Review

Regulation of TGF-β signaling by Smad7

Xiaohua Yan, Ziying Liu, and Yeguang Chen*

State Key Laboratory of Biomembrane and Membrane Biotechnology, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, China

*Correspondence address. Tel: +86-10-62795184; Fax: +86-10-62794376; E-mail: ygchen@tsinghua.edu.cn

Transforming growth factor (TGF-β) is a pleiotropic cytokine regulating a variety of cellular processes such as cell growth, differentiation, apoptosis, migration, cell adhesion, and immune response. In the well-understood classical TGF-β signaling pathway, TGF-β activates Smad signalling via its two cell surface receptors such as TβRII and ALK5/TβRI, leading to Smad-mediated transcriptional regulation. In addition, TGF-β may also activate other signaling pathways like mitogen-activated protein kinase, PI3K, etc. The signaling of TGF-β is finely regulated at different levels. Inhibitory Smads, including Smad6 and Smad7, are key regulators of TGF-β/bone morphogenetic protein (BMP) signaling by negative feedback loops. They can form stable complexes with activated type I receptors and thereby blocking the phosphorylation of R-Smads, or recruit ubiquitin E3 ligases, such as Smurf1/2, resulting in the ubiquitination and degradation of the activated type I receptors. Besides, these inhibitory Smad proteins also inhibit TGF-β/BMP signaling in the nucleus by interacting with transcriptional repressors, such as histone deacetylases, Hoxc-8, and CtBP, or disrupting the formation of the TGF-β-induced functional Smad-DNA complexes. Smad7 is in turn regulated by different stimuli, including TGF-β, IFN-γ, TNF-α as well as ultraviolet and TPA, and mediates the crosstalk between TGF-β and other signaling pathways. Deregulation of Smad7 expression has been associated with various human diseases, such as tissue fibrosis, inflammatory disease as well as carcinogenesis. Overexpression of Smad7 has been shown to antagonize TGF-β-mediated fibrosis, carcinogenesis, and inflammation, suggesting a therapeutic potential of Smad7 to treat these diseases.

Keywords TGF-β; signal transduction; Smad7; feedback loop; crosstalk

Received: November 7, 2008 Accepted: February 9, 2009

Introduction

Transforming growth factor (TGF-β) family cytokines have been found to play diverse roles in regulating growth, differentiation, immune response as well as development in multi-organ systems. Up to now, more than 30 factors have been discovered to belong to the TGF-β superfamily, which is generally divided into two subfamilies. One of them is consisted of TGF-β, activin, Nodal, myostatin, inhibin, etc. The other one includes BMPs, anti-mullerian hormone (AMH, or MIS), as well as many growth and differentiation factors (GDFs) [1,2].

There are three species of TGF-β (TGF-β1, TGF-β2, and TGF-β3) in mammalian cells. TGF-βs are synthesized via inactive precursors, which cannot bind to their receptors until being activated. After released from cells, they associate with latency-associated protein (LAP) and form a small inactive complex. In the extracellular matrix, this complex is bound by latent TGF-β-binding protein (LTBP), a component of the extracellular matrix that is necessary for the secretion and storage of TGF-β [3]. The latent TGF-β can be activated either by enzymatic proteolysis, executed by plasmin, integrin, or thrombin, or through a conformational change [4,5].

Overview of TGF-β Signaling Pathways

Once activated, the TGF-β homodimer transduces its signal by bringing together two types of serine/threonine kinase receptors—two type I receptors and two type II receptors. ALK5 (TβRI) and TβRII are specific for TGF-β. Upon TGF-β binding, ALK5 is phosphorylated and activated by the constitutively active TβRII at the GS region, which is important for the activation of its
kinase domain as well as the recruitment of R-Smads. The phosphorylation of R-Smads at the C-terminal SSXS motif by activated type I receptor is an essential step for signal transduction. Of these R-Smads, Smad2 and 3 are engaged in signal transduction of TGF-β, activin, and Nodal group cytokines, whereas Smad1, 5, and 8 are necessary for the other groups including BMPs and GDFs. Once activated, R-Smads form complexes with a common Smad (Co-Smad, Smad4), enter the nucleus and regulate transcription of target genes along with different cofactors [6]. Both R-Smads and Smad4 are characterized by two conserved regions known as MH1 domain at the N-terminus and MH2 domain at the C-terminus, respectively, which are joined together by a linker region. The MH1 domain of R-Smads and Smad4 plays important roles in cytoplasmic anchoring, nuclear import, DNA binding, and regulation of gene transcription, while the MH2 domain is responsible for Smad–receptor interaction, Smad hetero-complex formation, cytoplasmic anchoring as well as transactivation of target genes [7].

Besides the canonical Smad-mediated signaling pathway (Fig. 1), it has long been recognized that TGF-β can also regulate some cellular or physiological processes independent of Smad proteins. There is evidence showing that Smad4 is not indispensable for the development of the mammary gland, liver, or pancreas in mice [2,8]. We have demonstrated that the nucleocapsid (N) protein of severe acute respiratory syndrome-associated coronavirus can bind to Smad3, interfere with the complex formation between Smad3 and Smad4, and promote Smad3–p300 complex formation in the nucleus [9], indicating a novel mode of Smad3 effect in a Smad4-independent manner. TIF1γ is also found to selectively associate with phosphorylated Smad2/3 in hematopoietic, mesenchymal, and epithelial cells in response to TGF-β and transduce the signal independent of Smad4 to promote erythrogenesis [10]. In addition, TGF-β can also activate mitogen-activated protein kinase (MAPKs) (ERK1/2, JNK, p38), PI3K, protein phosphatase 2A, Rho family proteins as well as the epithelial polarity protein Par6, but the underlying mechanisms are not fully understood. Lee et al. [11] reported that upon TGF-β stimulation, activated TGF-β type I receptor (TβRI) recruits and directly phosphorylates ShcA protein on tyrosine and serine residues, activating...
ERK MAPK signaling pathway. Recently, the ubiquitin E3 ligase TRAF6 has also been reported to interact with TβRI, and this interaction is required for TGF-β-induced auto-ubiquitination of TRAF6 as well as subsequent activation of TAK1-p38 MAPK, which leads to apoptosis [12, 13]. This plasticity of TGF-β signaling allows TGF-β to exert pleiotropic influences.

**Negative Regulation of TGF-β Signaling by Smad7**

As mentioned above, TGF-β is a pleiotropic cytokine that regulates embryonic development and cellular homeostasis mainly through the canonical Smad-mediated signaling pathway, which appears to be relatively simple and consists of only a few essential components. However, the signal transduction is finely regulated both temporally and spatially at different components. Many proteins have been shown to play key roles in regulating TGF-β signaling, either affecting the link between the receptor and downstream Smads or Co-Smads, activation, and translocation, as well as transcription in the nucleus. Many proteins have been identified to be associated with the receptors, R-Smads, or Co-Smad, and regulate TGF-β family signaling either in the cytoplasm or in the nucleus. In addition to R-Smads and Co-Smad, there is a third Smad protein family, namely the I-Smads (Smad6 and Smad7), which have been documented to play key roles in regulating signal transduction of TGF-β family cytokines. I-Smads are transcriptionally induced by TGF-β family cytokines and regulate these signaling pathways negatively, thus establishing an important negative feedback loop. Of these two I-Smads, Smad7 is a general antagonist of TGF-β family, while Smad6 is specific for BMP signaling. Smad6 and Smad7 also have conserved the C-terminal MH2 domain, but unlike R-Smads or Co-Smad, they lack the N-terminal MH1 domain and the phosphorylation site by the type I receptors at the C-terminal tail. The N-terminus of these two I-Smads shares a similarity of only 36.7%. Both the N-terminus and MH2 domain of Smad7 are essential for its specific inhibition of TGF-β/activin signaling [14, 15]. I-Smads can also bind to DNA. For instance, we recently demonstrated that Smad7 could function in the nucleus by binding to the DNA elements containing the minimal Smad-binding element (SBE) CAGA box. By single-molecule force spectroscopy, our results revealed that Smad7 had similar binding strength to the oligonucleotides corresponding to the CAGA-containing activin responsive element (ARE) and the PAI-1 promoter, as that of Smad4 although, unlike R-Smad or Co-Smad, Smad7 also exhibits a binding activity to the mutant ARE with the CAGA sequence substituted. Interestingly, distinct from other Smad proteins, the MH2 domain, but not the N-terminal region, of Smad7 is responsible for DNA binding [16, 17].

I-Smads can antagonize TGF-β family signaling through various mechanisms (Fig. 2). First, Smad7 is shown to form a stable complex with type I receptors, therefore leading to inhibition of R-Smad phosphorylation and the hetero-complex formation between R-Smads and Co-Smad [18]. Smad7 also recruits the HECT type of E3 ubiquitin ligases, Smurf1 and Smurf2. It binds to Smurfs in the nucleus and translocates into the cytoplasm in response to TGF-β and recruits the ubiquitin ligases to the activated type I receptor ALK5/TβRI, leading to the degradation of the receptor through the proteasomal pathway. Smad7 itself is also degraded in this process. Besides, the E3 ligases Nedd4-2 and WWP1/Tiu1 can also promote the degradation of type I receptor as well as R-Smads and Smad4, in which process Smad7 serves as an adaptor protein [19]. By employing similar mechanism, Smad6 interferes with BMP signaling. Moreover, Shi et al. [20] reported that the phosphatase GADD34-PP1c could be recruited to ALK5/TβRI by Smad7 and in turn dephosphorylate the receptor. Protein phosphatase 1a was also reported to have similar effect to dephosphorylate ALK1 in endothelial cells. It could be recruited by Smad7 to ALK1, and thereby controlled TGF-β/ALK1-induced Smad1/5 activation [21]. In addition, Smad6 has been shown to bind to the phosphorylated Smad1 in competition with Smad4 [22].

It has been known that Smad6 can interfere with BMP signaling both in the cytoplasm and in the nucleus. Smad6 acts as a transcriptional repressor by interacting with Hoxc-8, or binding to DNA and recruiting transcriptional co-repressor histone deacetylases (HDACs) or CtBP to inhibit the transcription of target genes [23–26]. Recently, we reported that in some cell lines, such as Hep3B, HeLa, human normal lung epithelial Mv1Lu mutant L17, and human normal lung epithelial HPL-1 cells, most Smad7 proteins retain in the nucleus even under TGF-β stimulation, and Smad7 can exert its inhibitory effect on TGF-β signaling in the nucleus [16]. Forced expression of Smad7 in the nucleus potently repressed the transcriptional activity of TGF-β, and the inhibitory effect of Smad7 can be independent of TβRI, as demonstrated in TβRI-deficient R1B/L17 cells. Furthermore, Smad7 is able to bind DNA in vivo and in vitro with the
MH2 domain and disrupt the formation of functional Smad–DNA complexes [16,17].

Several proteins have been shown to interact with Smad7 and regulate TGF-β signaling (Table 1). A WD protein, STRAP, which associates with the type I and II receptors, can also interact with Smad7, stabilizing the complex between Smad7 and type I receptor, thus inhibiting TGF-β signaling synergistically with Smad7 [27]. AIP4, which is an E3 ubiquitin ligase and induces the degradation of Smad7, may negatively regulate TGF-β signaling without affecting the turnover of the type I receptor [28]. Instead, AIP4 may enhance the interaction between Smad7 and activated ALK5/TβRI.

Regulation of the stability of Smad7 protein has been used as another means to influence TGF-β signaling. Arkadia facilitates TGF-β signaling by promoting the degradation of Smad7 and Axin may act as an adaptor between Arkadia and Smad7 [29,30]. Jab1/CSN5, which is a component of the COP9 signalosome complex, also regulates the stability of Smad7 and releases Smad7-mediated suppression of TGF-β signaling [31]. Hic-5/ARA55 [32], Yes-associated protein (YAP65) [33], the salt-inducible kinase (SIK) [34], and Crk-associated substrate lymphocyte type (Cas-L) [35] are identified to be binding partners of Smad7 and regulate TGF-β signaling either positively or negatively. FKBP12, which is a cytoplasmic protein that binds to the immunosuppressant drugs, Tacrolimus (FK506) and rapamycin, has been found to interact with the GS region of type I receptors and inhibit signal transduction of TGF-β family [36]. Interestingly, it can serve as an adaptor for the Smad7–Smurf1 complex and promote the ubiquitination and degradation of TβRI [37].

Post-translational modification plays a pivotal role in regulating the function of proteins. As mentioned above, ubiquitination plays an important role in the regulation of the stability of I-Smads, and TGF-β receptors. In addition, Smad7 interacts with p300, a histone acetylase, and could be acetylated at the same lysine residues where ubiquitination occurs. Smad7 can also interact with

| Table 1 Smad7-binding partners | Function | References |
|--------------------------------|----------|------------|
| AIP4                           | Stabilizing the complex between ALK5 and Smad7 and inhibiting TGF-β signaling | [19] |
| Arkadia                       | Degradation of Smad7 | [20] |
| Axin                           | An adaptor between Smad7 and Arkadia | [21] |
| Cas-L (Crk-associated substrate lymphocyte type) | Facilitating TGF-β signaling by interfering with the inhibitory effect of Smad7 | [26] |
| FKBP12                         | An adaptor between ALK5 and Smad7–Smurf1 | [27] |
| GADD34-PP1c                    | Dephosphorylation of ALK5 | [16] |
| HDACs                          | Dephosphorylation and inhibition of TGF-β signaling | [34,35] |
| Hic-5/ARA55                    | Degradation of Smad7 | [23] |
| Jab1/CSN5                      | Degradation of Smad7 | [22] |
| Nedd4-2                        | Degradation of the type I receptors, R-Smads and Smad4 | [14] |
| p300/CBP                       | Acetylation and stabilization of Smad7 | [34,35] |
| Phosphatase 1α                 | Dephosphorylation of ALK1 | [17] |
| SIK (the salt-inducible kinase) | Degradation of TβRI | [25] |
| SIRT1                          | Deacetylation of Smad7 and inhibition of TGF-β signaling | [33] |
| Smurf1/2                       | Degradation of the type I receptors, R-Smads, Smad4 and Smad7 | [14] |
| STRAP                          | Stabilizing the complex between ALK5 and Smad7 and inhibiting TGF-β signaling | [18] |
| TRAFs; TAK1                    | Activation of p38 MAPK signaling pathway by TGF-β with Smad7 as an adaptor; inhibition of NF-κB signaling by dissembling the TRAF–TAK1–TAB2/3 complex via Smad7 | [12,42,80] |
| Yes-associated protein (YAP65)  | facilitating the recruitment of Smad7 to activated TβRI | [24] |
| WWP1/Tiul1                     | Degradation of the type I receptors, R-Smads and Smad4 | [14] |
HDACs as well as SIRT1, and is deacetylated by these enzymes [38–40]. The acetylation of Smad7 inhibits its ubiquitination and proteasome-mediated degradation, so the degradation of Smad7 is regulated by the balance between acetylation, deacetylation, and ubiquitination.

**Smad7 Connects Other Signaling Pathways With the TGF-β Pathway**

Smad7 is an important regulator of TGF-β, activin, Nodal, and BMPs signaling via a negative feedback circuit. It is transcriptionally induced by TGF-β and BMPs. A perfect SBE has been identified in the promoter of human Smad7, and both Smad2/3 and Smad4 are involved in its transcriptional induction by TGF-β [41]. However, full induction of Smad7 by Smad proteins needs other transcriptional co-activators, such as CBP/p300, FoxH1, TFE-3 (transcription factor mE3), CBFA (PEBP2/core-binding factor A) and ATF2, and so on [42]. AP1 and SP1 were also found to promote the transcription of Smad7, indicating that other signaling pathways may be involved in the transcriptional regulation of Smad7 (Fig. 2) [43]. Similarly, Smad6 is also transcriptionally induced by BMP/Smad signaling pathway. Besides CREB, other factors, such as OAZ and Runx2, also upregulate the expression of Smad6 [44,45]. And the transcriptional co-repressor Ski was reported to inhibit the transcription of both Smad6 and Smad7 [46].

In addition to TGF-β/BMP signaling, the transcription of I-Smads can also be induced by inflammatory cytokines, such as interleukin 1, IFN-γ, and TNF-α [47]. EGF, ultraviolet irradiation, lamina shear stress as well as EGF or TPA treatment can induce Smad7 expression in a cell-line-dependent manner, but the mechanisms remain elusive [47,48]. Interestingly, Smad7 was shown to disrupt the formation of TRAF2–TAK1–TAB2/3 complex and inhibit TNF-α/NF-κB signaling [49]. Likewise, Smad6 binds to Pellino-1, an adaptor protein of mammalian interleukin 1 receptor-associated kinase 1 (IRAK1), and thereby promoting TGF-β-mediated...
anti-inflammatory effects [50]. Since TGF-β has an anti-inflammatory activity, and usually acts against those cytokines, I-Smads induction by one kind of cytokine may repress the signaling of another. So they mediate the balance and crosstalk of these signaling pathways.

TGF-β can activate MAPKs, including ERK, JNK, and p38 signaling in a cell-specific manner. Accumulating evidence shows that Smad7 may play roles in this process. Mazars et al. [51] reported that Smad7 could activate JNK signaling and is essential for JNK-mediated apoptosis. TGF-β induces apoptosis of prostate cancer cells by activating p38 MAPK, and Smad7 may serve as a scaffold protein in the process [52,53]. In prechondrogenic cells, Smad7 inhibits chondrocytic differentiation possibly by downregulating BMP-activated p38 MAPK pathway [52,53]. Smad7 was also reported to be involved in TGF-β-dependent activation of Rho GTPases Cdc42 and RhoA [54]. All these data indicate that I-Smads not only act as antagonists of TGF-β/BMP signaling, but also stand at the crossroad of diverse signaling pathways.

There are multiple cross-talking steps between TGF-β and Wnt signaling. β-catenin, a key signal transducer of Wnt signaling, has functional interaction with Smad7. The association of Smad7 with β-catenin regulates the transcription of c-myc, which is important for TGF-β induced apoptosis of human prostate cancer PC-3U cells [55]. In addition, Smad7 transgenic mice exhibit perturbed hair follicle morphogenesis and differentiation and accelerated sebaceous gland morphogenesis due to β-catenin degradation by the ubiquitin E3 ligase Smurf2 (recruited by Smad7) and thereby decreased Wnt signaling [56]. In contrast, a recent report showed that Smad7 stabilizes β-catenin binding to E-cadherin and modulates cell–cell adhesion [57]. Therefore, Smad7 can regulate the activity of β-catenin in a cellular context-dependent way.

The Role of Smad7 in TGF-β Mediated Physiology and Pathology

Although Smad7 is a well-documented key antagonist of TGF-β, it has been shown to promote TGF-β-mediated apoptosis of human prostatic carcinoma cells, podocytes, Mv1Lu, MDCK, and COS7 cells by activating MAPK signaling or repressing NF-κB signaling [58]. On the other hand, Smad7 can also inhibit apoptosis induced by TGF-β in some cell lines, such as B cells and gastric epithelial cells [59,60], suggesting that the function of Smad7 is context dependent.

TGF-β plays a key role in fibrosis of different tissues, such as skin, the liver, kidney, eye, lung as well as cardiovascular system. It induces the Smad3-dependent transcription of fibrillar collagens, inhibits the degradation of ECM by downregulating the expression of matrix degrading enzymes, and increases the expression of metalloproteinase (MMP) inhibitors, together leading to the accumulation of ECM. Elevated TGF-β level and decreased Smad7 level are often present in tissues where an uncontrolled fibrotic response occurs. Therefore, inhibition of Smad3 by overexpression of Smad7 dramatically reduced fibrotic responses of the kidney, lung and liver in animal models, indicating an important anti-fibrotic effect of Smad7 by antagonizing TGF-β/Smad3 signaling pathway [61–64].

In addition to pro-fibrotic activity, TGF-β also has a major role in the regulation of immune cell functions. TGF-β1 knockout mice showed a multifocal, mixed inflammatory cell response and tissue necrosis, leading to organ failure and death [65]. Consistently, blocking TGF-β signaling via specific deletion of TGF-β type II receptor in T cells leads to disruption of T-cell development as well as homeostasis, resulting in autoimmune inflammation and death at last [66]. TGF-β also regulates the differentiation and activation of many other leukocytes, including B cells, NK cells, dendritic cells, monocytes/macrophages, granulocytes, and mast cells [67]. Thus, TGF-β is a key regulator in immune system homeostasis, and dysfunction of TGF-β may results in disorders of this system, such as autoimmunity or inflammatory bowel disease. In patients with inflammatory bowel disease, the phosphorylation level of Smad3 is very low, and the formation of Smad3–Smad4 complex is also affected, so TGF-β signaling is disrupted despite the abundance of TGF-β in the inflamed gut [68,69]. Instead, Smad7 level is elevated in the tissues with inflammatory bowel disease. When Smad7 was reduced by anti-sense oligonucleotides, Smad3 activation was restored and the inflammation was subsequently inhibited by endogenous TGF-β [70]. Conversely, Smad7 may also mediate the anti-inflammatory activity of TGF-β in other situations by inhibiting NF-κB signaling upon activation by inflammatory cytokines like interleukine 1 or TNF-α. Smad7 has also been shown to disrupt the TRAF6–TAK1–TAB2/3 signaling complex or to induce the expression of IkB, which induces the degradation of NF-κB sub-units by the proteosomal pathway, therefore resulting in
inhibition of NF-κB signaling. In addition, Smad7 has been shown to be a key negative regulator of the renal inflammatory response [62,68,71,72]. These results suggest that Smad7 may have both anti-fibrotic and anti-inflammatory functions.

TGF-β has anti-proliferative effects in epithelial cells. However, it can also promote tumorigenesis by modulating processes such as cell invasion, metastasis, immune regulation, and microenvironment modification that cancer cells may exploit for their own good [2]. Smad7 has been shown to play a role in these processes. A genome-wide association study showed that common alleles of Smad7 influenced the risk of colorectal cancer [73]. As a key negative regulator of TGF-β signaling, Smad7 has been reported to inhibit the formation of osteolytic metastases by human breast cancer and melanoma when overexpressed [74–76]. In addition, it also inhibits endometrial carcinomas, thyroid follicular tumors, and hepatocellular carcinomas [77–79]. Azuma et al. [80] reported that overexpression of Smad7 in mouse mammary carcinoma JygMC(A) cells inhibits their metastasis via upregulation of E-cadherin and downregulation of N-cadherin, leading to a reduction of cell migration [81]. In other cases, Smad7 can promote tumorigenesis. For instance, Smad7 blocks TGF-β-mediated growth inhibition and inhibits apoptosis in pancreatic cancer as well as FET cells [82,83]. Liu et al. [84] also reported that in a xenograft model, when primary keratinocytes were co-transfected with Smad7 and H-ras, mixed with dermal fibroblasts, and grafted into nude mice, they progressed into skin squamous cell carcinomas, while control cells did not. Since Smad7 has been shown to degrade β-catenin by recruiting Smurf2, and reduced Wnt signaling leads to spontaneous skin cancer in mice [85], it would be interesting to examine the relationship among Smad7, Wnt signaling, and skin cancer. On the other hand, enhanced Wnt signaling contributes to many different types of cancers. Therefore, the role of Smad7 in the process of tumor formation is very complicated and varies depending on tumor types and their microenvironments [86].

Conclusions

TGF-β is a cytokine of crucial importance that regulates diverse cellular and physiological processes, such as cell proliferation, differentiation, apoptosis, adhesion, and migration. To maintain the cellular or/and organic homeostasis, the TGF-β signaling pathway is precisely regulated at different levels. Dysfunction or deregulation of TGF-β signaling has been associated with different human diseases, such as fibrosis, inflammatory diseases, and tumorigenesis. Smad7 regulates TGF-β signaling via a negative feedback loop and mediates the crosstalk between TGF-β and other signaling pathways. Smad7 also plays an important role in pathological processes and has both anti-fibrotic and anti-inflammatory activities, suggesting that overexpression of Smad7 may have therapeutic potential to treat fibrosis and inflammation.

Acknowledgements

The authors wish to apologize to the investigators whose outstanding work was not cited here because of space limitation. The authors would also like to thank Mr Martin Ting Ma for assisting in manuscript preparation.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (30430360, 30671033, and 30600096), the Major State Basic Research Development Program of China (2004CB720002, 2006CB943401, and 2006CB910102), and the National High Technology Research and Development Program of China (2006AA02Z172).

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