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TGF-β Signaling

Dual Role in Tumor Suppression and Oncogenesis

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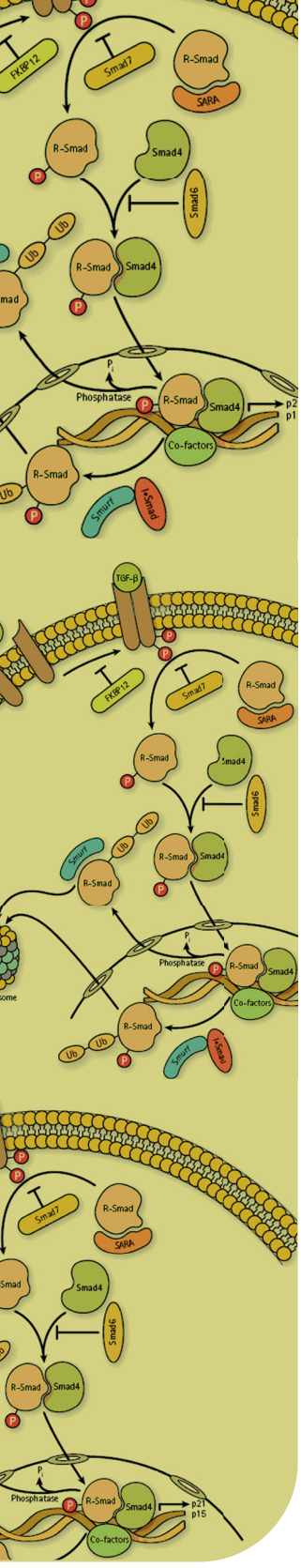

Transforming growth factor-β (TGF-β), a member of the TGF superfamily of proteins that includes activins, inhibins, and bone morphogenetic proteins (BMPs), regulates a wide array of cellular processes including cell differentiation, cellular senescence, immune response, wound healing, and apoptosis. TGF-β signaling promotes growth and development during early embryogenesis. However, in mature tissues many cells respond to TGF-β with either a cytostatic or apoptotic response. TGF-β signaling involves its binding to the TGF-β receptor type II (TGF-βRII), which allows it to recruit TGF-β receptor type I (TGF-β RI) and assemble it as a heterodimeric receptor complex. TGF-βRII phosphorylates TGF-βRI in the glycine-serine rich region (GS sequence) and activates the serine/threonine kinase activity of TGF-βRI, which in turn phosphorylates receptor-linked Smad (Small mothers against decapentaplegic) proteins. To prevent any spontaneous phosphorylation of Smads, the inhibitor molecule FKBP12 binds to the GS region of the TGF-βRI and blocks the access of TGF-βRII to this domain. This inhibitory effect is removed upon TGF-β binding to the receptor. The TGF-β receptor complex is internalized by lipid-raft and clathrin endocytotic pathways. The clathrin endocytotic pathway is considered to be essential for activation of the TGF-β signaling cascade. The lipid-raft pathway for TGF-β receptor internalization negatively regulates TGF-β signaling by inducing receptor complex degradation.

The activated TGF-βRI in the internalized complex phosphorylates specific Smad proteins. At least nine different Smad proteins have been reported in vertebrates that are classified as: (a) receptor-activated Smads (R-Smads): Smad1, Smad2, Smad3, Smad5, and Smad8; (b) co-mediator Smads: Smad4 and Smad10; and (c) inhibitory Smads (I-Smads): Smad6 and Smad7. R-Smads2 and 3 are involved in TGF-β and activin signaling, whereas R-Smads 1, 5, and 8 are mediators of BMP signaling. R-Smads and Co-Smads are predominantly located in the cytoplasm and their activity is modulated by adaptor proteins, such as Smad anchor for receptor activation (SARA) and ELF. SARA is reported to associate with endosomal membranes via lipid-binding FYVE domains. It binds Smad2 and Smad3 and facilitates their interaction with TGF-β receptors. Smad2 and Smad3 are directly phosphorylated by TGF-βRI, which changes their conformation and releases these R-Smads from the receptor complex. The C-terminal phosphoserines of R-Smads are recognized by the Mad Homology 2 (MH2) domain of Smad4 that enables them to form a heterodimeric complex (R-Smad/Co-Smad). This complex then translocates to the nucleus where Smad proteins bind to their cognate DNA binding sites with low affinity. This binding is further enhanced in the presence of transcriptional co-activators.

EMD

Antibodies for TGF-β Signaling Pathways

Product	Cat. No.	Comments	Size
Anti-Smad1 Rabbit pAb	566416	Polyclonal, immunoaffinity purified IgG. Recognizes the ~60 kDa human Smad1 protein. **IB**	100 µg
PhosphoDetect™ Anti-Smad1 (pSer$^{463/465}$) Rabbit pAb	566411	Polyclonal, protein A purified IgG. Recognizes the ~65 kDa Smad1 protein phosphorylated at Ser$^{463/465}$ in human and mouse. **IB**	50 µg
PhosphoDetect™ Anti-Smad2 (pSer$^{465/467}$) Rabbit pAb	566415	Polyclonal IgG, protein A and immunoaffinity purified. Recognizes the ~58 kDa Smad2 protein phosphorylated at Ser$^{465/467}$. Reacts with human, mink, and mouse. **IB**	50 µl
Anti-Smad3 (206-220) Rabbit pAb	566414	Polyclonal IgG, protein A purified. Recognizes the ~50 kDa Smad3 protein in human, mouse, and rat. **IB**	50 µg
PhosphoDetect™ Anti-Smad3 (pSer$^{423/425}$)/ Smad1 (pSer$^{463/465}$) Rabbit pAb	PK1012	Polyclonal IgG, protein A and immunoaffinity purified. Recognizes the ~58 kDa Smad3 protein phosphorylated at Ser^{433} and Ser^{435} in TGF-β-treated HeLa cells and PC3 cells. **IB, IC**	50 µl
Anti-Smad5 Rabbit pAb	ST1104	Polyclonal IgG, protein A and immunoaffinity purified. Recognizes the ~60 kDa Smad5 protein in SK-N-M, COS, and PC12 cells.Reacts with human, monkey, mouse, and rat. **IB**	50 µl
Anti-Sp1 (520-534) Rabbit pAb	PC701	Polyclonal IgG, immunoaffinity purified. Recognizes the ~105 kDa Sp1 protein in HeLa and K562 nuclear cell extracts. Reacts with human. **ELISA, IB**	100 µl
Anti-TGF-β1 (Ab-1) Mouse mAb (9016.2)	GF33L	Monoclonal IgG1, purified. This antibody neutralizes the biological activities of TGF-β1 and TGF-β1.2. **ELISA, IB, NT**	100 µg
Anti-TGF-β3 (Ab-1) Mouse mAb (236-5.2)	GF16	Monoclonal IgG1, purified. Recognizes TGF-β2 and TGF-β3 in umbilical cord cells. Does not cross-react with TGF-β1. Reacts with human, mouse, and rat. **FS, IB, IP, PS**	200 µg

ELISA: enzyme-linked immunosorbent assay; **FS**: frozen sections; **IB**: immunoblotting; **IC**: immunocytochemistry; **IP**: immunoprecipitation; **NT**: neutralization; **PS**: paraffin sections

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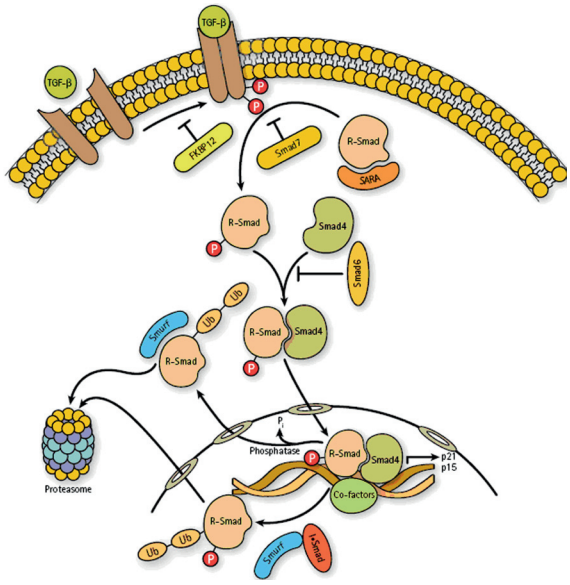
Both Smad3 and Smad4 bind to DNA sequences known as the Smad-binding elements (SBE); however, Smad2 participates in DNA-bound complexes via its interaction with Smad4. Genes for cyclin-dependent kinase inhibitors, p21 and p15, which mediate growth inhibitory processes, are up-regulated by TGF-β stimulation. TGF-β-induced growth inhibition is also achieved by down-regulation of c-Myc, which further amplifies the expression of p21 and p15.

The transcriptional induction of inhibitory Smad6 and Smad7 is under the control of TGF-β, which provides a negative feedback loop for attenuation of TGF-β signaling. Smad6 antagonizes TGF-β signaling by blocking the interaction of Smad2 and Smad4, whereas Smad7 negatively regulates TGF-β signaling by recruiting Smurf1 or Smurf2 to the Smad7-TGF-β-receptor complex. It has also been suggested that Smad7 blocks the activity of all R-Smads, but Smad6 preferentially blocks bone morphogenetic protein-activated Smads. Inhibitory Smads are also known to reside in the nucleus where they associate with Smurfs, a group of E3 ligases. Following TGF-β stimulation, the Smad7-Smurf complex is exported to the cytoplasm where it targets TGF-β receptors. By binding to TGF-βRII, Smad7 inhibits recruitment and phosphorylation of R-Smads. In the nucleus, Smads are ubiquitinated by the E3 ligases and are exported to cytoplasm for proteasomal degradation. Even in the cytoplasm R-Smads can be ubiquitinated by Smurfs and degraded in the proteasome complex. This keeps the pool of R-Smads at low levels. Nuclear Smurfs can also ubiquitinate phosphorylated R-Smads, which can then be degraded in the nucleus. Smad shuttling between the cytoplasm and the nucleus is dependent on phosphorylation of R-Smads by TGF-βR1 kinase in the cytoplasm and dephosphorylation by R-Smad phosphatases in the nucleus. However, a majority of activated R-Smad is believed to be recycled following its dephosphorylation.

TGF-β plays a significant role in maintaining the quiescent state of stem cells. Low concentrations of TGF-β are reported to prevent the rapid loss of CD34, common marker hematopoietic stem cells. Several mutations in the TGF-β signaling pathway are reported to cause neoplastic proliferation of primitive stem/progenitor cells in human tissues. Blocking of autocrine and endogenous TGF-β is shown to trigger cell cycling and proliferation of undifferenciated stem/progenitor cells.

Apart from its role as a growth inhibitor and tumor suppressor, TGF-β also promotes tumor metastasis during late stages of tumor development. TGF-β also modulates cell invasion, immune regulation, and microenvironment modification that cancer cells exploit to their advantage. Dysregulated TGF-β signaling has been implicated in the pathogenesis of human solid tumors. Several key proteins involved in tumor metastasis, such as Snail, Slug (zinc-finger transcriptional repressors) and Smad-interacting protein 1 (SiP1), are positively regulated by TGF-β. Any disruption in TGF-β signaling, either by mutational inactivation or by down regulation of expression of any of the signaling components involved, can lead to tumor development.

Inactivating mutations in TGF-βRII are observed in about 25% of all colon cancer cases, and a reduction in tumorigenicity and restoration of TGF-β-mediated growth arrest are reported in human colon cancer cell lines stably transfected with wild-type TGF-βRII. Mutations in Smad2 and Smad4 can also disrupt TGF-β signaling, leading to tumor development. Smad4, initially identified as DPC4 (deleted in pancreatic carcinoma locus 4), undergoes biallelic loss in about 50% of all pancreatic cancers. Of current terests in preventing tumor growth and metastasis are the development of molecules for inhibition or sequestration of TGF-β, blocking the kinase activity of TGF-βRI, and inhibition of downstream Smad signaling.



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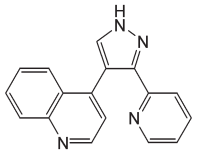
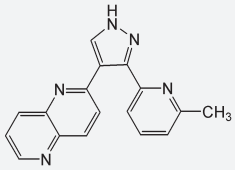
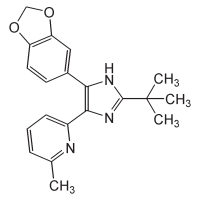
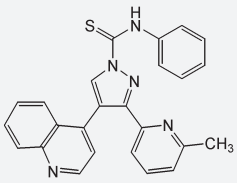
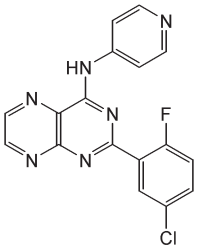
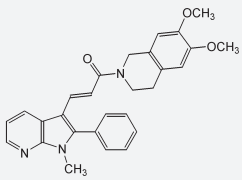
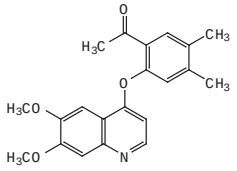
TGF-β Receptor I Kinase Inhibitors

Product	Structure	Additional Information
TGF-β Receptor I Kinase Inhibitors **([3-(Pyridin-2-yl)-4-(4-quinonyl)]-1H-pyrazole)** A cell-permeable, potent, selective, ATP-competitive inhibitor of TGF-β Receptor I kinase (IC_{50} = 51 nM). *Purity: 97% by HPLC. M.W. 272.3.*		Cat. No. 616451 5 mg Ref: Sawyer, J.S., et al. 2003. *J. Med. Chem.* **46**, 3953; Singh, J., et al. 2003. *Bioorg. Med. Chem. Lett.* **13**, 4355.
TGF-β RI Kinase Inhibitor II **(2-(3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl)-1, 5-naphthyridine)** A cell-permeable, potent, selective, and ATP-competitive inhibitor of TGF-β type I receptor (ALK5; IC_{50} = 23 nM, 4 nM and 18 nM for ALK5 binding, ALK5 auto-phosphorylation and TGF-β cellular assay in HepG2 cells, respectively). *Purity: 95% by HPLC. M.W. 287.3.*		Cat. No. 616452 1 mg Ref.: Gellibert, F., et al. 2004. *J. Med. Chem.* **47**, 4494.
TGF-β RI Inhibitor III **(2-(5-Benzo[1,3]dioxol-4-yl-2-tert-butyl-1H-imidazol-4-yl)-6-methylpyridine, HCl)** A cell-permeable, potent, selective, ATP-competitive, reversible inhibitor of activin receptor-like kinase 4 (IC_{50} = 129 nM), 5 (IC_{50} = 47 nM), and 7. *Purity: 97% by HPLC. M.W. 371.9.*		Cat. No. 616453 2 mg Ref.: Hu, T., et al. 2005. *Am. J. Physiol Renal Physiol.* **289**, F816; DaCosta Byfield, S., et al. 2004. *Mol. Pharmacol.* **65**, 744.
TGF-β RI Inhibitor IV **(3-(6-Methylpyridin-2-yl)-4-(4-quinolyl)-1-phenylthiocarbamoyl-1H-pyrazole)** A cell-permeable, selective inhibitor of ALK-4/5/7-mediated (IC_{50} = 45, 12, and 7.5 nM, respectively) signaling in Mv1Lu R4-2 transfectants expressing TGF-β Receptor I. *Purity: 98% by HPLC. M.W. 421.5.*		Cat. No. 616454 2 mg Ref.: Tojo, M., et al. 2005. *Cancer Sci.* **11**, 791.
TGF-β RI Inhibitor V **(2-(5-Chloro-2-fluorophenyl)pteridin-4-yl)pyridin-4-yl amine)** A cell-permeable, potent, selective, reversible inhibitor of TGF-β RI/ ALK5 kinase (EC_{50} = 48 nM). *Purity: 97% by HPLC. M.W. 352.8.*		Cat. No. 616456 2 mg Ref.: Ge, R., et al. 2006. *Clin. Cancer Res.* **12**, 4315; Kapoun, A. M., et al. 2006. *Mol. Pharmacol.* **70**, 518; Jian, H., et al. 2006. *Genes Dev.* **20**, 666; Uhl, M., et al. 2004. *Cancer Res.* **64**, 7954.
Smad3 Inhibitor, SIS3 **(6,7-Dimethyl-2-((2E)-3-(1-methyl-2-phenyl-1H-pyrrolo [2,3-b]pyridin-3-yl-prop-2-enoyl))-1,2,3,4-tetrahydroisoquinoline)** A cell-permeable, selective inhibitor of TGF-β1-dependent Smad3 phosphorylation and Smad3-mediated cellular signaling. *Purity: 93% by HPLC. M.W. 453.5.*		Cat. No. 566405 1 mg Ref.: Jinnin, M., et al. 2006. *Mol. Pharmacol.* **69**, 597.
TGF-β RI Kinase Inhibitor VII **(1-(2-((6,7-Dimethoxy-4-quinolyl)oxy)-(4,5-dimethylphenyl)-1-ethanone)** A cell-permeable, potent, ATP-competitive inhibitor of ALK5/TGF-β Receptor I kinase activity (IC_{50} = 0.63 µM). Blocks TGF-β-induced Smad2 phosphorylation and Smad2/3 transcription activity (IC_{50} = 0.5 and 0.37 µM, respectively) in human lung cancer epithelial cell line A549. *Purity: 97% by HPLC. M.W. 351.4.*		Cat. No. 616458 5 mg Ref.: Shimizu, T., et al. 2008. *J. Med. Chem.* **51**, 3326

TGF-β Family Proteins

Product	Cat. No.	Comments	Size
Activin A, Human, Recombinant, CHO Cells	114700	Recombinant, human activin A (disulfide-linked homodimer of two 116-amino acid residue βA subunits) expressed in CHO cells. Inhibits endogenous IL-6 production. *Biological activity: ED_{50} = 0.5-2.0 ng/ml*	5 µg
TGF-β1, Human Platelets	616450	Native TGF-β1 that is shown to promote apoptosis in resting human B lymphocytes, glioma cells, and trigeminal neurinomal cells. *Biological activity: ED_{50} = 40-10 pg/ml.*	1 µg
TGF-β1, Human, Recombinant, CHO Cells	616455	Recombinant, human TGF-β1 expressed in CHO cells. *Biological activity: ED_{50} = 0.04-0.1 ng/ml.*	2 µg
TGF-β1, Porcine Platelets	616460	Native TGF-β1 from porcine platelets. *Biological activity: ED_{50} = 0.1-0.2 ng/ml.*	1 µg
TGF-β2, Human, Recombinant, *E. coli*	PF017	A full length, recombinant, human TGF-β2 that is reactive in many cell types. *Biological activity: ED_{50} = 0.1-0.3 ng/ml.*	1 µg
TGF-β3, Human, Recombinant, *S. frugiperda*	PF073	Recombinant, human TGF-β3 expressed in *S. frugiperda* insect cells. *Biological activity: EC_{50} = 0.01-0.03 ng/ml.*	2 µg

Other Related Products

Smurf2 Ligating Enzyme, Human, Recombinant, *S. frugiperda*

Recombinant, human Smurf2 expressed in *S. frugiperda* insect cells using a baculovirus expression system. Smurf2 is an E3 ubiquitin ligase with 83% homology to Smurf1. Smurf2 is known to be constitutively associated with Smad7. *Purity: 98% by SDS-PAGE.*

Cat. No 662079 10 µg

SMAD3, GST-Fusion, Human, Recombinant, *E. coli*

Full-length, recombinant, human SMAD3 fused at the N-terminus to GST and expressed in *E. coli.* SMAD3 is a receptor-associated SMAD phosphorylated by TGF-β 1R kinase. *Purity: 90% by SDS-PAGE.*

Cat. No. 566418 50 µg

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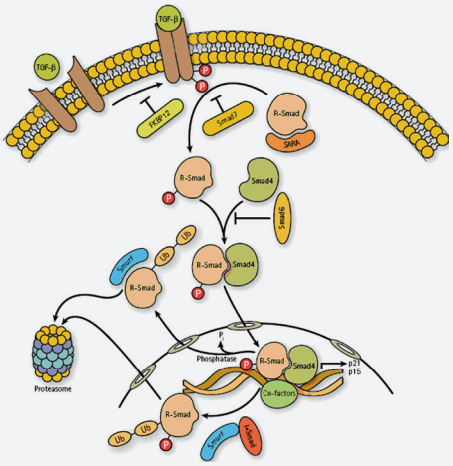
TGF-β Signaling

Dual Role in Tumor Suppression and Oncogenesis



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