Systematic Review

Medical Applications of Transforming Growth Factor- β

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ABSTRACT

Transforming growth factor- β (TGF- β) proteins and their antagonists have entered clinical trials. These multi-functional regulators of cell growth and differentiation induce extracellular matrix proteins and suppress the immune system making TGF- β s useful in treatment of wounds with impaired healing, mucositis, fractures, ischemia-reperfusion injuries, and autoimmune disease. In diseases such as keloids, glomerulonephritis and pulmonary fibrosis, excessive expression of TGF- β has been implicated as being responsible for accumulation of detrimental scar tissue. In these conditions, agents that block TGF- β have prevented or reversed disease. Similarly, in carcinogenesis, blocking TGF- β activity may be valuable in stimulating an immune response towards metastasis. As these blocking agents receive approval, we will likely have new therapies for previously recalcitrant diseases.

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TGF- β SYNTHESIS

Transforming growth factor- β (TGF- β) is a family of related proteins that regulate many cellular processes including growth, differentiation, extracellular matrix formation and immunosuppression.¹⁻⁴ TGF- β protein is produced by nearly all normal cells and functions through a complex cell surface receptor system.⁵ The three mammalian isoforms of TGF- β (TGF- β s 1, 2, and 3) have similar but distinct functions and are approximately 70% identical in amino acid sequence. The TGF- β s are extremely important in the regulation of physiological homeostasis with loss of TGF- β activity being implicated in the pathogenesis of ovarian cancer,⁶ pancreatic cancer,⁷ colon cancer,⁸ and squamous cell carcinoma⁹ since TGF- β is a potent growth inhibitor. Mutations preventing TGF- β function are also causal for hereditary hemorrhagic telangiectasia,¹⁰ and corneal dystrophy.¹¹ Over activity of TGF- β leads to Camurati-Engelmann Disease of bone,¹² glomerulonephritis,¹³ scar formation,¹⁴ keloids,¹⁵ pulmonary fibrosis,¹⁶ and liver cirrhosis.¹⁷

The TGF- β proteins are synthesized as pre-pro-peptides which are secreted from cells in a primarily inactive form called latent TGF- β ¹⁸ consisting of a 25 kD mature region surrounded by the pro region, which is also called the latency

Figure 1. TGF- β latency complex. The structure of the inactive TGF- β complex is shown with the TGF- β dimer interacting with the latency associated peptide (LAP) and the latent TGF- β binding protein (LTBP). Arrow indicates cleavage site.

associated peptide (figure 1). Latent TGF- β resides in the extracellular spaces between cells and is available for response to cellular stress or changes in physiology. Activation of TGF- β cleaves the pro region and separates the latency peptide from the active molecule in a highly regulated manner. Agents known to activate TGF- β include, acidic microenvironments, plasmin, plasmin-like proteases, and α v β 6 integrin.

The structure of TGF- β has been determined using a combination of nuclear magnetic resonance and x-ray crystal diffraction (figure 2).¹⁹⁻²⁴ Amino acids 45 and 47 interact with α ₂-macroglobulin, an abundant serum protein that binds and inactivates TGF- β .^{25,26} Amino acids 67 and 68 determine the affinity of the TGF- β isoforms for the GPI-linked protein on vascular endothelial cells.²⁷ Amino acids 92-98 regulate binding of TGF- β to the TGF- β type II receptor (T β RII),²⁸ with amino acid 94 directly binding T β RII.^{29,30}

SIGNALING PATHWAYS

TGF- β signals through a complex membrane bound receptor and binding protein system^{31,32} which can include β -glycan,³³ the type I and II receptors,³⁴ endoglin,³⁵ an uncharacterized glycosyl phosphatidylinositol-linked protein,³⁶ and BAMBI, a naturally occurring truncated type I receptor.³⁷ Both endoglin and β -glycan can present TGF- β to the type II receptor which complexes with and phosphorylates the type I receptor. The type I (T β RI) and II (T β RII) receptors are transmembrane serine/threonine specific kinases with T β RI interacting with and phosphorylating intracellular molecules. Intracellular signaling requires the Smad family of proteins in which Smad2 and Smad3 are directly phosphorylated by T β RI and go on to form a complex with Smad4.^{3,4,31,32} This complex directly enters the nucleus where it interacts with other transcription factors to regulate gene transcription. In contrast, Smad7 inhibits phosphorylation of Smad2 and Smad3 in a negative feedback loop preventing over-stimula-

Figure 2. Structure of TGF- β . The crystal structure of TGF- β is shown. Amino acids important in regulating binding of TGF- β to receptors and binding proteins have been highlighted.

tion of the cell by TGF- β . TGF- β signaling is further regulated by the interaction of Smad proteins with a variety of transcriptional activators and repressors³⁸ as well as by complex interactions of the Smad and MAP kinase signaling pathways.³⁹

Through the use of microarray technology, much progress has been made in identifying the genes that are regulated by TGF- β .⁴⁰ β ig-h3 (TGF- β induced gene), a secreted protein that inhibits tumor formation when overexpressed, is another important protein synthesized by cells in response to TGF- β .⁴¹

TGF- β AND DISEASE

Mutations of TGF- β or components of its signal pathway are frequently found in disease (table 1). Patients with Camurati-Engelmann have a missense mutation in the latency associated peptide which changes one of three different amino acids, producing constitutively active TGF- β .^{12,42} This results in dysregulation of TGF- β synthesis, uncoupling bone formation and resorption at the level of the osteoblasts and osteoclasts.

Mutations of β ig-h3, a protein involved in corneal morphogenesis, have been identified as a cause of six autosomal dominant corneal dystrophies. These include Groenouw corneal dystrophy type I, superficial variant of granular corneal dystrophy, lattice corneal dystrophy type I, lattice corneal dystrophy type IIIA, Avellino corneal dystrophy, and Reis-Bucklers corneal dystrophy.⁴³ It appears that mutant β ig-h3 protein self-polymerizes and/or incorrectly binds to other corneal components resulting in the abnormal deposits found in these dystrophies.⁴⁴

Hereditary hemorrhagic telangiectasia (HHT) type 1 or Osler-Weber-Rendu disease is caused by mutations of endoglin.¹⁰ In HHT type II, mutations of activin receptor-like kinase 1 (ALK1) have been identified.⁴⁵ ALK1, a membrane bound receptor that is closely related to T β RI, binds TGF- β and signals a TGF- β response. However, the preferred ligand for ALK1 is activin, a protein structurally similar to TGF- β , but distinct in its function. In both types of HHT, the endothelial

cells would be expected to respond poorly to TGF- β , which could contribute to the abnormal vessel formation or altered cell adhesion properties leading to the vascular anomalies seen in this disorder.

Mutations of Smad4 have been identified as the cause of familial autosomal dominant juvenile polyposis in a subset of patients.⁴⁶ Other families with this disease carry mutations in the gene encoding bone morphogenetic protein receptor 1⁴⁷ which is a membrane bound receptor similar to T β RI. The preferred ligand for this mutated receptor is bone morphogenetic protein, a protein similar to TGF- β with growth regulating activity. T β RII mutations have been identified in approximately 90% of colon polyps with DNA repair defects⁴⁸ and 70% of gastric cancers⁴⁹ with repair defects. In these patients, DNA replication errors occur due to mutations in the genes that check the copied strand of DNA for mistakes. As a result, inactivating mutations accumulate in a stretch of 10 adenine bases in T β RII, since the replication machinery does not carefully check this repetitive sequence. Additionally, deletion or inactivation of Smad4 has been shown in 30-50% of colorectal and pancreatic cancers.^{7,50} In all these conditions a disruption in the TGF- β signaling pathway compromises the cell's ability to respond to the growth inhibitory effects of TGF- β , thereby promoting tumorigenesis.

TGF- β KNOCKOUT MICE

Mice lacking the gene for the TGF- β s or their regulatory and signaling proteins have been generated using homologous recombination and the role of TGF- β in development and disease has been studied. Approximately 60% of mice lacking TGF- β 1 die *in utero* due to defective yolk sac vasculogenesis and hematopoiesis, which is consistent with the role of TGF- β in embryogenesis. This defect is due to inappropriate endothelial cell differentiation.⁵¹ The remaining mice develop to term, but die in about week 3 to 4 from a rapid wasting syndrome.^{52,53} At birth, the knockout mice are indistinguishable from wild type litter mates, but soon develop severe, multifocal organ-dependent mixed inflammatory cell infiltration into heart, stomach, liver, diaphragm, lung, salivary gland and pancreas. These mice also have elevated antibody levels to dsDNA, ssDNA, and Sm ribonuclear protein.⁵⁴ Treatment of these mice with anti-inflammatory and immune suppressive agents such as rapamycin reduces the severity of inflammation. In addition to immune system defects, the mice that are born experience delayed wound healing,⁵⁵ ineffective remodeling of bone,⁵⁶ and increased mitochondria in the liver in response to stress.⁵⁷

TGF- β 2 knockout mice exhibit primarily developmental defects in contrast to TGF- β 1 mice.⁵⁸ These include defects in epithelial-mesenchymal interactions, cell growth, extracellular matrix production and tissue remodeling, and affect the function of cardiac, lung, craniofacial, limb, spinal column, eye, inner ear and urogenital tissues. Analysis of eyes of

Table 1. Genetic diseases of the TGF- β system.

Camurati-Engelmann	Latency associated peptide
Corneal dystrophies	β ig-h3
Hereditary hemorrhagic telangiectasis type I	Endoglin
Hereditary hemorrhagic telangiectasis type II	Activin receptor-link kinase 1
Autosomal dominant juvenile polyposis	Smad4 bone morphogenetic protein receptor I

TGF- β 2 knockout mice show that extracellular matrix proteins, including collagen I and keratocan, are diminished and the stroma is thinner.⁵⁹ TGF- β 3 deficient mice also exhibit disruptions in epithelial-mesenchymal interactions as evidenced by the appearance of abnormal lung development and cleft palate.⁶⁰ Unlike other models of cleft palate, these mice do not develop other craniofacial abnormalities.

Mice with deletions of genes in various components of the TGF- β signaling pathway develop additional pathological phenotypes. T β RI mice die at mid-gestation exhibiting defects in vascular development of the yolk sac and placenta with absence of red blood cells.⁶¹ T β RII mutants developed pituitary tumors when treated with chronic estradiol.⁶² Mice lacking Smad3 live until 8 months and die of defects in immune function.⁶³ These mice also have an imbalance between osteoblasts and osteoclasts resulting in osteopenia⁶³ and accelerated healing of cutaneous incisional wounds.⁶⁴ Exposure of these mice to radiation-induced injury causes significantly less epidermal acanthosis and dermal influx of mast cells, macrophages, and neutrophils than wild type littermates, demonstrating that these mice have a significantly reduced fibrotic response.⁶⁵ Smad4 mice present with inflammatory polyps in the glandular stomach and duodenum consistent with previous reports that Smad4 mutations are involved in a subset of familial juvenile polyposis.⁶⁶

POSSIBLE USE OF TGF- β LIGANDS FOR THERAPEUTIC INTERVENTION

The well-characterized abilities of TGF- β to promote healing in both hard and soft tissues, as well as its potent immunosuppressive effects, have provided the basis for the use of TGF- β ligands as potential therapeutic agents in several disease models. Topical application of TGF- β improves the rate of healing and wound strength in cutaneous wounds in a wide variety of animal models of impaired healing including animals treated with corticosteroids, antineoplastic agents, or radiation, as well as diabetic or aged animals.⁶⁷ In clinical trials TGF- β 2 and TGF- β 3 treatment of venous stasis and pressure ulcers, respectively, has been shown to improve healing.^{68,69} In a hamster model of chemotherapy-induced oral mucositis, application of TGF- β 3 reduces the severity and duration of the resulting mucositis,⁷⁰ and clinical trials of TGF- β 3 to treat this condition are underway.⁷¹ TGF- β has also been shown to accelerate the repair of bone defects. In canine models, both TGF- β 1 and TGF- β 2 have been effective in increasing bone formation when applied to defects in the alveolar ridge and in the humerus, respectively.^{72,73} In keeping with its healing properties, TGF- β also can protect tissues from ischemia-reperfusion injury in several animal models. In rat and rabbit models of stroke, administration of TGF- β before or even 2 h after insult reduces the infarct size,⁷⁴ while intravenous administration of TGF- β following coronary artery occlusion, but before reperfusion reduces cardiac necrosis.^{75,76} Recent studies are investigating improved

delivery systems for TGF- β . Pang et al.⁷⁷ report that mice receiving adenovirus overexpressing TGF- β 1 showed a smaller infarct volume after middle cerebral artery occlusion followed by reperfusion.

The potent immunosuppressive effects of TGF- β make it a potential therapeutic agent in the treatment of autoimmune diseases. Indeed, treatment of rodents with TGF- β 1 during the latter part of the induction phase of acute experimental allergic encephalomyelitis (EAE) (a model of multiple sclerosis) and collagen-induced arthritis prevents the development and/or exacerbation of disease symptoms.^{78,79} Again, novel delivery systems for administration of TGF- β are being developed. A genetically engineered retrovirus transduced with cDNA for latent TGF- β delays and ameliorates EAE development,⁸⁰ and intranasal administration of a TGF- β 1 plasmid prevents the development of T helper cell type 1-mediated experimental colitis.⁸¹ Additionally, intramuscular injections of adenoviral TGF- β 1 into rodent recipients of lung transplants attenuates acute rejection.⁸²

ANTAGONISTS OF TGF- β FOR DISEASE TREATMENT

Even though TGF- β has great potential as a therapeutic agent, there are a number of fibroproliferative disorders where unregulated expression of TGF- β plays a causal role in the condition. Numerous immunohistochemical studies have demonstrated the overexpression of TGF- β in glomerulonephritis, pulmonary fibrosis, liver cirrhosis, and keloids suggesting that molecules that antagonize TGF- β may be useful in the treatment of these diseases.⁸³ In addition, since many tumors express increased amounts of TGF- β , agents that block TGF- β may be valuable in stimulating an immune response toward metastases.⁸⁴ Mechanisms to block TGF- β activity include soluble T β RII fragments,⁸⁵ decorin,⁸⁶ trani-last,⁸⁷ neutralizing antibodies,⁸⁸ threonine kinase inhibitors,⁸⁹ and RNA expression inhibitors such as anti-sense expression vectors or blocking oligonucleotides.⁹⁰

Impressive results have been obtained in animal models of fibrosis using TGF- β antagonists. Glomerulonephritis in rats has been essentially cured using antibodies to TGF- β ,⁹¹ administration of decorin protein,⁹² and gene therapy using a decorin gene expressed in muscle tissue that circulates to act on kidney.¹³ Moreover, anti-TGF- β 2 antibody and a TGF- β 1 antisense oligonucleotide attenuate kidney fibrosis in the diabetic rat and in unilateral ureteral obstructions, respectively.^{88,93} Hepatic fibrosis in rodents can be inhibited by soluble T β RII administered intraperitoneally⁹⁴ or by intramuscular injections of an adenovirus expressing the ectodomain of T β RII fused to the Fc portion of IgG.⁹⁵ Adenoviral soluble T β RII can also inhibit constrictive remodeling after coronary angioplasty in pigs.⁹⁶ Pulmonary fibrosis has been successfully treated using adenovirus expression of decorin in the airway.⁸⁶ Administration of TGF- β antibodies prevents skin and lung fibrosis in murine sclerodermatous graft-vs-host

disease.⁹⁷ Fibrotic scar tissue is successfully treated using antibodies to TGF- β 1 or antibodies to TGF- β 2, but paradoxically, topical application of TGF- β 3 protein.¹⁴ Both decorin⁹⁸ and TGF- β antibodies⁹⁹ attenuate gliotic scar formation following injury to the rat CNS. In a mouse model of intestinal radiation enteropathy, intraperitoneal injection of soluble T β RII preserves mucosal surface area with less intestinal wall fibrosis than in controls.¹⁰⁰ Mice injected intramuscularly with an adenovirus vector expressing the soluble extra-cellular TGF- β binding domain of T β RII are protected from developing corneal opacification, edema, and angiogenesis induced by silver nitrate.⁸⁵

Several rodent carcinogenesis models have demonstrated the efficacy of TGF- β antagonists to inhibit tumor growth and metastasis. Antisense oligonucleotides to TGF- β 2 inhibit the growth of mouse malignant melanomas⁸⁴ and fibrosarcomas.¹⁰¹ TGF- β antibody inhibits metastasis of tumorigenic human xenotransplants in nude mice,¹⁰² while ectopic expression of decorin in rat C6 glioma cells inhibits tumor formation.¹⁰³ Furthermore, oral administration of tranilast, an anti-allergic compound which can inhibit TGF- β , inhibits the growth of experimental 9L rat gliomas.¹⁰⁴ A phase I clinical trial of TGF- β antagonists for the treatment of metastatic cancer is currently in progress.¹⁰⁵ In this trial, autologous tumor cells are removed from patients and treated *in vitro* with TGF- β 2 anti-sense DNA to suppress TGF- β expression from tumor cells. This treatment blocks the immunosuppressive activity of TGF- β enhancing immune system recognition of tumor cells. TGF- β 2 blocked cells are then injected back into the patient as antigens for the immune system. Five patients with progressive glioblastoma multi-form and one with progressive gliosarcoma are enrolled in the trial and treated with 2 to 7 subcutaneous injections of approximately 1×10^7 modified tumor cells. TGF- β levels were decreased by 50%-98%. Two patients had partial regressions and one patient had stable disease following therapy supporting further clinical evaluation of TGF- β modified anti-sense tumor cells for treatment of incurable metastatic disease.

THERAPEUTIC APPLICATIONS: PROBLEMS AND PERSPECTIVES

The preceding sections highlight the many types of diseases in which alterations in the TGF- β signaling pathway may have pathological consequences. While restoration of the proper flux through the pathway by administration of either ligand or antagonists may alleviate the pathology, accomplishing this in only the affected tissue or cell type will be difficult. Normal homeostatic actions of TGF- β in uncompromised cells may also be altered by treatment agents leading to unwanted and unexpected complications. The pleiotropic actions of TGF- β are illustrated by its role in carcinogenesis, where TGF- β can have tumor suppressor, as well as pro-oncogenic activities.¹⁰⁶ TGF- β normally inhibits growth of epithelial cells, but tumorigenesis is often accompanied by a

loss of responsiveness to this growth inhibition coupled with increased production of TGF- β , which in turn facilitates pro-oncogenic effects of TGF- β on stroma. These effects can include increased cell motility, enhanced angiogenesis and suppression of immune surveillance. An ideal treatment for cancer would involve restoring TGF- β signaling in epithelial cells and inhibiting its action in stromal cells, necessitating the development of cell type specific delivery systems.

Since the mode of delivery of TGF- β ligand or antagonist will be critical in affecting only the desired system, local, as opposed to systemic, delivery is probably preferable. For example, in a phase I trial for treatment of chronic progressive multiple sclerosis, intravenous administration of active TGF- β 2¹⁰⁷ resulted in anemia and reversible nephrotoxicity in some patients with no change in expanded disability status score of magnetic resonance imaging lesions during treatment. In contrast, murine experimental allergic encephalomyelitis was ameliorated by administration of myelin basic protein-activated T cells transduced with latent TGF- β 1.⁸⁰ In this regard administration of adenoviral vectors of TGF- β or TGF- β antagonists is being widely used in animal models.^{13,77,81,95,96} The administration of active-vs.-latent TGF- β is also an important consideration. Active TGF- β has a much shorter half-life than the latent form,¹⁰⁸ and active TGF- β may result in more side effects, since it is not subject to the activation step which is tightly controlled *in vivo*. Additional complications of administration of active TGF- β are its reported inactivation by proteases present in wound fluid from venous leg ulcers.¹⁰⁹ This proteolytic degradation of ligand may have contributed to the inefficacy of topical TGF- β 3 in treating chemotherapy-induced oral mucositis in patients with lymphomas and solid tumors.⁷¹

In spite of these problems, some current clinical modalities may be acting by decreasing TGF- β signaling. The success of angiotensin-converting-enzyme inhibitors in treating diabetic nephropathy, interferon- α in treating hepatic fibrosis, azathioprine and prednisone in treating autoimmune hepatitis, and interferon- γ in treating pulmonary fibrosis is due, in part, to the ability of these agents to reduce serum levels of TGF- β .¹¹⁰ The search for small molecules that target the TGF- β system is currently underway using combinatorial chemistry and high-throughput drug screening.

CONCLUSION

TGF- β and its antagonists have tremendous potential for the treatment of diseases that currently do not respond well to conventional therapy. The development of additional analogs of TGF- β and antagonists of TGF- β , as well as further studies into the cell biology of this important cytokine, may enable us to develop new therapeutics.

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