

Transforming Growth Factor- β Signaling Through the Smad Pathway: Role in Extracellular Matrix Gene Expression and Regulation

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Transforming growth factor (TGF)- β represents a prototype of multifunctional cytokine. Its broad activities include, among others, context-specific inhibition or stimulation of cell proliferation, control of extracellular matrix (ECM) synthesis and degradation, control of mesenchymal–epithelial interactions during embryogenesis, mediation of cell and tissue responses to injury, control of carcinogenesis, and modulation of immune functions. Regulation of production and turnover of ECM components is essential for tissue homeostasis and function. TGF- β exerts its effects on cell proliferation, differentiation, and migration in part through its capacity to modulate the deposition of ECM components. Specifically, TGF- β isoforms have the ability to induce the expression of ECM proteins in mesenchymal cells, and to stimulate the production of protease inhibitors that prevent enzymatic breakdown of the ECM. Deregulation of these functions is associated with abnormal connective tissue deposition, as observed, for example, during scarring or fibrotic processes. In this review we discuss the current understanding of the signaling mechanisms used by TGF- β to elicit its

effects on target genes, focusing primarily on Smad proteins and their role in the transcriptional regulation of ECM gene expression. Other signaling mechanisms, such as the MAP/SAP kinase or Ras pathways, although potentially important for transmission of some of the TGF- β signals, will not be described. Transforming growth factor- β (TGF- β) plays a critical role in the regulation of extracellular matrix gene expression. Its overexpression is believed to contribute to the development of tissue fibrosis. The recent identification of Smad proteins, TGF- β receptor kinase substrates that translocate into the cell nucleus to act as transcription factors, has increased our understanding of the molecular mechanisms underlying TGF- β action. This review focuses primarily on the mechanisms underlying Smad modulation of gene expression and how they relate to wound healing. Potential implications for the development of therapeutic approaches against tissue fibrosis are discussed. *Key words: IGF- β /gene expression regulation/intracellular signaling/Smad proteins/target genes. J Invest Dermatol 118:211–215, 2002*

THE TGF- β SUPER-FAMILY, STRUCTURE AND ACTIVATION

The TGF- β super-family of growth factors includes the various forms of TGF- β , bone morphogenic proteins (BMP), nodals, activins, the anti-Mullerian hormone, and many other structurally related factors found in vertebrates, insects, and nematodes (Massagué, 1998). There are three mammalian isoforms of TGF- β (TGF- β 1–3), structurally nearly identical, with a knot motif composed of six cysteine residues joined together by three intrachain disulfide bonds that stabilize β -sheet bands. One free cysteine forms an interchain disulfide bond with an identical monomeric chain to generate the mature TGF- β dimer.

TGF- β are secreted as latent precursor molecules (LTGF- β) requiring activation into a mature form for receptor binding and

subsequent activation of signal transduction pathways. The LTGF- β molecules consist of 390–414 amino acids. They contain an amino-terminal hydrophobic signal peptide region, the latency-associated peptide (LAP) region, of 249 residues, and the C-terminal, potentially bioactive region that contains 112 amino acids per monomer. LTGF- β is usually secreted as a large latent complex covalently bound via the LAP region to LTGF- β -binding protein (LTBP; Roberts, 1998) or as a small latent complex without LTBP. The LAP confers latency to the complex, whereas LTBP serves to bind TGF- β to the ECM and to enable its proteolytic activation (Nunes *et al*, 1997).

Activation of TGF- β is a complex process involving conformational changes of LTGF- β , induced by either cleavage of the LAP by various proteases such as plasmin, thrombin, plasma transglutaminase, or endoglycosylases, or by physical interactions of the LAP with other proteins, such as thrombospondin-1, leading to the release of bioactive, mature, TGF- β (Roberts, 1998).

TGF- β /Smad SIGNAL TRANSDUCTION MACHINERY

TGF- β receptors Upon activation, TGF- β superfamily members initiate their cellular action by binding to serine/

Manuscript received August 8, 2001; revised October 12, 2001; accepted for publication October 18, 2001.

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Abbreviations: LTBP, LTGF- β -binding protein; LTGF- β , latent precursor molecules.

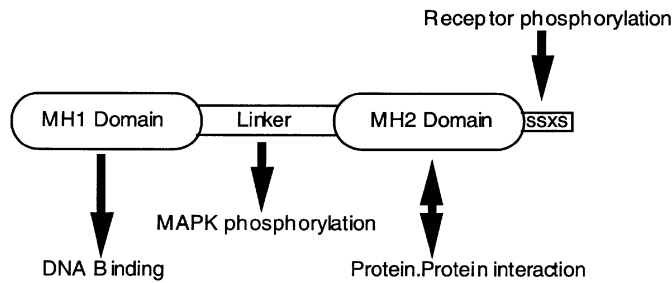


Figure 1. Structural domains of an R-Smad. R-Smad consist of two conserved globular domains known as MH1 (Mad homology 1) and MH2 domains, linked by a linker region. In the basal state, R-Smad remain in an inactive conformation through an auto-inhibitory MH1/MH2 interaction. Phosphorylation of the C-terminal SSXS motif by activated T β RI results in R-Smad activation, heteromerization with Smad4, and subsequent translocation into the cell nucleus. The Smad3 domain in MH1 recognizes the DNA sequence CAGAC; the MH2 domain is involved in protein/protein interactions with co-Smad, transcriptional coactivators and corepressors.

threonine kinase receptors. The TGF- β receptor family consists of two structurally similar subfamilies, type I and type II receptors, with small cysteine-rich extracellular regions and intracellular portions consisting mainly of their kinase domains. Type I receptors have a region rich in glycine and serine residues (GS domain) preceding the receptor kinase domain (Huse *et al*, 1999). To exert their signal, type II and type I receptors act in sequence: TGF- β first binds to the type II receptor (T β RII), which occurs in the cell membrane in an oligomeric form with intrinsic kinase activity; TGF- β type I receptor (T β RI) is then recruited and phosphorylated in its GS domain by T β RII, leading to activation of its kinase activity and subsequent intracellular signaling (Massagué, 1998; Piek *et al*, 1999). Betaglycan, a transmembrane proteoglycan also known as T β RIII (Lopez-Casillas *et al*, 1991), allows high-affinity binding of TGF- β to T β RII but does not itself transduce signal (Rodriguez *et al*, 1995; Brown *et al*, 1999).

Smad proteins Following ligand activation, signaling from T β RI to the nucleus occurs predominantly by phosphorylation of cytoplasmic mediators belonging to the Smad family (Piek *et al*, 1999; Massagué and Chen, 2000; Massagué and Wotton, 2000). Type I receptors specifically recognize and phosphorylate the ligand-specific receptor-activated Smad (R-Smad, Fig 1).

The latter are recruited to activated T β RI by a membrane bound cytoplasmic protein called SARA (Smad Anchor for Receptor Activation; Tsukazaki *et al*, 1998). R-Smad include Smad1, Smad5, and Smad8 downstream of the BMP, and Smad2 and Smad3 downstream of TGF- β and activin. They all consist of two conserved Mad-homology (MH) domains that form globular structures separated by a linker region (Shi *et al*, 1997). The N-terminal MH1 domain has DNA-binding activity, whereas the C-terminal MH2 domain has protein-binding properties. Phosphorylation of R-Smad by type I receptors occurs principally on two serine residues within a conserved -SS(M/V)S-motif at their C-terminus (Massagué and Chen, 2000; Massagué and Wotton, 2000).

Upon phosphorylation by T β RI, R-Smad form heteromeric complexes with co-Smad, such as Smad4. Co-Smad act as a convergent node in the Smad pathways downstream of the TGF- β superfamily receptors, complexing R-Smad, regardless of the TGF- β ligand specificity of the latter. R-Smad/Smad4 complexes are then translocated into the nucleus by a mechanism involving the cytoplasmic protein importin (Xiao *et al*, 2000; Kurisaki *et al*, 2001). They may then function as transcription factors, binding DNA either directly or in association with other DNA binding proteins (Piek *et al*, 1999; Massagué and Chen, 2000; Massagué and Wotton, 2000). Maximal affinity of recombinant Smad3 and Smad4 to DNA is observed with the 5 bp sequence, CAGAC (Shi

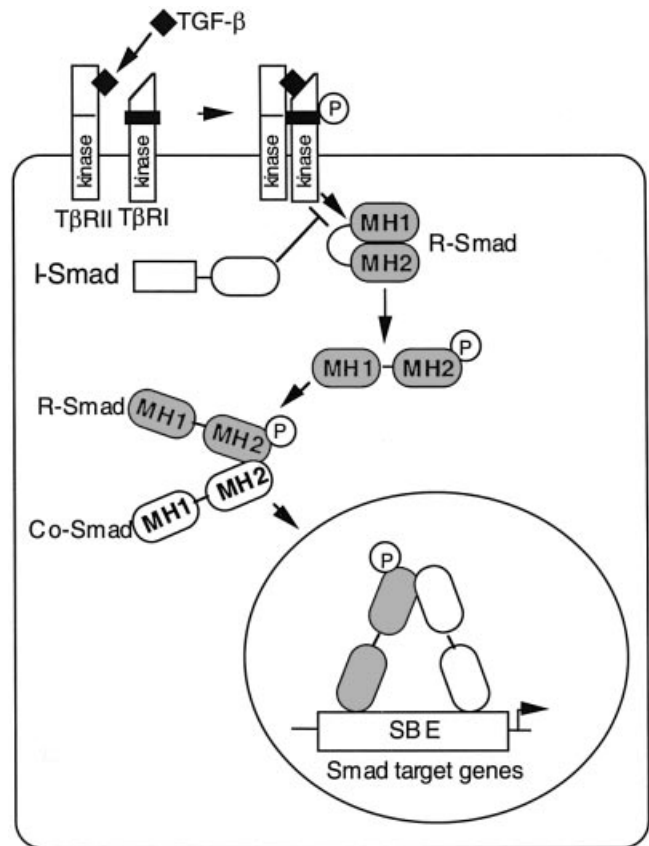


Figure 2. Schematic representation of the Smad signaling cascade downstream of the TGF- β receptors. Initiation of signaling occurs when TGF- β binds to the serine/threonine kinase known as T β RII. Ligand-bound T β RII complexes with and phosphorylates the transducer receptor, T β RI, which becomes activated. Activated T β RI then recruits and phosphorylates R-Smad, leading to the association of the latter with a co-Smad, such as Smad4, and translocation of the formed heterocomplex into the nucleus where it acts as a transcriptional regulator of target genes.

et al, 1998; Zawel *et al*, 1998). Smad2, on the other hand, does not bind DNA directly, requiring a nuclear DNA-binding protein of the Fast family to bind DNA, in association with Smad4, and activate transcription in response to TGF- β and activin (Chen *et al*, 1997; Labbé *et al*, 1998; Liu *et al*, 1999).

A third group of Smad proteins, the inhibitory Smad (I-Smad), such as Smad6 or Smad7, prevent R-Smad phosphorylation and subsequent nuclear translocation of R-Smad/Smad4 heterocomplexes (Imamura *et al*, 1997; Nakao *et al*, 1997).

Following target gene transcription, Smad complexes are released from the chromatin and undergo ubiquitination, followed by proteasomal degradation (Zhu *et al*, 1999).

A summary of the various steps of TGF- β signaling through the Smad cascade is provided in Fig 2.

TRANSCRIPTIONAL CROSS-TALKS

Several cross-signaling mechanisms have been described that implicate Smad proteins. For example, Smad3 interacts with the vitamin D receptor to mediate the effect of TGF- β on vitamin D₃-induced transcription (Yanagisawa *et al*, 1999). Smad3 also interacts with TFE3 and Sp1 to activate transcription from the *PAI-1* and *p21^{cip1}* promoters, respectively (Hua *et al*, 1998; Moustakas and Kardassis, 1998). Smad/AP-1 interactions have also been reported, which may lead to either additive (Zhang *et al*, 1998; Liberati *et al*, 1999; Verrecchia *et al*, 2001c) or antagonistic (Verrecchia *et al*, 2000; Verrecchia *et al*, 2001c) activities on gene transactivation.

The outcome is dependent on the structure of target promoters, as not only the presence of AP-1- and/or Smad-specific *cis*-elements, but also their respective positions, may influence the transcriptional response (Liberati *et al*, 1999; Verrecchia *et al*, 2001c). For example, in a promoter context exhibiting Smad-specific boxes distant from functional AP-1 sites, such as in the *PAI-1* and *c-jun* promoters, transcriptional cooperation between Smad and Jun has been observed, without formation of Smad/Jun heterocomplexes on DNA (Liberati *et al*, 1999; Verrecchia *et al*, 2001c). In the context of the *COL7A1* promoter that possesses an AP-1 site located within a bipartite Smad-specific TGF- β -response element, inhibition of Smad-driven transactivation by Jun members was observed, again without formation of Smad/Jun heterocomplexes on DNA. In the latter case, Jun was shown to act as a direct repressor of Smad function, binding to and blocking transcriptional activation domains intrinsic to Smad3, and preventing its binding to DNA (Verrecchia *et al*, 2001c).

Another possibility for Smad to interfere with other transcription factors occurs through direct interactions with transcriptional coactivators such as the Ski-interacting protein (SKIP) or CBP/p300. The latter proteins modify transcription either by altering chromatin structure so that the underlying DNA sequences are exposed to the transcriptional apparatus (Workman and Kingston, 1998), or by directly recruiting the RNA polymerase II holoenzyme to the promoter (Snowden and Perkins, 1998). Smad-CBP/p300 interaction is required for transcriptional activation of several TGF- β -dependent promoters (Feng *et al*, 1998; Janknecht *et al*, 1998; Nishihara *et al*, 1998; Pouponnot *et al*, 1998; Shen *et al*, 1998). SKIP, a nuclear hormone receptor coactivator, enhances TGF- β -dependent transactivation by interacting with Smad2 and Smad3 proteins (Leong *et al*, 2001).

In addition, several nuclear proteins, including the viral oncoprotein E1A (Nishihara *et al*, 1999), the proto-oncogenes *c-Ski* and *SnoN* (Akiyoshi *et al*, 1999; Stroschein *et al*, 1999), *TGIF* (Wotton *et al*, 1999), *Snip-1* (Kim *et al*, 2000), *SIP1* (Verschueren *et al*, 1999), and *c-Jun* (Verrecchia *et al*, 2000) compete for R-Smad/Smad4 binding to CBP or p300, thereby interfering with Smad-dependent gene expression.

TGF- β /Smad MODULATION OF ECM GENE EXPRESSION

Despite extensive research directed toward the elucidation of the role played by Smad proteins downstream of TGF- β , very few direct Smad target genes have been characterized. Indeed, it was shown that transactivation of the fibronectin gene downstream of TGF- β is a JNK-specific, Smad-independent, mechanism (Hoccevar *et al*, 1999), suggesting that pathways that alternate to the Smad cascade also play an important part in the modulation of ECM gene expression by TGF- β .

Recently, using a combined cDNA microarray/promoter transactivation approach, we have identified several new Smad gene targets among which are *COL1A1*, *COL3A1*, *COL5A2*, *COL6A1*, *COL6A3*, and *TIMP-1*. Most notably, these data indicate that the Smad signaling pathway is crucial for simultaneous activation of several fibrillar collagen genes by TGF- β . About 60 other ECM-related genes were also identified as immediate-early genes targets downstream of TGF- β (Verrecchia *et al*, 2001a).

Our group identified the first described genuine Smad binding sequence (SBS) within the human *COL7A1* promoter (Vindevooghel *et al*, 1998a, b). Specifically, TGF- β upregulation of *COL7A1* gene expression is mediated by rapid and transient binding of a Smad-containing complex to the region -496/-444 of the *COL7A1* promoter, a bipartite element consisting of a CAGA tandem repeat in its 5' end, and a Medea-like (*Drosophila* Smad4 homolog) binding site in 3', both framing a potential AP-1 binding site whose integrity is not necessary for either Smad-driven transactivation or Smad/DNA complex formation (Verrecchia *et al*, 2001c). A few other ECM-related genes whose expression is modulated by TGF- β have also been identified as potential Smad

targets, and CAGA-like elements have been identified within their promoter regions, that bind Smad complexes. These genes include *PAI-1*, *COL1A2*, and the $\beta 5$ integrin gene (*INTB5*) (Chen *et al*, 1998; Denler *et al*, 1998; Lai *et al*, 2000). In these three cases, TGF- β -induced promoter activation may also involve functional interactions between Smad and Sp1, a mechanism that has also been shown to be critical for activation of *p21/WAF1/CIP1* (Pardali *et al*, 2000) and *p15/Ink4B* (Feng *et al*, 2000) by TGF- β in the control of cell proliferation.

Regarding *COL1A2*, initial observations demonstrated that a 135 bp region of the *COL1A2* promoter within 330 bp of the transcription start site confers responsiveness to TGF- β (Inagaki *et al*, 1994). The minimal TGF- β -response element was further refined to the region between nucleotides -271 and -235 (Chung *et al*, 1996). The latter contains potential overlapping *cis*-element for Smad, AP-1, and NF- κ B (Chung *et al*, 1996; Chen *et al*, 1998; Kouba *et al*, 1999). Cooperation between Smad3 and Sp1, the latter binding upstream elements, to transactivate the *COL1A2* promoter has been described (Zhang *et al*, 2000; Inagaki *et al*, 2001). The role of this cooperation in mediating the TGF- β effect is unclear, as elimination of the corresponding Sp1 sites upstream of the Smad-specific CAGACA motif does not prevent TGF- β -driven promoter transactivation (Chung *et al*, 1996). Also, it has been shown that Smad-p300/CBP interactions are a key event in TGF- β -driven collagen gene transactivation (Ghosh *et al*, 2000), and that interferon- γ -driven antagonistic activity against TGF- β effect is integrated at the level of p300/CBP, the latter being sequestered by interferon-induced Stat1 α (Ghosh *et al*, 2001).

Besides playing a part in the regulation of the expression of ECM components, Smad have recently been identified as capable of mediating the inhibitory activity of TGF- β on interstitial collagenase (matrix metalloproteinase-1) gene activation by pro-inflammatory cytokines (Yuan and Varga, 2001). In that study, the authors demonstrate that Smad3 and Smad4, but not Smad1 or Smad2, overexpression block IL-1-driven transactivation of the matrix metalloproteinase-1 promoter, both by and via p300 sequestration.

Smad SIGNALING IN WOUND HEALING AND FIBROSIS

Numerous studies have documented the ability of exogenous TGF- β to improve wound healing (Roberts and Sporn, 1993). Recent work involving inactivation of the Smad3 gene in mice has allowed to explore specifically the contribution of the Smad pathway to tissue repair (Ashcroft *et al*, 1999). In contrast to predictions made on the basis of the ability of exogenous TGF- β to improve wound healing, Smad3-null (Smad3^{ex8/ex8}) mice paradoxically show accelerated cutaneous wound healing compared with wild-type mice, characterized by an increased rate of re-epithelialization and significantly reduced local infiltration of monocytes. Smad3^{ex8/ex8} keratinocytes show altered patterns of growth and migration, and Smad3^{ex8/ex8} monocytes exhibit a selectively blunted chemotactic response to TGF- β . From these data, it may therefore be concluded that Smad3 may mediate *in vivo* signaling pathways that are inhibitory to wound healing. These data suggest that "natural" wound healing may involve suppression of the Smad3 level, and that complete loss of this signaling intermediate further accelerates the wound healing process. In the same study, it was shown that reduced deposition of ECM in Smad3^{-/-} mice could be reversed with the addition of exogenous TGF- β 1, which implies that ECM gene regulation by TGF- β in fibroblasts *in vivo* may also occur via Smad-independent pathways. Also, this report indicates that disruption of the Smad3 pathway *in vivo*, coupled with exogenous TGF- β signaling through intact alternate pathways, may be of therapeutic benefit in accelerating all aspects of impaired wound healing.

Type I collagen and ECM accumulation is one of the hallmarks of fibrotic conditions. For example, affected skin areas from patients with systemic sclerosis exhibit abnormal accumulation of various ECM components, predominantly type I and type III collagens, but

also types V and VII, as well as various proteoglycans. Accumulation of ECM proteins in fibrotic conditions is accompanied by elevated mRNA steady-state levels of fibrillar collagens (Kähäri *et al.*, 1984; Ohta and Uitto, 1987). It is generally accepted that transcriptional activation of ECM-related genes, and in particular that of collagen genes, is a critical event underlying the progressive nature of tissue fibrosis, as is observed in cutaneous lesions from systemic sclerosis or keloid scars (reviewed in Jimenez and Saitta, 1999; Uitto and Kouba, 2000). An essential factor contributing to the metabolic modulation of ECM genes expression is TGF- β , although other factors such as connective tissue growth factor, whose expression is controlled by TGF- β in a Smad-dependent manner (Holmes *et al.*, 2001; Leask *et al.*, 2001), which may contribute to some of the TGF- β effects on extracellular matrix gene expression and subsequent development of tissue fibrosis.

INTERFERING WITH FIBROTIC PROCESSES

It is reasonable to hypothesize that a better understanding of the mechanisms of TGF- β -mediated upregulation of ECM gene expression in fibrotic conditions will provide promising and novel approaches to the therapy of these incurable diseases. In this context, it should be noted that the transcription factor Sp1 is able to interact physically with Smad proteins, leading for example to the activation of the p21, p15, and COL1A2 genes in response to TGF- β (Moustakas and Kardassis, 1998; Feng *et al.*, 2000; Inagaki *et al.*, 2001). Sp1 is also important for the basal expression of various collagens, as well as for the expression of TGF- β itself (Geiser *et al.*, 1993). Altering the function of Sp1 may therefore represent an interesting alternative to interfere with the pro-fibrotic activities of TGF- β . In this context, we have recently demonstrated that Sp1 gene targeting in fibroblasts broadly inhibits ECM gene expression, as measured using differential hybridization of cDNA microarrays, as well as various promoter transactivation assays (Verrecchia *et al.*, 2001b). In addition, using a transgenic mouse model carrying a COL1A2 promoter/luciferase reporter transgene, we have shown that decoy Sp1-binding oligonucleotides inhibit COL1A2 promoter activity *in vivo*. Such an approach, once optimized, may represent an interesting therapeutic alternative toward the treatment of fibrotic disorders.

Altering the Smad pathway may also be achieved at the level of transcriptional coactivators, such as p300. For example, it has been shown that RelA, cJun, and Stat1 α are able to inhibit Smad-driven promoter transactivation by competing with Smad for limiting amounts of cellular p300 (Bitzer *et al.*, 2000; Ghosh *et al.*, 2000; Verrecchia *et al.*, 2000). These mechanisms may explain, at least in part, the antagonistic activity of tumor necrosis factor- α and interferon- γ on TGF- β -driven COL1A2 expression (Chung *et al.*, 1996; Verrecchia *et al.*, 2000; Ghosh *et al.*, 2001).

Other approaches may include local overexpression of the inhibitory Smad, Smad7, targeting of connective tissue growth factor in situations where it is proven to be the mediator of the TGF- β effect on ECM deposition. Several options have been discussed in a recent review (Branton and Kopp, 1999).

CONCLUSIONS AND PERSPECTIVES

Tremendous progress has been accomplished over the past several years in the understanding of the initial steps of TGF- β intracellular signaling. The identification of Smad proteins as direct links between the cell surface and the nucleus has allowed for the elucidation of critical events leading to gene activation by TGF- β . Several hurdles remain before the TGF- β /Smad pathway can be targeted directly in situations such as tissue fibrosis or impaired wound healing. The cell-type specificity of Smad3 effects, together with the identification of alternate signaling pathways for TGF- β remain of critical importance. For example, even though fibrillar collagens have been identified as direct Smad3/4 target genes, it has been demonstrated that Smad3-deficient mice heal faster than their wild-type counterparts, due to accelerated re-epithelialization and

reduced monocytic influx at the site of injury. In these mice, exogenously added TGF- β can still activate type I collagen gene expression. These data suggest that, *in vivo*, Smad3 may have effects that are inhibitory to wound healing. Also, they indicate that disruption of the Smad3 pathway *in vivo*, coupled with exogenous TGF- β signaling through intact alternate pathways, may be of therapeutic benefit in accelerating all aspects of impaired wound healing.

REFERENCES

- Akiyoshi S, Inoue H, Hanai J, Kusanagi K, Nemoto N, Miyazono K, Kawabata M: c-Ski acts as a transcriptional co-repressor in transforming growth factor-beta signaling through interaction with smads. *J Biol Chem* 274:35269-35277, 1999
- Ashcroft GS, Yang X, Glick AB *et al.*: Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat Cell Biol* 1:260-266, 1999
- Bitzer M, von Gersdorff G, Liang D, Dominguez-Rosales A, Beg AA, Rojkind M, Bottinger EP: A mechanism of suppression of TGF-beta/SMAD signaling by NF-kappa B/RelA. *Genes Dev* 14:187-197, 2000
- Border WA, Noble NA, Yamamoto T, Harper JR, Yamaguchi Y, Pierschbacher MD, Ruoslahti E: Natural inhibitor of transforming growth factor-beta protects against scarring in experimental kidney disease. *Nature* 360:361-364, 1992
- Branton MH, Kopp JB: TGF-beta and fibrosis. *Microbes Infect* 1:1349-1365, 1999
- Brown CB, Boyer AS, Runyan RB, Barnett JV: Requirement of type III TGF-beta receptor for endocardial cell transformation in the heart. *Science* 283:2080-2082, 1999
- Chen SJ, Artlett CM, Jimenez SA, Varga J: Modulation of human alpha1(I) procollagen gene activity by interaction with Sp1 and Sp3 transcription factors *in vitro*. *Gene* 215:101-110, 1998
- Chen X, Weisberg E, Fridmacher V, Watanabe M, Naco G, Whitman M: Smad4 and FAST-1 in the assembly of activin-responsive factor. *Nature* 389:85-89, 1997
- Chung KY, Agarwal A, Uitto J, Mauviel A: An AP-1 binding sequence is essential for regulation of the human alpha2(I) collagen (COL1A2) promoter activity by transforming growth factor-beta. *J Biol Chem* 271:3272-3278, 1996
- Denler S, Itoh S, Vivien D, ten Dijke P, Huet S, Gauthier JM: Direct binding of Smad3 and Smad4 to critical TGF-beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. *EMBO J* 17:3091-3100, 1998
- Feng XH, Zhang Y, Wu RY, Derynck R: The tumor suppressor Smad4/DPC4 and transcriptional adaptor CBP/p300 are coactivators for smad3 in TGF-beta-induced transcriptional activation. *Genes Dev* 12:2153-2163, 1998
- Feng XH, Lin X, Derynck R: Smad2, Smad3 and Smad4 cooperate with Sp1 to induce p15 (Ink4B) transcription in response to TGF-beta. *EMBO J* 19:5178-5193, 2000
- Geiser AG, Busam KJ, Kim SJ *et al.*: Regulation of the transforming growth factor-beta 1 and -beta 3 promoters by transcription factor Sp1. *Gene* 129:223-228, 1993
- Ghosh AK, Yuan W, Mori Y, Varga J: Smad-dependent stimulation of type I collagen gene expression in human skin fibroblasts by TGF-beta involves functional cooperation with p300/CBP transcriptional coactivators. *Oncogene* 19:3546-3555, 2000
- Ghosh AK, Yuan W, Mori Y, Chen S, Varga J: Antagonistic regulation of type I collagen gene expression by interferon-gamma and transforming growth factor-beta. Integration at the level of p300/CBP transcriptional coactivators. *J Biol Chem* 276:11041-11048, 2001
- Hocevar BA, Brown TL, Howe PH: TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway. *EMBO J* 18:1345-1356, 1999
- Holmes A, Abraham DJ, Sa S, Shiwen X, Black CM, Leask A: CTGF and SMADs. maintenance of scleroderma phenotype is independent of SMAD signaling. *J Biol Chem* 276:10594-10601, 2001
- Hua X, Liu X, Ansari DO, Lodish HF: Synergistic cooperation of TFE3 and smad proteins in TGF-beta-induced transcription of the plasminogen activator inhibitor-1 gene. *Genes Dev* 12:3084-3095, 1998
- Huse M, Chen YG, Massagué J, Kuriyan J: Crystal structure of the cytoplasmic domain of the type I TGF beta receptor in complex with FKBP12. *Cell* 96:425-436, 1999
- Imamura T, Takase M, Nishihara A, Oeda E, Hanai J, Kawabata M, Miyazono K: Smad6 inhibits signalling by the TGF-beta superfamily. *Nature* 389:622-626, 1997
- Inagaki Y, Truter S, Ramirez F: Transforming growth factor-beta stimulates alpha 2(I) collagen gene expression through a cis-acting element that contains an Sp1-binding site. *J Biol Chem* 269:14828-14834, 1994
- Inagaki Y, Nemoto T, Nakao A, Dijke Pt P, Kobayashi K, Takehara K, Greenwel P: Interaction between GC box binding factors and Smad proteins modulates cell lineage-specific alpha 2(I) collagen gene transcription. *J Biol Chem* 276:16573-16579, 2001
- Janknecht R, Wells NJ, Hunter T: TGF-beta-stimulated cooperation of smad proteins with the coactivators CBP/p300. *Genes Dev* 12:2114-2119, 1998
- Jimenez SA, Saitta B: Alterations in the regulation of expression of the alpha 1(I) collagen gene (COL1A1) in systemic sclerosis (scleroderma). *Springer Semin Immunopathol* 21:397-414, 1999

- Kähäri VM, Vuorio T, Nanto-Salonen K, Vuorio E: Increased type I collagen mRNA levels in cultured scleroderma fibroblasts. *Biochim Biophys Acta* 781:183-186, 1984
- Kim RH, Wang D, Tsang M, et al: A novel smad nuclear interacting protein, SNIP1, suppresses p300-dependent TGF-beta signal transduction. *Genes Dev* 14:1605-1616, 2000
- Kouba DJ, Chung KY, Nishiyama T, et al: Nuclear factor-kappa B mediates TNF-alpha inhibitory effect on alpha 2(I) collagen (COL1A2) gene transcription in human dermal fibroblasts. *J Immunol* 162:4226-4234, 1999
- Kurisasi A, Kose S, Yoneda Y, Heldin CH, Moustakas A: Transforming growth factor-beta induces nuclear import of Smad3 in an Importin-beta1 and Ran-dependent manner. *Mol Biol Cell* 12:1079-1091, 2001
- Labbé E, Silvestri C, Hoodless PA, Wrana JL, Attisano L: Smad2 and Smad3 positively and negatively regulate TGF beta-dependent transcription through the forkhead DNA-binding protein FAST2. *Mol Cell* 2:109-120, 1998
- Lai CF, Feng X, Nishimura R, Teitelbaum SL, Avioli LV, Ross FP, Cheng SL: Transforming growth factor-beta up-regulates the beta 5 integrin subunit expression via Sp1 and Smad signaling. *J Biol Chem* 275:36400-36406, 2000
- Leask A, Sa S, Holmes A, Shiwen X, Black CM, Abraham DJ: The control of ccn2 (ctgf) gene expression in normal and scleroderma fibroblasts. *Mol Pathol* 54:180-183, 2001
- Leong GM, Subramaniam N, Figueroa J, Flanagan JL, Hayman MJ, Eisman JA, Kouzmenko AP: Ski-interacting protein interacts with smad proteins to augment transforming growth factor-beta-dependent transcription. *J Biol Chem* 276:18243-18248, 2001
- Liberati NT, Datto MB, Frederick JP, Shen X, Wong C, Rougier-Chapman EM, Wang XF: Smad bind directly to the Jun family of AP-1 transcription factors. *Proc Natl Acad Sci USA* 96:4844-4849, 1999
- Liu B, Dou CL, Prabhu L, Lai E: FAST-2 is a mammalian winged-helix protein which mediates transforming growth factor beta signals. *Mol Cell Biol* 19:424-430, 1999
- Lopez-Casillas F, Cheifetz S, Doody J, Andres JL, Lane WS, Massagué J: Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-beta receptor system. *Cell* 67:785-795, 1991
- Massagué J: TGF-beta signal transduction. *Annu Rev Biochem* 67:753-791, 1998
- Massagué J, Chen YG: Controlling TGF-beta signaling. *Genes Dev* 14:627-644, 2000
- Massagué J, Wotton D: Transcriptional control by the TGF-beta/Smad signaling system. *EMBO J* 19:1745-1754, 2000
- Moustakas A, Kardassis D: Regulation of the human p21/WAF1/Cip1 promoter in hepatic cells by functional interactions between Sp1 and Smad family members. *Proc Natl Acad Sci USA* 95:6733-6738, 1998
- Nakao A, Afrakhte M, Moren A, et al: Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signalling. *Nature* 389:631-635, 1997
- Nishihara A, Hanai JI, Okamoto N, Yanagisawa J, Kato S, Miyazono K, Kawabata M: Role of p300, a transcriptional coactivator, in signalling of TGF-beta. *Genes Cells* 3:613-623, 1998
- Nishihara A, Hanai J, Imamura T, Miyazono K, Kawabata M: E1A inhibits transforming growth factor-beta signaling through binding to Smad proteins. *J Biol Chem* 274:28716-28723, 1999
- Nunes I, Gleizes PE, Metz CN, Rifkin DB: Latent transforming growth factor-beta binding protein domains involved in activation and transglutaminase-dependent cross-linking of latent transforming growth factor-beta. *J Cell Biol* 136:1151-1163, 1997
- Ohta A, Uitto J: Procollagen gene expression by scleroderma fibroblasts in culture. Inhibition of collagen production and reduction of pro alpha 1(I) and pro alpha 1(III) collagen messenger RNA steady-state levels by retinoids. *Arthritis Rheum* 30:404-411, 1987
- Pardali K, Kurisasi A, Moren A, ten Dijke P, Kardassis D, Moustakas A: Role of Smad proteins and transcription factor Sp1 in p21 (Waf1/Cip1) regulation by transforming growth factor-beta. *J Biol Chem* 275:29244-29256, 2000
- Piek E, Heldin CH, Ten Dijke P: Specificity, diversity, and regulation in TGF-beta superfamily signaling. *FASEB J* 13:2105-2124, 1999
- Poupponnot C, Jayaraman L, Massagué J: Physical and functional interaction of SMADs and p300/CBP. *J Biol Chem* 273:22865-22868, 1998
- Roberts AB: Molecular and cell biology of TGF-beta. *Miner Electrolyte Metab* 24:111-119, 1998
- Roberts AB, Sporn MB: Physiological actions and clinical applications of transforming growth factor-beta (TGF-beta). *Growth Factors* 8:1-9, 1993
- Rodriguez C, Chen F, Weinberg RA, Lodish HF: Cooperative binding of transforming growth factor (TGF)-beta 2 to the types I and II TGF-beta receptors. *J Biol Chem* 270:15919-15922, 1995
- Shen X, Hu PP, Liberati NT, Datto MB, Frederick JP, Wang XF: TGF-beta-induced phosphorylation of Smad3 regulates its interaction with coactivator p300/CREB-binding protein. *Mol Biol Cell* 9:3309-3319, 1998
- Shi Y, Hata A, Lo RS, Massagué J, Pavletich NP: A structural basis for mutational inactivation of the tumour suppressor Smad4. *Nature* 388:87-93, 1997
- Shi Y, Wang YF, Jayaraman L, Yang H, Massagué J, Pavletich NP: Crystal structure of a Smad MH1 domain bound to DNA. Insights on DNA binding in TGF-beta signaling. *Cell* 94:585-594, 1998
- Snowden AW, Perkins ND: Cell cycle regulation of the transcriptional coactivators p300 and CREB binding protein. *Biochem Pharmacol* 55:1947-1954, 1998
- Sporn MB, Roberts AB: TGF-beta: problems and prospects. *Cell Regul* 1:875-882, 1990
- Stroschein SL, Wang W, Zhou S, Zhou Q, Luo K: Negative feedback regulation of TGF-beta signaling by the SnoN oncoprotein. *Science* 286:771-774, 1999
- Tsukazaki T, Chiang TA, Davison AF, Attisano L, Wrana JL: SARA, a FYVE domain protein that recruits Smad2 to the TGFbeta receptor. *Cell* 95:779-791, 1998
- Uitto J, Kouba D: Cytokine modulation of extracellular matrix gene expression: relevance to fibrotic skin diseases. *J Dermatol Sci* 24(Suppl. 1):S60-69, 2000
- Verrecchia F, Pessah M, Atfi A, Mauviel A: Tumor necrosis factor-alpha inhibits transforming growth factor-beta/Smad signaling in human dermal fibroblasts via AP-1 activation. *J Biol Chem* 275:30226-30231, 2000
- Verrecchia F, Chu ML, Mauviel A: Identification of novel TGF-beta/Smad gene targets in dermal fibroblasts using a combined cDNA microarray/promoter transactivation approach. *J Biol Chem* 276:17058-17062, 2001a
- Verrecchia F, Rossert J, Mauviel A: Blocking sp1 transcription factor broadly inhibits extracellular matrix gene expression in vitro and in vivo: implications for the treatment of tissue fibrosis. *J Invest Dermatol* 116:755-763, 2001b
- Verrecchia F, Vindevoghel L, Lechleider RJ, Uitto J, Roberts AB, Mauviel A: Smad3/AP-1 interactions control transcriptional responses to TGF-beta in a promoter-specific manner. *Oncogene* 20:3332-3340, 2001c
- Verschueren K, Remacle JE, Collart C, et al: SIP1, a novel zinc finger/homeodomain repressor, interacts with Smad proteins and binds to 5'-CACCT sequences in candidate target genes. *J Biol Chem* 274:20489-20498, 1999
- Vindevoghel L, Lechleider RJ, Kon A, de Caestecker MP, Uitto J, Roberts AB, Mauviel A: SMAD3/4-dependent transcriptional activation of the human type VII collagen gene (COL7A1) promoter by transforming growth factor beta. *Proc Natl Acad Sci USA* 95:14769-14774, 1998a
- Vindevoghel L, Kon A, Lechleider RJ, Uitto J, Roberts AB, Mauviel A: Smad-dependent transcriptional activation of human type VII collagen gene (COL7A1) promoter by transforming growth factor-beta. *J Biol Chem* 273:13053-13057, 1998b
- Workman JL, Kingston RE: Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu Rev Biochem* 67:545-579, 1998
- Wotton D, Lo RS, Lee S, Massagué J: A Smad transcriptional corepressor. *Cell* 97:29-39, 1999
- Xiao Z, Liu X, Lodish HF: Importin beta mediates nuclear translocation of Smad 3. *J Biol Chem* 275:23425-23428, 2000
- Yanagisawa J, Yanagi Y, Masuhiro Y, et al: Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science* 283:1317-1321, 1999
- Yuan W, Varga J: Transforming growth factor-{beta} repression of matrix metalloproteinase-1 transcription in dermal fibroblasts involves Smad3. *J Biol Chem* 276:38502-38510, 2001
- Zawel L, Dai JL, Buckhaults P, Zhou S, Kinzler KW, Vogelstein B, Kern SE: Human Smad3 and Smad4 are sequence-specific transcription activators. *Mol Cell* 1:611-617, 1998
- Zhang W, Ou J, Inagaki Y, Greenwel P, Ramirez F: Synergistic cooperation between Sp1 and Smad3/Smad4 mediates transforming growth factor beta1 stimulation of alpha 2(I)-collagen (COL1A2) transcription. *J Biol Chem* 275:39237-39245, 2000
- Zhang Y, Feng XH, Derynck R: Smad3 and Smad4 cooperate with c-Jun/c-Fos to mediate TGF-beta-induced transcription. *Nature* 394:909-913, 1998
- Zhu H, Kavsak P, Abdollah S, Wrana JL, Thomsen GH: A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature* 400:687-693, 1999