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Review

# Fine Tuning and Cross-talking of TGF-β Signal by Inhibitory Smads

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**Transforming Growth Factor (TGF)-β family, including TGF-β, bone morphorgenic protein (BMP), and activn, plays an important role in essential cellular functions such as proliferation, differentiation, apoptosis, tissue remodeling, angiognesis, immune responses, and cell adhesions. TGF-β predominantly transmits the signals through serine/threonine receptor kinases and cytoplasmic proteins called Smads. Since the discovery of TGF-β in the early 1980s, the dysregulation of TGF-β/Smad signaling has been implicated in the pathogenesis of human diseases. Among signal transducers in TGF-β/Smad signaling, inhibitory Smads (I-Smads), Smad6 and Smad7, act as major negative regulators forming autoinhibitory feedback loops and mediate the cross-talking with other signaling pathways. Expressions of I-Smads are mainly regulated on the transcriptional levels and post-translational protein degradations and their intracellular levels are tightly controlled to maintain the homeostatic balances. However, abnormal levels of I-Smads in the pathological conditions elicit the altered TGF-β signaling in cells, eventually causing TGF-β-related human diseases. Thus, exploring the molecular mechanisms about the regulations of I-Smads may provide the therapeutic clues for human diseases induced by the abnormal TGF-β signaling.**

**Keywords:** Cross-talk, Inhibitory Smads, Negative regulation, TGF-β-related diseases, TGF-β signaling

## Introduction

Transforming growth factor-β (TGF-β) is a multifunctional cytokine which regulates essential cellular functions, including proliferation, differentiation, apoptosis, motility and adhesion (Roberts and Sporn, 1990; Massague, 1998). Over the past two decades, it has been known that TGF-β plays a pivotal role in physiological circumstances such as embryonic development and wound healing (Roberts and Sporn, 1993; Whitman, 1998), and that alterations of TGF-β signaling in pathological conditions cause a variety of human disease, including cancers and tissue fibrosis (Blobe *et al.*, 2000; de Caestecker MP, 2000).

The predominant pathway of TGF-β signaling is mediated by heterodimeric receptors with serine/threonine receptor kinases and cytoplasmic proteins called Smads (Heldin *et al.*, 1997; Derynck *et al.*, 1998; Massague and Chen, 2000). TGF-β binds to serine/threonine kinase receptors consisting of both type I (TβRI) and type II (TβRII) and then activated type I receptor transmits intracellular signals through the phosphorylation of receptor-regulated Smad proteins (R-Smads), Smad2 and Smad3. Activated R-Smads form heteromeric complexes with a common partner, Smad4 (Co-Smad). Following their binding to Smad4, the complex translocates into the nucleus to activate the transcription of various target genes (Massague and Wotton, 2000). However, the Smads-mediated signals induced by TGF-β are tightly regulated by negative-feedback mechanisms via inhibitory Smads (I-Smads) (Hayashi *et al.*, 1997; Imamura *et al.*, 1997; Nakao *et al.*, 1997). These carefully controlled TGF-β signals are altered in human diseases under pathophysiological conditions (Blobe *et al.*, 2000). In general, abnormal production of TGF-β or genetic mutations of TGF-β signaling components have been related with human diseases, particularly cancers (Markowitz *et al.*, 1995; Myeroff *et al.*, 1995; Hahn *et al.*, 1996; Thiagalingam *et al.*, 1996; de Caestecker *et al.*, 2000; Akhurst and Derynck, 2001; Pasche, 2001). Recent studies revealed that the aberrant expressions of I-Smads play an important role in TGF-β-associated diseases (Monteleone *et al.*, 2001; Dong *et al.*, 2002). In addition, emerging evidences show that inhibitory Smads mediate the cross-talks between TGF-β signal and other cellular signaling pathway (Ulloa *et al.*, 1999; Bitzer *et al.*, 2000), and that expressions of I-Smads are stimulated by various stimuli (Topper *et al.*, 1997; Patil *et al.*, 2000; Quan *et al.*, 2001). These data implicate that the regulation of I-Smads may be

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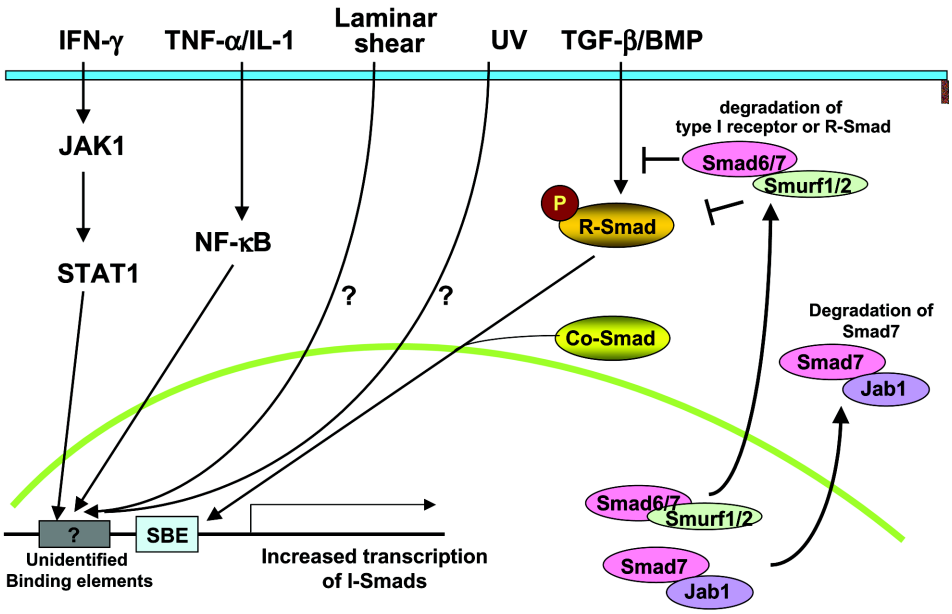


**Fig. 1.** Expressions of I-Smads by various extracellular stimuli and post-translational degradations of I-Smads. I-Smads, Smad6 and Smad7, are basically turned on by TGF-β/BMP itself through binding of activated R-Smads/Co-Smad complexes to Smad binding element (SBE) in the promoter regions of I-Smad genes. Smad6 and Smad7 protein are translocated into cytoplasm by Smurf1/2 proteins, which have the activity of E3 ubiquitin ligase. Smad7/Smurf complexes are recruited to activated type I receptor and induce its degradation, resulting in the inhibition of TGF-β signaling. Also, Smurf proteins indirectly bind to R-Smads through the association with I-Smads and induce the degradation of R-Smads. Jab1 protein translocates the Smad7 protein into cytoplasm and induces the degradation of only Smad7, resulting in reactivating TGF-β signaling. In addition to TGF-β superfamily ligands, I-Smads are induced by various stimuli, including IFN-γ, TNF-α, IL-1, laminar shear stress, and ultraviolet radiation. Increased expressions of I-Smads play an important role in cross-talking of TGF-β signaling with other signaling pathways. Binding elements for STAT1 and NF-κB in the promoter region of Smad7 still remains unclear. The mechanisms of transcriptional activations of I-Smads by UV and lamina shear stress are not described.

one of targets for modulation of TGF-β/Smad signaling in certain human diseases. In this review, we summarize the recent advances made in understanding the roles of inhibitory Smads in TGF-β biology.

## Negative regulation of TGF-β/Smad signaling by I-Smads

The duration and intensity of TGF-β/Smad responses are critically regulated in the physiological conditions and provide important determinants of signaling specificity. Therefore, the excessive or deficient TGF-β signal causes various human diseases (Blobe *et al.*, 2000; Monteleone *et al.*, 2001; Dong *et al.*, 2002). One important form of regulation occurs through the action of inhibitory Smads (I-Smad). Until now, Smad6 and Smad7 have been identified as members of I-Smads in the TGF-β superfamily signaling pathway (Hayashi *et al.*, 1997; Imamura *et al.*, 1997; Nakao *et al.*, 1997; Hata *et al.*, 1998). I-Smads function as intracellular antagonists of TGF-β signaling through stable interactions with activated serine/threonine receptors, preventing the phosphorylation of R-Smads (Hayashi *et al.*, 1997; Imamura *et al.*, 1997; Nakao *et al.*, 1997) and also interfering with the complex formation of R-Smads with Co-Smad (Hata *et al.*, 1998) (Fig. 1).

I-Smads are structurally related to R-Smads and Co-Smad, particularly in the MH2 domain (Hayashi *et al.*, 1997; Imamura *et al.*, 1997; Nakao *et al.*, 1997). However, their N-terminal regions of I-Smads are highly distinct from those of other Smads; I-Smads lack a C-terminal SSXS motif, which is phosphorylated following receptor activation. Thus, I-Smads are not phosphorylated upon treatment of TGF-β superfamily ligands. The observation that I-Smads intrinsically act as negative regulators forming signaling circuit, distinguishes this regulatory mechanism from other cellular signaling pathways in higher eukaryotes.

Through extensive studies on I-Smads, however, it appears that Smad6 and Smad7 differ in the inhibitory effects they exert on the signaling initiated by the TGF-β family. Smad6 preferentially inhibits by bone morphogenetic proteins (BMPs) whereas Smad7 inhibits signaling by all TGF-β family members including TGF-β, activin, and BMP (Itoh *et al.*, 1998; Ishisaki *et al.*, 1999).

The expression and duration of I-Smads are basically regulated by two mechanisms in cells: post-translational protein degradation and transcriptional level (Fig. 1). I-Smads physically interact with the type I receptor (TβRI) which is activated by type II receptor (TβRII) kinases (Hayashi *et al.*,

1997; Imamura *et al.*, 1997; Nakao *et al.*, 1997; Souchelnytskyi *et al.*, 1998). Recent studies have showed that inhibitory effects of I-Smads are facilitated by HECT type of E3 ubiquitin ligase Smurf1 and Smurf2 (Kavsak *et al.*, 2000; Ebisawa *et al.*, 2001; Murakami *et al.*, 2003). Smurfs were originally identified as E3 ligases that interact with R-Smads and induce their degradations (Zhu *et al.*, 1999). Recent data has shown that Smad7 interacts with Smurf1 and Smurf2, recruiting them to TβRI complexes and inducing the degradation of activated TβRI (Kavsak *et al.*, 2000; Ebisawa *et al.*, 2001). Thus, binding of I-Smads to TβRI complexes and recruitment of Smurf proteins eventually inhibit cellular signalings induced by TGF-β through degradations of the activated type I receptor. Moreover, it has been reported that the Smurf1 protein induces degradation of BMP-activated R-Smads indirectly through I-Smads (Murakami *et al.*, 2003). In contrast to Smurfs, Jab1/CSN5, which is the fifth component of the COP9 signalosome, has been recently shown to degrade Smad7 through ubiquitin-proteasomal pathway, reactivating TGF-β signaling upon its suppression by the inhibitory Smad7 (Kim *et al.*, 2004). These accumulated data suggest that the mechanism of protein degradations for I-Smads is an important step for regulating the intracellular levels of I-Smads, and the alteration of this mechanism under certain pathological conditions may be one of the reasons affecting TGF-β signaling.

To maintain the homeostatic balances for TGF-β/Smad signaling, expressions of I-Smads are also regulated in the transcriptional level (Fig. 1). I-Smads are basically turned on by TGF-β itself in the TGF-β/Smad signaling circuit (Nakao *et al.*, 1997). TGF-β transmits the signals via R-Smads to modulate the various target genes and also induce the expression of I-Smads to negatively regulate the signals (Nakao *et al.*, 1997; Afrakhte *et al.*, 1998; Takase *et al.*, 1998). Promoter analysis of I-Smad genes revealed that Smad binding elements (SBE) are important for expression of their genes (Nagarajan *et al.*, 1999; Denissova *et al.*, 2000; Ishida *et al.*, 2000; von Gersdorff *et al.*, 2000), suggesting that I-Smads are target genes induced by TGF-β. Therefore, the increased expressions of I-Smads, which are induced by TGF-β itself, ultimately form an autoinhibitory feedback loop to inhibit TGF-β/Smad signaling.

Based on these results, it is thought that aberrant expressions of I-Smads, due to altered protein degradation or transcription, cause the diseases related to TGF-β signaling. These aberrant expressions of I-Smads, especially Smad7, were found in human diseases such as inflammatory bowel disease and scleroderma (Monteleone *et al.*, 2001; Dong *et al.*, 2002). Thus, fine-tunings of the intracellular levels of I-Smads on the transcriptional and post-translational levels appear to be essential for regulations of TGF-β/Smad signaling.

## Transcriptional regulation of I-Smads

Most importantly, I-Smads are induced by TGF-β, activin and BMP through the activated R-Smads (Afrakhte *et al.*, 1998; Nagarajan *et al.*, 1999; Denissova *et al.*, 2000; Ishida *et al.*, 2000; von Gersdorff *et al.*, 2000). However, the regulatory mechanisms for induction of I-Smads are not fully understood. Smad7, which has been studied the most, is transiently induced by activated Smad2/3 within a few hours after treatment of TGF-β (Nakao *et al.*, 1997), completing the autoinhibitory feedback signaling circuit; the promoter region of the Smad7 gene contains a consensus Smad3-Smad4 binding element (SBE), a palindromic sequences of GTCTAGAC (Nagarajan *et al.*, 1999; Denissova *et al.*, 2000; von Gersdorff *et al.*, 2000). It has also been reported that the cooperation of Smad, Sp1, and AP-1 transcription factors are required for efficient expression of Smad7 (Brodin *et al.*, 2000). More recently, it has been reported that the Ski protein inhibits the Smad7 promoter basal activity in an SBE-dependent manner together with Smad4 (Denissova and Liu, 2004). In contrast, the regulatory mechanism of the Smad6 gene has been less studied than that of Smad7. The Smad6 gene is preferentially regulated by BMP signaling through the SBE element (Ishida *et al.*, 2000). That is, the expression of Smad6 gene is induced by BMP-activated Smad1/5 and its promoter contains a 28 bp GC-rich sequence, which is identified as Smad-responsive binding elements (Ishida *et al.*, 2000). It is noteworthy that a characterized SBE element in Smad6 gene was found through the analysis of the mouse Smad6 promoter whereas the functional promoter of the human Smad6 gene has not been reported until now. Furthermore, other transcription factors that cooperatively bind to the Smad6 promoter are not clearly defined. Recent studies show that CREB cooperates with BMP-stimulated Smad signaling to enhance transcription of the Smad6 promoter (Ionescu *et al.*, 2004). Interestingly, Smad6 also appears to be transiently induced by TGF-β and activin, compared with sustained expression of Smad6 by BMP (Afrakhte *et al.*, 1998). However, there is no evidence concerning whether the induction of the Smad6 gene by TGF-β and activin requires the activated Smad2/3 and a previously characterized SBE element. Therefore, these differences of regulatory mechanisms between two inhibitory Smads suggest the possibility that Smad6 and Smad7 might have distinct roles in *in vivo* as well as inhibitory actions against TGF-β superfamily signaling.

## Cross-talking of TGF-β signaling with other cellular signals by I-Smad

The interesting feature for the expression of I-Smads is that I-

Smads are induced by various extracellular stimuli and the induction of I-Smads mediates the interconnection between TGF-β signaling and other cellular signaling pathways (Fig. 1). Expression of the Smad7 gene has been extensively studied in this regard. The pleiotropic cytokine IFN-γ exerts opposite effects on diverse cellular functions modulated by TGF-β. These opposite effects between the two signaling pathways are mediated by increased expression of Smad7 (Ulloa *et al.*, 1999). The induction of Smad7 via the activation of the JAK1 and STAT1 prevents the interaction of Smad3 with the TGF-β receptor, resulting in downregulation of the TGF-β signal (Ulloa *et al.*, 1999). Because inflammatory cells at sites of tissue injury secrete TGF-β and IFN-γ, these antagonistic interactions between the two signals by the increased Smad7 may play an important role in the maintenance of tissue homeostasis. TNF-α and Interleukine-1, well-known pro-inflammatory cytokines, also induce the expression of Smad7 gene through the RelA subunit of NF-κB, which in turn blocks TGF-β/Smad signaling (Bitzer *et al.*, 2000). In summary, these results provide the possibility that the regulation of I-Smad genes, in particular Smad7, plays a key role in mediating the cross-talks and balances between inflammatory and TGF-β signals.

In addition to IFN-γ and TNF-α, the Smad7 gene is induced by other stimuli. Activation of CD40 induces Smad7 through NF-κB, resulting in the suppression of TGF-β-induced growth inhibition and apoptosis in B-lymphocytes (Patil *et al.*, 2000). Moreover, ultraviolet irradiation induces the expression of Smad7 to block the cellular responses to TGF-β (Quan *et al.*, 2001), although the reason for induction of Smad7 still remains unknown.

It has been first reported that expression of Smad6 as well as Smad7 is induced by lamina shear stress in vascular endothelial cells (Topper *et al.*, 1997). However, the reason that Smad6 is induced by this stimulus is still unclear although Smad6 knock-out mice show that Smad6 is important in development and homeostasis of the cardiovascular system (Galvin *et al.*, 2000). A recent study shows that phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), a well-known PKC activator, downregulates Smad6 mRNA whereas it upregulates Smad7 mRNA in lung fibroblasts (Tsunobuchi *et al.*, 2004). These results suggest the possibility that PKC signal pathway may cross-talk with TGF-β signaling by differentially regulating the expression of these I-Smads. In contrast to Smad7, little is known about Smad6's role as a mediator for cross-talking with other signaling pathways. Therefore, the extensive studies of the regulation for Smad6 expression might be promising in revealing new roles of Smad6 protein in TGF-β superfamily signaling.

## Interacting proteins with I-Smads and additional activities of I-Smads

In addition to intrinsic roles as the intracellular antagonist in TGF-β superfamily signaling and as the mediators of cross-talkings with other signaling pathways, the possibilities that I-Smads might have distinct functions in cellular signaling have been proposed.

The unanswered question for I-Smads is that they are localized in the nucleus but not in the cytoplasm in many cells. Thus, to interfere with TGF-β signaling, I-Smads have to translocate into the cytoplasm (Fig. 1). Actually, it has been reported that Smad7 is exported from the nucleus to the cytoplasm upon treatment of TGF-β (Itoh *et al.*, 1998). Also, this nuclearcytoplasmic shuttling of Smad7 appears to be mediated by Smurf proteins with nuclear export signal (NES) (Kavsak *et al.*, 2000; Tajima *et al.*, 2003) or Jab1/CSN5 (Kim *et al*., 2004). Recently, it was reported that the WW domain-containing protein 1 (WWP1), which is structurally related with Smurf, associates with Smad7 and induces its nuclear export (Komuro *et al.*, 2004). However, this phenomenon is not always observed in many cells and little is known about the mechanism of nuclear export of Smad6.

These observations on cellular localizations of I-Smads imply the possibility that I-Smads might be nuclear functions. Few reports showed that Smad6 acts as a transcriptional corepressor (Bai *et al*., 2000; Bai and Cao, 2002; Lin *et al.*, 2003). For example, Smad6 represses BMP-induced Id1 transcription through recruiting transcriptional corepressor C-terminal binding protein (CtBP) (Lin *et al.*, 2003). Other results reveal that Smad6 interacts with class I histone deacetylase (HDAC) as well as homeobox (Hox) c-8 proteins, ultimately inhibiting transcription (Bai *et al.*, 2000; Bai and Cao, 2002). These results suggest a novel mechanism for transcriptional regulation by Smad6.

Another activity of I-Smads that can be distinguished from an inhibitory role may be related to apoptosis (Landstrom *et al.*, 2000; Lallemand *et al.*, 2001; Mazars *et al.*, 2001). Smad7 has been shown to potentiate apoptosis in prostate carcinoma cells (Landstrom *et al.*, 2000). Ectopic expression of Smad7 induces apoptosis in PC-3U cells and the expression of Smad7 antisense mRNA suppresses TGF-β-induced cell death in these cells (Landstrom *et al.*, 2000). These results imply that Smad7 might act as an apoptosis-inducing protein. Furthermore, Smad7 has been known to specifically induce the JNK signaling cascade required for Smad7-induced apoptosis (Mazars *et al.*, 2001). The Smad7-induced activation of JNK is not involved in the inhibitory function of Smad7, but required for apoptosis (Mazars *et al.*, 2001). On the contrary, however, another group showed that Smad7 is involved in anti-apoptosis in WEHI 231 B-lymphocytes (Patil *et al.*, 2000). Thus, the function of Smad7 is still controversial during the apoptotic process and seems to be cell-type dependent.

Compared with Smad7, little is known about the effects of Smad6 during apoptosis. In a study of Kimura *et al.* (2000), the authors showed that ectopic expression of Smad6 is resistant to BMP2-induced apoptosis in mouse hybridoma MH60 cells. These effects are due to blocking BMP2-induced

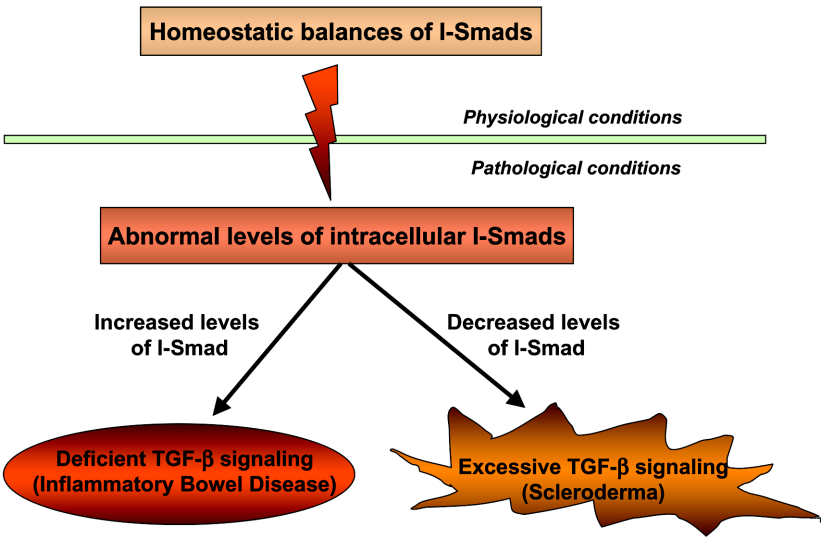


**Fig. 2.** TGF-β-associated diseases by abnormal levels of intracellular I-Smads. Excessive levels or decreased levels of intracellular I-Smads under pathological conditions cause deficient TGF-β signaling or enhanced TGF-β signaling, respectively. These aberrant TGF-β signalings could be important for certain TGF-β-associated diseases such as inflammatory bowel disease and scleroderma.

TAK1 activation and p38 phosphorylation through direct binding of Smad6 to TAK1 (Kimura *et al.*, 2000). Although the possible role of Smad6 in apoptosis is less studied than Smad7, it appears to be interesting that the effects of Smad6 are investigated during BMP or TGF-β-related developmental process.

## TGF-β-associated diseases by aberrant expressions of I-Smad

An excessive or deficient expression of I-Smads, which are responsible for fine-tuning of TGF-β signaling, disrupts the balanced activity of TGF-β cytokine under pathophysiological conditions. These abnormal expressions of I-Smads are hypothesized as one of the reasons causing TGF-β-associated diseases (Fig. 2). This hypothesis is now supported by the results that aberrant expressions of Smad7 are observed in chronic inflammatory bowel disease (IBD) and scleroderma in humans (Monteleone *et al.*, 2001; Dong *et al.*, 2002). Monteleone *et al.* (2001) demonstrated that there is marked overexpression of Smad7 in IBD mucosa and purified mucosal T cells. This is associated with a reduction in Smad3 phosphorylation, exhibiting defective TGF-β signaling (Monteleone *et al.*, 2001). Furthermore, blocking Smad7 with a specific antisense oligonucleotide restores TGF-β signaling and importantly allows TGF-β1 to inhibit pro-inflammatory cytokine productions by isolated mucosal lamina propia mononuclear cells (Monteleone *et al.*, 2001). Thus, these results demonstrate that blocking TGF-β signaling by overexpressed Smad7 helps maintain the chronic production of pro-inflammatory cytokines that drives the inflammatory process in IBD, and that inhibition of Smad7 enables endogeneous TGF-β to downregulate this response. However, the underlying mechanism of Smad7 overexpression in IBD patient remains unknown. Particularly, these novel and important findings provoke the interesting questions about how the hyperactivity of the intestinal NF-κB system in IBD patient is related to the overexpression of Smad7.

Another interesting result about aberrant expression of Smad7 was observed in the skin lesions of scleroderma (Dong *et al.*, 2002). Dong *et al.* reported that the basal level and TGF-β-inducible expression of Smad7 are selectively decreased whereas Smad3 expression is increased both in scleroderma skin and in explanted scleroderma fibroblast in culture. This deficient expression of Smad7 increases the phosphorylation of Smad2 and Smad3 and the transcription of the PAI-1 gene, eventually showing the enhanced TGF-β signaling in scleroderma fibroblasts. Interestingly, *in vitro* adenoviral gene transfer with Smad7 restores normal TGF-β signaling in scleroderma fibrobasts (Dong *et al.*, 2002). Thus, the authors suggest that the deficient Smad7 expression play a key role in hyperresponsiveness to TGF-β in fibroblasts and development of the sclerotic skin diseases. The deficiency of Smad7 is presumably due to transcriptional inhibition of Smad7 gene or excessive degradation of Smad7 protein. However, the molecular basis of Smad7 deficiency remains unknown in scleroderma.

TGF-β has two important properties in inflammation; anti-inflammatory and pro-fibrotic activities. In the studies of IBD and scleroderma, a scenario may be delineated that an excessive or decreased expression of Smad7 modulates the responsiveness of the cells to TGF-β, resulting in the augmented inflammation by deficient TGF-β signaling and the augmented fibrotic responses by enhanced TGF-β signaling, respectively (Fig. 2). Thus, exploring the mechanisms

of Smad7 expression or degradation may provide clues for developing therapeutic molecules in these TGF-β-associated diseases.

Aberrant expression of I-Smads is also observed in certain cancers. Recent study demonstrates that Smad7 plays an important role in the development of gastric carcinoma and that overexpression of Smad7 may be a significant independent prognostic indicator for clinical outcome in patients with gastric carcinoma (Kim *et al.*, 2004). Smad7 overexpression is associated with poor outcome in gastric carcinomas, indicating that Smad7 expression may present one of the novel mechanisms for TGF-β resistance in human gastric carcinoma. Increased expression of Smad6 and Smad7 has been described in human pancreatic cancers (Kleeff *et al.*, 1999a; Kleeff *et al.*, 1999b), but the mechanisms underlying these changes in I-Smad expression still remains unresolved.

In contrast to Smad7, the importance of Smad6 under pathophysiological conditions is not clearly demonstrated at the moment. However, a possible role of Smad6 has been revealed in Smad6-mutant mice (Galvin *et al.*, 2000). Targeted insertion of the LacZ reporter demonstrated that Smad6 expression is largely restricted to the heart and blood vessels, and that Smad6 mutant mice have multiple cardiovascular abnormalities with hyperplasia of the cardiac valves and outflow tract septation defects (Galvin *et al.*, 2000). These results indicate that Smad6 plays an important role in the regulation of endocardial cushion transformation.

Distinct roles of I-Smads associated with human diseases have been described in renal cells, in murine models of renal disease and in human glomerular diseases (Schiffer *et al.*, 2002). Smad7 is upregulated in podocytes in all examined glomerular diseases (Schiffer *et al.*, 2002). Smad7 expression inhibits both Smad2- and Smad3-mediated TGF-β signaling in podocytes, but only Smad3 signaling in mesangial cells (Schiffer *et al.*, 2002). In contrast, Smad6 is only upregulated in the mesangium in human glomerular diseases (Schiffer *et al.*, 2002). Smad6 enhances Smad3-mediated signaling in mesangial cells, but has no effect on TGF-β/Smad signaling and injury in podocytes (Schiffer *et al.*, 2002). These results provide evidence for cell type-dependent differential roles of Smad6 and Smad7 in glomerular pathology.

These recent studies strongly support the possibility that abnormality of the intracellular levels or aberrant expression of I-Smads disrupt cellular homeostasis of TGF-β/Smad signaling and thus cause TGF-β-associated diseases in human (Fig. 2).

## Conclusion

Since the TGF-β superfamily has multiple effects under physiological and pathological conditions, modulation of TGF-β signaling has been a fascinating therapeutic target of TGF-β-related clinical diseases. In this regard, many researchers are interested in the regulation of I-Smads. I-Smads are involved in both negative regulation of TGF-β superfamily signaling and cross-talking with other cellular signaling. As shown in IBD and scleroderma, aberrant expression of I-Smads disrupts the balanced TGF-β signaling, resulting in human diseases. Based on recent progress in TGF-β biology, exploring the regulatory mechanism of I-Smads in transcriptional level, nuclear/cytoplasmic translocations, and protein degradations may provide important clues for regulating the level of I-Smads. Although extensive studies about I-Smads have been performed, the regulatory mechanisms of I-Smad are not fully understood, in particular, the transcriptional regulation and the cellular localization of I-Smad. Thus, understanding these basic mechanisms of I-Smads may provide valuable ways for treating clinical diseases caused by the TGF-β superfamily in the future.

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