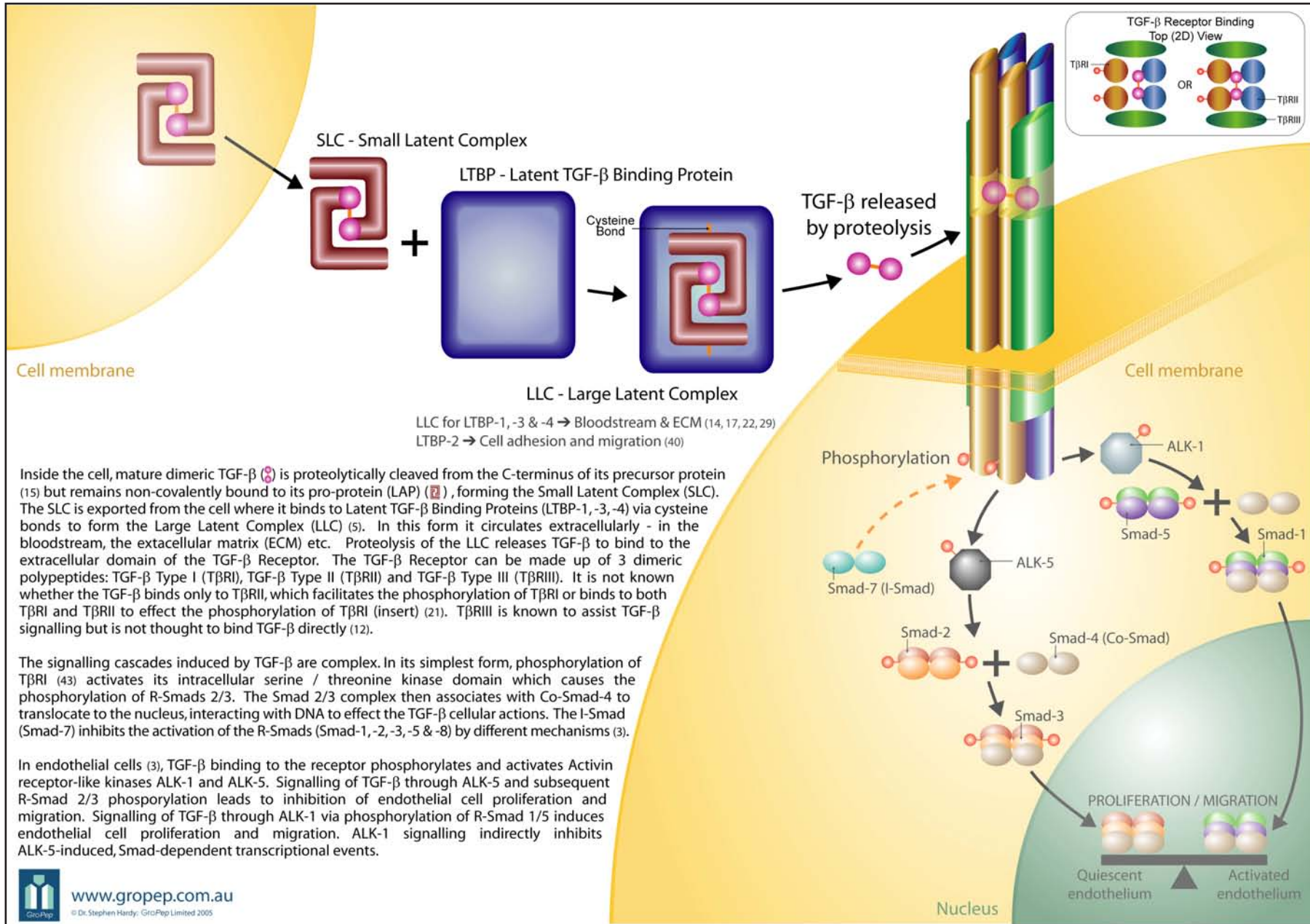
T β RIII, also known as betaglycan, is a highly glycosylated transmembrane protein with a large extracellular domain and a short cytoplasmic tail that lacks kinase activity. Increased expression of T β RIII is associated with enhanced TGF- β signalling, suggesting an essential role for T β RIII in TGF- β signalling (12). There are two ligand binding sites on the membrane-anchored T β RIII ectodomain, one on the N-terminal and one on the C-terminal (15,16) and it would seem that T β RIII acts as a co-receptor for TGF- β . T β RIII appears to function by selectively binding via its cytoplasmic domain to the auto-phosphorylated T β RII, and this promotes the preferential formation of an auto-phosphorylated T β RII and T β RI complex. T β RIII then dissociates from this active signalling complex (12).

TGF- β Receptor Signalling

TGF- β receptor signalling occurs through heteromeric complexes of two trans-membrane serine / threonine kinase receptors (10, 21, 28, 44). The binding of TGF- β is achieved by the T β RII receptor forming a heteromeric complex with the T β RI receptor, aided by the T β RIII receptor, resulting in the phosphorylation and activation of T β RI (43). The activated T β RI then signals through Smads. Smad proteins are nuclear effectors for TGF- β receptors and are divided into Receptor-regulated R-Smads, Common mediator Co-Smads and Inhibitory I-Smads (3). R-Smads and Co-Smad 4 share two conserved domains, termed Mad homology (MH) MH1 and MH2. Both the MH1 and MH2 domains can interact with select sequence-specific transcription factors (3). With the exception of Smad 2, the Smad MH1 domains can bind to DNA, whereas the MH2 domains mediate Smad oligomerization and Smad-Receptor interactions (3). R-Smads are phosphorylated by T β RI at their extreme C-terminal serine residues. R-Smads 2,3 or 1,5, combine with Co-Smad 4 to translocate the signal from the cytoplasm to the nucleus. I-Smad 7 is quickly induced upon TGF- β stimulation and competes with R-Smads, thus regulating their activation by T β RI by recruiting ubiquitin ligases and/or phosphatases to the activated T β RI (3). It would appear (6) that each member of the TGF- β superfamily binds to a characteristic combination of Type I and Type II receptors, which in turn activate specific sub-groups of Smads.

In endothelial cells (3), TGF- β binding to the receptor phosphorylates and activates Activin receptor-like kinases ALK-1 and ALK-5. Signalling of TGF- β through ALK-5 and subsequent Smad 2/3 phosphorylation leads to inhibition of endothelial cell proliferation and migration. Signalling of TGF- β through ALK-1 via phosphorylation of Smad 1/5 induces endothelial cell proliferation and migration. ALK-1 signalling indirectly inhibits ALK-5-induced, Smad-dependent transcriptional events.

TGF-β synthesis, export, association with extracellular components, receptor binding and signalling pathways



Some Biological Actions of TGFβs:

1. Cell Cycle Inhibition

Inhibition of the cell cycle by TGF-β is thought to be mediated in part by down-regulation of proliferative proteins, such as c-myc, coupled with up-regulation of cell cycle inhibitory proteins, such as p15^{INK4b}, p21^{CIP1} or p27^{KIP}. Regulation of these genes by TGF-β signalling occurs both at the transcriptional and post-transcriptional level (8, 41).

2. Inflammation and Repair

The TGF-β family is known to be involved in both down- and up-regulation of inflammatory bowel disease, ulcerative colitis, venous leg ulcers and other wound repair and orthopedic joint diseases (2).

3. Angiogenesis

TGF-β1 plays an important role in the development of the vascular system, in vascular remodelling and can inhibit the activities of other angiogenic factors in endothelial proliferation and migration (2).

4. Cancer

TGF-β plays a dual role in tumorigenesis. On the one hand it inhibits the growth of epithelial and endothelial cells (28) and induces cell cycle inhibitors, such as p15^{INK4B} and p21^{WAF1/CIP} (10,19). On the other hand it can accelerate the malignant process during the late stages of tumorigenesis (11,13).

Most carcinomas are characterized by a loss of the normal growth-inhibitory and apoptotic responses to TGF-β (33). This lack of responsiveness is usually the result of loss of TGF-β receptor expression or defects in downstream signalling events.

TGF-β is over-expressed in various epithelial tumors in which it can suppress immune surveillance (25), facilitate tumor invasion (13) and promote development of metastases (32).

Increased levels of TGF-β also appear to be involved in the epithelial – mesenchymal transitions (EMT) associated with the invasive / metastatic phase of tumor growth (13,32).

SUMMARY

The TGF-β family regulates a diverse range of biological processes. While many aspects of their molecular and cellular biology are understood, their signalling pathways and mechanisms that mediate their effects on target cells are only beginning to be deciphered. In particular, more information is required regarding the circumstances and mechanisms whereby TGF-βs and their signalling processes can be altered from acting as inhibitors of growth to being promoters of tumor cell proliferation.

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Human TGF- β 3 (Receptor Grade)

Transforming Growth Factor- β 3 (TGF- β 3) is one of a family of transforming growth factors identified in mammals. TGF- β s mediate their activity by high affinity binding to the TGF- β type II receptor which signals through a serine/threonine kinase pathway.

Source: Produced recombinantly in *E. coli*
Purity: > 95 % by H.P.L.C.
Molecular Weight: 25,427 daltons

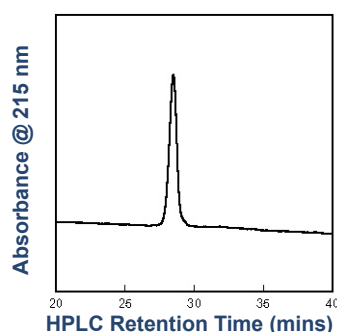
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Human TGF- β 2 Antibody, Monoclonal

Human TGF- β 2 monoclonal antibody (IgG1): Ideal for immunoblotting and immunohistochemistry.

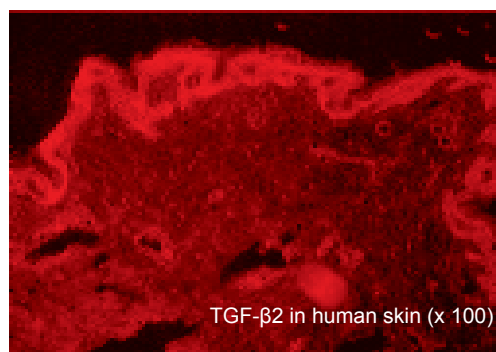
Specificity: Recognises human and bovine TGF- β 2.
Does not inhibit the binding of TGF- β 2 to the TGF- β 2 Type 2 Receptor in mink lung epithelial cells.
Does not recognize human TGF- β 1 or TGF- β 3.
Not a neutralizing antibody

Titre: For immunoblotting, use at 1/1,000
For immunohistochemistry, use at 1/10 to 1/50

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Product Code:	MAB1
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Human TGF- β 3 Antiserum

Rabbit antisera against an epitope of human TGF- β 3: Ideal for EIA.

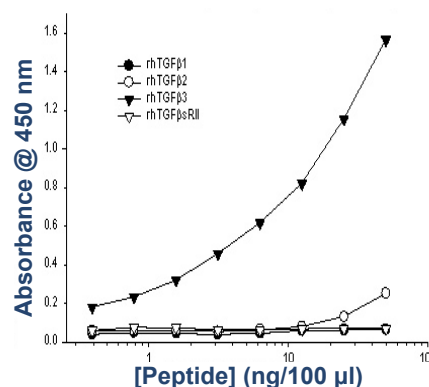
Cross Reactivity: Does not cross-react with human TGF- β 1 or TGF- β 2.
Titre: For EIA, use at 1/4,000.

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Product Code:	PAAM1
Quantity:	100 μl
Cost:	US\$220

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Useful Reviews:

- Govinden, R. and Bhoola, K. D. Genealogy, expression, and cellular function of transforming growth factor- β . *Pharmacol. Therapeutics*, **98**, (2003), 257-265.
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