



Transforming Growth Factor- β Signaling in Bladder Fibrosis*

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Abstract

Anumanthan G, Pope IV JC. Transforming Growth Factor- β signaling in bladder fibrosis. *ARBS Annu Rev Biomed Sci* 2008;10:1-5. In human tissues, normal homeostasis requires intricately balanced interactions between cells and the network of secreted proteins known as the extracellular matrix. These cooperative interactions involve numerous cytokines acting through specific cell-surface receptors. When the balance between the cells and the extracellular matrix is perturbed, disease can result. This is clearly evident in the interactions mediated by the cytokine transforming growth factor- β (TGF- β). TGF- β signaling has been studied extensively in fibrotic disease of lung, liver, skin, and kidney. However, little is known about the role of TGF- β in bladder fibrosis. This review focuses on the mechanisms underlying TGF- β expression and how it relates to fibrotic processes in bladder.

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1. Introduction

Transforming growth factor- β (TGF- β) belongs to a superfamily of structurally related polypeptides that are involved in various biological processes, including cell growth, differentiation, angiogenesis, apoptosis, and extracellular matrix remodeling (Massague, 1998). There are three isoforms of TGF- β : TGF- β 1, TGF- β 2 and TGF- β 3. Each TGF- β isoform is synthesized as part of a large precursor molecule containing a propeptide region in addition to TGF- β . TGF- β 1 messenger RNA (mRNA) is expressed in endothelial and hematopoietic, and connective tissue cells; TGF- β 2 in neuronal and epithelial cells; TGF β 3 mRNA primarily expressed in mesenchymal cells. All three isoforms are highly conserved in mammals. These isoforms differ in their binding affinity for TGF- β receptors. And the deletion of individual isoforms in mice results in different phenotypes.

The multifunctional effects of TGF- β in cellular actions occur by binding to its serine threonine kinase receptors. Sequence comparisons indicate that these receptors fall into two subgroups, designated as type I (T β RI) and type II (T β RII). Transforming growth factor- β 1 initiates signals by binding to T β RII which stabilizes the heteromeric complex with T β RI. As a result, T β RI is transphosphorylated within a glycine serine-rich domain (GS-domain). The activated T β RI then propagates the signals to intracellular signal mediators including Smad proteins (Massague & Wotton, 2000; Anumanthan *et al.*, 2005).

Smad proteins are classified according to their structure and function in signaling by TGF- β family members. They are characterised by homologous regions at their N- and C-termini known as Mad homology (MH-1 and MH-2) domains, respectively. A divergent linker region separates these domains. Both structural and functional differences provide the basis for a division of the Smads into three groups: receptor regulated (R-Smad), common-mediator, and inhibitory Smads. R-Smads including Smad1, 2, 3, 5, 8, and 9, act as direct substrates of specific type I receptors and are activated by phosphorylation of serine residues at the carboxyl terminus. Thus, Smad2 and Smad3 mediate signalling by TGF- β and activin, while Smad1 and presumably Smad5, Smad8, and Smad9 are similarly modified through bone morphogenetic protein (BMP) exposure. Transforming growth factor- β /activin receptor-phosphorylated Smads (R-Smads) oligomerize with the common mediator Smad4 (Co-Smad), and after nuclear import, they regulate gene expression positively or negatively by binding to DNA or by interacting with transcription factors (Shi & Massague, 2003). A distinct class of distantly related Smads, including Smad6 and Smad7, has been identified as inhibitors of TGF- β signaling pathways. Originally, it was assumed that the main signaling occurs via Smad proteins, which translocate into the nucleus to regulate gene transcription (Feng & Derynck, 2005) (Fig. 1).

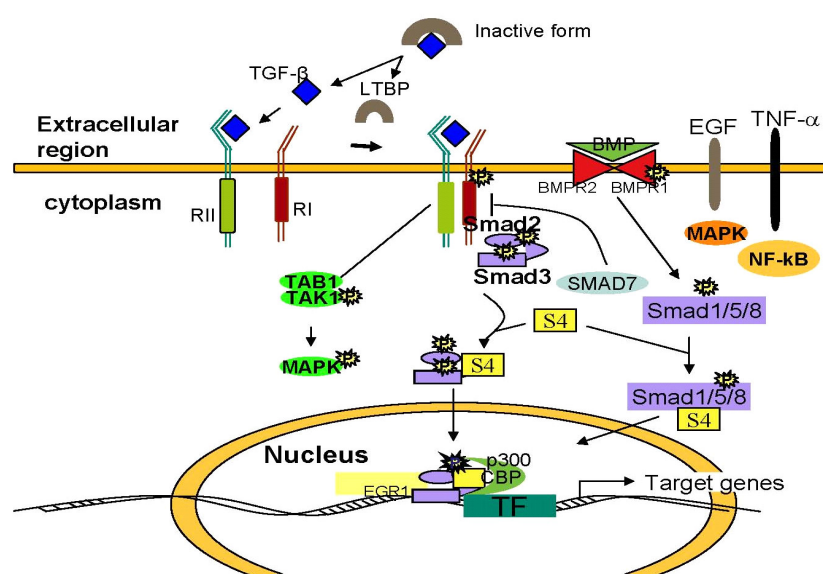


Figure 1. The Transforming Growth Factor- β (TGF- β) signalling pathway.

Role of Smad3 in fibrosis: Smad3 is a key mediator for regulation of collagen synthesis and other profibrotic responses to TGF- β . Smad3 null mutant mice have been generated in different laboratories by targeted disruption of exon 1, or exon 2 or exon 8 in the Smad3 gene (Zhao *et al.*, 2002; Lakos *et al.*, 2004). Mice with homozygous deletions of the Smad3 gene were viable and survive into adulthood, with impaired mucosal immunity, defective neutrophil chemotaxis, and abnormal receptor-induced activation of thymocytes and peripheral T cells (Yang *et al.*, 1999). In lungs, lack of Smad3 attenuated bleomycin-induced lung fibrosis, despite a robust early inflammatory response and macrophage accumulation in the lungs (Zhao *et al.*, 2002). However the role of Smad3 regulation in bladder fibrosis is unknown.

More recently, a parallel signaling pathway of TGF- β became evident. TGF- β activates other signalling cascades, including the Erk, JNK and p38 MAPK kinase pathways. The pathway operates independently of Smad proteins and rather involves one or more members of the family of mitogen-activated protein kinases (MAPKs) (Roberts, 1998). Studies using Smad4-deficient cells, or dominant-negative Smads, support the possibility of MAPK pathway activation that is independent from Smads (Derynck & Zhang, 2003). The mechanisms of Erk, JNK or p38 MAPK activation by TGF- β and its biological consequences are poorly characterized. Further characterization of this signaling pathways of interactions will provide insight into the activation of MAPK pathways by TGF- β ligands. In addition, recent evidence for a role of the Rho pathway in the pathogenesis of radiation-induced enteritis suggest that inhibition of Rho pathway by pravastatin, an inhibitor of Rho isoprenylation, may also promise opportunities for new therapeutic perspectives. The identification of alternate signalling pathways for TGF- β remains critically important and shed some light on alternate mechanisms by which TGF- β may affect connective tissue remodeling.

2. Fibrosis

Fibrosis is characterized by excessive scarring due to excessive production, deposition, and contraction of extracellular matrix. This process usually occurs over many months and years, and can lead to organ dysfunction. Fibrotic disease represents one of the largest groups of disorders for which there is no effective therapy and thus represents a major unmet medical need. The lack of appropriate antifibrotic therapies arises primarily because the etiology of fibrotic disease is unknown.

Tissue fibrosis is generally considered to arise when normal wound healing response fails to terminate (Eckes *et al.*, 2000; Gabbiani, 2003). After injury, new connective tissue needs to be synthesized. During this process, mesenchymal fibroblasts become "activated" in that they proliferate and migrate into the wound and synthesize elevated levels of matrix proteins, including collagen and fibronectin. The fibroblasts present in a wound are a specialized form of fibroblasts termed myofibroblasts. Myofibroblasts are present in abundance within fibrotic lesions and thus contribute to the excessive scarring observed in lesions of fibrotic disease (Gabbiani, 2003)

TGF- β is known to play a major role in the differentiation of myofibroblasts, the synthesis of many other matrix components such as collagens, fibronectin and proteoglycans, and the synthesis of protease inhibitors which play a role in matrix degradation.

2.1. Over expression of TGF- β results in fibrosis of the kidney, liver, skin and lung

Pathologic fibrosis is mediated by TGF- β . TGF- β production by the damaged tissue is increased before the production of increased extracellular matrix. Levels of TGF- β and of TGF- β mRNA are increased in fibrotic organs. Exogenous TGF- β induces fibrosis independently of tissue damage and inhibitors of TGF- β -receptor binding reduce or abolish fibrosis. Finally tissue-specific overexpression of TGF- β 1 in transgenic mice results in fibrosis in those organs (Sanderson *et al.*, 1995; Kopp *et al.*, 1996).

3. Bladder Fibrosis

Severe functional bladder obstruction can occur in children with spina bifida, leading to both urinary incontinence and kidney failure. Adult patients can also develop bladder fibrosis from neurogenic bladder disease and benign prostatic hyperplasia. Physiologic impairment of urinary flow likely causes increased bladder wall stress from retained urine and over distension of bladder muscle. This increased mechanical stress causes a cascade of cellular events which results in secondary changes in bladder wall architecture. The most prominent histopathologic findings are muscular hypertrophy/ hyperplasia and increased extracellular matrix protein deposition.

In adult bladder, increased TGF- β expression plays major role in fibrosis, smooth muscle atrophy and diminished compliance. There are a limited number of studies exploring the role of TGF- β in bladder disease. An understanding of the mechanism of TGF- β signaling in bladder tissue development will allow the creation of new potential therapeutic options to treat bladder fibrosis.

Extracellular matrix provides essential functions, including structural support, cellular adhesion, and barrier to the movement of fluid and macromolecules. Each tissue type has particular matrix assemblies that contribute to specialized tissue functions. These specialized tissue functions are accomplished by particular extracellular matrix proteins. TGF- β regulates the expression of many matrix proteins. Under normal circumstances, the degradation and remodeling of matrix proteins are in balance, controlled by the cellular actions of TGF- β and other cytokines. TGF- β plays a central role in this balance by stimulating the expression of matrix components such as fibronectin, collagens, and matrix proteoglycans. TGF- β also promotes extracellular matrix accumulation by inducing inhibitors of matrix degrading metalloproteinases and plasminogen-activator inhibitor. Numerous studies in animals investigating the role of excessive TGF- β activity in fibrotic disease have suggested that intervening in the TGF- β signaling pathway may provide a useful therapeutic target for tissue fibrosis.

Attempts to block the effects of excessive TGF- β activity have involved the use of neutralizing TGF- β antibodies and natural TGF- β inhibitors (e.g., decorin), both of which inhibit TGF- β binding to its receptors, and gene therapy with the inhibitory Smads or dominant negative TGF- β receptors, both of which block TGF- β signaling (Isaka *et al.*, 1996; Qi *et al.*, 1999). One popular method of TGF- β signaling inhibition in fibrosis model is targeting the expression and function of TGF- β ligands. Strategies such as RNA interference (RNAi), receptor kinase inhibitors and drug treatment are among the most commonly used recent approaches to prevent TGF- β ligand activation of downstream signaling. However, studies using transgenic mice showed deletion of T β RII in males Tgfbr2(fspko) leads to a hypertrophied lamina propria and muscularis externa with myofibroblast differentiation and proliferation. But, female homozygous Tgfbr2(fspko), bladders appeared the same as those of wild-type male and female controls. Moreover, this model also suggests a role for stromal TGF- β signaling with estrogens and androgens in bladder fibrosis. It supports the fact that, in animal models, sex steroids, especially estrogen, have been shown to modulate the expression of TGF- β (Takahashi *et al.*, 1994). Research in our laboratory has shown promise with the use of the Angiotensin-II receptor inhibitor losartan to modulate bladder outlet obstruction induced bladder fibrosis through TGF- β signaling in a rat bladder outlet obstruction model (unpublished data). In fact, the efficacy of angiotensin-converting-enzyme inhibitors and Angiotensin receptor inhibitors in decreasing renal and bladder fibrosis directly correlates with decreased TGF- β levels (Gobet *et al.*, 1999; Miyakita *et al.*, 2007). Inhibition of ECM production induced by TGF- β is a potentially useful means for treatment of bladder fibrosis. In addition, reports also cited TGF- β induced p38 MAPK activation has an important role in collagen production (Varela-Rey *et al.*, 2002). Therefore, a small molecule that can inhibit TGF- β action and reduce ECM production is expected to be a good therapeutic medicine against bladder fibrosis.

4. Conclusion

TGF- β is known to play a major role in the process of tissue fibrosis. Recent literatures show that TGF- β signaling is a potential target for future therapeutic intervention in fibrosis. In addition, a better understanding of the cellular factors, the cytokines and growth factors, which regulate bladder smooth muscle growth and collagen production under normal and pathological condition, are warranted.

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