Journal of Cellular Biochemistry 102:593–608 (2007)

TGF-β Signaling: A Tale of Two Responses

Rod A. Rahimi and Edward B. Leof*

Department of Biochemistry and Molecular Biology, Thoracic Diseases Research Unit, Mayo Clinic Cancer Center, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, Minnesota 55905

Abstract Transforming growth factor-β (TGF-β) regulates a wide variety of cellular processes including cell growth, apoptosis, differentiation, migration, and extracellular matrix production among others. The canonical signaling pathway induced by the TGF-β receptor complex involves the phosphorylation of Smad proteins which upon activation accumulate in the nucleus and regulate transcription. Interestingly, the cellular response to TGF-β can be extremely variable depending on the cell type and stimulation context. TGF-β causes epithelial cells to undergo growth arrest and apoptosis, responses which are critical to suppressing carcinogenesis, whereas it can also induce epithelial-mesenchymal transition and mediate fibroblast activation, responses implicated in promoting carcinogenesis and fibrotic diseases. However, TGF-β induces all these responses via the same receptor complex and Smad proteins. To address this apparent paradox, during the last few years a number of additional signaling pathways have been identified which potentially regulate the different cellular responses to TGF-β. The identification of these signaling pathways has shed light onto the mechanisms whereby Smad and non-Smad pathways collaborate to induce a particular cellular phenotype. In this article, we review TGF-β signaling in epithelial cells and fibroblasts with a focus on understanding the mechanisms of TGF-β versatility. J. Cell. Biochem. 102: 593–608, 2007.

Key words: TGF-β; signaling; Smad; non-Smad; epithelia; growth arrest; apoptosis; EMT; fibroblasts

Transforming growth factor-β (TGF-β) was originally identified as a component of 'sarcoma growth factor' secreted from Moloney sarcoma virus infected 3T3 cells that could mediate transformation (anchorage-independent cell growth) of non-neoplastic rat kidney NRK and murine AKR-2B fibroblasts [DeLarco and Todaro, 1978; Moses et al., 1981; Anzano et al., 1983]. Since the discovery of TGF-β, a superfamily of related signaling proteins has been identified that regulate a wide variety of biological processes including cell growth, apoptosis, differentiation, migration, extracellular matrix (ECM) production, angiogenesis, immunity, and development [Massagué et al., 2000; Patterson and Padgett, 2000; ten Dijke et al., 2002]. Members of the TGF-β superfamily are characterized by the presence of common sequence and structural features that include the unique positioning of seven cysteine residues resulting in the formation of a core structure known as the 'cysteine knot' [Sun and Davies, 1995]. The TGF-β superfamily is composed of the TGF-β isoforms (TGF-β1, 2, and 3), Activins, Nodals, bone morphogenetic proteins (BMPs), growth and differentiation factor (GDF), and Mullerian inhibitory substance (MIF).

The active form of TGF-β is a 25 kDa dimer in which the two polypeptides interact via a disulfide bond and hydrophobic interactions. TGF-β is initially synthesized as a precursor protein which is proteolytically processed into an inactive (latent) form composed of mature TGF-β non-covalently bound to the amino-terminal precursor remnant termed latency-associated peptide (LAP) [Munger et al., 1997]. After being secreted by the cell, latent TGF-β can be activated via a variety of mechanisms [Yang et al., 2007]. Once activated, TGF-β family members initiate signaling by interacting with and complexing two receptor serine/threonine kinases referred to as the type I and type II receptors. Ligand binding mediates formation of a heterotetrameric complex in

Grant sponsor: Public Health Service; Grant numbers: GM54200, GM55816; Grant sponsor: National Institutes of General Medical Sciences; Grant sponsor: Mayo Foundation.

*Correspondence to: Edward B. Leof, Stabile 8-58, Mayo Clinic, 200 First Street, SW, Rochester, MN 55905.
E-mail: leof.edward@mayo.edu

Received 21 June 2007; Accepted 27 June 2007

DOI 10.1002/jcb.21501

which the constitutively active type II receptor phosphorylates the glycine-serine rich domain in the juxtamembrane region of the type I receptor resulting in activation of the kinase. The most well characterized mechanism whereby TGF-β superfamily members initiate signal transduction is via the phosphorylation and activation of the Smad proteins by the type I receptor. Phosphorylated Smad proteins accumulate in the nucleus where they collaborate with other transcription factors to regulate gene expression [Massagué et al., 2005].

The cellular response to many TGF-β superfamily members can be variable, promoting as well as antagonizing a variety of responses including proliferation, apoptosis, and differentiation depending on the cell type and stimulation context [Siegel and Massague, 2003]. For instance, TGF-β causes epithelial cells to undergo growth arrest and apoptosis, responses which suppress carcinogenesis, whereas it can also induce epithelial-mesenchymal transition (EMT) and mediate fibroblast activation, responses implicated in promoting carcinogenesis and fibrotic diseases [Siegel and Massague, 2003]. Interestingly, TGF-β induces all of these responses via the same type I and II receptors and the same Smad proteins. The diversity of cellular responses to TGF-β has been a driving force for identifying additional complexity to TGF-β signaling. Not surprisingly, our model of TGF-β signaling has dramatically increased in complexity from the simple receptor-Smad-gene regulation model. Knowledge of the TGF-β signaling pathways which mediate particular cellular responses will allow for the development of therapeutics which target certain TGF-β responses but not others. In this article, we will review the current models of TGF-β signaling in epithelial cells and fibroblasts with a focus on understanding how the same growth factor and receptor can induce such different responses. We hope this article will provide a framework for future investigation into these important signaling proteins.

CANONICAL RECEPTOR-Smad SIGNALING PATHWAY

The TGF-β superfamily members utilize a variety of type I and type II receptors. There are seven known mammalian type I receptors termed ALK1-7 (activin receptor-like kinase) and five type II receptors. TGF-β utilizes ALK5 and TβR-II receptors in the vast majority of cell types. There are additional receptors for TGF-β including the type III (TβR-III) and type V (TβR-V). TβR-III, also known as betaglycan, is a proteoglycan containing glycoprotein that functions as a co-receptor by presenting ligand to TβR-II [Blobe et al., 2001; Eickelberg et al., 2002]. TβR-V, also known as IGFBP-3R or LRP-1, is a receptor for TGF-β and IGFBP-3 and is co-expressed with TβR-I, TβR-II, and TβR-III in most cell types [O'Grady et al., 1991, 1992; Leal et al., 1997, 1999; Liu et al., 1997; Huang et al., 2003]. An additional layer of complexity is provided by ligand traps, proteins which regulate the accessibility of TGF-β for its cognate receptors [Shi and Massagué, 2003]. As mentioned above, the best characterized mechanism whereby TGF-β initiates signal transduction is via ALK5-mediated activation of the Smad proteins. There are eight Smad proteins in mammals that can be grouped into three classes based on function: the receptor-activated Smads (R-Smads: Smad1, 2, 3, 5, and 8), the Common mediator Smad (Co-Smad: Smad4), and the Inhibitory Smads (I-Smads: Smad6 and 7). It is generally accepted that the TGF-β/Activin/Nodal subfamily activates Smad2 and 3 whereas the BMP/GDF/MIS subfamily functions through Smad1, 5, and 8. However, there are exceptions to this classification. For instance, in endothelial cells TGF-β can induce phosphorylation of Smad1/5 and Smad2 via utilization of ALK1 and ALK5, respectively [Goumans et al., 2002]. While it is unclear whether similar mechanisms are operative in other cell types, the ultimate determinant of which R-Smads are activated is dependent upon the type I receptor utilized, and more specifically, a region within the type I receptor referred to as the L45 loop [Feng and Derynck, 1997; Chen et al., 1998; Persson et al., 1998].

Upon ligand binding, the type I receptor directly phosphorylates R-Smads on the C-terminal Ser-Ser-X-Ser (SSXS) motif. This phosphorylation is facilitated by other proteins including SARA (Smad anchor for receptor activation) and HRS/HGS (hepatocyte growth factor-regulated tyrosine kinase substrate) [Tsukazaki et al., 1998; Miura et al., 2000]. Both SARA and HRS/HGS contain the phospholipid binding FYVE domain and function as adaptors to aid in recruitment of Smad2 and 3 to the TGF-β receptor complex. In addition to SARA and HRS/HGS, cPML is another recently

identified adaptor that promotes ALK5 mediated R-Smad phosphorylation [Lin et al., 2004]. In addition to the need for adaptor proteins to generate the active receptor complex, R-Smad phosphorylation is coupled to TGF-β receptor internalization [Hayes et al., 2002; Penheiter et al., 2002; Le Roy, 2005, p. 2529]. Understanding the relation of R-Smad activation and receptor endocytic activity is critical as it directly impacts on defining potential targets for pathologies resulting from diminished/enhanced TGF-β signaling.

Once phosphorylated, the R-Smads dissociate from the receptor/SARA complex and form an oligomeric complex with Smad4 whereupon they translocate to the nucleus and interact with Smad binding elements (SBE: GTCT) or GC-rich sequences present in certain promoters [Shi et al., 1998; Zawel et al., 1998; Ishida et al., 2000]. SBEs are often adjacent to binding sites for other transcription factors and Smad proteins have been shown to cooperate with a wide variety of transcription factors to regulate gene expression [Massagué et al., 2005]. However, this canonical model has been challenged by various studies. For instance, there is evidence that SARA is not required for Smad3 activation [Goto et al., 2001]. A mutant Smad3 which is incapable of binding to SARA retains its ability to be phosphorylated by ALK5, oligomerize with Smad4, shuttle to the nucleus, and regulate transcription [Goto et al., 2001]. In addition, homo-oligomers of type I and type II receptors have been identified in both the absence or presence of ligand [Henis et al., 1994; Gilboa et al., 1998]. Finally, Levy and Hill have demonstrated that a subset of Smad-dependent TGF-β target genes do not require Smad4 [Levy and Hill, 2005]. Thus, although the majority of Smad signaling can be accounted by the aforementioned canonical model, it is clear that there are still a number of component and regulatory interactions to be defined.

In contrast to the R-Smads and Co-Smad, the I-Smads (Smad6 and 7) lack the C-terminal sites for phosphorylation by the type I receptor and negatively regulate TGF-β signaling. Smad7 inhibits the TGF-β/activin and BMP pathways whereas Smad6 appears to only inhibit the BMP pathway [Hayashi et al., 1997; Imamura et al., 1997; Nakao et al., 1997; Hata et al., 1998]. Smad6 and 7 inhibit signaling via distinct mechanisms. Smad6 competes with Smad4 for binding receptor phosphorylated Smad1, thereby creating Smad1/6 complexes unable to regulate transcription [Hata et al., 1998]. In contrast, Smad7 competes with R-Smads for interacting with the type I receptor [Hayashi et al., 1997; Nakao et al., 1997]. Smad7 is also able to mediate TGF-β receptor ubiquitination and degradation by recruiting Smurf ubiquitin ligases [Kavsak et al., 2000; Suzuki et al., 2002]. Interestingly, Smad6 and 7 can be upregulated by the TGF-β superfamily members which they regulate. Smad7 expression is induced by TGF-β, activin, and BMP signaling whereas Smad6 expression is induced by BMP [Nakao et al., 1997; Afrakhte et al., 1998; Ishisaki et al., 1998; Ishida et al., 2000; Benchabane and Wrana, 2003]. These studies demonstrate how TGF-β superfamily signaling requires the integration of both positive and negative signals for appropriate cellular responses.

THE ANTI-TUMOR RESPONSE

Growth Arrest and Apoptosis

One of the most well characterized cellular responses to TGF-β is growth arrest. TGF-β is able to induce growth arrest in a variety of cell types including epithelial, endothelial, hematopoietic, neural, and certain types of mesenchymal cells. TGF-β stimulation can induce signaling at any stage in the cell cycle but only induces arrest at G1 [Massague and Gomis, 2006]. TGF-β stimulation of epithelial cells has been shown to induce a G1 cell cycle arrest by activating various anti-proliferative responses such as the transcriptional upregulation of the cyclin-dependent kinase (CDK) inhibitors $p21^{Cip1/WAF1}$ and $p15^{Ink4b}$ [Hannon and Beach, 1994; Datto et al., 1995]. The upregulation of these cell cycle inhibitors is dependent on Smads as well as the FoxO transcription factors [Seoane et al., 2004; Gomis et al., 2006a,b]. FoxO transcription factors belong to the large family of Forkhead transcription factors which have been shown to be critical for numerous cellular processes including metabolism, cell division, and cell survival [Wijchers et al., 2006]. TGF-β induction of $p21^{CIP1/WAF1}$ in epithelial cells requires the formation of a Smad-FoxO complex whereas $p15^{INK4b}$ requires the formation of a Smad-FoxO-C/EBPβ complex [Seoane et al., 2004; Gomis et al., 2006b]. Along with inducing the upregulation of CDK inhibitors, TGF-β also induces downregulation

of cdc25A, a phosphatase that removes inhibitory phosphoryl groups from CDKs [Iavarone and Massague, 1997; Iavarone and Massague, 1999].

In addition to regulating the activity of CDK complexes in cycling epithelial cells, TGF-β causes transcriptional repression of the pro-growth transcription factor c-Myc and the inhibitors of differentiation Id1, Id2, and Id3 [Pietenpol et al., 1990; Alexandrow et al., 1995; Kang et al., 2003; Siegel and Massague, 2003]. TGF-β mediated transcriptional repression of c-Myc and Id1 is Smad-dependent, though different Smad co-factors are utilized to repress the expression of these two genes. For instance, c-Myc repression requires formation of a Smad-E2F4/5-C/EBPβ transcriptional complex on a TGF-β inhibitory element (TIE) within the c-Myc promoter [Chen et al., 2001; Gomis et al., 2006b]. Interestingly, TGF-β mediated down-regulation of c-Myc is required for TGF-β induced $p15^{INK4b}$ upregulation [Warner et al., 1999; Massagué et al., 2000]. This process involves the transcription factor Miz-1 which associates with the transcription initiator element within the $p15^{INK4b}$ gene [Seoane et al., 2001; Staller et al., 2001]. c-Myc and its binding partner Max associate with Miz-1 and prevent recruitment of the co-activator CBP/p300. Upon initiation of TGF-β signaling, Smad-mediated downregulation of c-Myc liberates Miz-1 and allows it to associate with Smad3/4 complexes which recognize a SBE within the $p15^{INK4b}$ promoter. TGF-β also induces Sp1 recruitment to the $p15^{INK4b}$ promoter via Smad4 which collaborates with Miz-1 to induce transcription of $p15^{INK4b}$ [Seoane et al., 2001; Staller et al., 2001]. On the other hand, transcriptional repression of Id1 requires the transcriptional repressor ATF3 which complexes with Smad proteins. ATF3 itself is upregulated in a Smad-dependent manner and thus a similar two-step mechanism is required for Id1 repression [Kang et al., 2003].

In addition to Smad-dependent induction of growth arrest in epithelial cells, TGF-β also activates non-Smad pathways that inhibit proliferation. ALK5 has been shown to associate with the Bα subunit of phosphatase 2A (PP2A) [Griswold-Prenner et al., 1998]. In EpH4 polarized mammary epithelial cells, TGF-β stimulation leads to PP2A mediated dephosphorylation and inactivation of the serine/threonine kinase p70 S6K, a known regulator of ribosome biogenesis and cell growth [Petritsch et al., 2000]. As such, both the Smad and PP2A pathways need to be overcome to prevent growth inhibition in response to TGF-β [Petritsch et al., 2000]. It would not be unexpected to see this paradigm extended to numerous aspects of TGF-β action.

Along with ALK5 and TβR-II mediated signals leading to growth arrest, there is evidence that the TGF-β type V receptor (TβR-V) also plays a role in inducing growth inhibition of epithelial cells [Liu et al., 1997; Huang et al., 2003; Tseng et al., 2004]. TβR-V exhibits Ser/Thr kinase activity and has been shown to complex with ALK5 upon TGF-β treatment [Liu et al., 1994; Liu et al., 1997]. CHO cells lacking TβR-V do not exhibit growth inhibition in response to TGF-β while reintroduction of TβR-V can rescue the response [Tseng et al., 2004]. TβR-V also mediates growth inhibition in response to IGFBP-3 which involves dephosphorylation of the insulin receptor substrate (IRS) proteins, well characterized adaptor proteins that mediate insulin and insulin-like growth factor receptor signaling [Huang et al., 2004]. Murine 32D myeloid cells, which lack endogeneous IRS proteins, do not exhibit growth inhibition in response to IGFBP-3 but stable transfection of IRS-1 or IRS-2 confers sensitivity to growth inhibition [Huang et al., 2004]. While these studies suggest that TβR-V plays a role in TGF-β-dependent growth arrest, the precise mechanism remains unclear.

In addition to inducing epithelial cell growth arrest, TGF-β has been shown to induce a number of pro-apoptotic responses [Siegel and Massague, 2003]. The role of Smad proteins in TGF-β mediated apoptosis is well documented. For instance, in gastric carcinoma cells TGF-β induces caspase-8 activation via Smad3 up-regulation of the Fas receptor [Kim et al., 2004], while in hepatoma cells, Smads have been shown to transcriptionally induce death associated protein kinase (DAPK) [Jang et al., 2002]. In addition, several studies have suggested that PI3K-Akt signaling may regulate TGF-β mediated apoptosis by modulating Smad activation. One proposed mechanism has Akt binding and sequestering Smad3 from the transcriptional machinery [Conery et al., 2004; Remy et al., 2004]. In contrast, a more recent study suggests that Akt inhibits Smad3 function and subsequent apoptosis not via direct association, but by activating mammalian

Target of Rapamycin (mTOR) [Song et al., 2006]. Moreover, there is evidence that an upstream activator of Akt, phosphoinositide-dependent kinase 1 (PDK1), inhibits Smad function and TGF-β mediated apoptosis [Seong et al., 2007]. It is clear that a number of players in the PI3K-Akt pathway regulate TGF-β mediated apoptosis in various cell types. Additional studies are required to determine which of these mechanisms play a predominant role in vivo.

Another mechanism whereby TGF-β mediates apoptosis is by regulating the expression of Bcl-2 family members [Motyl et al., 1998; Francis et al., 2000; Chipuk et al., 2001; Ohgushi et al., 2005; Yano et al., 2006; Ramjaun et al., 2007]. The Bcl-2 family consists of at least 17 proteins which can be grouped as either pro-apoptotic or anti-apoptotic [Adams and Cory, 2007]. The relative expression of pro-apoptotic and anti-apoptotic members determines the eventual cellular outcome. For instance, in prostate cancer cells, TGF-β downregulates Bcl-xL, an anti-apoptotic Bcl-2 family member, and cells stably overexpressing Bcl-xL exhibit resistance to TGF-β induced apoptosis but not growth arrest [Chipuk et al., 2001]. In addition, TGF-β induces upregulation of the pro-apoptotic BH3-only proteins Bim and Bmf via a Smad4- and p38-dependent mechanism also requiring the transcription factor RUNX3 [Ohgushi et al., 2005; Yano et al., 2006; Ramjaun et al., 2007]. RUNX3 recognizes binding sites within the Bim promoter and Runx3−/− as well as Bim−/− gastric epithelial cells exhibit reduced TGF-β induced apoptosis compared to wild-type cells despite similar expression of TGF-β receptors [Yano et al., 2006]. Clearly, Smad proteins can mediate apoptosis via a variety of mechanisms including transcriptionally upregulating pro-apoptotic proteins and downregulating pro- survival proteins. However, there also is evidence that the Smad proteins are not required for TGF-β mediated apoptosis.

Several non-Smad pathways mediating TGF-β apoptosis have been identified. The most well characterized of these pathways include the MAP kinase members JNK and p38. Interestingly, TβR-II has an additional role in TGF-β mediated JNK signaling aside from its well established function in ALK5 activation. TβR-II interacts with the pro-apoptotic adaptor protein Daxx which upon TGF-β treatment leads to Daxx phosphorylation by HIPK2 and activation of the MAP kinase kinases MKK4 and 7, resulting in JNK-dependent apoptosis [Perlman et al., 2001; Hofmann et al., 2003]. In addition, TGF-β can activate p38 and JNK via the MAPKKK family member TGF-β-activated kinase 1 (TAK1) [Yamaguchi et al., 1995]. Although the mechanism remains poorly defined, there is evidence that the TGF-β receptor complex activates TAK1 via HPK1 and TAB1 [Shibuya et al., 1996; Wang et al., 1997].

Numerous studies have shown that TGF-β induces a large number of tumor suppressive signaling events in epithelial cells via both Smad and non-Smad pathways (Figs. 1 and 2). The importance of understanding TGF-β signaling in epithelia is underscored by the considerable evidence that these pathways can be subverted during oncogenesis. For instance, brain tumor progression to advanced stages is associated with loss of TGF-β cytostasis which is mediated in part by the expression of FoxG1, an Smad-FoxO antagonist, and enhanced PI3K-Akt signaling [Seoane et al., 2004]. Also, metastatic breast cancer cells which exhibit evasion of the TGF-β cytostatic program express high levels of the C/EBPβ inhibitory isoform LIP and are unable to downregulate c-Myc expression [Chen et al., 2001; Gomis et al., 2006b]. Future investigations will undoubtedly reveal additional layers of regulatory complexity as well as entirely new pathways whereby TGF-β modulates epithelial cell biology and shed more light on the mechanisms cancer cells utilize to evade TGF-β growth inhibition.

THE ONCOGENIC AND FIBROGENIC RESPONSE

Epithelial-Mesenchymal Transition (EMT)

During the process of carcinogenesis, cells often lose the anti-proliferative or apoptotic response to TGF-β. Moreover, during the advanced stages of carcinogenesis, TGF-β (as well as other superfamily members) has been shown to induce a type of transdifferentiation termed EMT. The hallmarks of EMT include the disruption of cell−cell and cell−matrix interactions, degradation of the surrounding ECM, and actin reorganization whereby the cell becomes more migratory. While EMT is a normal physiological process necessary for proper development, the pathologic induction

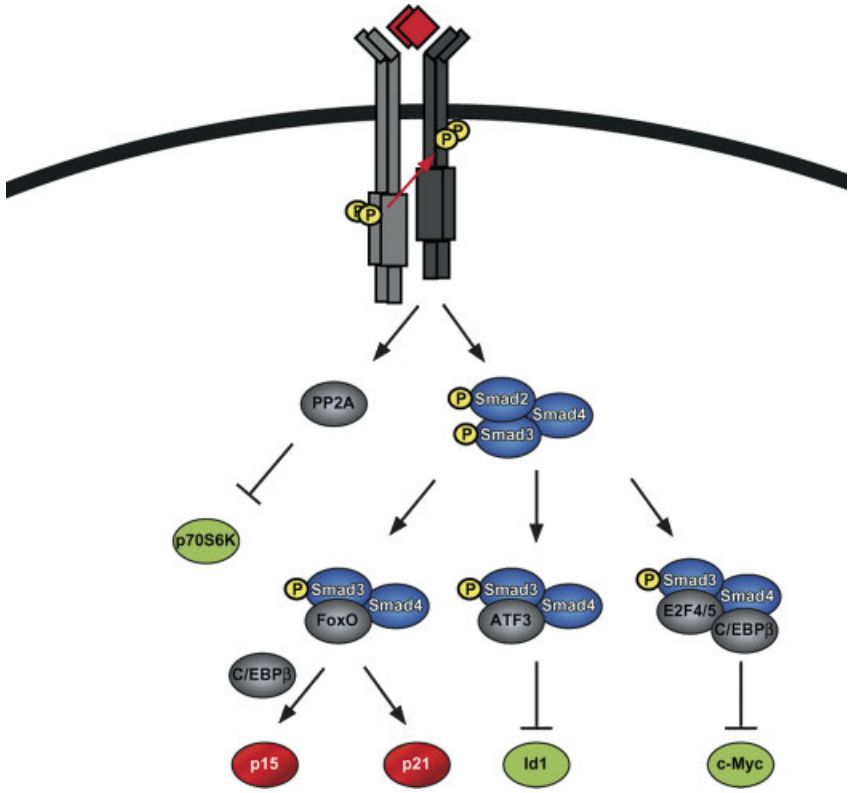

Fig. 1. TGF-β signaling mediating growth arrest of epithelial cells. TGF-β (red diamonds) mediates oligomerization of TβR-II (gray) and ALK5 (black) resulting in TβR-II phosphorylating and activating ALK5. Activated ALK5 phosphorylates Smad2 and 3 which oligomerize with Smad4. The Smad complexes collaborate with other transcription factors including FoxO, ATF3, E2F4/5, and C/EBPβ to transcriptionally regulate genes critical in cell cycle progression. In addition, the TβR-II/ALK5 complex can cause growth arrest via the Smad-independent pathway involving dephosphorylation of p70 S6K via the recruitment of PP2A. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of EMT is associated with carcinoma metastasis and fibrotic diseases [Condeelis and Segall, 2003; Gotzmann et al., 2004; Lee et al., 2006; Radisky et al., 2007]. Once cancer cells acquire a capacity to evade the tumor suppression of TGF-β and induce EMT, there is a selective advantage for the cancer cells to produce and/or activate TGF-β and further promote the carcinogenic process by activating fibroblasts in the surrounding stroma and inhibiting anti-tumor immune responses. As such, understanding the signaling pathways regulating EMT is critical.

A number of studies have shown that Smad proteins are required for TGF-β mediated EMT. A mutant TGF-β type I receptor (ALK5) that is unable to bind Smads, but retains MAPK signaling fails to induce EMT [Yu et al., 2002; Itoh et al., 2003]. Similarly, Smads have been shown to be required for TGF-β mediated EMT both in vitro and in vivo [Oft et al., 2002; Li et al., 2003; Saika et al., 2004; Tian et al., 2004; Valcourt et al., 2005]. For instance, Smad3 has been shown to upregulate the zinc-finger transcription factor Snail, which represses the transcription of E-cadherin [Cho et al., 2007]. Interestingly, in prostate cancer cells TGF-β induces actin cytoskeleton rearrangement via Smad7 mediated activation of Cdc42 [Edlund et al., 2004]. While the mechanism whereby Smad7 regulates Cdc42 is unclear, this is further evidence that the I-Smads may have broader functions than TGF-β signaling attenuation.

However, as is common in the TGF-β field, there are studies which have generated alternative conclusions. For instance, reduction of Smad4 expression via RNA interference in human keritinocytes and pancreatic cancer cells reveals that Smad4 is required for TGF-β mediated growth arrest and migration, but not for EMT [Levy and Hill, 2005]. This result is consistent with the reports showing that TGF-β mediated upregulation of the EMT promoting

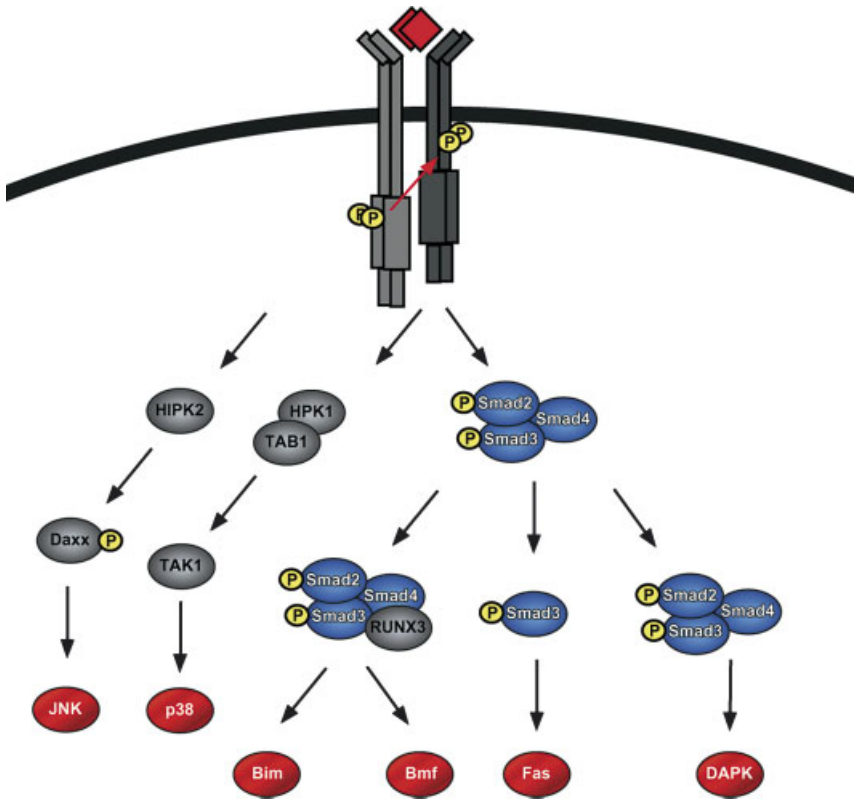

Fig. 2. TGF-β signaling mediating apoptosis of epithelial cells. Activated R-Smads collaborate with other transcription factors to transcriptionally regulate genes critical to cell survival including Bim, Bmf, Fas, and DAPK. Furthermore, the TβR-II/ALK5 complex activates a number of non-Smad pathways such as JNK and p38 which can cause apoptosis in the absence of Smad signaling. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

transcription factor Snail is independent of Smad4 [Peinado et al., 2003; Medici et al., 2006]. However, several other studies have demonstrated a requirement for Smad4 in TGF-β mediated EMT [Valcourt et al., 2005; Medici et al., 2006]. While this may simply reflect the myriad of potential mechanisms by which different cell types induce EMT, further investigation of these processes is critical as they provide potential targets for therapeutic intervention.

In addition to the Smad requirement for EMT, a number of non-Smad pathways have also been shown to be necessary. In epithelial cells, ALK5 has been shown to interact with the tight junction protein Par6 [Ozdamar et al., 2005]. Par6 phosphorylation by TβR-II regulates tight junction integrity via recruiting the ubiquitin ligase Smurf1, subsequently resulting in ubiquitination and degradation of RhoA GTPase and tight junction dissolution [Ozdamar et al., 2005]. This study demonstrates a number of important findings. First, previous work on EMT has primarily focused on the mechanisms of adherens junction dissolution, but much less is known regarding the disassembly of tight junctions; second, the identification of Par6 as a substrate for TβR-II provides an additional target downstream of the TGF-β receptor complex; and third, a potential mechanism integrating the proposed scaffolding function of Par6 with an important biological response to TGF-β is defined. Whether TβR-II has additional substrates that play a role in EMT remains unclear, as does the potential mechanisms regulating TβR-II substrate accessibility.

Additional non-Smad pathways have been shown to be critical for EMT including activation of ERK and PI3K signaling [Bakin et al., 2000; Peinado et al., 2003; Medici et al., 2006; Cho et al., 2007; Wang et al., 2007]. TGF-β induced ERK and PI3K activation has been shown to upregulate Snail expression [Carver et al., 2001; Peinado et al., 2003]. Furthermore, PI3K-Akt signaling promotes EMT by Akt phosphorylation

and inactivation of GSK-3β which allows for formation of LEF-1/β-Catenin complexes and upregulation of EMT promoting genes [Medici et al., 2006]. Similarly, Wang et al. have shown that the metastasis-associated phosphatase PRL-3 can promote EMT by downregulating the PI3K negative regulator PTEN [Wang et al., 2007]. These studies demonstrate that ERK and PI3K signaling plays an important role in determining the epithelial response to TGF-β. However, it is currently unclear how the TGF-β receptor complex couples to Ras and PI3K. The TβR-II receptor has been reported to interact constitutively with the p85 subunit of PI3K and TGF-β stimulation induces an association with ALK5 [Yi et al., 2005]. However, this interaction was determined to be indirect and the adaptor(s) utilized to couple the receptor complex to p85 is unknown [Yi et al., 2005]. While much progress has been made in understanding the Smad and non-Smad pathways required for EMT (Fig. 3), defining additional regulatory mechanisms whereby an epithelial cell growth inhibitor induces the transdifferentiation to a more aggressive mesenchymal phenotype is fundamental.

Fibroblast Activation

TGF-β was originally identified for its ability to induce anchroage-independent growth of non-neoplastic rat kidney fibroblasts and murine AKR-2B fibroblasts [DeLarco and Todaro, 1978; Moses et al., 1981; Anzano et al., 1983]. The subsequent discovery that TGF-β induces growth arrest of most cell types including epithelial, endothelial, hematopoietic, and neural cells suggested that the initial pro-growth response in fibroblasts may not have been a bona fide physiological response. In addition, TGF-β induces growth arrest of primary mouse embryonic fibroblasts [Datto et al., 1999]. However, a number of studies have demonstrated that fibroblasts isolated from adult organisms do not growth arrest, but instead proliferate in response to TGF-β [Kay

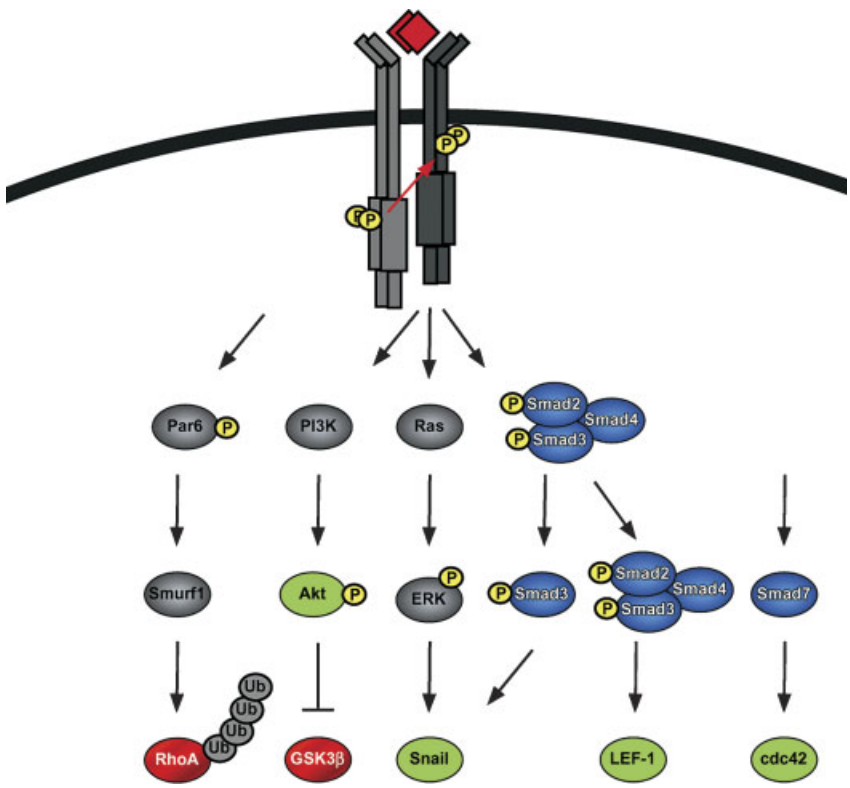

Fig. 3. TGF-β signaling mediating epithelial-to-mesenchymal transition (EMT). Activated R-Smads transcriptionally regulate genes which play an important role in EMT such as Snail and LEF-1. In addition, Smad7 can cause activation of Cdc42 independent of its ability to attenuate receptor activation of R-Smads. Finally, TGF-β activates non-Smad pathways such as Ras and PI3K as well as Par6 which play important roles in promoting EMT. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

et al., 1998; Dkhissi et al., 1999; Strutz et al., 2001; Khalil et al., 2005; Pelaia et al., 2007]. Along with mediating fibroblast proliferation, TGF-β also induces fibroblasts to differentiate into myofibroblasts, which have been shown to be present in areas of fibrosis and appear to be key mediators of ECM production in vivo [Kuhn and McDonald, 1991; Phan, 2002]. This morphological transformation to myofibroblasts is associated with upregulation of the smooth muscle isoform of α-actin (α-SMA) and the reorganization of the actin cytoskeleton, resulting in a spindle shape morphology [Sime et al., 1997]. Numerous studies have demonstrated that TGF-β is one of the most, if not primary, pro-fibrotic cytokine in vivo [George et al., 1999; Qi et al., 1999; Nakamura et al., 2000; Ueno et al., 2000; Bissell, 2001; Flanders, 2004]. Adenoviral or IL-13 mediated overexpression of TGF-β induces severe lung fibrosis in animal models [Sime et al., 1997; Lee et al., 2001]. Furthermore, administration of TGF-β neutralizing antibodies has been shown to prevent skin and lung fibrosis in a murine scleroderma model and soluble TGF-β type II receptor attenuates bleomycin-induced lung fibrosis as well as injury-induced liver fibrosis [George et al., 1999; McCormick et al., 1999; Wang et al., 1999]. These studies underscore the critical role of TGF-β in organ fibrosis, and therefore make the signaling pathways activated by this cytokine potential therapeutic targets.

The role of Smad signaling in TGF-β driven fibrosis has been demonstrated using Smad3 null mice which are resistant to TGF-β mediated pulmonary fibrosis [Bonniaud et al., 2004]. Interestingly, these mice also develop increased lung spaces and emphysema [Bonniaud et al., 2004]. This study, demonstrating the importance of Smad proteins in the fibroblast response to TGF-β, has been complemented by a number of in vitro studies. Overexpression of Smad proteins induces upregulation of α-SMA, actin cytoskeleton reorganization, and morphological transformation of fibroblasts independent of TGF-β [Evans et al., 2003; Hu et al., 2003]. These results are consistent with studies demonstrating that a constitutively activate ALK5 causes actin stress fiber formation in fibroblasts while a constitutively active receptor with a mutant L45 loop unable to associate with Smads has no effect [Vardouli et al., 2005]. Similarly, several studies have demonstrated a role of R-Smads in TGF-β mediated regulation of ECM components. For instance, TGF-β stimulated α2(I)-collagen expression occurs via cooperation between Smad3/4, Sp1, CBP/p300, and Egr-1 [Ghosh et al., 2000; Zhang et al., 2000; Chen et al., 2006]. In addition, upregulation of the ECM glycoprotein Tenascin-C has been shown to require Smad3 as well as the transcription factors Sp1, Ets1, and CBP/p300 [Jinnin et al., 2004]. Finally, TGF-β represses expression of matrix metalloproteinase-1 (MMP-1) via Smad3/4 with dominant negative Smad3 or 4 mutants abrogating this response [Yuan and Varga, 2001]. While the R-Smads are clearly critical for increased matrix deposition in response to TGF-β, whether they are differentially regulated in epithelial cells and fibroblasts is unclear.

Given that activation of R-Smads occur in all cell types stimulated by TGF-β, inhibition of these proteins in disease states is likely to have a variety of undesirable side effects. One approach to this problem would be to identify non-Smad pathways which are activated in a cell type specific manner. In that regard, recent studies have identified a number of distinct non-Smad pathways required for the fibroblast response to TGF-β. TGF-β activates p21 activated kinase 2 (PAK2) and Akt in fibroblasts but not epithelial cells independently of Smad proteins [Wilkes et al., 2003; Wilkes et al., 2005]. Although TGF-β mediates PAK2 and Akt activation via PI3K, PAK2 and Akt are independently regulated by PI3K [Wilkes et al., 2005]. While the targets downstream of Akt have not as yet been identified, pharmacological inhibition of PI3K or expression of a dominant negative PAK2 prevents TGF-β mediated fibroblast proliferation and morphological transformation [Wilkes et al., 2005]. Moreover, one of the critical effectors downstream of PAK2 is the non-receptor tyrosine kinase c-Abl [Plattner et al., 2003; Wilkes and Leof, 2006]. As TGF-β was shown to stimulate c-Abl kinase activity independent of Platelet derived growth factor (PDGF) and the c-Abl inhibitor Imatinib/ Gleevec attenuated bleomycin-induced lung fibrosis and ureter obstruction induced kidney fibrosis [Daniels et al., 2004; Wang et al., 2005], these preclinical findings have been extended to a Phase II clinical trial testing the efficacy of imatinib versus placebo for the treatment of idiopathic pulmonary fibrosis (http://clinicaltrials.gov/ct/show/NCT00131274?order=1).

In addition to directly modulating the fibroblast response, TGF-β also induces the expression of other pro-fibrotic cytokines via both Smad and non-Smad pathways. TGF-β upregulates connective tissue growth factor (CTGF) which is known to induce fibroblast proliferation and ECM production [Moussad and Brigstock, 2000]. Induction of CTGF requires Smad3/4 which recognize a SBE within the CTGF promoter [Holmes et al., 2001]. TGF-β has also been shown to increase the expression of the peptide endothelin-1 (ET-1) through a Smad-independent, JNK-AP1-dependent pathway [Shi-Wen et al., 2006]. Interestingly, ET-1 itself activates JNK via TAK1, suggesting that TGF-β stimulation of fibroblasts may lead to an ET-1 autocrine loop that further promotes ECM production [Shi-Wen et al., 2006]. In addition to these cytokines, TGF-β also transcriptionally induces PDGF and Fibroblast growth factor-2 (FGF-2) [Leof et al., 1986; Strutz et al., 2001; Khalil et al., 2005]. While the precise mechanism(s) whereby TGF-β increases the expression of a number of pro-fibrotic mediators is unclear, these findings are consistent with its role as a critical regulator of fibrosis in vivo and underscore the importance of understanding cytokine crosstalk. Figure 4 is a schematic summary of TGF-β signaling in fibroblasts.

CONCLUDING REMARKS

Over the last several years, a great deal of progress has been made in our understanding of the signal transduction pathways that mediate the various cellular responses to TGF-β. It is now clear that the integration of Smad and non-Smad signaling pathways determines

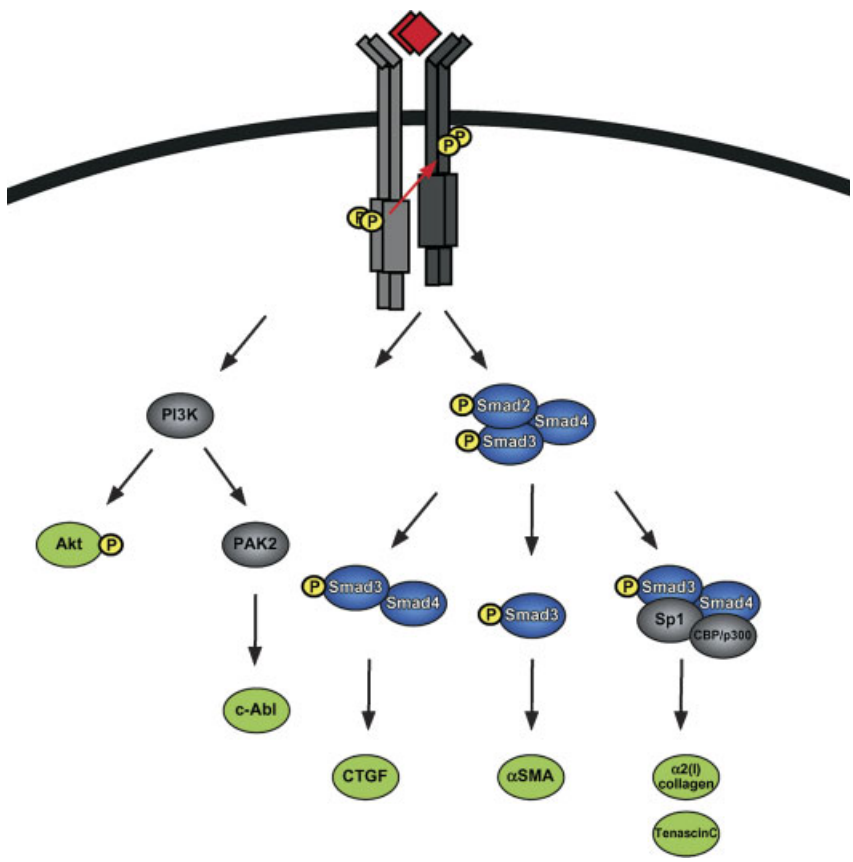

Fig. 4. TGF-β signaling mediating fibroblast activation. Activated R-Smads transcriptionally upregulate αSMA which is associated with myofibroblast differentiation. Activated Smad3 and 4 collaborate with transcription factors Sp1 and CBP/p300 to induce production of extracellular matrix components α2(I)collagen and TenascinC. Smad3 and 4 also upregulate the cytokine CTGF which promotes fibroblast activation. The TβR-II/ALK5 complex activates a number of non-Smad pathways such as PI3K-Akt and PI3K-PAK2-c-Abl which promote fibroblast proliferation and myofibroblast differentiation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the cellular responses to TGF-β. Moreover, the realization that Smad2 and Smad3 can execute different functions and may be distinctly regulated has further increased the complexity of TGF-β signaling [Brown et al., 2007]. For instance, Smad3, but not Smad2, associates with FoxO transcription factors and ATF3 to regulate $p21^{Cip1/WAF1}$ and Id1 expression, respectively [Kang et al., 2003; Seoane et al., 2004]. In addition, E1A-like inhibitor of differentiation (EID-2), which attenuates TGF-β mediated upregulation of the CDK inhibitors $p21^{Cip1/WAF1}$ and $p15^{Ink4b}$, preferentially binds Smad3 and inhibits Smad3/4 complex formation but not Smad2/4 complex formation [Lee et al., 2004]. For a review on the differential regulation and function of Smad2 and Smad3 see [Brown et al., 2007]. It will be interesting to determine if these Smad regulatory mechanisms are cell type specific or influence the particular epithelial response to TGF-β.

The mechanisms regulating non-Smad pathways also require additional elucidation. Of primary interest is the relation between TGF-β and PI3K-Akt. Though PI3K-Akt signaling has been implicated in promoting EMT, recent evidence indicates that PI3K-Akt activation by TGF-β primarily occurs in fibroblasts, not epithelia [Wilkes et al., 2005]. As many of TGF-β′s cytostatic responses require FoxO transcription factors which are known to be inhibited by Akt via direct phosphorylation [Greer and Brunet, 2005], PI3K activation may be a critical factor in determining the cellular response to TGF-β. In that regard, while phosphotyrosine mediated engagement of the SH2 domains within the p85 regulatory subunit of PI3K is a common means for receptor tyrosine kinase activation of PI3K, how TGF-β receptor Ser/Thr kinase activity leads to PI3K activity remains unclear. Since PI3K appears to have a central role in TGF-β mediated proliferation, apoptosis, growth arrest, and/or EMT, defining the mechanism(s) and cell context of TGF-β receptor-p85 coupling is essential.

It is an exciting time for the TGF-β signaling field. Our current models lead to a number of interesting questions, the answers to which will reveal in greater detail the mechanisms underlying critical cellular responses to TGF-β, and hopefully lead to the development of more effective therapeutics for a number of human diseases.

REFERENCES

Adams JM, Cory S. 2007. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 26:1324–1337.

Afrakhte M, Moren A, Jossan S, Itoh S, Sampath K, Westermark B, Heldin CH, Heldin NE, ten Dijke P. 1998. Induction of inhibitory Smad6 and Smad7 mRNA by TGF-beta family members. Biochem Biophys Res Commun 249:505–511.

Alexandrow MG, Kawabata M, Aakre M, Moses HL. 1995. Overexpression of the c-Myc oncoprotein blocks the growth-inhibitory response but is required for the mitogenic effects of transforming growth factor b1. Proc Natl Acad Sci USA 92:3239–3243.

Anzano MA, Roberts AB, Smith JM, Sporn MB, De Larco JE. 1983. Sarcoma growth factor from conditioned medium of virally transformed cells is composed of both type alpha and type beta transforming growth factors. Proc Natal Acad Sci USA 80:6264–6268.

Bakin AV, Tomlinson AK, Bhowmick NA, Moses HL, Arteaga CL. 2000. Phosphatidylinositol 3-kinase function is required for transforming growth factor b-medicated epithelial to mesenchymal transition and cell migration. J Biol Chem 275:36803–36810.

Benchabane H, Wrana JL. 2003. GATA- and Smad1-dependent enhancers in the Smad7 gene differentially interpret bone morphogenetic protein concentrations. Mol Cell Biol 23:6646–6661.

Bissell DM. 2001. Chronic liver injury, TGF-b, and cancer. Exp Mol Med 33:179–190.

Blobe GC, Schiemann WP, Pepin MC, Beauchemin M, Moustakas A, Lodish HF, O'Connor-McCourt MD. 2001. Functional roles for the cytoplasmic domain of the type III transforming growth factor beta receptor in regulating transforming growth factor beta signaling. J Biol Chem 276:24627–24637.

Bonniaud P, Kolb M, Galt T, Robertson J, Robbins C, Stampfli M, Lavery C, Margetts PJ, Roberts AB, Gauldie J. 2004. Smad3 null mice develop airspace enlargement and are resistant to TGF-beta-mediated pulmonary fibrosis. J Immunol 173:2099–2108.

Brown KA, Pietenpol JA, Moses HL. 2007. A tale of two proteins: Differential roles and regulation of Smad2 and Smad3 in TGF-beta signaling. J Cell Biochem 101:9–33.

Carver EA, Jiang R, Lan Y, Oram KF, Gridley T. 2001. The mouse snail gene encodes a key regulator of the epithelial-mesenchymal transition. Mol Cell Biol 21: 8184–8188.

Chen Y-G, Hata A, Lo RS, Wotton D, Shi Y, Pavletich N, Massagué J. 1998. Determinants of specificity in TGF-b signal transduction. Genes Dev 12:2144–2152.

Chen CR, Kang Y, Massague J. 2001. Defective repression of c-myc in breast cancer cells: A loss at the core of the transforming growth factor beta growth arrest program. Proc Natl Acad Sci USA 98:992–999.

Chen SJ, Ning H, Ishida W, Sodin-Semrl S, Takagawa S, Mori Y, Varga J. 2006. The early-immediate gene EGR-1 is induced by transforming growth factor-beta and mediates stimulation of collagen gene expression. J Biol Chem 281:21183–21197.

Chipuk JE, Bhat M, Hsing AY, Ma J, Danielpour D. 2001. Bcl-xL blocks transforming growth factor-beta 1-induced

apoptosis by inhibiting cytochrome c release and not by directly antagonizing Apaf-1-dependent caspase activation in prostate epithelial cells. J Biol Chem 276:26614–26621.

Cho HJ, Baek KE, Saika S, Jeong MJ, Yoo J. 2007. Snail is required for transforming growth factor-beta-induced epithelial-mesenchymal transition by activating PI3 kinase/Akt signal pathway. Biochem Biophys Res Commun 353:337–343.

Condeelis J, Segall JE. 2003. Intravital imaging of cell movement in tumours. Nat Rev Cancer 3:921–930.

Conery AR, Cao Y, Thompson EA, Townsend CMJ, Ko TC, Luo K. 2004. Akt interacts directly with Smad3 to regulate the sensitivity to TGF-beta induced apoptosis. Nat Cell Biol 6:366–372.

Daniels CE, Wilkes MC, Edens M, Kottom TJ, Murphy SJ, Limper AH, Leof EB. 2004. Imatinib mesylate inhibits the profibrogenic activity of TGF-b and prevents bleomycin-mediated lung fibrosis. J Clin Invest 114:1303–1316.

Datto MB, Li Y, Panus JF, Howe DJ, Xiong Y, Wang XF. 1995. Transforming growth factor beta induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. Proc Natl Acad Sci USA 92:5545–5549.

Datto MB, Frederick JP, Pan L, Borton AJ, Zhuang Y, Wang XF. 1999. Targeted disruption of Smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction. Mol Cell Biol 19:2495–2504.

DeLarco JE, Todaro GJ. 1978. Growth factors from murine sarcoma virus-transformed cells. Proc Natl Acad Sci USA 75:4001–4005.

Dkhissi F, Raynal S, Jullien P, Lawrence DA. 1999. Growth stimulation of murine fibroblasts by TGF-beta1 depends on the expression of a functional p53 protein. Oncogene 18:703–711.

Edlund S, Landstrom M, Heldin CH, Aspenstrom P. 2004. Smad7 is required for TGF-beta-induced activation of the small GTPase Cdc42. J Cell Sci 117:1835–1847.

Eickelberg O, Centrella M, Reiss M, Kashgarian M, Wells RG. 2002. Betaglycan inhibits TGF-b signaling by preventing type I-type II receptor complex formation. J Biol Chem 277:823–829.

Evans RA, Tian YC, Steadman R, Phillips AO. 2003. TGF-beta1-mediated fibroblast-myofibroblast terminal differentiation—the role of smad proteins. Exp Cell Res 282: 90–100.

Feng XH, Derynck R. 1997. A kinase subdomain of transforming growth factor-beta (TGF-beta) type I receptor determines the TGF-beta intracellular signaling specificity. EMBO J 16(13):3912–3923.

Flanders KC. 2004. Smad3 as a mediator of the fibrotic response. Int J Exp Path 85:47–64.

Francis JM, Heyworth CM, Spooncer E, Pierce A, Dexter TM, Whetton AD. 2000. Transforming growth factor-beta 1 induces apoptosis independently of p53 and selectively reduces expression of Bcl-2 in multipotent hematopoietic cells. J Biol Chem 275:39137–39145.

George J, Roulot D, Koteliansky VE, Bissell DM. 1999. In vivo inhibition of rat stellate cell activation by soluble transforming growth factor beta type II receptor: A potential new therapy for hepatic fibrosis. Proc Natl Acad Sci USA 96:12719–12724.

Ghosh AK, Yuan W, Mori Y, Varga J. 2000. 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.

Gilboa L, Wells RG, Lodish HF, Henis YI. 1998. Oligomeric structure of type I and type II transforming growth factor beta receptors—homodimers form in the ER and persist at the plasma membrane. J Cell Biol 140:767–777.

Gomis RR, Alarcon C, He W, Wang Q, Seoane J, Lash A, Massague J. 2006. A FoxO-Smad synexpression group in human keratinocytes. Proc Natl Acad Sci USA 103: 12747–12752.

Gomis RR, Alarcon C, Nadal C, Van Poznak C, Massague J. 2006. C/EBPbeta at the core of the TGFbeta cytostatic response and its evasion in metastatic breast cancer cells. Cancer Cell 10:203–214.

Goto D, Nakajima H, Mori Y, Kurasawa K, Kitamura N, Iwamoto I. 2001. Interaction between Smad anchor for receptor activation and Smad3 is not essential for TGF-beta/Smad3-mediated signaling. Biochem Biophys Res Commun 281:1100–1105.

Gotzmann J, Mikula M, Eger A, Schulte-Hermann R, Foisner R, Beug H, Mikulits W. 2004. Molecular aspects of epithelial cell plasticity: Implications for local tumor invasion and metastasis. Mutat Res 566:9–20.

Goumans M-J, Valdimarsdottir G, Itoh S, Rosendahl A, Sideras P, ten Dijke P. 2002. Balancing the activation state of the endothelium via two distinct TGF-b type I receptors. EMBO J 21:1743–1753.

Greer EL, Brunet A. 2005. FOXO transcription factors at the interface between longevity and tumor suppression. Oncogene 24:7410–7425.

Griswold-Prenner I, Kamibayashi C, Maruoka EM, Mumby MC, Derynck R. 1998. Physical and functional interactions between type I transforming growth factor beta receptors and Balpha, a WD-40 repeat subunit of phosphatase 2A. Mol Cell Biol 18:6596–6604.

Hannon GJ, Beach D. 1994. p15Ink4B is a potential effector of TGF-ß-induced cell cycle arrest. Nature 371:257–261.

Hata A, Lagna G, Massague J, Hemmati-Brivanlou A. 1998. Smad6 inhibits BMP/Smad1 signaling by specifically competing with the Smad4 tumor suppressor. Genes Dev 12:186–197.

Hayashi H, Abdollah S, Qiu Y, Cai J, Xu Y-Y, Grinnell BW, Richardson MA, Topper JN, Gimbrone MA, Wrana JL, Falb D. 1997. The MAD-related protein Smad7 associates with the TGFb receptor and functions as an antagonist of TGFb signaling. Cell 89:1165–1173.

Hayes S, Chawla A, Corvera S. 2002. TGFß receptor internalization into EEA1-enriched early endosomes: Role in signaling to Smad2. J Cell Biol 158:1239–1249.

Henis YI, Moustakas A, Lin HY, Lodish HF. 1994. The types II and III transforming growth factor-beta receptors form homo-oligomers. J Cell Biol 126:139–154.

Hofmann TG, Stollberg N, Schmitz ML, Will H. 2003. HIPK2 regulates transforming growth factor-beta-induced c-Jun NH(2)-terminal kinase activation and apoptosis in human hepatoma cells. Cancer Res 63: 8271–8277.

Holmes A, Abraham DJ, Sa S, Shiwen X, Black CM, Leask A. 2001. CTGF and SMADs, maintenance of scleroderma phenotype is independent of SMAD signaling. J Biol Chem 276:10594–10601.

Hu B, Wu Z, Phan SH. 2003. Smad3 mediates TGF-beta-induced alpha-smooth muscle actin expression. Am J Respir Crit Care Med 29:397–404.

Huang SS, Ling TY, Tseng WF, Huang YH, Tang FM, Leal SM, Huang JS. 2003. Cellular growth inhibition by IGFBP-3 and TGF-beta1 requires LRP-1. FASEB J 17:2068–2081.

Huang SS, Leal SM, Chen CL, Liu IH, Huang JS. 2004. Identification of insulin receptor substrate proteins as key molecules for the TbetaR-V/LRP-1-mediated growth inhibitory signaling cascade in epithelial and myeloid cells. FASEB J 18:1719–1721.

Iavarone A, Massague J. 1997. Repression of the CDK activator Cdc25A and cell-cycle arrest by cytokine TGF-beta in cells lacking the CDK inhibitor p15. Nature 387: 417–422.

Iavarone A, Massague J. 1999. E2F and histone deacetylase mediate transforming growth factor beta repression of cdc25A during keratinocyte cell cycle arrest. Mol Cell Biol 19:916–922.

Imamura T, Takase M, Nishihara A, Oeda E, Hanai J, Kawabata M, Miyazono K. 1997. Smad6 inhibits signalling by the TGF-b superfamily. Nature 389:622–626.

Ishida W, Hamamoto T, Kusanagi K, Yagi K, Kawabata M, Takehara K, Sampath TK, Kato M, Miyazono K. 2000. Smad6 is a Smad1/5-induced smad inhibitor. Characterization of bone morphogenetic protein-responsive element in the mouse Smad6 promoter. J Biol Chem 275: 6075–6079.

Ishisaki A, Yamato K, Nakao A, Nonaka K, Ohguchi M, ten Dijke P, Nishihara T. 1998. Smad7 is an activin-inducible inhibitor of activin-induced growth arrest and apoptosis in mouse B cells. J Biol Chem 273:24293–24296.

Itoh S, Thorikay M, Kowanetz M, Moustakis A, Itoh F, Heldin CH, ten Dijke P. 2003. Elucidation of Smad requirement in transforming growth factor-beta type I receptor-induced responses. J Biol Chem 278:3751–3761.

Jang CW, Chen CH, Chen CC, Chen JY, Su YH, Chen RH. 2002. TGF-beta induces apoptosis through Smad-mediated expression of DAP-kinase. Nat Cell Biol 4:51–58.

Jinnin M, Ihn H, Asano Y, Yamane K, Trojanowska M, Tamaki K. 2004. Tenascin-C upregulation by transforming growth factor-beta in human dermal fibroblasts involves Smad3, Sp1, and Ets1. Oncogene 23:1656–1667.

Kang Y, Chen CR, Massague J. 2003. A self-enabling TGFbeta response coupled to stress signaling: Smad engages stress response factor ATF3 for Id1 repression in epithelial cells. Mol Cell 11:915–926.

Kavsak P, Rasmussen RK, Causing CG, Bonni S, Zhu H, Thomsen GH, Wrana JL. 2000. Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGF beta receptor for degradation. Mol Cell 6:1365–1375.

Kay EP, Lee MS, Seong GJ, Lee YG. 1998. TGF-betas stimulate cell proliferation via an autocrine production of FGF-2 in corneal stromal fibroblasts. Curr Eye Res 17: 286–293.

Khalil N, Xu YD, O'Connor R, Duronio V. 2005. Proliferation of pulmonary interstitial fibroblasts is mediated by transforming growth factor-beta1-induced release of extracellular fibroblast growth factor-2 and phosphorylation of p38 MAPK and JNK. J Biol Chem 280:43000–43009.

Kim SG, Jong HS, Kim TY, Lee JW, Kim NK, Hong SH, Bang YJ. 2004. Transforming growth factor-beta 1 induces apoptosis through Fas ligand-independent activation of the Fas death pathway in human gastric SNU-620 carcinoma cells. Mol Biol Cell 15:420–434.

Kuhn C, McDonald JA. 1991. The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. Am J Pathol 138:1257–1265.

Le Roy C, Wrana JL. 2005. Clathrin- and non-clathrin-mediated endocytic regulation of cell signalling. Nat Rev Mol Cell Biol 6:112–126.

Leal SM, Liu Q, Huang SS, Huang JS. 1997. The type V transforming growth factor b receptor is the putative insulin-like growth factor-binding protein 3 receptor. J Biol Chem 272:20572–20576.

Leal SM, Huang SS, Huang JS. 1999. Interactions of high affinity insulin-like growth factor-binding proteins with the type V transforming growth factor-beta receptor in mink lung epithelial cells. J Biol Chem 274:6711–6717.

Lee CG, Homer RJ, Zhu Z, Lanone S, Wang X, Koteliansky V, Shipley JM, Gotwals P, Noble P, Chen Q, Senior RM, Elias JA. 2001. Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor beta(1). J Exp Med 194:809–821.

Lee HJ, Lee JK, Miyake S, Kim SJ. 2004. A novel E1A-like inhibitor of differentiation (EID) family member, EID-2, suppresses transforming growth factor (TGF)-beta signaling by blocking TGF-beta-induced formation of Smad3–Smad4 complexes. J Biol Chem 279:2666–2672.

Lee JM, Dedhar S, Kalluri R, Thompson EW. 2006. The epithelial-mesenchymal transition: New insights in signaling, development, and disease. J Cell Biol 172: 973–981.

Leof EB, Proper JA, Goustin AS, Shipley GD, DiCorleto PE, Moses HL. 1986. Induction of c-sis mRNA and activity similar to platelet-derived growth factor by transforming growth factor beta: A proposed model for indirect mitogenesis involving autocrine activity. Proc Natl Acad Sci USA 83:2453–2457.

Levy L, Hill CS. 2005. Smad4 dependency defines two classes of transforming growth factor b (TGF-b) target genes and distinguishes TGF-b-induced epithelial-mesenchymal transition from its antiproliferative and migratory responses. Mol Cell Biol 25:8108–8125.

Li Y, Yang J, Dai C, Wu C, Liu Y. 2003. Role for integrin-linked kinase in mediating tubular epithelial to mesenchymal transition and renal interstitial fibrogenesis. J Clin Invest 112:503–516.

Lin HK, Bergmann S, Pandolfi PP. 2004. Cytoplasmic PML function in TGF-beta signalling. Nature 431:205–211.

Liu Q, Huang SS, Huang JS. 1994. Kinase activity of the type V transforming growth factor beta receptor. J Biol Chem 269:9221–9226.

Liu Q, Huang SS, Huang JS. 1997. Function of the type V transforming growth factor b receptor in transforming growth factor b-induced growth inhibition of mink lung epithelial cells. J Biol Chem 272:18891–18895.

Massague J, Gomis RR. 2006. The logic of TGFbeta signaling. FEBS Lett 580:2811–2820.

Massagué J, Blain SW, Lo RS. 2000. TGFß signaling in growth control, cancer, and heritable disorders. Cell 103: 295–309.

Massagué J, Seoane J, Wotton D. 2005. Smad transcription factors. Genes Dev 19:2783–2810.

McCormick LL, Zhang Y, Tootell E, Gilliam AC. 1999. Anti-TGF-beta treatment prevents skin and lung fibrosis in murine sclerodermatous graft-versus-host disease: A model for human scleroderma. J Immunol 163:5693–5699.

Medici D, Hay ED, Goodenough DA. 2006. Cooperation between snail and LEF-1 transcription factors is essential for TGF-beta1-induced epithelial-mesenchymal transition. Mol Biol Cell 17:1871–1879.

Miura S, Takeshita T, Asao H, Kimura Y, Murata K, Sasaki Y, Hanai J-I, Beppu H, Tsukazaki T, Wrana JL, Miyazono K, Sugamura K. 2000. Hgs (Hrs), a FYVE domain protein, is involved in Smad signaling through cooperation with SARA. Mol Cell Biol 20:9346–9355.

Moses HL, Branum EL, Proper JA, Robinson RA. 1981. Transforming growth factor production by chemically transformed cells. Cancer Res 41:2842–2848.

Motyl T, Grzelkowska K, Zimowska W, Skierski J, Wareski P, Ploszaj T, Trzeciak L. 1998. Expression of bcl-2 and bax in TGF-beta 1-induced apoptosis of L1210 leukemic cells. Eur J Cell Biol 75:367–374.

Moussad EE, Brigstock DR. 2000. Connective tissue growth factor: What's in a name? Mol Genet Metab 71:276–292.

Munger JS, Harpel JG, Gleizes PE, Mazzieri R, Nunes I, Rifkin DB. 1997. Latent transforming growth factor-beta: Structural features and mechanisms of activation. Kidney Int 51:1376–1382.

Nakamura T, Sakata R, Ueno T, Sata M, Ueno H. 2000. Inhibition of transforming growth factor beta prevents progression of liver fibrosis and enhances hepatocyte regeneration in dimethylnitrosamine-treated rats. Hepatology 32:247–255.

Nakao A, Afrakhte M, Morén A, Nakayama T, Christian JL, Heuchel R, Itoh S, Kawabata M, Heldin N-E, Heldin C-H, ten Dijke P. 1997. Identification of Smad7, a TGFb-inducible antagonist of TGF-b signalling. Nature 389: 631–635.

Oft M, Akhurst RJ, Balmain A. 2002. Metastasis is driven by sequential elevation of H-ras and Smad2 levels. Nat Cell Biol 4:487–494.

O'Grady P, Kuo M-D, Baldassare JJ, Huang SS, Huang JS. 1991. Purification of a new type high molecular weight receptor (Type V receptor) of transforming growth factor b (TGF-b) from bovine liver. J Biol Chem 266:8583–8589.

O'Grady P, Liu Q, Huang SS, Huang JS. 1992. Transforming growth factor beta (TGF-beta) type V receptor has a TGF-beta-stimulated serine/threonine-specific autophosphorylation activity. J Biol Chem 267:21033–21037.

Ohgushi M, Kuroki S, Fukamachi H, O'Reilly LA, Kuida K, Strasser A, Yonehara S. 2005. Transforming growth factor beta-dependent sequential activation of Smad, Bim, and caspase-9 mediates physiological apoptosis in gastric epithelial cells. Mol Cell Biol 25:10017–10028.

Ozdamar B, Bose R, Barrios-Rodiles M, Wang HR, Zhang Y, Wrana JL. 2005. Regulation of the polarity protein Par6 by TGFb receptors controls epithelial cell plasticity. Science 307:1603–1609.

Patterson GI, Padgett RW. 2000. TGF beta-related pathways. Roles in *Caenorhabditis elegans* development. Trends Genet 16:27–33.

Peinado H, Quintanilla M, Cano A. 2003. Transforming growth factor beta-1 induces snail transcription factor in epithelial cell lines. J Biol Chem 278:21113–21123.

Pelaia G, Gallelli L, D'Agostino B, Vatrella A, Cuda G, Fratto D, Renda T, Galderisi U, Piegari E, Crimi N, Rossi F, Caputi M, Costanzo FS, Vancheri C, Maselli R, Marsico SA. 2007. Effects of TGF-beta and glucocorticoids on map kinase phosphorylation, IL-6/IL-11 secretion and cell proliferation in primary cultures of human lung fibroblasts. J Cell Physiol 210:489–497.

Penheiter SG, Mitchell H, Garamszegi N, Edens M, Doré JJE, Jr., Leof EB. 2002. Internalization-dependent and -independent requirements for transforming growth factor ß receptor signaling via the Smad pathway. Mol Cell Biol 22:4750–4759.

Perlman R, Schiemann WP, Brooks MW, Lodish HF, Weinberg RA. 2001. TGF-beta-induced apoptosis is mediated by the adapter protein Daxx that facilitates JNK activation. Nat Cell Biol 3:708–714.

Persson U, Izumi H, Souchelnytskyi S, Itoh S, Grimsby S, Engstrom U, Heldin CH, Funa K, ten Dijke P. 1998. The L45 loop in type I receptors for TGF-beta family members is a critical determinant in specifying Smad isoform activation. FEBS Lett 434:83–87.

Petritsch C, Beug H, Balmain A, Oft M. 2000. TGF-beta inhibits p70 S6 kinase via protein phosphatase 2A to induce G(1) arrest. Genes Dev 14:3093–3101.

Phan SH. 2002. The myofibroblast in pulmonary fibrosis. Chest 122:286S–289S.

Pietenpol JA, Holt JT, Stein RW, Moses HL. 1990. Transforming growth factor beta 1 suppression of c-myc gene transcription: Role in inhibition of keratinocyte proliferation. Proc Natl Acad Sci USA 87:3758–3762.

Plattner R, Irvin BJ, Guo S, Blackburn K, Kazlauskas A, Abraham RT, York JD, Pendergast AM. 2003. A new link between the c-Abl tyrosine kinase and phosphoinositide signalling through PLC-gammal. Nat Cell Biol 5:309–319.

Qi Z, Atsuchi N, Ooshima A, Takeshita A, Ueno H. 1999. Blockade of type beta transforming growth factor signaling prevents liver fibrosis and dysfunction in the rat. Proc Natl Acad Sci USA 96:2345–2349.

Radisky DC, Kenny PA, Bissell MJ. 2007. Fibrosis and cancer: Do myofibroblasts come also from epithelial cells via EMT? J Cell Biochem 101:830–839.

Ramjaun AR, Tomlinson S, Eddaoudi A, Downward J. 2007. Upregulation of two BH3-only proteins, Bmf and Bim, during TGF beta-induced apoptosis. Oncogene 26:970–981.

Remy I, Montmarquette A, Michnick SW. 2004. PKB/Akt modulates TGF-beta signalling through a direct interaction with Smad3. Nat Cell Biol 6:358–365.

Saika S, Kono-Saika S, Ohnishi Y, Sato M, Muragaki Y, Ooshima A, Flanders KC, Yoo J, Anzano M, Liu CY, Kao WW, Roberts AB. 2004. Smad3 signaling is required for epithelial-mesenchymal transition of lens epithelium after injury. Am J Pathol 164:651–663.

Seoane J, Pouponnot C, Staller P, Schader M, Eilers M, Massague J. 2001. TGFbeta influences Myc, Miz-1 and Smad to control the CDK inhibitor p15INK4b. Nat Cell Biol 3:400–408.

Seoane J, Le HV, Shen L, Anderson SA, Massague J. 2004. Integration of Smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation. Cell 117:211–223.

Seong HA, Jung H, Kim KT, Ha H. 2007. 3-Phosphoinositide-dependent protein kinase-1 (PDK1) negatively reg-

ulates TGF-beta -induced signaling in a kinase-dependent manner through physical interaction with Smad proteins. J Biol Chem 282:12272–12289.

Shi Y, Massagué J. 2003. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113:685–700.

Shi Y, Wang YF, Jayaraman L, Yang H, Massague J, Pavletich NP. 1998. Crystal structure of a Smad MH1 domain bound to DNA: Insights on DNA binding in TGF-beta signaling. Cell 94:585–594.

Shibuya H, Yamaguchi K, Shirakabe K, Tonegawa A, Gotoh Y, Ueno N, Irie K, Nishida E, Matsumoto K. 1996. TA B1: An activator of the TAK1 MAPKKK in TGF-beta signal transduction. Science 272:1179–1182.

Shi-Wen X, Rodriguez-Pascual F, Lamas S, Holmes A, Howat S, Pearson JD, Dashwood MR, du Bois RM, Denton CP, Black CM, Abraham DJ, Leask A. 2006. Constitutive ALK5-independent c-Jun N-terminal kinase activation contributes to endothelin-1 overexpression in pulmonary fibrosis: Evidence of an autocrine endothelin loop operating through the endothelin A and B receptors. Mol Cell Biol 26:5518–5527.

Siegel PM, Massague J. 2003. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer 3:807–821.

Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J. 1997. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. J Clin Invest 100:768–776.

Song K, Wang H, Krebs TL, Danielpour D. 2006. Novel roles of Akt and mTOR in suppressing TGF-beta/ALK5-mediated Smad3 activation. EMBO J 25:58–69.

Staller P, Peukert K, Kiermaier A, Seoane J, Lukas J, Karsunky H, Moroy T, Bartek J, Massague J, Hanel F, Eilers M. 2001. Repression of p15INK4b expression by Myc through association with Miz-1. Nat Cell Biol 3:392–399.

Strutz F, Zeisberg M, Renziehausen A, Raschke B, Becker V, van Kooten C, Muller G. 2001. TGF-beta 1 induces proliferation in human renal fibroblasts via induction of basic fibroblast growth factor (FGF-2). Kidney Int 59:579–592.

Sun PD, Davies DR. 1995. The cysteine-knot growth factor superfamily. Annu Rev Biophys Biomol Struct 24:269–291.

Suzuki C, Murakami G, Fukuchi M, Shimanuki T, Shikauchi Y, Imamura T, Miyazono K. 2002. Smurf1 regulates the inhibitory activity of Smad7 by targeting Smad7 to the plasma membrane. J Biol Chem 277: 39919–39925.

ten Dijke P, Goumans M-J, Itoh H, Itoh S. 2002. Regulation of cell proliferation by Smad proteins. J Cell Physiol 191:1–16.

Tian F, Byfield SD, Parks WT, Stuelten CH, Nemani D, Zhang YE, Roberts AB. 2004. Smad-binding defective mutant of transforming growth factor beta type I receptor enhances tumorigenesis but suppresses metastasis of breast cancer cell lines. Cancer Res 64:4523–4530.

Tseng WF, Huang SS, Huang JS. 2004. LRP-1/TbetaR-V mediates TGF-beta1-induced growth inhibition in CHO cells. FEBS Lett 562:71–78.

Tsukazaki T, Chiang TA, Davison AF, Attisano L, Wrana JL. 1998. SARA, a FYVE domain protein that recruits smad2 to the TGFß receptor. Cell 95:779–791.

Ueno H, Sakamoto T, Nakamura T, Qi Z, Astuchi N, Takeshita A, Shimizu K, Ohashi H. 2000. A soluble transforming growth factor beta receptor expressed in muscle prevents liver fibrogenesis and dysfunction in rats. Hum Gene Ther 11:33–42.

Valcourt U, Kowanetz M, Niimi H, Heldin CH, Moustakas A. 2005. TGF-beta and the Smad signaling pathway support transcriptomic reprogramming during epithelial-mesenchymal cell transition. Mol Biol Cell 16:1987–2002.

Vardouli L, Moustakas A, Stournaras C. 2005. LIM-kinase 2 and cofilin phosphorylation mediate actin cytoskeleton reorganization induced by transforming growth factor-beta. J Biol Chem 280:11448–11457.

Wang W, Zhou G, Hu MC, Yao Z, Tan TH. 1997. Activation of the hematopoietic progenitor kinase-1 (HPK1)-dependent, stress-activated c-Jun N-terminal kinase (JNK) pathway by transforming growth factor beta (TGF-beta)-activated kinase (TAK1), a kinase mediator of TGF beta signal transduction. J Biol Chem 272:22771–22775.

Wang Q, Wang Y, Hyde DM, Gotwals PJ, Koteliansky VE, Ryan ST, Giri SN. 1999. Reduction of bleomycin induced lung fibrosis by transforming growth factor beta soluble receptor in hamsters. Thorax 54:805–812.

Wang S, Wilkes MC, Leof EB, Hirschberg R. 2005. Imatinib mesylate blocks a non-Smad TGFb pathway and reduces fibrogenesis in experimental obstructive nephropathy. FASEB J 19:1–11.

Wang H, Quah SY, Dong JM, Manser E, Tang JP, Zeng Q. 2007. PRL-3 down-regulates PTEN expression and signals through PI3K to promote epithelial-mesenchymal transition. Cancer Res 67:2922–2926.

Warner BJ, Blain SW, Seoane J, Massague J. 1999. Myc downregulation by transforming growth factor-beta required for activation of the p15Ink4b G1 arrest pathway. Mol Cell Biol 19:5913–5922.

Wijchers PJ, Burbach JP, Smidt MP. 2006. In control of biology: Of mice, men and Foxes. Biochem J 397:233–246.

Wilkes MC, Leof EB. 2006. Transforming growth factor beta activation of c-Abl is independent of receptor internalization and regulated by phosphatidylinositol 3-kinase and PAK2 in mesenchymal cultures. J Biol Chem 281:27846–27854.

Wilkes MC, Murphy SJ, Garamszegi N, Leof EB. 2003. Cell-type-specific activation of PAK2 by transforming growth factor ß independent of Smad2 and Smad3. Mol Cell Biol 23:8878–8889.

Wilkes MC, Mitchell H, Gulati-Penheiter S, Doré JJ, Suzuki K, Edens M, Sharma DK, Pagano RE, Leof EB. 2005. Transforming growth factor-b activation of phosphatidylinositol 3-kinase is independent of Smad2 and Smad3 and regulates fibroblast responses via p21-activated kinase-2. Cancer Res 65:10431–10440.

Yamaguchi K, Shirakabe K, Shibuya H, Irie K, Oishi I, Ueno N, Taniguchi T, Nishida E, Matsumoto K. 1995. Identification of a member of the MAPKKK family as a potential mediator of TGF-ß signal transduction. Science 270:2008–2011.

Yang Z, Mu Z, Dabovic B, Jurukovski V, Yu D, Sung J, Xiong X, Munger JS. 2007. Absence of integrin-mediated TGFbeta1 activation in vivo recapitulates the phenotype of TGFbeta1-null mice. J Cell Biol 176:787–793.

Yano T, Ito K, Fukamachi H, Chi XZ, Wee HJ, Inoue K, Ida H, Bouillet P, Strasser A, Bae SC, Ito Y. 2006. The RUNX3 tumor suppressor upregulates Bim in gastric epithelial cells undergoing transforming growth factor beta-induced apoptosis. Mol Cell Biol 26:4474–4488.

Yi JY, Shin I, Arteaga CL. 2005. Type I transforming growth factor beta receptor binds to and activates phosphatidylinositol 3-kinase. J Biol Chem 280:10870–10876.

Yu L, Hébert MC, Zhang YE. 2002. TGF-b receptor-activated p38 MAP kinase mediates Smad-independent TGF-b responses. EMBO J 21:3749–3759.

Yuan W, Varga J. 2001. Transforming growth factor-beta repression of matrix metalloproteinase-1 in dermal fibroblasts involves Smad3. J Biol Chem 276:38502–38510.

Zawel L, Da JL, Buckhaults P, Zhou SB, Kinzler KW, Vogelstein B, Kern SE. 1998. Human Smad3 and Smad4 are sequence-specific transcription activators. Mol Cell 1:611–617.

Zhang W, Ou J, Inagaki Y, Greenwel P, Ramirez F. 2000. 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.