Pin1 Down-regulates Transforming Growth Factor-β (TGF-β) Signaling by Inducing Degradation of Smad Proteins

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Transforming growth factor-β (TGF-β) is crucial in numerous cellular processes, such as proliferation, differentiation, migration, and apoptosis. TGF-β signaling is transduced by intracellular Smad proteins that are regulated by the ubiquitin-proteasome system. Smad ubiquitin regulatory factor 2 (Smurf2) prevents TGF-β and bone morphogenetic protein signaling by interacting with Smads and inducing their ubiquitin-mediated degradation. Here we identified Pin1, a peptidyl-prolyl cis-trans isomerase, as a novel protein binding Smads. Pin1 interacted with Smad2 and Smad3 but not Smad4; this interaction was enhanced by the phosphorylation of (S/T)P motifs in the Smad linker region. (S/T)P motif phosphorylation also enhanced the interaction of Smad2/3 with Smurf2. Pin1 reduced Smad2/3 protein levels in a manner dependent on its peptidyl-prolyl cis-trans isomerase activity. Knockdown of Pin1 increased the protein levels of endogenous Smad2/3. In addition, Pin1 both enhanced the interaction of Smurf2 with Smads and enhanced Smad ubiquitination. Pin1 inhibited TGF-β-induced transcription and gene expression, suggesting that Pin1 negatively regulates TGF-β signaling by down-regulating Smad2/3 protein levels via induction of Smurf2-mediated ubiquitin-proteasomal degradation.

Members of transforming growth factor-β (TGF-β) family, including TGF-βs, activin, and bone morphogenetic proteins (BMPs), are multifunctional proteins that regulate proliferation, differentiation, migration, and apoptosis (1). Ligation of TGF-β family members by type I and type II serine/threonine kinase receptors transduces the signals to intracellular Smad proteins (2–4). After TGF-β binding, the TGF-β type II receptor (TBR-II) phosphorylates the TGF-β type I receptor (TβR-I), which in turn phosphorylates the TGF-β-specific receptor-regulated Smads Smad2 and Smad3 at C-terminal SXS motifs. Phosphorylated Smad2 and Smad3 form a complex with Smad4, a common partner Smad. This complex translocates into the nucleus, where it regulates the transcription of target genes in cooperation with transcriptional activators and/or repressors.

Smad proteins possess a conserved N-terminal Mad homology 1 (MH1) domain and a C-terminal MH2 domain connected by a linker region. The MH1 domain binds DNA, whereas the MH2 domain is important for receptor binding, nuclear import, and transcription (5). The linker region contains several serine and threonine residues that can be phosphorylated by kinases. Mitogen-activated protein kinases (MAPKs), which are activated by growth factors and cytokines, modulate Smad signaling (3, 4, 6, 7). In the BMP signaling pathway, extracellular signal-regulated kinase (Erk) phosphorylates the linker region of Smad1 to inhibit Smad1 nuclear accumulation and transcriptional activity (8). Smad1 mutant mice lacking the MAPK phosphorylation sites revealed their importance in BMP signaling (9). In the TGF-β signaling pathway, activated MAPKs inhibit TGF-β signaling by inducing the cytoplasmic retention of Smad2 and/or Smad3 via phosphorylation of the linker region (8, 10–12). Conversely, Kamaraju and Roberts (13) reported that phosphorylation of the Smad3 linker region by p38 MAPK was required for maximal transcriptional activation and growth inhibition by TGF-β.

TGF-β family signaling is regulated by proteasomal proteolysis, which is mediated by HECT (homologous to the E6-association protein C terminus)-type E3 ubiquitin family ligases, Smad ubiquitin regulatory factor 1 (Smurf1) and Smurf2, a Smurf1-related protein (14–19). Smurf1, which was originally identified as an E3 ubiquitin ligase whose WW domain interacts with the PY motif in the linker region of Smad1 and Smad5, induces degradation of Smads (19). Sapkota et al. (20) reported that phosphorylation of the Smad1 linker region facilitates the interaction with Smurf1 to enhance Smurf1-dependent polyu-
biquitination of Smad1. Therefore, phosphorylation of the linker region of receptor-regulated Smads is crucial in the regulation of TGF-β family signaling. The molecular mechanism by which phosphorylation of receptor-regulated Smad linker regions exerts these effects remains unclear.

Here we identified Pin1, a peptidyl-prolyl cis-trans isomerase (PPIase), as a novel binding partner for Smad2/3. Pin1 interacted with phosphorylated (S/T)P (p(S/T)P) motifs in the linker regions of Smad2 and Smad3. This interaction inhibited TGF-β signaling by down-regulating Smad2/3 protein levels via the induction of Smurf2-mediated ubiquitin-proteasomal degradation. Our findings revealed that Pin1 participates in the complex regulation of the Smad2/3 linker region; thus, altered expression of Pin1 in cancer cells may adversely affect TGF-β signaling.

Experimental Procedures

Cell Cultures—COS7 cells, HaCaT cells, 293T cells, and MDA-MB-231 cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 units/ml penicillin G, and 100 μg/ml streptomycin. Mouse embryonic fibroblasts derived from Pin1 knock-out mice (Pin1−/− mouse embryonic fibroblasts (MEFs)) have been described previously (21). Cells were grown in a 5% CO₂ atmosphere at 37 °C.

Constructs and Chemicals—Wild-type human Pin1 (Pin1WT) as well as the R68A/R69A (Pin1RA) and W34A (Pin1WA) Pin1 mutant constructs have been described previously (22). A catalytically inactive C113A mutant of Pin1 (Pin1CA) and various human Smad2 and Smad3 mutants were generated by point mutagenesis. Wild-type human Smad2, wild-type and deletion mutants of human Smad3, wild-type Smad4, wild-type and CA mutant Smurf2, ubiquitin, and constitutively active Ras (RasG12V) has been described previously (25). TGF-β3 was acquired from Novartis (East Hanover, NJ). EGF was obtained from R&D Systems (Minneapolis, MN), whereas Juglone was purchased from Calbiochem (Darmstadt, Germany), and Pin1 antibodies were previously described (26). We also utilized commercially available antibodies against Myc (9E10; BD Biosciences), FLAG (M2) (Sigma-Aldrich), HA (InvivoGen; San Diego, CA), Smad2/3 (BD Transduction Laboratories), and α-tubulin (DM1A) (Sigma-Aldrich).

RNA Interference and Oligonucleotides—Steady small interfering RNAs (siRNA) specific for Pin1, Pin1-1 (sense, 5′-CCGCCAACAGCGACGAGGUGGUGCCAAA-3′), and Pin1-2 (sense, 5′-GCCCGGAGCAUGACCAAGGGCUA-3′) were purchased from Invitrogen. siRNAs were transfected into 293T or MDA-MB-231 cells using Lipofectamine 2000 (Invitrogen) or Lipofectamine RNAiMax (Invitrogen) according to the manufacturer’s protocols.

GST Pull-down Assay—We subcloned Pin1 and derived mutants into the pGEX-4T-1 vector (GE Healthcare, Buckinghamshire, England). Following expression in Escherichia coli DH5α, glutathione S-transferase (GST) fusion proteins were purified using glutathione-Sepharose beads (Amersham Biosciences, Uppsala, Sweden). COS7 cells were transfected with tagged Smad2,-3, or -4 wild-type and mutant constructs. After 24 h, cells were lysed in 1% IGEPAL CA630 in lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 100 units/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). Cell lysates were incubated with 20 μl of GST-Pin1 wild-type or mutant proteins or GST alone for 1.5 h at 4 °C. Bound proteins were analyzed by SDS-PAGE followed by immunoblotting.

Immunoprecipitation, Immunoblotting, and Ubiquitination Assay—We transfected cDNAs into cells using FuGENE 6 (Roche Applied Science) according to the manufacturer’s protocols. After 24 h, cells were lysed in 1% IGEPAL CA630 in lysis buffer and incubated with antibodies for 1.5–16 h at 4 °C. Immune complexes were precipitated with protein G-Sepharose beads for 30 min and analyzed by immunoblotting. Endogenous proteins were immunoprecipitated with anti-Smad2/3 antibody, which had been preincubated for 6 h with Dynabeads M-280 sheep anti-mouse IgG (Invitrogen). To assess ubiquitination, 293T cells were preincubated with 2.5 μM lactacystin (Wako, Osaka, Japan) 24 h prior to cell harvest to inhibit proteasomal degradation.

Quantification of Immunoblotting Data—We used the Multi Gauge version 3.0 software (Fujifilm, Tokyo, Japan) for quantification of immunoblotting data. All quantification data were obtained from raw image data acquired by LAS-3000 mini luminoimage analyzer.

Luciferase Assay—Cells were transiently transfected with the indicated combinations of promoter-reporter constructs and expression plasmids. The total amount of transfected DNA was adjusted to a constant quantity using empty vector. After 24–48 h, cells were lysed, and luciferase activity was measured with the Dual-Luciferase reporter system (Promega, Madison, WI). pGL4.75-SV40-hRluc (Promega) was used for normalization. (CAGA)₉-MLP-Luc2 was generated from (CAGA)₉-MLP-Luc (27) by replacing the plasmid backbone of pGL3 (Promega) with that of pGL4 (Promega).

Quantitative Real-time Reverse Transcription-PCR—Quantitative real-time reverse transcription-PCR was performed as described previously (28). Briefly, we extracted total RNA from cells using TRizol reagent (Invitrogen). First-strand cDNAs were synthesized using PrimeScript reverse transcriptase (TaKaRa Bio, Shiga, Japan) with oligo(dT). Real-time PCR was performed using SYBR Green PCR master mix (Invitrogen) on an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) using the following PCR primers: human fibronectin1 (sense, 5′-GAGCCATGTTGTTATCAGATCT-3′), antisense, 5′-AGTTATTTCTGGTCTGTGCTA-3′), human Smad7 (sense, 5′-CCTAGCCGACCTGGGAACTA-3′), antisense, 5′-CCAGATAATTGTTCCCCCTGT-3′), mouse connective tissue growth factor (CTGF) (sense, 5′-GAAG- TAAGGGAACGGAACACTA-3′; antisense, 5′-ATAGTTCCCTCCACGGTAGT-3′). A dissociation curve consisting of a single peak confirmed the specificity of detected signals. In each experiment, all samples were run in duplicate. Values were normalized to those obtained for either human HPRT1 (sense, 5′-TTTGCTTTCCTGGTCAAGC-3′; antisense, 5′-GCTT-GCGACCGTACCAT-3′) or mouse HPRT1 (sense, 5′-CCAGACGACCTGGTCAAGC-3′; antisense, 5′-GCTT-GCGACCGTACCAT-3′) and human HPRT1 (sense, 5′-GAAG-
FIGURE 1. TGF-β-induced interaction of Smad2/3 with Pin1. A, in vitro interaction of GST-Pin1 with Smad2/3. COS7 cells were transfected with the indicated plasmids 24 h before harvest. Cell lysates were incubated with GST-Pin1 for 1.5 h and subjected to GST pull down followed by immunoblotting (IB) with an anti-Myc antibody. The top panel displays input protein expression and the interaction. The lower panel shows the amount of GST or GST-Pin1 visualized by Coomassie Brilliant Blue (CBB) staining. B, the effect of phosphorylation of the four (S/T)P motifs in the Smad linker region on the interaction of Smad3 with Pin1. Wild-type (WT) FLAG-Smad3 and alanine mutants of the linker phosphorylation sites (T179A, S204A/S208A, S213A, and 4A) of FLAG-Smad3 were overexpressed in COS7 cells with c.a.TjR-i-HA in the presence or absence of HA-RasG12V. Cell lysates were subjected to GST pull-down assay using GST-Pin1 followed by immunoblotting. The top panel displays the interaction, whereas the second panel shows GST-Pin1 expression by CBB staining. The lower three panels indicate input protein expression. C, interaction of Smad3 with Pin1 in vivo. COS7 cells were transfected with the indicated plasmids. Cell lysates were subjected to immunoprecipitation (IP) with anti-FLAG antibodies and immunoblotting. The top panel demonstrates the interaction, whereas the lower four panels display expression of the input proteins. The graph in the bottom panel shows the quantification of Pin1-bound Smad3 (top panel), where lane 1 was regarded as a background signal. Asterisks indicate significant differences (p < 0.05). D, interaction between endogenous Smad2/3 and Pin1 proteins. MDA-MB-231 cells were treated with 1 ng/ml TGF-β3 and 100 ng/ml EGF for 1.5 h in the presence of 3 μM Juglone. Cell lysates were immunoprecipitated with an anti-Smad2/3 antibody or control IgG and then by using anti-mouse IgG Dynabeads M-280. Bound proteins were analyzed by immunoblotting. The top panel demonstrates the interaction, whereas the second panel displays immunoprecipitated Smad2/3. The lower two panels show input protein expression. The asterisk indicates a nonspecific band.
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5′-CTGGTTAAGCAGTACAGCCCA-3′; antisense, 5′-GGTCTTTTTCACCAGCAGCT-3′).

**Statistical Analysis**—The results of the quantitative real-time reverse transcription-PCR experiments and quantifications of immunoblotting were expressed as means ± S.D. Statistical significance was determined using the Tukey-Kramer method.

**RESULTS AND DISCUSSION**

**Pin1 Interacts with Smad2 and Smad3 but Not Smad4**—We previously reported the identification of several Smad2-binding proteins, such as JunB and c-Ski, by yeast-two-hybrid screening of a human cDNA library with full-length Smad2 as bait (29). This screen also determined that Pin1, a PPIase, interacted with Smad2 (data not shown). To confirm the interaction between Pin1 and Smad proteins, we incubated GST-Pin1 with lysates from COS7 cells transfected with Myc<sub>e</sub>-epitope-tagged Smad2 (Myc<sub>e</sub>-Smad2), Myc<sub>e</sub>-Smad3, or Myc<sub>e</sub>-Smad4. GST pull-down served to isolate Pin1; we then assayed for associated Smads by immunoblotting with an anti-Myc antibody. GST-Pin1 bound Smad2 and Smad3 but not Smad4 (Fig. 1A); this interaction was potentiated by co-expression of constitutively active TGF-β type 1 receptor (c.a.TβR-I).

To determine the site of Pin1 binding to Smad3, we generated various deletion mutants of FLAG-epitope-tagged Smad3 (FLAG-Smad3). In a GST pull-down assay, Pin1 interacted with full-length and the linker + MH2 domain Smad3 but not with the MH2 or MH1 + linker domains of Smad3 (supplemental Fig. S1), suggesting that both the linker and the MH2 domains of Smad3 are important for the Pin1-Smad3 interaction. Pin1 specifically binds the p(S/T)P motifs of substrates through its WW domain (30); there are four conserved (S/T)P motifs in the Smad3 linker region (30). Thus, four p(S/T)P motifs in the Smad3 linker region are essential for the interaction of Pin1 with Smad3. Pin1 WT but not Pin1 WA, which fails to bind to Smads (supplemen-
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tal Fig. S5), did not affect Smad3 protein levels (Fig. 2A). Similar results were obtained using Smad2 (supplemental Fig. S6). Pin1 thus appeared to cause down-regulation of Smad2/3 proteins through physical interaction. We also found that knockdown of Pin1 resulted in increase of endogenous Smad2/3 proteins in MDA-MB-231 (Fig. 2B), human embryo kidney 293 (supplemental Fig. S7A), and human fibrosarcoma HT1080 (supplemental Fig. S7B) cells. Notably, a PPIase mutant of Pin1 (Pin1CA) with lower enzymatic activity (supplemental Fig. S5) decreased Smad3 protein level less efficiently than Pin1WT (Fig. 2A). Consistently, Juglone, a Pin1 inhibitor, abrogated the Pin1-mediated decreases in Smad3 protein levels (Fig. 2C), although Juglone may have some nonspecific effects other than inhibition of PPIase activity. These results indicate that Pin1 decreases Smad2/3 protein levels in a manner dependent on PPIase activity.

Pin1 Enhances the Interaction of Smad2/3 with Smurf2 and Induces Smurf2-mediated Ubiquitination—MAPKs inhibit BMP signaling by phosphorylating the linker region of Smad1 to induce Smurf1-mediated ubiquitin-dependent degradation of Smad1 (20). We examined the effect of Smad linker phosphorylation on the interaction of Smurf2 with Smad2 and Smad3. Because Smurf2WT, but not Smurf2CA, a catalytic inactive mutant of Smurf2, induced degradation of Smad2 and Smad3 (supplemental Fig. S8), we performed the experiment using Smurf2CA. Co-expression of either RasG12V or c.a.T/H9252R-I enhanced interaction of FLAG-Smurf2CA with Myc6-Smad2 and Myc6-Smad3 (Fig. 3A). Binding enhancement seemed to result from linker phosphorylation as Myc6-Smad2/3–4A failed to bind to FLAG-Smurf2CA even in the presence of both RasG12V and c.a.T/H9252R-I. We confirmed the endogenous interaction of Smad2/3 with Smurf2 in the presence of TGF-β and EGF (Fig. 3B). The Smurf2-mediated ubiquitina-

FIGURE 3. The effect of Pin1 on the interaction of Smad2/3 with Smurf2 and on Smurf2-mediated ubiquitination of Smad2/3. A, the effects of c.a.T/JR-I and/or RasG12V on the interaction of Smad2 or Smad3 with Smurf2CA, a catalytically inactive Smurf2 mutant. COS7 cells were transfected with the indicated plasmids. Cell lysates derived from these transfectants were subjected to immunoprecipitation (IP) with an anti-FLAG antibody and then subjected to immunoblotting (IB). The top panel demonstrates the interaction between Smad2/3 and Smurf2, whereas the second panel displays immunoprecipitated Smurf2. The lower three panels show expression of each protein. B, interaction of endogenous Smad2/3 and Smurf2 proteins. HaCaT cells were stimulated with 1 ng/ml TGF-β3 and 100 ng/ml EGF for 6.5 h in the presence of 10 μM lactacystin. Cells were lysed and subjected to immunoprecipitation with an anti-Smad2/3 antibody and then subjected to immunoblotting. The top panel displays the interaction, whereas the second panel shows the immunoprecipitated Smad2/3. The lower two panels indicate expression of each protein. C, the effect of Pin1 on the interaction between Smad2/3 and Smurf2. Immortalized Pin1−/− MEFs infected with control (empty) and Pin1-encoding (Pin1) adenoviruses were treated with or without TGF-β3 (1 ng/ml) and EGF (100 ng/ml) for 1.5 h in the presence of 10 μM lactacystin. Smad2/3-bound Smurf2 was detected by immunoprecipitation and immunoblotting. The top panel demonstrates the interaction, whereas the second panel shows immunoprecipitated Smad2/3. The lower three panels indicate expression of each protein. The graph in the bottom panel shows the quantification of Smad2/3-bound Smurf2 (top panel). The asterisk indicates significant difference from all the other data (p < 0.05). D, the effect of Pin1 knockdown on the Smurf2-mediated ubiquitination of Smad3. 293T cells were transfected with control or Pin1-targeting siRNAs with the indicated plasmids in the presence of c.a.T/JR-I. After lysis, Smad3 ubiquitination was determined by immunoprecipitation with an anti-FLAG antibody followed by immunoblotting with an anti-Myc antibody (top panel). The lower four panels display expression of each protein. FLAG-UB, FLAG-ubiquitin.
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**FIGURE 4.** The effect of Pin1 on TGF-β-induced transcription of target genes. A. COS7 cells were transfected with the indicated expression plasmids together with promoter-reporter constructs. After cell lysis, luciferase activity was determined and normalized to Renilla luciferase activity. Experiments were performed in duplicate. The error bars indicate the S.D. B, MDA-MB-231 cells were transfected with control or Pin1-targeting (Pin1) siRNAs with promoter-reporter and/or FLAG-Smad3 constructs. Cells were treated with the indicated concentrations of TGF-β for 24 h before harvest and normalized to Renilla luciferase activity. Experiments were performed in duplicate. The error bars show the S.D. C and D, MDA-MB-231 cells transfected with control or Pin1 siRNAs were treated with TGF-β (0.3 ng/ml) for 24 h (C) or 1 h (D). Expression of fibronectin1 (C) or Smad7 (D) was determined by quantitative real-time PCR. Values were normalized to those determined for Hprt1 mRNA expression. Experiments were performed in duplicate. The error bars indicate the S.D. E, the effect of Pin1 on the TGF-β-induced CTGF expression in MEFs. Immortalized Pin1−/− MEFs infected with control (Empty vector) and Pin1-encoding (Pin1) adenoviruses were treated with TGF-β (0.3 ng/ml) or left untreated for 4 h. Expression of CTGF was determined by quantitative real-time PCR. Values were normalized to those determined for Hprt1 mRNA expression. Experiments were performed in duplicate. The error bars indicate the S.D. Asterisks indicate significant differences (p < 0.05), n.s., not significant.

Significant expression of Mycα-Smad2/3−4A was profoundly reduced (data not shown). Thus, linker phosphorylation of Smad2/3 was required for Smurf2-mediated ubiquitination. We examined the effect of Pin1 on the interaction of Smurf2 with Smad2/3 in MEFs. MEFs deficient in Pin1 (Pin1−/− MEFs) and Pin1−/− MEFs infected with a Pin1-encoding adenovirus (21) were lysed and subjected to immunoprecipitation with an anti-Smad2/3 antibody. Restoration of Pin1 expression in Pin1−/− MEFs enhanced the interaction of Smurf2 with Smad2/3 in the presence of exogenous TGF-β and EGF (Fig. 3C). These results suggest that phosphorylation of the Smad linker region enhances the interaction of Smurf2 with Smad2/3. We examined the effect of Pin1 on Smurf2-mediated ubiquitination of Smad3 in 293T cells expressing abundant Pin1 (data not shown). We transfected Mycα-Smad3, FLAG-ubiquitin (Fig. 3D, FLAG-Ub), and HA-Smurf2 into 293T cells with or without an siRNA specific for Pin1. Smad3 ubiquitination was detected by immunoprecipitation with an anti-FLAG antibody followed by immunoblotting with an anti-HA antibody. Pin1 knockdown reduced the ubiquitination of Smad3 induced by Smurf2 in vivo (Fig. 3D), suggesting that endogenous Pin1 enhances the ubiquitination of Smad3 by Smurf2.

**Pin1 Negatively Regulates TGF-β Signaling**—We examined the effect of Pin1 on TGF-β-induced transcription and gene expression. Overexpression of Pin1 suppressed c.a.TβR-I-induced transcriptional activity in a dose-dependent manner (Fig. 4A). siRNA-mediated Pin1 knockdown enhanced TGF-β-induced reporter gene transcription (Fig. 4B) and up-regulated TGF-β-induced mRNA expression of several target genes, including fibronectin1 (Fig. 4C) and Smad7 (Fig. 4D), in MDA-MB-231 cells. Moreover, TGF-β induced mRNA for connective tissue growth factor (CTGF), a TGF-β-induced cytokine, in Pin1−/− MEFs, and adenoviral Pin1 expression decreased the effect of TGF-β on CTGF production (Fig. 4E). These results indicate that Pin1 enhanced the ubiquitin-proteasomal degradation of Smad2/3 induced by Smurf2 that down-regulates TGF-β signaling.

Pin1 is a member of parvulin family of PPIases that specifically recognizes p(S/T)P motifs. The enzymes catalyze the cis-trans isomerization of bound peptide at a site preceding proline (30, 33). Our observations thus suggest that Pin1 induces conformational change in the linker regions of Smad2/3 through binding to the phosphorylated (S/T)P motifs, which likely enables Smurf2 to interact with Smad2/3 via the PY motif and cause their degradation.

The phosphorylation of (S/T)P motifs by proline-directed kinases is a major regulatory mechanism for the control of various cellular processes. Pin1 serves as a post-phosphorylation regulatory factor in multiple signaling pathways. It has been
reported that Smurf1 negatively regulates BMP signaling through ubiquitin-dependent degradation of Smad1/5. Smurf1 binds to Smad1 via PY motif, and the interaction is enhanced by phosphorylation of (S/T)P motifs (20). In the study, Sapkota et al. (20) suggested that phosphorylation of (S/T)P motifs enables Smurf1 binding by unmasking the PY motif or by providing additional Smurf1 docking sites that cooperate with the PY motif. In the present study, we suggest that phosphorylation of (S/T)P motifs facilitates binding of Smads to Pin1 and conformational change in Smads by Pin1, and the conformational change induces interaction of Smads with Smurfs. Although we cannot exclude the possibility of other mechanisms by which Pin1 regulates TGF-β signaling, down-regulation of Smad proteins would be one of the major mechanisms of inhibition of TGF-β signaling by Pin1.

Pin1 is overexpressed in many cancer tissues. Such overexpression often correlates with increases in cyclin D1 and β-catenin (34–37). On the other hand, dysregulation of TGF-β signaling is often observed in cancers. Our study adds a new role to Pin1 and provides new insight into the mechanisms by which cancers impair TGF-β signaling.

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