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Review Article

Therapeutic strategies against TGF-β signaling pathway in hepatic fibrosis

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Abstract: Hepatic fibrosis is the common wound-healing response to chronic liver injury. In this process, activation of hepatic stellate cells is characteristic of cell proliferation and migration, production of collagen and other extracellular matrix (ECM) molecules, and contraction after transforming into myofibroblasts. It has been shown that the fibrogenic process is prominently regulated by transforming growth factor-β1 (TGF-β1) and that the specific blockade of TGF-β1/Smad3 signaling may therapeutically intervene the fibrosis of various tissues. In this review, we attempt to integrate recent advances in the understanding of the mechanisms underlying TGF-β1/Smad3 pathway modulation of ECM gene expression in the context of liver fibrosis, discuss intervention strategies targeting the blockade of related signal pathways, and look into novel ways to the safe and efficacious prevention and treatment of hepatic fibrosis.

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Liver fibrogenesis represents the common response of the liver to toxic, infectious, or metabolic agents and is characterized by excessive accumulation of extracellular matrix (ECM) caused by both increased synthesis and deposition of newly formed components, and decreased or unbalanced degradation of ECM (1, 2), ultimately leading to cirrhosis and many complications: portal hypertension, liver failure, and hepatocellular carcinoma. Both clarification of the molecular mechanisms underlying pathological fibrosis and the development of effective therapy are of clinical importance. A central role in fibrogenesis has been assigned to transforming growth factor-β1 (TGF-β1), which is a particularly well-studied cytokine and widely regarded as a pro-fibrogenic agent in liver injury, particularly after chronic liver injury (3, 4).

The multiple biological actions of TGF-β1, which involves in a variety of biological processes including development, cell growth, differentiation, cell adhesion, migration, ECM deposition, and the immune response, contribute to the regulation of the production, degradation, and accumulation of ECM proteins in a direct or indirect manner. TGF-β1 may play a pivotal role in the fibroproliferative changes that follow tissue damage in many vital organs and tissue, including liver, lung, kidney, skin, heart, and arterial wall (5–7). Fibrotic pathologies are associated with increased levels of TGF-β1 that initially recruit inflammatory cells and fibroblasts into an area of injury and then stimulate these cells to produce cytokines and ECM, because TGF-β1 not only enhances ECM synthesis but also inhibits ECM degradation by downregulating expression of matrix-degrading enzymes and promoting expression of matrix metalloproteinase (MMP) inhibitors (8, 9). In addition, TGF-β1 also appears to induce tissue inhibitor of metalloproteinases-1 (TIMP-1) (9).

High levels of TGF-β1 are often found in hepatic fibrosis and it has been implicated as a mediator of fibrosis in many liver diseases (10). Liver fibrosis occurs as a consequence of the transdifferentiation of hepatic stellate cells (HSCs) into myofibroblasts and is associated with an increased expression and activation of TGF-β1. It is believed that release of TGF-β1 by necrotic hepatocytes may be one of the first signals to activate adjacent quiescent HSCs, resulting in their transdiffertiation into proliferatve, fibrogenic, and contractile myofibroblasts.

Blocking TGF-β1 signaling has been implicated in numerous animal models of fibrosis as well as in several human fibrotic diseases, including radiation fibrosis (7, 11). For example, antagonizing TGF-β1 pathway using neutralizing antibodies, soluble type II receptors, antisense oligonucleotides and RNA interference technologies, and small molecular inhibitors has been shown to perturb various types of TGF-β-mediated fibrosis (7, 11–13). So TGF-β1 signaling can be designated as anti-inflammatory and potentially anti-fibrogenic targets (14). These findings have major translational implications for therapeutic strategies aimed at TGF-β1/Smad3 signaling. Indeed, TGF-β1 is crucial in rat liver fibrogenesis *in vivo*, implicating that inhibition of TGF-β is effective in not only preventing fibrosis, but also preserving organ function.

According to these characteristics, there are three lines of evidence that TGF-β1 has a causal role in pathological fibrosis. Firstly, TGF-β1 levels are elevated in diseased organs and are often specifically localized to fibrotic areas. secondly, administration of exogenous TGF-β1 to laboratory animals leads to the development of fibrosis in some organs, and to cultured cells leads to excel ECM deposition. finally, anti-TGF-β1/Smad3 therapies could lessen experimentally induced fibrosis.

An interesting question is whether anti-TGF-β1/Smad3 intervention can prevent fibrogenesis or halt the progression of fibrosis in an organ in which fibrosis is already well established. In this review we will focus on recent insight into the molecular effects of anti-fibrogenesis through the blocking of TGF-β signaling specificity, especially the TGF-β1/Smad3 pathway, explore the therapeutic strategies for suppressing the action of several key proteins in the cascade and discuss the advantages and disadvantages of antagonists to the TGF-β pathway.

TGF-β/Smads signaling pathway

TGF-β superfamily consists of over 40 ligands including three TGF-β isoforms, activins, inhibitins, bone morphogenetic proteins (BMPs), growth and differentiation factors, anti-mullerian hormone (AMH), myostatin, and others (15). Mammal cells express three highly homologous TGF-β isoforms such as TGF-β1–3. They have similar biological activities *in vitro* with noticeably different biological responses *in vivo*, although many members of the superfamily signal through a similar pathway. It is thought that TGF-β1 plays the most significant role in wound healing and organs fibrogenesis (16).

Briefly, two types of transmembrane protein kinases, types I and II receptors, mediate signaling by TGF-β. Ligands interact with a homodimer of type II receptors (TβRII), which recruit and activate type I receptors (TβRI, activin receptor-like kinases, ALKs). The TβRII kinase then phosphorylates TβRI in a glycine- and serine-rich juxtamembrane region called the GS box, which is one of the critical events in TGF-β signaling and serves as the initiation point for the downstream events. Activated ALKs phosphorylate a subset of the downstream signaling molecules, the receptor-activated Smads (R-Smads), which enables their binding to the common Smad (Co-Smads). And R-Smad/Co-Smad complex is shuttled into the nucleus where it can interact with various transcription factors and regulate transcription of many target genes (16, 17) (Fig. 1).

Five type II receptors (BMPRII, ActRII, ActRIIB, TβRII, and AMHR), seven type I receptors (ALK1–ALK7), five R-Smads (Smad1, Smad2, Smad3, Smad5, and Smad8), one Co-Smad (Smad4), and two I-Smads (Smad6 and Smad7) are recognized in mammalian cells. ALK1, ALK2, ALK3, and ALK6 phosphorylate and activate Smad1, Smad5, and Smad8, whereas ALK4, ALK5, and ALK7 phosphorylate and activate Smad2 and Smad3 (15, 18, 19).

TGF-β1 is a secreted homodimeric protein that regulates numerous cellular responses such as proliferation, differentiation, migration, and apoptosis, and initiates TβRII–ALK5–Smad2/3 pathway in most cells (19). Reports have pointed out a cooperative genetic interaction between Smad2 and Smad3 (20, 21). Although both Smad2 and Smad3 mediate signals from extracellular TGF-β and activin, it is thought that there may be other accessory proteins involved in regulating the balance of Smad2/Smad3 signaling (21).

It has recently been proved that TGF-β1/Smad3 signaling is mediated via two Smads pathways in endothelial cells: TGF-β1 may activate the two TβRI (ALK1 and ALK5) and then activated ALK5 phosphorylates the Smad2/3 protein while activated Smad1/5 signals through the ALK1 pathway. In the regulation of target gene transcription, ALK5 induces various types of collagen (including COL1A2, COL3A1, COL5A2, COL6A1, COL6A3, and COL7A1), TIMP, PAI-1, and other ECM genes, while ALK1 regulates *c-myc* gene transcription (9, 22–26). In endothelial cells, ALK5 inhibits their migration and proliferation, whereas ALK1 stimulates both the processes (18). These results indicate that TGF-β1 can initiate ALK5–Smad2/3 and ALK1–Smad1/5 signaling pathway.

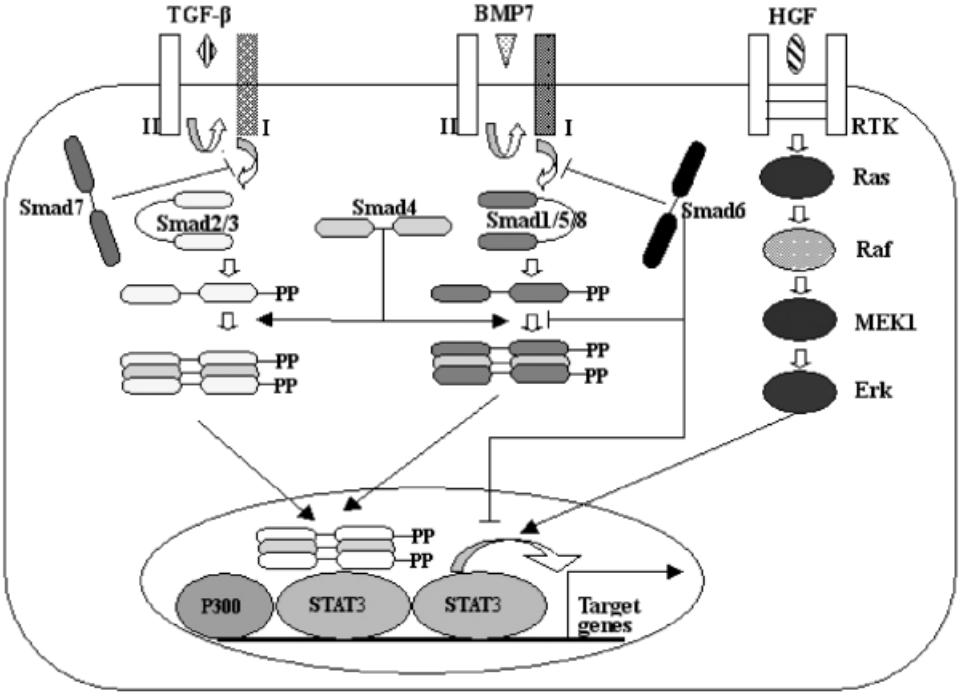

Fig. 1. A cartoon diagram of transforming growth factor-β1 (TGF-β)/Smad, bone morphogenetic protein (BMP)-7/Smad and HGF (ERK/MAPK) signaling pathways. TGF-β/Smad pathway: I and II represent types I and II TGF-β receptor, i.e. TβRI activin receptor-like kinase (ALK5) and TβRII, respectively. BMP-7/Smad pathway: I and II represent types I and II BMP receptor, i.e. BMPRIB and ActRII, respectively. ERK/MAPK pathway: RTK represents receptor tyrosine kinase. The folded Smad indicates the unstimulated one and the unfolded Smad indicates the stimulated one. PP indicates phosphorylation. Red, Smad7 (I-Smads); yellow, Smad2 and Smad3; green, co-Smad (Smad4); black, Smad6 (I-Smads); pink, Smad1, Smad5 and Smad8; blank, R-Smads (1–3, 5, 8).

As an I-Smad, Smad7 binds competitively to the type I receptor, blocks recruitment and phosphorylation of Smad2/3, and recruits E3-ubiquitin ligase for degradation of types I and II receptor complexes (27–29). In addition, Smad7 has been shown to recruit a complex of GADD34 and the catalytic subunit of protein phosphatase 1 to dephosphorylate and inactivate ALK5 (30). A body of evidence shows that Smad7 expression is induced by TGF-β1, enabling rapid downregulation of the TGF-β1 response in turn (31–33).

TGF-β levels in hepatic fibrosis

High levels of TGF-β1 are often found in many fibrotic tissues. The cytokine has been implicated as a mediator of fibrosis in many liver diseases (34). In fibrotic diseases of all tissues, regions of ECM excessive deposition show high expression of TGF-β1 (35). Accordingly, the TGF-β1 gene is upregulated in response to tissue injury, which correlates with the fibrogenic activities (7).

In liver disease

In patients with fibrosis and cirrhosisa, significantly high level of TGF-β1 was detected. For example, the expression of TGF-β1 mRNA in peripheral blood mononuclear cells of alcoholic cirrhosis patients was remarkably increased (36), matching with the high protein level in the loci from other reports (37, 38).

The study on 38 children patients with cystic fibrosis liver disease (CFLD) indicated that activated HSCs were the preeminent mediators (39). The dominant stimulus for ECM deposition by HSCs was mediated by the cytokine TGF-β1. Active HSCs were demonstrated as the cellular source of excess collagen production in the fibrosis surrounding the bile ducts and the advancing edge of scar tissue. It was shown that bile duct epithelial cells predominantly expressed TGF-β1 protein and mRNA. Thus, TGF-β1 expression was significantly correlated with both hepatic fibrosis and percentage of portal tracts showing histological abnormalities associated with CFLD.

In fibrotic liver, from patients and fibrotic rats resulted from bile duct ligation and scission, high levels of TGF-β1 RNA were present in most mesenchymal liver cells, in most inflammatory cells, and in few bile duct epithelial cells (40). Two models of hepatic fibrogenesis, one induced by the administration of carbon tetrachloride and the other by infection with schistosoma, increased concentrations of TGF-β1 mRNA and TGF-β1 in perisinusoidal cells paralleled the increased

expression of collagens (41). In addition, total TGF-β was found to increase in porcine serum-induced rat models (42). These suggested that the effects of TGF-β in liver regeneration and fibro genesis might be central.

In cultured HSCs

The steady advances in basic research exploring mechanisms of hepatic fibrosis have fueled tremendous progress in elucidating its pathophysiology. Central among these has been the identification of HSCs and their myofibroblastic counterparts as key sources of an array of mediators, matrix molecules, proteases, and their inhibitors that orchestrate the wound-healing response in liver. Methods to isolate and characterize HSC have provided tools to explore the pathogenesis of hepatic fibrosis in cultured cells of this type, animal models and human diseases (43).

Transcriptional upregulation of the TGF-β gene has been demonstrated in culture-activated HSCs (44). In the process of HSCs conversion to myofibroblasts, TGF-β1 mRNA and proteins show a sustaining increase *in vitro* (42, 45), corresponding with the characteristics of HSCs *in vitro* from fibrotic models induced by ligation of the bile duct (38). Rat HSCs in early primary culture exhibit a strikingly high expression of TGF-β, while they sustain a new peak of the cytokine after partial hepatectomy. In liver fibrotic models induced by ligation of the biliary duct, mRNA of TGF-β was found to increase expression only in HSCs (3).

Others

TGF-β1 plays a central role in diverse forms of wound healing. Evidence supporting this view comes from data indicating that elevated levels of plasma TGF-β correlate with increased fibrogenesis, including lung, kidney, skin, wound healing, eyes, and others fibrotic diseases (46–51).

Fibrogenesis induced by exogenous TGF-β

As already noted, TGF-β1 causes the ECM deposition by simultaneously stimulating cells to increase the synthesis of matrix proteins, decrease the production of matrix-degrading proteases, increase the production of inhibitors of these proteases, and modulate the expression of integrins in a manner that increases cellular adhesion to the matrix. Potential evidence provided by a large number of studies is that administration of exogenous TGF-β1 could induce fibrotic lesions in local tissues and organs *in vivo* or ECM deposition *in vitro* in cultured cells.

Effects of TGF-β1 on fibroblasts

HSCs are the major source of ECM components, and the activation of them is a crucial event in liver fibrogenesis, in which TGF-β1 is one of the key cytokines in the process. TGF-β1 treatment stimulates expression of ECM molecules but inhibits proliferation and induces phenotypic transdifferentiation *in vitro* (52). In addition, TGF-β1 stimulates collagen fiber formation in M cells (53). With regard to other fibroblasts, such as chick embryo backskin fibroblasts, it synthesized new ECM by exogenous TGF-β *in vitro* (54). Furthermore, the mRNA of type I collagen, the predominant matrix component in injured liver, is increased in cultured rat hepatocytes incubated with TGF-β1 (41).

Effects of TGF-β1 *in vivo*

Delivery of exogenous TGF-β1 by various means to tissues could result in severe fibrosis in experimental animals, and hepatic fibrosis was seen, which facilitates the understanding of TGF-β1 action and liver fibrogenic mechanisms. Intravenous administration of recombinant TGF-β1 to rats induced fibrotic lesions in the liver, kidney, pancreas, bone, heart, thymus, stomach, and cecum (55).

Because transgenic models are important and a further answer to the function of TGF-β1, which may also provide an appropriate paradigm for testing therapeutic interventions aimed at neutralizing the detrimental effects of this important cytokine, a large majority employed overexpressed transgenic models. For example, transgenic mice overexpressing a constitutively active human TGF-β1 developed fibrosis of the liver, kidney, and adipose tissue, and exhibited a severe reduction in body fat. Expression of the transgene in hepatocytes resulted in increase of collagen production, altered lobular organization, increased hepatocyte turnover, and, in extreme cases, hemorrhage and thrombosis (56). With a fusion gene consisting of a cDNA coding for the mature form of TGF-β1 under the control of the C-reactive protein gene promoter, a transgenic model with highly TGF-β1 expression was developed, which formed severe liver fibrosis in 6 weeks (57).

Other reports showed that transfection of the TGF-β1 gene into normal mice or rats resulted in the rapid development of liver fibrosis and glomerulosclerosis, which was characterized by ECM deposition including types I, II and IV collagen, and FN (58–60). With expression of high levels of the TGF-β1 in the livers and plasma, transgenic mice died because of hepatic

fibrosis and apoptotic death of hepacytes. The fibrotic process was characterized by deposition of collagen around individual hepatocytes and within the space of Disse in a radiating linear pattern. One line even developed glomerulonephritis and renal failure, arteritis and myocarditis, as well as atrophic changes in pancreas and testis (58).

In the other tissues, such as lung (61), renal (60, 62), and wounded skin (48), increase in the tissue levels of TGF-β1 induced fibrogenic effects, including deposition of collagen, FN, elastin, and other ECM proteins.

These studies establish TGF-β1 as a primary mediator in pathological fibrosis, although fibrosis occurred in some but not all tissues in transgenic models of TGF-β1 overexpression, and the significance of local vs circulating TGF-β1 remains unknown.

Anti-fibrogenic approaches

Building of tissue requires coordination of several important cellular actions including fibrogenesis, proliferation, migration, contraction, fibrolysis, and apoptosis. Once an imbalance appears in processes of fibrogenesis and fibrolysis, it may lead to scar formation and tissue fibrosis. At the cellular level, these interrelated events are orchestrated by numerous factors including many cytokines and the ECM. An important early change in the local ECM in response to injury appears to be the addition of EDA fibronectin (6, 63) that is secreted from and endothelial cells stimulated by TGF-β. HSCs functions that may contribute to the formation of permanent scar include proliferation, migration, contraction, and angiogenesis.

The steady advances in basic research exploring mechanisms of hepatic fibrosis have fueled tremendous progress in elucidating its pathophysiology. Central among these has been the identification of HSCs and their myofibroblastic counterparts as key sources of an array of mediators, matrix molecules, proteases, and their inhibitors that orchestrate the wound-healing response in liver (64, 65). One of the responses to injury is migration of activated HSCs termed 'myofibroblasts' to the wound area, in which many cytokines maybe involved. TGF-β has been implicated in multiple facets of the injury response. The most direct evidence in the involvement of TGF-β in fibrosis comes from *in vivo* and *in vitro* experiments, where agents that block TGF-β function reduce the fibrotic response in the liver and other tissues. For example, administrations of TGF-β antibodies or antisense oligonucleotides, and soluble TβRII are beneficial in liver, lung, renal, skin, and other tissue fibrosis. But the most compellent example of events is that loss of Smad3 might be resistant to fibrogenesis. Based on these and other advances in understanding the fundamentals of the injury response and TGF-β1–Smad3 signal pathway, several strategies now exist for preventing or halting fibrogenesis, ranging from agents that block TGF-β signaling to small molecular compounds.

Indeed, there are a number of reports of cellular or animal models of fibrosis where loss of TGF-β1–Smad3 regulation results in a diminished fibrotic response. The results of these studies will be summarized below.

Disruption of TGF-β1 protein

Overwhelming evidence has demonstrated that sustained overexpression of TGF-β1 is a key mediator of fibrotic disease in all tissues. Because TGF-β1 protein, as an extrocellular ligand, initiates the TGF-β1–Smads signal cascade it became a study target early. Several lines of evidence point to TGF-β as a key cytokine that initiates and terminates tissue repair and whose sustained production underlies the development of tissue fibrosis, which suggested that abolishment of TGF-β1 action could halt TGF-β1–Smad3 signaling and distinctly inhibit deposition of ECM *in vitro* and *in vivo*. The antibody of TGF-β earlier showed therapeutic intervention, and injecting anti-TGF-β antibody inhibited fibrogenic and inflammatory action of TGF-β1 in the liver, lung, kidney, skin, brain, joint, arterial wall, and other tissues (7).

In culture-activated HSCs, anti-TGF-β1 antibody treatment or the administration of adenoviruses constitutively expressing an antisense mRNA complementary to the 3′ coding sequence of TGF-β1 was able to attenuate the synthesis of TGF-β1 (66) and the production of collagen (42, 66). In addition, adenoviral expressing an antisense complementary to the rat TGF-β1 mRNA treatment could abolish the diverse effects of direct TGF-β1 function in ongoing liver fibrogenesis of rat models induced by ligation of bile duct (67).

TGF-β1 antibody could significantly reduce accumulation of collagen in bleomycin-induced mice lung (68) and prevent fibrotic complication in the lungs of mice with a murine sclerodermatous graft-vs-host disease (47). Furthermore, TGF-β antibody inhibited both the radiation-induced reduction in clonogenic activity of rat lung fibroblasts and the radiation-induced terminal differentiation of progenitor fibroblasts to

postmitotic fibrocytes, which are essential cellular processes in the development of radiation-induced fibrosis in the lung (12).

A neutralizing monoclonal antibody against TGF-β1 attenuated kidney hypertrophy and the enhanced expression of ECM gene in experimentally diabetic mice (69). Another work showed that the antibody of TGF-β is a promising agent to prevent renal tubular fibrosis and apoptosis in unilateral ureteral obstruction (UUO) (70). In chronic progressive anti-thymocyte serum nephritis, the TGF-β/Smad signaling was upregulated, whereas TGF-β blockade by antibody suppressed the progression of renal scarring, at least in part, via inhibition of activated TGF-β/Smad signaling (71). Earlier reports showed that an intraperitoneal therapy of murine anti-TGF-β monoclonal antibody significantly reduced blood pressure, proteinuria, and the degree of glomerulosclerosis and renal medullary fibrosis in Dahl salt-sensitive rats (72). High glucose-induced activation of Smad signaling and collagen synthesis proved to be TGF-β dependent, and blocked by a neutralizing TGF-β antibody too (73).

Systemic sclerosis is a connective tissue disorder characterized by fibroblast proliferation, excessive accumulation of ECM by fibroblasts, and its deposition on the affected skin. Anti-TGF-β1 antibody could effectively prevent this disorder in an experimental model (74), and a human anti-TGF-β1 monoclonal antibody, the CAT-192, showed safety, tolerability, and pharmacokinetics traits in clinical trials. In a murine model, neutralizing TGF-β antibody could prevent the skin fibrosis (47). Wounds treated with neutralizing antibody to TGF-β1 not only showed a lower inflammatory response, but also reduced early ECM deposition and later cutaneous scar formation in adult rodents (48). A free myocutaneous gracilis flap was transplanted from the groin to the neck region in experimental rats, before polyclonal TGF-β1 antibodies were injected into the neck region of them. The results showed that collagen types I–IV fibers significanly reducted in the transition area (75), and indicated that anti-TGF-β1 antibody treatment might improve the healing of free flaps in the graft beds prone to excessive fibrosis.

Furthermore, in experimental models, neutralization of TGF-β1 by anti-TGF-β1 antibody resulted in attenuation of thyroid, corneal, stromal, empyema and pleural fibrosis (76–78). Recently, experimental findings also suggest that Smad-binding peptide aptamers can be developed to selectively inhibit TGF-β-induced gene expression (79). These data above indicate that many tissues or organs fibrogenic disorders are subjected to common mechanisms that partially involve TGF-β1 overexpression, and that deletion of TGF-β1 activity could halt various fibrogenic processes in all tissues.

Blockage of TβRII

For the type II TGF-β receptor, three strategies to block its signaling have efficaciously prevented fibrogenesis in the levels of cell and experimental models. One uses a 'dominant-negative' receptor strategy, in which a TβRII with a truncated cytoplasmic domain is delivered through a certain vector in which the adenovirus is generally used. The second approach is administration of a 'soluble TGF-β receptor', which consists of the extracellular portion of the TβRII spliced into chimeric IgG. Thirdly, the disruption of the gene interest is a consensus and authoritative technology, which includes gene knockout and knockdown, and the latter consists of both antisense and RNA interfence methods.

Firstly, the 'dominant-negative receptor' strategy takes advantage of the high transfection of the adenovirus vector and the no-kinase action of the truncated TβRII. The adenovirus holds particular interest for the liver because of the fact that this vector homes predominantly in the liver after its intravenous administration (80). The strategy was proved to be able to abolish all the diverse signaling from TGF-β in arterial endothelial cells, lung epithelial cell, smooth muscle cells, and rat HSCs (81, 82). This type of receptor treatment could not only enhance hepatocyte regeneration *in vivo* (82), but also therapeutically attenuate liver fibrosis in dimethylnitrosamine (DMN)-induced rats (82–84). Using this approach, investigators not only decreased pancreatic fibrosis and protected the pancreas against chronic injury by preventing acinar cell apoptosis (85), but also inhibited luminal loss after percutaneous transluminal cornary angioplasty (PTCA) in porcine coronary arteries, which was caused by local deposition of collagen (86).

Secondly, the soluble TβRII, consisting of the extracellular portion of the TβR-II fused to the Fc or other portion of IgG, presumably functions as a competitive binder of TGF-β, preventing interaction of the cytokine with its normal receptor. As a distinct advantage over a small peptide competitor, which would be expected to disappear within a few minutes of injection, the IgG can circulate for several days. The intervening vehicle was often used the adenovirus vector mentioned above, too.

The soluble receptor not only bound TGF-β and blocked TGF-β-signaling *in vitro*, but also

abrogated the fibrogenetic effect of TGF-β in animals subjected to bile duct ligation, DMN, and CCl_4 treatment (84, 87, 88). There is now unequivocal and mounting evidence that fibrotic diseases of other tissues can be prevented or attenuated with this strategy, including hamster's lung fibrosis induced by bleomycin and experimental renal disease (89–91).

Finally, although knockdown of TβRII gene led to a lethal inflammatory disease in adult animals (92), the gene knockdown by administration of an antisense TβRII recombinant plasmid was proved to halt formation of rat liver fibrosis induced by pig serum (93), and a small interference RNA (SiRNA), as a new tool for silencing of targeting gene, targeting TβRII could not only knockdown the TβR-II gene but also prevent ocular inflammation and scar formation *in vitro* and *in vivo* (94).

Inhibition of type I receptor

In most mammal cell types, TGF-β1 signals through ALK5, the TGF-β type I receptor, although TGF-β1 can bind to and transduce signals through ALK1 and ALK5 in other cell types (95, 96). It is notable that ALK5-deficient endothelial cells show an impaired TGF-β–ALK1 response (96), implicating that activated ALK5 is very important in TGF-β1–Smads signaling pathway and that disruption of TβRI biological action will effectively block TGF-β–Smad2/3 signal cascade. For example, administration of an antisense TβRI recombinant plasmid was proven to halt formation of rat liver fibrosis induced by pig serum (93).

Small molecular inhibitors had been proven extremely useful for investigating signal transduction pathways and have the potential for development into therapeutics for inhibiting signal transduction pathways whose activities contribute to human diseases. A new class of water-soluble small molecular inhibitors, related to imidazole inhibitors of p38, have recently been shown to inhibit the kinase activity of ALK4, ALK5, and ALK7. An initial inhibitor in this class, SB-431542, inhibited TGF-β-induced phosphorylation and nuclear translocation of Smad3 as well as TGF-β-induced COL1A1 mRNA levels (97–99). The compound inhibits TGF-β1-induced FN and Col Iα1 in renal epithelial carcinoma A498 cells and selectively inhibits kinase activity of ALK5 with IC_{50} of 94 nM *in vitro*. Another similar compound, SB-505124, was found to selectively interfere with ALK5 more potently than SB-431542 and abrogate cell death induced by TGF-β1 (100).

Intriguingly, a recent study demonstrated that blocking TGF-β effect through ALK5 inhibition displayed a promising approach for the treatment of liver fibrosis (101). GW6604, another small molecular inhibitor of ALK5, could not only inhibit TGF-β1-induced transcription of PAI-1 *in vitro*, but also facilitate the regeneration of liver that had undergone partial hepatectomy in TGF-β1 overexpressing mice. In addition, the compound significantly decreased the deposition of ECM and meliorated liver dysfunction in a DMN-induced fibrotic model.

These results further suggested that inhibition of ALK5 is an attractive new approach to treat liver fibrosis by both preventing matrix deposition and promoting hepatocyte regeneration.

Deletion of Smad3

Smad3 protein has been identified as an intracellular mediator in the fibrogenic process via TGF-β, p38 MAPK, and ERK MAPK signaling pathways (102, 103), and increasing evidence implicates Smad3 as at least a partial mediator of the fibrosis (21). For example, Smad3 completely mediates the expression of Col Iα1 in activated HSCs (104). In addition, Smad3 overexpression increased FN promoter activity, an effect that was enhanced by treatment with TGF-β. The TGF-β-stimulated activity of FN promoter was prevented by transfection with either a dominant-negative Smad3 or Smad7 in mesangial cells (105). The use of animals with a targeted deletion of Smad3 showed that most of the fibrogenic activities of TGF-β were mediated by Smad3. The loss of Smad3 interferes with TGF-β-mediated induction of epithelial–mesenchymal cell transition (EMT) and the expression of genes for collagens, PAI-1 and TIMP (21, 106). Smad3 null inflammatory cells and fibroblasts also do not respond to the chemotactic effects of TGF-β and do not autoinduce TGF-β secretion (21).

Although TGF-β signaling plays a prominent role in liver fibrogennesis by HSCs and myofibroblasts during chronic liver injury, other cytokines also are involved in the process of fibrogenesis. For example, PDGF could lead to activation of p38 MAPK through binding PDGF receptors, and activated p38 MAPK pathway phosphorylates Smad3 at the linker region (102). It is suggested that basal Smad3 activation in myofibroblasts might be induced partially by PDGF via p38 MAPK signaling. Also, bodies of evidence indicate that MAPK and Smad3 pathway can interact with different outcomes, depending on the cellular context, although

Smad3 phosphorylation by p38 MAPK was unable to activate the transcription of PAI-1 (102). It is perfect that anti-fibrogenesis focuses at the Smad3 level, because the deactivated Smad3 could block not only TGF-β–Smad3 but also p38 MAPK–Smad3 and even other undefined pathways.

Another work, which explored the contribution of Smad3 in mediating TGF-β fibrotic responses in an acute liver injury model and in cultured HSCs, showed that hepatic Col Iα1 and α2 mRNA of Smad3 homozygous knockout mice were significantly reduced than that of wild-type mice 72 h after CCl_4 was induced. In culture-activated HSCs from Smad3 null mice, mRNA of Col Iα1 was decreased (104).

Smad3 null mice could resist lung fibrosis transient induced by using adenoviral vector-mediated gene transfer TGF-β, associated with high spontaneous presence of MMP-9 and MMP-12 in the lung (107). Smad3 deficiency attenuated renal fibrosis, inflammation, and apoptosis after UUO. The deposition of types I and III collagens was significantly reduced in the obstructed kidney of Smad3 null mice. In addition, the numbers of myofibroblasts, macrophages, and CD4/CD8 T cells infiltrated into the kidney were significantly attenuated in the obstructed kidney of these mice (108). These results suggest that the Smad3 pathway plays a pivotal role in ECM metabolism, and involves pathogenic mechanisms mediating tissue destruction and fibrogenesis.

The study, which investigated the potential role of Smad3 in Smad3-null mice using a bleomycin-induced model of skin fibrosis, showed that lesional skin from null mice attenuated fibrosis, reduced synthesis and accumulation of collagen, and inhibited collagen gene transcription *in situ*, compared with wild-type mice, and that CTGF and α-SMA expressions in lesional skin were also significantly attenuated (109). Furthermore, the α-smooth muscle actin (α-SMA) was reduced in corneal repair tissue of Smad3$^{-/-}$ mice, although the expression of FN was unaffected (110).

Taken together, these data showed Smad3 as a pivotal mediator for TGF-β in modulating fibrogenic function and implicated that inhibition of Smad3 protein function might be a potential and effective therapeutic strategy to anti-fibrogenesis.

Other strategies

Besides the TGF-β signaling pathway, other pathways and factors have been found to involve tissue fibrosis. They include BMP-7, hepatic growth factor (HGF), protease inhibitors, and others. BMP-7, a member of TGF-β superfamily, has been shown to impede the progression of fibrotic disease and decrease interstitial volume, and prevent glomerular sclerosis. Experimental evidence suggests that administration of BMP-7 may be an effective treatment to restore or preserve renal histology and renal function (111). As KCP, an enhancer of BMP signaling, binds to BMP-7 and enhances binding to the type I receptor, Smad1-dependent transcription and phosphorylated Smad1 (P-Smad1) levels are increased. Further experiments indicated that Kcp(−/−) animals displayed reduced levels of P-Smad1, and became more susceptible to developing renal interstitial fibrosis (112) (BMP-7/Smads pathway in Fig. 1).

HGF has been identified to have antifibrogenic activity in rats. Several investigations demonstrated that HGF could inhibit stellate cell activation, decrease the mRNA levels of procollagenα2 (I), α1 (III), and α1 (IV), and influence the expression of α-smooth muscle actin, desmin, and TGF-β1. Furthermore, HGF plays antifibrogenic roles even after liver fibrosis has been established (113). Subsequently, Liu et al. found that SnoN physically interacted with activated Smad2 to form a transcriptionally inactive complex, which in turn superseded the profibrotic action of TGF-β1. The results suggest that HGF can interplay with TGF-β signaling pathway and block EMT through the upregulation of Smad transcriptional co-repressor SnoN expression (114). In addition, HGF stimulates or induces proteases involved in the breakdown of ECM proteins including MT1-MMP, uPA, and some MMPs, whereas it reduces TIMP-1 and TIMP-2 expression. Recently, Watanabe et al. reported that the addition of exogenous HGF significantly decreased TNF-α, IL-6, and collagen synthesis after bleomycin injury. Thus, HGF is likely to be against fibrosis in multiple ways (113, 115) (HGF pathway in Fig. 1).

Similarly, some protease and chemical inhibitors have been investigated *in vitro* and *in vivo*. In fibrotic models, TGF-β expression was suppressed and the tissue fibrosis was reduced by chymase inhibitors. Obviously, chymase inhibitors may be useful for preventing fibrosis via inhibition of TGF-β activation (116). Interestingly, Pirfenidone (PFD) is a recently developed antifibrotic agent. Chemically, PFD is a substituted pyridine molecule 5-metyl-1-phenil-2-(1H)-pyridine). This small molecule can prevent or even revert ECM accumulation, as found in pulmonary fibrosis, peritoneal sclerosis, cardiac fibrosis, and progressive renal fibrosis. PFD notably inhibits the stellate hepatic cell proliferation in rats, significantly reduces the mRNA level of

TIMP-1, and suppresses the increment in TGF-β (117).

Upregulation of Smad7 expression

The interaction of R-Smads with their receptors is governed by several different regulators including Smad7. Smad7 is termed an inhibitory Smad because it competes with R-Smads for the type I receptor and blocks signal transduction (27–29). The introduction of Smad7 inhibits experimental fibrogenesis *in vivo*, while many studies with isolated HSC indicate that the underlying mechanisms involve inhibition of TGF-β signaling. The steady advances in basic research exploring mechanisms of hepatic fibrosis have indicated that the specific blockade of TGF-β/Smad3 signaling by expression of Smad7 may provide a new therapeutic potential for tissue fibrosis.

The most exciting report may be that Smad7 overexpresson by adenovirus carrying Smad7 cDNA displays inhibited collagen and α-SMA expression and reduced hydroxyproline content in the common bile duct ligation rat livers (118). Smad7 expression totally blocked TGF-β signal transduction by inhibiting Smad2/3 phosphorylation, and nuclear translocation of activated Smads complexes resulted in decreased collagen I expression. Exogenous Smad7 also abrogated TGF-β-dependent proliferation inhibition of HSCs and destructed the fibrillar organization of the actin cytoskeleton (118).

The intratracheal administration of an adenovirus expressing Smad7 could prevent bleomycin-induced lung fibrosis in mice (119). Similar results from another study showed that the ectopic overexpression of Smad7 prevents the downregulation of surfactant protein C gene expression and blocks the phosphorylation of Smad2 induced by TGF-β in cultured lung (28).

Like liver fibrosis, TGF-β-mediated renal fibrosis is regulated positively by Smad3, but negatively by Smad7. The levels of Smad7 protein display a progressive decrease in UUO nephropathy in mice, whereas Smurf1 and Smurf 2 with eliminated Smad7 in turn show increased levels (120). The exogenous Smad7 introduced by recombinant adenovirus vector or ultrasound-mediated inducible gene transfection significantly inhibited renal fibrosis on UUO-induced renal fibrosis in rats (121, 122). In messangial cells, the reporter gene showed that overexpression of Smad7 effectively blocked the activity of FN, Col Iα2 promoter, and other effects stimulated by TGF-β (33,105). Furthermore, the overexpression of inhibitory Smad7 in renal and vascular cells had been shown to suppress high glucose-induced Smad2 and Smad3 activation and collagen synthesis (73).

Smad3 phosphorylation and PAI-1 expression were augmented in systemic sclerosis (SSc) fibroblasts as compared with normal fibroblasts, while adenovirus-mediated overexpression of Smad7 restored normal PAI-1 production in SSc fibroblasts (123). In addition, adenovirus-mediated transient expression of Smad7 prevented the injury-induced EMT of lens epithelial cells and the sealing of capsular broken with fibrous tissue (124). Furthermore, anti-TGF-β effects of IFN-ã on diverse cellular functions, specifically anti-liver fibrosis, might be through inducing the expression of Smad7 (125, 126).

Gene silencing of TGF-β/Smad3 pathway

A potent strategy to anti-fibrosis

As new approaches to gene therapy, except the transgenic approach, there are two methods that interfere with the manufacture of proteins in mammal cells such as antisense and RNA interference. Numerous experiments have shown that gene silencing may be a new way to treat fibrosis.

Antisense and ribozyme oligonucleotides

Introducing a neutralizing 'antisense' version of RNA can modify TGF-β mRNA bioactivity, showing anti-fibrogenic characterization. An antisense complementary to the 3′ portion of rat TGF-β1 mRNA, expressed by an adenoviral vector, could abolish the diverse effects of direct TGF-β1 function in ongoing liver fibrogenesis (67). The TβRII gene knockdown by administration of antisense TβR-II recombinant plasmids was proven to halt formation of rat liver fibrosis induced by pig serum (93). A recent study showed that TGF-β1 expression was significantly downregulated in activated HSCs by U1 snRNA chimeric ribozymes, and that U1 snRNA chimeric ribozymes reduced the synthesis and deposition of Col I in transfected HSC-T6 cells. In addition, the ribozyme expressed by adenoviral vector could alleviate fibrotic pathology in rats treated with carbon tetrachloride (127).

In UUO rat kidneys, it has been revealed that TGF-β1 antisense oligodeoxynucleotides treatment dramatically decreased TGF-β1 and type I collagen mRNA levels, and significantly reduced the interstitially fibrotic area of the obstructed kidneys (128, 129). Because TGF-β2 is the predominant form in the eyes (49), antisense oligonucleotides against TGF-β2 remarkably reduced postoperative scarring and improved surgical outcome of eyes in two different animal models

(130, 131). In addition, the ribozyme oligonucleotides to actively cleave the targeted TGF-β gene suppressed the TGF-β1 expression *in vivo* (132), reduced collagen synthesis and their mRNA level, and significantly inhibited neointimal formation after vascular injury in the rat carotid artery model. The results indicated that ribozyme gene therapy, like antisense oligonucleotides, could act as a new avenue for the treatment of diverse diseases caused by TGF-β1 overexpression (132).

Although antisense phosphorothioate oligonucleotides and ribozymes anti-TGF-β1 could reverse the character of tissue fibrosis *in vitro* and improve fibrotic pathology *in vivo*, their effectiveness, stability, specificity, and security are still in debate, whereas RNA interference may be a new way to inhibit tissue fibrosis.

RNA interference (RNAi)–small interfering RNA (siRNA)

The discovery of RNAi has opened an unanticipated new window on gene expression regulation. RNAi technology has been employed as a facile and effective tool for function valuation and target gene identification (133, 134). A large number of studies with animal models have confirmed that RNAi may conceivably be exploited for human therapeutics in the future, possibly bringing greater clinical excition than have the so far disappointing antisense endeavors (134, 135).

As a new technology for silencing of targeting gene, siRNA targeting Smad3 could not only inhibit Smad3 transcription and nuclear localization, but prevent TGF-β1-induced growth arrest and promoter activity in murine B-cell lymphoma lines and mink lung epithelial cell line (Mv1Lu) (135). A common siRNA targeting the cognate sequence of Smad3 mRNAs from human, rat, and mouse species designed by our group could effectively decrease levels of Smad3 mRNA and collagen I mRNA in cultured rat HSC-T6 cell line. This approach had also had similar applications for other tissues, including eyes, skin, kidney, and lung. For example, siRNAs targeting TβRII could not only knockdown the TβR-II gene but also prevent ocular inflammation and scar formation *in vitro* and *in vivo* (94).

Moreover, the knockdown of TGF-β1 and TGF-β2 by siRNA technology could prevent the downregulation of NKG2D on immune cells mediated by LNT-229 glioma cell supernatant, strongly enhance MICA expression in the glioma cells and promote tumor recognition and lysis by CD8(+) T and NK cells. TGF-β silencing could also lead to a less migratory and invasive glioma cell phenotype *in vitro* (136).

The antisense oligonucleotides, ribozymes, and RNAi can reverse fibrotic pathology *in vitro* and *in vivo*, indicating that TGF-β1 might be a novel candidate for therapeutic strategies against hepatic fibrosis.

Conclusion and perspectives

Overwhelming evidence has demonstrated that sustained overexpression of TGF-β is a key mediator of fibrotic tissues. The potentially fibrogenic effects of TGF-β include stimulation of matrix synthesis, inhibition of matrix degradation, and modulation of matrix receptor expression. In addition, TGF-β1 also alters the number and expression of various integrins on some cells, and potentially enhances their adhesion to the ECM (137, 138). Hence, anti-TGF-β1/Smad3 intervention might be therapeutic in already-established fibrotic livers, not only by suppressing fibrosis, but also by facilitating hepatocyte regeneration (82).

Although TGF-β1/Smad3 antagonists have been shown to be effective anti-fibrogenic agents, they need detailed safety testing and efficacy evaluation. Firstly, besides TGF-β, many signaling agents, including platelet-derived growth factor (PDGF), endothelin (ET), TIMP, and TNF-α, are likely to contribute to liver injury and repair (6, 65, 102, 139). And it has been proposed that the combination of connective tissue growth factor (CTGF) and TGF-β is needed for optimal matrix synthesis (6, 140). Second, inhibition of the TGF-β/ALK5 pathway may activate TGF-β/ALK1 in endothelial cells (18) and BMP/Smad1 pathways in HSCs (141). Third, the anti-proliferative action of TGF-β has been documented largely with particular evidence in HSCs and rats (4). It has been shown that inhibition of TGF-β during liver fibrogenesis leads to increased HSCs proliferation (87). Moreover, it should be emphasized even more that up to now there are no long-term animal experiments available systematically studying side effects of systemic TGF-β inhibition. Thus, it is attractive to speculate that the high dose of antagonists of TGF-β signaling might potentially stimulate proliferation of HSC (53, 142) and offset their antifibrotic effects such as precancerous risks, various autoimmunopathies, atherosclerotic lesions, and chronic inflammatory diseases (143). Finally, TGF-β is a potent immunosuppressive agent and plays a role in the pathogenesis of chronic inflammatory diseases, so that blocking TGF-β may stimulate the development of autoimmune diseases (14, 143–145).

Considered together, one concern is that long-term administration of TGF-β antagonists, including antibody, soluble receptor, small molecular inhibitors, and siRNAs, might increase the immunoinflammatory axis of diseases and result in the exacerbation of inflammation and the unexpected injury of the liver and other organs. In addition, because TGF-β/Smads signaling forms cross-talk networks with other signal pathways, inhibition of the signaling certainly affects the intricate networks and leads to unwanted side effects. Furthermore, a practical consideration with any method of blocking TGF-β can result in growth dysregulation (146) and increase risks of neoplasia issue (147, 148). All of them require careful planning in any clinical trial, although a report has showed an attractive experimental result that a soluble TβRII antagonist has potential for long-term clinical use in preventing metastasis (149, 150).

Because Smad3 plays such a critical role in mediating the pathology of fibrotic disease, inhibition of Smad3 may be a prime target for intervention in fibrotic conditions (102–110). A therapeutic agent that blocks only signaling through Smad3 might be ideal to inhibit fibrosis with minimal side effects. Concern about the safety, efficacy, and possible side effects of gene therapy strategies or systemic cytokine administration to inhibit fibrotic disease makes the use of small molecule inhibitors of Smad3 attractive therapeutic agents. And traditional Chinese herb extracts may be a new area, because several traditional Chinese herbs have been demonstrated by some reports to have preventive effects on hepatic fibrosis (140, 151). One possible mechanism is that those herb extracts might be involved in inhibiting TGF-β1/Smad3 signaling pathway (152).

In addition, as knowledge advances concerning the details of the pathways subserving individual responses to TGF-β/Smad3 signaling, it will improve the targeting, and presumably the safety, of intervention based on modulating the effect of this important cytokine.

References

1. Friedman S L, Maher J J, Bissell D M. Mechanisms and therapy of hepatic fibrosis: report of the AASLD single topic basic research conference. Hepatology 2000; 32: 1403–8.
2. Wells R G. The role of matrix stiffness in hepatic stellate cell activation and liver fibrosis. J Clin Gastroenterol 2005; 39: S158–61.
3. Bissell D M, Wang S S, Jarnagin W R, Roll F J. Cell-specific expression of transforming growth factor-beta in rat liver. Evidence for autocrine of hepatocyte proliferation. J Clin Invest 1995; 96: 447–55.
4. Bissell D M, Roulot D, George J. Transforming growth factor-β and the liver. Hepatology 2001; 34: 859–67.
5. Massague J, Chen Y G. Controlling TGF-β signaling. Genes Dev 2000; 14: 627–44.
6. Leask A, Abraham D J. TGF-β signaling and the fibrotic response. FASEB J 2004; 18: 816–27.
7. Border W A, Noble N A. Transforming growth factor β in tissue fibrosis. N Engl J Med 1994; 331: 1286–92.
8. Ogawa K, Chen F, Kuang C, Chen Y. Suppression of matrix metalloproteinase-9 transcription by transforming growth factor-β is mediated by a nuclear factor-κB site. Biochem J 2004; 381: 413–22.
9. Verrecchia F, Chu M L, Mauviel A. Identification of novel TGF-β/Smad gene targets in dermal fibroblasts using a combined cDNA microarray/promoter transactivation approach. J Biol Chem 2001; 276: 17058–62.
10. Jonsson J R, Clouston A D, Ando Y, Kelemen L I, Horn M J, Adamson M D, et al. Angiotensin-converting enzyme inhibition attenuates the progression of rat hepatic fibrosis. Gastroenterology 2001; 121: 148–55.
11. Martin M, Lefaix J, Delanian S. TGF-β1 and radiation fibrosis: a master switch and a specific therapeutic target? Int J Radiat Oncol Biol Phys 2000; 47: 277–90.
12. Hakenjos L, Bamberg M, Rodemannm H P. TGF-β1-mediated alterations of rat lung fibroblast differentiation resulting in the radiation-induced fibrotic phenotype. Int J Radiat Biol 2000; 76: 503–9.
13. de Gouville A C, Boullay V, Krysa G, Pilot J, Brusq J M, Loriolle F, et al. Inhibition of TGF-β signaling by an ALK5 inhibitor protects rats from dimethylnitrosamine-induced liver fibrosis. Br J Pharmacol 2005; 145: 166–77.
14. Monteleone G, Kumberova A, Croft N M, McKenzie C, Steer H W, MacDonald T T. Blocking Smad7 restores TGF-β1 signaling in chronic inflammatory bowel disease. J Clin Invest 2001; 108: 601–9.
15. Piek E, Heldin C H, ten Dijke P. Specificity, diversity, and regulation in TGF-β superfamily signaling. FASEB J 1999; 13: 2105–24.
16. Massague J, Chen Y G. Controlling TGF-β signaling. Genes Dev 2000; 14: 627–44.
17. Heldin C H, Miyazono K, ten Dijke P. TGF-β signaling from cell membrane to nucleus through SMAD proteins. Nature 1997; 390: 465–71.
18. ten Dijke P, Hill C S. New insights into TGF-β-Smad signaling. Trend Biochem Sci 2004; 29: 265–73.
19. Derynck R, Zhang Y E. Smad-dependent and Smad-independent pathways in TGF-β family signaling. Nature 2003; 425: 577–84.
20. Weinstein M, Yang X, Deng C X. Functions of mammalian Smad genes as revealed by targeted gene disruption in mice. Cytokine Growth Factor Rev 2000; 11: 49–58.
21. Flanders K C. Smad3 as a mediator of the fibrotic response. Int J Exp Pathol 2004; 85: 47–64.
22. Goumans M J, Lebrin F, Valdimarsdottir G. Controlling the angiogenic switch: a balance between two distinct TGF-β receptor signaling pathways. Trends Cardiovasc Med 2003; 13: 301–7.
23. Goumans M J, Valdimarsdottir G, Itoh S, Rosendahl A, Sideras P, ten Dijke P. Balancing the activation state of the endothelium via two distinct TGF-β type I receptors. EMBO J 2002; 21: 1743–53.
24. Ota T, Fujii M, Sugizaki T, Ishii M, Miyazawa K, Aburatani H, et al. Targets of transcriptional regulation by two distinct type I receptors for transforming growth

factor-β in human umbilical vein endothelial cells. J Cell Physiol 2002; 193: 299–318.
25. Vindevoghel L, Lechleider R J, Kon A, de Caestecker M P, Uitto J, Roberts A B, et al. SMAD3/4-dependent transcriptional activation of the human type VII collagen gene (COL7A1) promoter by transforming growth factor β. Proc Natl Acad Sci USA 1998; 95: 14769–74.
26. Dennler S, Itoh S, Vivien D, ten Dijke P, Huet S, Gauthier J M. Direct binding of Smad3 and Smad4 to critical TGF β-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. EMBO J 1998; 17: 3091–100.
27. Shi Y, Massague J. Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell 2003; 113: 685–700.
28. Zhao J, Shi W, Chen H, Warburton D. Smad7 and Smad6 differentially modulate transforming growth factor-β-induced inhibition of embryonic lung morphogenesis. J Biol Chem 2000; 275: 23992–7.
29. Ebisawa T, Fukuchi M, Murakami G, Chiba T, Tanaka K, Imamura T, et al. Smurf1 interacts with transforming growth factor-β type I receptor through Smad7 and induces receptor degradation. J Biol Chem 2001; 276: 2477–80.
30. Shi W, Sun C, He B, Xiong W, Shi X, Yao D, et al. GADD34-PP1c recruited by Smad7 dephosphorylates TGF-β type I receptor. J Cell Biol 2004; 164: 291–300.
31. Nakao A, Afrakhte M, Moren A, Nakayama T, Christian J L, Heuchel R, et al. Identification of Smad7, a TGF-β-inducible antagonist of TGF-β signaling. Nature 1997; 389: 631–5.
32. von Gersdorff G, Susztak K, Rezvani F, Bitzer M, Liang D, Bottinger E P. Smad3 and Smad4 mediate transcriptional activation of the human Smad7 promoter by transforming growth factor β. J Biol Chem 2000; 275: 11320–6.
33. Chen R, Huang C, Morinelli T A, Trojanowska M, Paul R V. Blockade of the effects of TGF-β1 on mesangial cells by overexpression of Smad7. J Am Soc Nephrol 2002; 13: 887–93.
34. Gressner A M, Weiskirchen R, Breitkopf K, Dooley S. Roles of TGF-β in hepatic fibrosis. Front Biosci 2002; 7: d793–807.
35. Branton M H, Kopp J B. TGF-β and fibrosis. Microbes Infect 1999; 1: 1349–65.
36. Chen W X, Li Y M, Yu C H, Cai W M, Zheng M, Chen F. Quantitative analysis of transforming growth factor β1 mRNA in patients with alcoholic liver disease. World J Gastroenterol 2002; 8: 379–81.
37. Takiya S, Tagaya T, Takahashi K, Kawashima H, Kamiya M, Fukuzawa Y, et al. Role of transforming growth factor β 1 on hepatic regeneration and apoptosis in liver diseases. J Clin Pathol 1995; 48: 1093–7.
38. Roulot D, Sevcsik A M, Coste T, Strosberg A D, Marullo S. Role of transforming growth factor β II receptor in hepatic fibrosis: studies of human chronic hepatitis C and experimental fibrosis in rats. Hepatology 1999; 29: 1730–8.
39. Lewindon P J, Pereira T N, Hoskins A C, Bridle K R, Williamson R M, Shepherd R W, et al. The role of hepatic stellate cells and transforming growth factor-β1 in cystic fibrosis liver disease. Am J Pathol 2002; 160: 1705–15.
40. Milani S, Herbst H, Schuppan D, Stein H, Surrenti C. Transforming growth factors beta 1 and beta 2 are differentially expressed in fibrotic liver disease. Am J Pathol 1991; 139: 1221–9.
41. Czaja M J, Weiner F R, Flanders K C, Giambrone M A, Wind R, Biempica L, et al. In vitro and in vivo association of transforming growth facto-β1 with hepatic fibrosis. J Cell Biol 1989; 108: 2477–82.
42. Okuno M, Moriwaki H, Imai S, Muto Y, Kawada N, Suzuki Y, et al. Retinoids exacerbate rat liver fibrosis by inducing the activation of latent TGF-β in liver stellate cells. Hepatology 1997; 26: 913–21.
43. Friedman S L. Liver fibrosis – from bench to bedside. J Hepatol 2003; 38: s38–53.
44. Kim Y, Ratziu V, Choi S G, Lalazar A, Theiss G, Dang Q, et al. Transcriptional activational transforming growth factor β1 and its receptors by the kruppel-like factor Zf9/Core promoter bingding protein and Sp1. J Biol Chem 1998; 273: 33750–8.
45. Bachem M G, Meyer D, Melchior R, Sell K M, Gressner A M. Activation of rat liver perisinusoidal lipocytes by transforming growth factors derived from myofibroblastlike cells. J Clin Invest 1992; 89: 19–27.
46. Cook D N, Brass D M, Schwartz D A. A matrix for new ideas in pulmonary fibrosis. Am J Respir Cell Mol Biol 2002; 27: 122–4.
47. McCormick L L, Zhang Y, Tootell E, Gilliam A C. Anti-TGF-β treatment prevents skin and lung fibrosis in murine sclerodermatous graft-versus-host disease: a model for human scleroderm. J Immunol 1999; 163: 5693–9.
48. Shah M, Revis D, Herrick S, Baillie R, Thorgeirson S, Ferguson M, et al. Role of elevated plasma transforming growth factor-β1 levels in wound healing. Am J Pathol 1999; 154: 1115–24.
49. Connor T B Jr, Roberts A B, Sporn M B, Danielpour D, Dart L L, Michels R G, et al. Correlation of fibrosis and transforming growth factor-β type 2 levels in the eye. J Clin Invest 1989; 83: 1661–6.
50. Shehata M, Schwarzmeier J D, Hilgarth M, Hubmann R, Duechler M, Gisslinger H. TGF-β1 induces bone marrow reticulin fibrosis in hariry cell leukemia. J Clin Invest 2004; 113: 676–85.
51. Lafyatis R, Thompson N L, Remmers E F, Flanders K C, Roche N S, Kim S J, et al. Transforming growth factor-β production by synovial tissues from rheumatoid patients and streptococcal cell wall arthritic rats. Studies on secretion by synovial fibroblast-like cells and immunohistologic localization. J Immunol 1989; 143: 1142–8.
52. Gressner A M. Transdifferentiation of hepatic stellate cells (Ito cells) to myofibroblasts: a key event in hepatic fibrogenesis. Kidney Int 1996; 54(Suppl.): S39–45.
53. Kato J, Ido A, Hasuike S, Uto H, Hori T, Hayashi K, et al. Transforming growth factor-β-induced stimulation of formation of collagen fiber network and anti-fibrotic effect of taurine in an in vitro model of hepatic fibrosis. Hepatol Res 2004; 30: 34–41.
54. Locci P, Baroni T, Lilli C, Martinese D, Marinucci L, Bellocchio S, et al. TGFβ and TGFα, antagonistic effect in vitro on extracellular matrix accumulation by chick skin fibroblasts at two distinct embryonic stages. Int J Dev Biol 1999; 43: 157–65.
55. Terrell T G, Working P K, Chow C P, Green J D. Pathology of recombinant human transforming growth factor -β 1 in rats and rabbits. Int Rev Exp Pathol 1993; 34(Part B): 43–67.
56. Clouthier D E, Comerford S A, Hammer R E. Hepatic fibrosis, glomerulosclerosis, and a lipodystrophy-like syndrome in PEPCK-TGF-β1 transgenic mice. J Clin Invest 1997; 100: 2697–713.
57. Kanzler S, Lohse A W, Keil A, Henninger J, Dienes H P, Schirmacher P, et al. TGF-β 1 in liver fibrosis: an

inducible transgenic mouse model to study liver fibrogenesis. Am J Physiol 1999; 276: G1059–68.
58. Sanderson N, Factor V, Nagy P, Kopp J, Kondaiah P, Wakefield L, et al. Hepatic expression of mature transforming growth factor β 1 in transgenic mice results in multiple tissue lesions. Proc Natl Acad Sci USA 1995; 92: 2572–6.
59. Imai E, Isaka Y, Fujiwara Y, Kaneda Y, Kamada T, Ueda N. Introduction of a foreign gene into the kidney in vivo: development of glomerulosclerosis by the transfection of genes for PDGF and TGF-β. Contr Nephrol 1994; 107: 205–15.
60. Kopp J B, Factor V M, Mozes M, Nagy P, Sanderson N, Bottinger E P, et al. Transgenic mice with increased plasma levels of TGF-β 1 develop progressive renal disease. Lab Invest 1996; 74: 991–1003.
61. Sime P J, Xing Z, Graham F L, Csaky K G, Gauldie J. Adenovector-mediated gene transfer of active transforming growth factor-β1 induces prolonged severe fibrosis in rat lung. J Clin Invest 1997; 100: 768–76.
62. Schnaper H W, Hayashida T, Hubchak S C, Poncelet A C. TGF-β signal transduction and mesangial cell fibrogenesis. Am J Physiol Renal Physiol 2003; 284: F243–52.
63. George J, Wang S S, Sevcsik A M, Sanicola M, Cate R L, Koteliansky V E, et al. Transforming growth factor-β initiates wound repair in rat liver through induction of the EIIIA-fibronectin splice isoform. Am J Pathol 2000; 156: 115–24.
64. Bataller R, Brenner D A. Liver fibrosis. J Clin Invest 2005; 115: 209–18.
65. Hui A Y, Friedman S L. Molecular basis of hepatic fibrosis. Exp Rev Mol Med 2003; 5: 1–23. Available from http://www.expertreviews.org/03005684h.htm.
66. Arias M, Lahme B, Van de Leur E, Gressner A M, Weiskirchen R. Adenoviral delivery of an antisense RNA complementary to the 3′ coding sequence of transforming growth factor-β1 inhibits fibrogenic activities of hepatic stellate cells. Cell Growth Differ 2002; 13: 265–73.
67. Arias M, Sauer-Lehnen S, Treptau J, Janoschek N, Theuerkauf I, Buettner R, et al. Adenoviral expression of a transforming growth factor-β1 antisense mRNA is effectie in preventing liver fibrosis in bile-duct ligated rats. BMC Gastroenterodogy 2003; 3(1): 29. Available from http://www.pubmedcentral.nih.gov/picrender.fcgi?artid = 270053 &blobtype = pdf.
68. Giri S N, Hyde D M, Hollinger M A. Effect of antibody to transforming growth factor β on bleomycin induced accumulation of lung collagen in mice. Thorax 1993; 48: 959–66.
69. Benigni A, Zoja C, Corna D, Zatelli C, Conti S, Campana M, et al. Add-on anti-TGF-β antibody to ACE inhibitor arresets progressive diabetic nephropathy in the rat. J Am Soc Nephrol 2003; 14: 1816–24.
70. Miyajima A, Chen J, Lawrence C, Ledbetter S, Soslow R A, Stern J, et al. Antibody to transforming growth factor-β ameliorates tubular apoptosis in unilateral ureteral obstruction. Kidney Int 2000; 58: 2301–13.
71. Fukasawa H, Yamamoto T, Suzuki H, Togawa A, Ohashi N, Fujigaki Y, et al. Treatment with anti-TGF-β antibody ameliorates chronic progressive nephritis by inhibiting Smad/TGF-β signaling. Kidney Int 2004; 65: 63–74.
72. Dahly A, Hosgland K M, Flasch A K, Jha S, Ledbetter S R, Roman R J. Antihypertensive effects of chronic anti-TGF-β antibody therapy in Dahl S rats. Am J Physiol Regul Integr Comp Physiol 2002; 283: R757–67.
73. Li J H, Huang X R, Zhu H J, Johnson R, Lan H Y. Role of TGF-β signaling in extracellular matrix production under high glucose conditions. Kidney Int 2003; 63: 2010–9.
74. Yamamoto T, Takagawa S, Katayama I, Nishioka K. Anti-sclerotic effect of transforming growth factor-β antibody in a mouse model of bleomycin-induced scleroderma. Clin Immunol 1999; 92: 6–13.
75. Schultze-Mosgau S, Wehrhan F, Rodel F, Amann K, Radespiel-Troger M, Kopp J, et al. Anti-TGFβ1 antibody for modulation of expression of endogenous transforming growth factor β1 to prevent fibrosis after plastic surgery in rats. Br J Oral Maxillofac Surg 2004; 42: 112–9.
76. Chen K, Wei Y, Sharp G C, Braley-Mullen H. Inhibition of TGFβ1 by anti- TGFβ1 antibody or lisinopril reduces thyroid fibrosis in granulomatous experimental autoimmune thyroiditis. J Immunol 2002; 169: 6530–8.
77. Jester J V, Barry-Lane P A, Petroll W M, Olsen D R, Cavanagh H D. Inhibition of corneal fibrosis by topical application of blocking antibodies to TGF β in the rabbit. Cornea 1997; 16: 177–87.
78. Kunz C R, Jadus M R, Kukes G D, Kramer F, Nguyen V N, Sasse S A. Intrapleural injection of transforming growth factor-β antibody inhibits pleural fibrosis in empyema. Chest 2004; 126: 1636–44.
79. Cui Q, Lim S K, Zhao B, Hoffmann F M. Selective inhibition of TGF-beta responsive genes by Smad-interacting peptide aptamers from FoxH1, Lef1 and CBP. Oncogene 2005; 24(24): 3864–74.
80. Davern T J. Molecular therapeutics of liver disease. Clin Liver Dis 2001; 5: 381–414.
81. Yamamoto H, Ueno H, Ooshima A, Takeshita A. Adenovirus-mediated transfer of a truncated transforming growth factor-β (TGF-β) type II receptor completely and specifically abolishes diverse signaling by TGF-β in vascular wall cells in primary culture. J Biol Chem 1996; 271: 16253–9.
82. Nakamura T, Sakata R, Ueno T, Sata M, Ueno H. Inhibition of transforming growth factor β prevents progression of liver fibrosis and enhances hepatocyte regeneration in dimethylnitrosamine-treated rats. Hepatology 2000; 32: 247–55.
83. Qi Z, Atsuchi N, Ooshima A, Takeshita A, Ueno H. Blockade of type β transforming growth factor signaling prevents liver fibrosis and dysfunction in the rat. Proc Natl Acad Sci USA 1999; 96: 2345–9.
84. Ueno H, Sakamoto T, Nakamura T, Qi Z, Astuchi N, Takeshita A, et al. A soluble transforming growth factor β receptor expressed in muscle prevents liver fibrogenesis and dysfunction in rats. Hum Gene Ther 2000; 11: 33–42.
85. Nagashio Y, Ueno H, Imamura M, Asaumi H, Watanabe S, Yamaguchi T, et al. Inhibition of transforming growth factor β decreases pancreatic fibrosis and protects the pancreas against chronic injury in mice. Lab Invest 2004; 84: 1610–8.
86. Kingston P A, Sinha S, David A, Castro M G, Lowenstein P R, Heagerty A M. Adenovirus-mediated gene transfer of a secreted transforming growth factor-β type II receptor inhibits luminal loss and constrictive remodelling after coronary angioplasty and enhances adventitial collagen deposition. Circulation 2001; 104: 2595–601.
87. George J, Roulot D, Koteliansky V E, Bissell D M. In vivo inhibition of rat stellate cell activation by soluble transforming growth factor β type II receptor: a potential new therapy for hepatic fibrosis. Proc Natl Acad Sci USA 1999; 96: 12719–24.
88. Yata Y, Gotwals P, Koteliansky V, Rockey D C. Dose-dependent inhibition of hepatic fibrosis in mice by

a TGF-β soluble receptor: implications for antifibrotic therapy. Hepatology 2002; 35: 1022–30.
89. Wang Q, Wang Y, Hyde D M, Gotwals P J, Koteliansky V E, Ryan S T, et al. Reduction of bleomycin induced lung fibrosis by transforming growth factor β soluble receptor in hamsters. Thorax 1999; 154: 805–12.
90. Haviv Y S, Takayama K, Nagi P A, Tousson A, Cook W, Wang M, et al. Modulation of renal glomerular disease using remote delivery of adenoviral-encoded soluble type II TGF-β receptor fusion molecule. J Gene Med 2003; 5: 839–51.
91. Zhou A, Ueno H, Shimomura M, Tanaka R, Shirakawa T, Nakamura H, et al. Blockade of TGF-beta action ameliorates renal dysfunction and histologic progression in anti-GBM nephritis. Kidney Int 2003; 64: 92–101.
92. Leveen P, Larsson J, Ehinger M, Cilio C M, Sundler M, Sjostrand L J, et al. Induced disruption of the transforming growth factor beta type II receptor gene in mice causes a lethal inflammatory disorder that is transplantable. Blood 2002; 100: 560–8.
93. Jiang W, Yang C Q, Liu W B, Wang Y Q, He B M, Wang J Y. Blockage of transforming growth factor β receptors prevents progression of pig serum-induced rat liver fibrosis. World J Gastroenterol 2004; 10: 1634–8.
94. Nakamura H, Siddiqui S S, Shen X, Malik A B, Pulido J S, Kumar N M, et al. RNA interference targeting transforming growth factor-β type II receptor suppresses ocular inflammation and fibrosis. Molecular Vision 2004; 10: 703–11.
95. Oh S P, Seki T, Goss K A, Imamura T, Yi Y, Donahoe P K, et al. Activin receptor-like kinase I modulates transforming growth factor-β1 signaling in the regulation of angiogenesis. Proc Natl Acad Sci USA 2000; 97: 2626–31.
96. Goumans M J, Valdimarsdottir G, Itoh S, Lebrin F, Larsson J, Mummery C, et al. Activin receptor-like kinase (ALK1) is an antagonistic mediator of lateral TGFβ/ALK5 signaling. Mol Cell 2003; 12: 817–28.
97. Laping N J, Grygielko E, Mathur A, Butter S, Bomberger J, Tweed C, et al. Inhibition of transforming growth factor (TGF)-β1-induced extracellular matrix with a novel inhibitor of the TGF-β type I receptor kinase activity: SB-431542. Mol Pharmacol 2002; 62: 58–64.
98. Inman G J, Nicolas F J, Callahan J F, Harling J D, Gaster L M, Reith A D, et al. SB-431542 is a potent and specific inhibitor of transforming growth factor-β superfamily type I activin receptor-like kinase (ALK) receptors ALK4, ALK5, and ALK7. Mol Pharmacol 2002; 62: 65–74.
99. Callahan J F, Burgess J L, Fornwald J A, Gaster L M, Harling J D, Harrington F P, et al. Identification of novel inhibitors of the transforming growth factor β 1 (TGF-β1) type I receptor (ALK5). J Med Chem 2002; 45: 999–1001.
100. DaCosta Byfield S, Major C, Laping N J, Roberts A B. SB-505124 is a selective inhibitor of transforming growth factor-β type I receptors ALK4, ALK5, and ALK7. Mol Pharmacol 2004; 65: 744–52.
101. de Gouville A C, Boullay V, Krysa G, Pilot J, Brusq J M, Loriolle F, et al. Inhibition of TGF-beta signaling by an ALK5 inhibitor protects rats from dimethylnitrosamine-induced liver fibrosis. Br J Pharmacol 2005; 145: 166–177.
102. Furukawa F, Matsuzaki K, Mori S, Tahashi Y, Yoshida K, Sugano Y, et al. p38 MAPK mediates fibrogenic signal through Smad3 phosphorylation in rat myofibroblasts. Hepatology 2003; 38: 879–89.
103. Hayashida T, Decaestecker M, Schnaper H W. Cross-talk between ERK MAP kinase and Smad signaling pathways enhances TGF-β-dependent responses in human messangial cells. FASEB J 2003; 17: 1576–8.
104. Schnabl B, Kweon Y O, Frederick J P, Wang X F, Rippe R A, Brenner D A. The role of SMad3 in mediating mouse hepatic stellate cell activation. Hepatology 2001; 34: 89–100.
105. Isono M, Chen S, Hong S W, Iglesias-de la Cruz M C, Ziyadeh F N. Smad pathway is activated in the diabetic mouse kidney and Smad3 mediates TGF-β-induced fibronectin in mesangial cells. Biochem Biophys Res Commun 2002; 296: 1356–65.
106. Valcourt U, Kowanetz M, Niimi H, Heldin C H, Moustakas A. TGF-β and the Smad signaling pathway support transcriptomic reprogramming during epithelial–mesenchymal cell transition. Mol Biol Cell 2005; 16: 1987–2002.
107. Bonniaud P, Kolb M, Galt T, Robertson J, Robbins C, Stampfli M, et al. Smad3 null mice develop airspace enlargement and are resistant to TGF-β-mediated pulmonary fibrosis. J Immunol 2004; 173: 2099–108.
108. Inazaki K, Kanamaru Y, Kojima Y, Sueyoshi N, Okumura K, Kaneko K, et al. Smad3 deficiency attenuates renal fibrosis, inflammation,and apoptosis after unilateral ureteral obstruction. Kidney Int 2004; 66: 597–604.
109. Lakos G, Takagawa S, Chen S J, Ferreira A M, Han G, Masuda K, et al. Targeted disruption of TGF-β/Smad3 signaling modulates skin fibrosis in a mouse model of scleroderma. Am J Pathol 2004; 165: 203–17.
110. Stramer B M, Austin J S, Roberts A B., Elizabeth Fini M. Selective reduction of fibrotic markers in repairing corneas of mice deficient in Smad3. J Cell Physiol 2005; 203: 226–32.
111. Wang S, Hirschberg R. BMP7 antagonizes TGF-β-dependent fibrogenesis in mesangial cells. Am J Physiol Renal Physiol 2003; 284: F1006–13.
112. Lin J, Patel S R, Cheng X, Cho E A, Levitan I, Ullenbruch M, et al. Kielin/chordin-like protein, a novel enhancer of BMP signaling, attenuates renal fibrotic disease. Nat Med 2005; 11: 387–93.
113. Watanable M, Ebina M, Orson F M, Nakamura A, Kubota K, Koinuma D, et al. Hepatocyte growth factor gene transfer to alveolar septa for effective suppression of lung fibrosis. Mol Ther 2005; 12: 58–67.
114. Yang J, Dai C, Liu Y. A novel mechanism by which hepatocyte growth factor blocks tubular epithelial to mesenchymal transition. J Am Soc Nephrol 2005; 16: 68–78.
115. Kadono Y, Shibahara K, Namiki M, Watanable Y, Seiki M, Sato H. Membrane type-matrix metalloproteinase is involved in the formation of hepatocyte growth factor/scatter factor-induced branching tubules in Mardin-Darby canine kidney epithelial cells. Biochem Biophys Res Commun 1998; 251: 681–7.
116. Takai S, Jin D, Muramatsu M, Okamoto Y, Miyazaki M. Therapeutic applications of chymase inhibitors in cardiovascular diseases and fibrosis. Eur J Pharmacol 2004; 501: 1–8.
117. Negri A L. Prevention of progressive fibrosis in chronic renal diseases: antifibrotic agents. J Nephrol 2004; 17: 496–503.
118. Dooley S, Hamzavi J, Breitkopf K, Wiercinska E, Said H M, Lorenzen J, et al. Smad7 prevents activation of hepatic stellate cells and liver fibrosis in rats. Gastroenterology 2003; 125: 178–91.
119. Nakao A, Fujii M, Matsumura R, Kumano K, Saito Y, Miyazono K, et al. Transient gene transfer and expression of Smad7 prevents bleomycin-induced lung fibrosis in mice. J Clin Invest 1999; 104: 5–11.

120. Fukasawa H, Yamamoto T, Togawa A, Ohashi N, Fujigaki Y, Oda T, et al. Down-regulation of Smad7 expression by ubiquity-dependent degradation contributes to renal fibrosis in obstructive nephropathy in mice. Proc Natl Acad Sci USA 2004; 101: 8687–92.
121. Terada Y, Hanada S, Nakao A, Kuwahara M, Sasaki S, Marumo F. Gene transfer of Smad7 using electroporation of adenovirus prevents renal fibrosis in post-obstructed kidney. Kidney Int 2002; 61: 94–8.
122. Hou C C, Wang W, Huang X R, Fu P, Chen T H, Sheikh-Hamad D, et al. Ultrasound-microbubble-mediated gene transfer of inducible Smad7 blocks transforming growth factor-β signaling and fibrosis in rat remnant kidney. Am J Pathol 2005; 166: 761–71.
123. Dong C, Zhu S, Wang T, Yoon W, Li Z, Alvarez R J, et al. Deficient Smad7 expression: a putative molecular defect in scleroderma. Proc Natl Acad Sci USA 2002; 99: 3908–13.
124. Saika S, Ikeda K, Yamanaka O, Sato M, Muragaki Y, Ohnishi Y, et al. Transient adenoviral gene transfer of Smad7 prevents injury-induced epithelial–mesenchymal transition of lens epithelium in mice. Lab Invest 2004; 84: 1259–70.
125. Ulloa L, Doody J, Massague J. Inhibition of transforming growth factor-β/SMAD signalling by the interferon-γ/STAT pathway. Nature 1999; 397: 710–13.
126. Soto P, Price-Schiavi S A, Carraway K L. SMAD2 and SMAD7 involvement in the post-translational regulation of Muc4 via the transforming growth factor-β and interferon-γ pathway in rat mammary epithelial cells. J Biol Chem 2003; 278: 20338–44.
127. Song Y H, Chen X L, Kong X J, Liu N Z, Li W, Wu X L, et al. Ribozymes against TGFβ1 reverse character of activated hepatic stellate cells in vitro and inhibit liver fibrosis in rats. J Gene Med 2005; 7: 965–76.
128. Isaka Y, Tsujie M, Ando Y, Nakamura H, Kaneda Y, Imai E, et al. Transforming growth factor-β1 antisense oligodeoxynucleotides block interstitial fibrosis in unilateral ureteral obstruction. Kidney Int 2000; 58: 1885–92.
129. Han D C, Hoffman B B, Hong S W, Guo J, Ziyadeh F N. Therapy with antisense TGF-β1 ologodeoxynucleotides reduces kidney weight and matrix mRNAs in diabetic mice. Am J Physiol Renal Physiol 2000; 278: F628–34.
130. Cordeiro M F, Mead A, Ali R R, Alexander R A, Murray S, Chen C, et al. Novel antisense oligonucleotides targeting TGF-β inhibit in vivo scarring and improve surgical outcome. Gene Ther 2003; 10: 59–71.
131. Shen Z J, Kim S K, Kwon O S, Lee Y S, Moon B J. Specific inhibition of transforming growth factor-β2 expression in human osteoblasts cells by antisense phosphorothioate oligonucleotides. Eur J Biochem 2001; 268: 2331–7.
132. Lin J S, Song Y H, Kong X J, Li B, Liu N Z, Wu X L, et al. Preparation and identification of anti-transforming growth factor β1 U1 small nuclear RNA chimeric ribozyme in vitro. Word J Gastroenterol 2003; 9: 572–7.
133. Yin J Q, Wang Y. siRNA-mediated gene regulation system: now and the future. Int J Mol Med 2002; 10: 355.
134. Tan F L, Yin J Q. Application of RNAi to cancer research and therapy. Front Biosci 2005; 10: 1946–60.
135. Imamura T, Kanai F, Kawakami T, Amarsanaa J, Ijichi H, Hoshida Y, et al. Proteomic analysis of the TGF-beta signaling pathway in pancreatic carcinoma cells using stable RNA interference to silence Smad4 expression. Biochem Biophys Res Commun 2005; 318(1): 289–96.
136. Yeom S Y, Jeoung D, Ha K S, Kim P H. Small interfering RNA (siRNA) targeted to Smad3 inhibits transforming growth factor-β signaling. Biotechnol Lett 2004; 26: 699–703.
137. Friese M A, Wischhusen J, Wick W, Weiler M, Eisele G, Steinle A, et al. RNA interference targeting transforming growth factor-β enhances NKG2D- mediated antiglioma immune response, inhibits glioma cell migration and invasiveness, and abrogates tumorigenicity in vivo. Cancer Res 2004; 64: 7596–603.
138. Munger J S, Huang X, Kawakatsu H, Griffiths M J, Dalton S L, Wu J, et al. The integrin αvβ6 binds and activates latent TGF β1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell 1999; 96: 319–28.
139. Bates R C, Bellovin D I, Brown C, Maynard E, Wu B, Kawakatsu H, et al. Transcriptional activation of integrin β6 during the epithelial-mesenchymal transition defines a novel prognostic indicator of aggressive colon carcinoma. J Clin Invest 2005; 115: 339–47.
140. Campbell J S, Hughes S D, Gilbertson D G, Palmer T E, Holdren M S, Haran A C, et al. Platelet-derived growth factor C induces liver fibrosis, steatosis, and hepatocellular carcinoma. Proc Natl Acad Sci USA 2005; 102: 3389–94.
141. Chujo S, Shirasaki F, Kawara S, Inagaki Y, Kinbara T, Inaoki M, et al. Connective tissue growth factor causes persistent proalpha2 (I) collagen gene expression induced by transforming growth factor-β in a mouse fibrosis model. J Cell Physiol 2005; 203: 447–56.
142. Shen H, Huang G, Hadi M, Choy P, Zhang M, Minuk G Y, et al. Transforming growth factor-β1 down-regulation of Smad1 gene expression in rat hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 2003; 285: G539–46.
143. Herrera B, Alvarez A M, Sanchez A, Fernandez M, Roncero C, Benito M, et al. Reactive oxygen species (ROS) mediates the mitochondrial- dependent apoptosis induced by transforming growth factor β in fetal hepatocytes. FASEB J 2001; 15: 741–51.
144. Gressner A M, Weiskirchen R. The tightrope of therapeutic suppression of active transforming growth factor-β: high enough to fall deeply? J Hepatol 2003; 39: 856–9.
145. Christ M, McCartney-Francis N L, Kulkarni A B, Ward J M, Mizel D E, Mackall C L, et al. Immune dysregulation in TGF-β1-deficient mice. J Immunol 1994; 153: 1936–46.
146. Yang X, Letterio J J, Lechleider R J, Chen L, Hayman R, Gu H, et al. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-β. EMBO J 1999; 18: 1280–91.
147. Matsuura I, Denissova N G, Wang G, He D, Long J, Liu F. Cyclin-dependent kinases regulate the antiproliferative function of Smads. Nature 2004; 430: 226–31.
148. Derynck R, Akhurst R J, Balmain A. TGF-β signaling in tumor suppression and cancer progression. Nat Genet 2001; 29: 117–29.
149. Kang Y, Hebron H, Ozbun L, Mariano J, Minoo P, Jakowlew S B. Nkx2.1 transcription factor in lung cells and a transforming growth factor-β1 heterozygous mouse model of lung carcinogenesis. Mol Carcinogen 2004; 40: 212–31.
150. Yang Y A, Dukhanina O, Tang B, Mamura M, Letterio J J, Macgregor J, et al. Lifetime exposure to a soluble TGF-β antagonist protects mice against metastasis without adverse side effects. J Clin Invest 2002; 109: 1607–15.
151. Xiao Y H, Liu D W, Li Q. Effects of drug serum of antifibrosis I herbal compound on calcium in hepatic stellate cell and its molecular mechanism. World J Gastroenterol 2005; 11: 1515–20.
152. Gaedeke J, Noble N A, Border W A. Curcumin blocks multiple sites of the TGF-β signalling cascade in renal cells. Kidney Int 2004; 66: 112–20.