

SUMO amplifies TGF- β signalling

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Transforming growth factor- β (TGF- β) stimulates phosphorylation of TGF- β type I receptor. This receptor is now shown to be sumoylated, leading to enhanced activation and modulation of the downstream Smad signalling pathway.

Phosphorylation of receptors and/or intracellular signalling molecules is the driving force in most signal transduction pathways. In addition, the actions of these signalling molecules are finely tuned by other post-translational modifications. Of these, the role of ubiquitination has been extensively studied in various signalling pathways. Another type of post-translational modification, sumoylation, has recently been shown to be important for certain signalling pathways¹. Sumoylation is mainly observed in nuclear and perinuclear proteins but has so far been shown only for a few types of cell-surface proteins, including ion channel proteins and glutamate and kinate receptors. On page 654 of this issue, Kang *et al.*² report that TGF- β type I receptor (T β RI) is sumoylated when the receptor is phosphorylated, causing enhancement of TGF- β signalling. This is the first growth factor receptor whose function has been shown to be modified by sumoylation.

TGF- β binds to two Ser-Thr kinase receptors: T β RI (also known as ALK-5) and type II (T β RII) receptors. When a ligand binds, T β RII transphosphorylates T β RI, leading to activation of intracellular signalling pathways, including the canonical Smad pathway and other non-Smad pathways³ (Fig. 1). Phosphorylation of T β RI occurs on the Gly-Ser rich region (GS region) located at the amino-terminal boundary of the Ser-Thr kinase domain, as well as on other Ser and Thr residues. Activated type I receptor kinase binds to the receptor-regulated Smads (R-Smads)

Smad2 and Smad3, inducing phosphorylation of their carboxy-terminal SSXS motif. R-Smads then interact with Smad4, translocate into the nucleus and regulate transcription of target genes. Interaction between T β RI kinase and R-Smads requires the L45 loop and GS region of T β RI to physically bind to the L3 loop and adjacent α -helix1 sequence in the C-terminal MH2 domain of R-Smads, causing phosphorylation of the SSXS motif. Thus, the phosphorylation cascade from the receptors to Smads is crucial for TGF- β signalling.

Covalent attachment of ubiquitin to Lys residues, is catalysed by three types of enzyme: E1 activating enzyme, E2 conjugating enzyme and specific E3 ubiquitin ligases. This controls the turnover of many signalling proteins. For example, ubiquitination of TGF- β receptors is induced by E3 ubiquitin ligases (for example, Smurf1 and Smurf2) and regulates the amplitude of TGF- β signalling through degradation of the receptors by the ubiquitin proteasome system^{4,5}. An inhibitory Smad (Smad7) interacts with the Smurfs and recruits them to T β RI for ubiquitin-dependent degradation of the receptor complexes. Ubiquitination also occurs on Smad proteins by the action of Smurfs and other E3 ligases⁶.

A number of ubiquitin-like proteins, including the small ubiquitin-like modifier (SUMO), use a system similar to ubiquitination. SUMO is activated by the E1 activating enzyme consisting of the Aos1/Uba2 heterodimer and transferred to the E2 enzyme Ubc9, followed by ligation to the substrates¹. E3 ligases for sumoylation have broad specificity, compared with those for ubiquitination, and several enzymes, including the PIAS family proteins, function as E3 ligases for sumoylation. This does not necessarily promote degradation of target proteins, and the functional consequences of sumoylation vary,

depending on target proteins. Smads are also post-translationally modified by sumoylation; in the case of Smad4, sumoylation protects it from ubiquitin-dependent degradation⁶.

In mammals, seven type I receptors transduce signals for ligands of the TGF- β family. Of these, T β RI/ALK-5, ActRIB/ALK-4 and ALK-7 are structurally related to each other. Among members of the TGF- β family, TGF- β 1, 2 and 3 bind to T β RI, whereas activins bind to ALK-4, and nodal binds to ALK-4 and ALK-7 (ref. 7). Although T β RI and ALK-4 both phosphorylate Smad2 and Smad3, their biological activities seem to be different. For example, TGF- β has potent growth inhibitory activity on epithelial cells, whereas activins do so less potently⁸. Interestingly, Kang *et al.*² report that sumoylation occurs only on T β RI but not on ALK-4 (nor on ALK-7 as it lacks the specific sumoylation residue), enhancing activation of the T β RI kinase and subsequent phosphorylation of Smad proteins. These findings suggest that sumoylation of the kinase domain induced by ligand stimulation is unique to T β RI, which may be responsible for specific effects of TGF- β , for example potent growth inhibition and stimulation of cancer metastasis.

How does sumoylation amplify the signalling activity of T β RI? Kang *et al.*² found that the kinase activities of both T β RII and T β RI are required for sumoylation of T β RI. Sumoylation may occur on multiple Lys residues, but Lys 389 is the primary sumoylation site. As Lys 389 is located on the surface of T β RI kinase and has the same orientation as the GS region and the L45 loop, sumoylation may increase the affinity of T β RI kinase for R-Smads. Smad7 also binds tightly to the T β RI kinase domain and competes with R-Smads for interaction with T β RI. Furthermore, Smad7 recruits Smurfs and the

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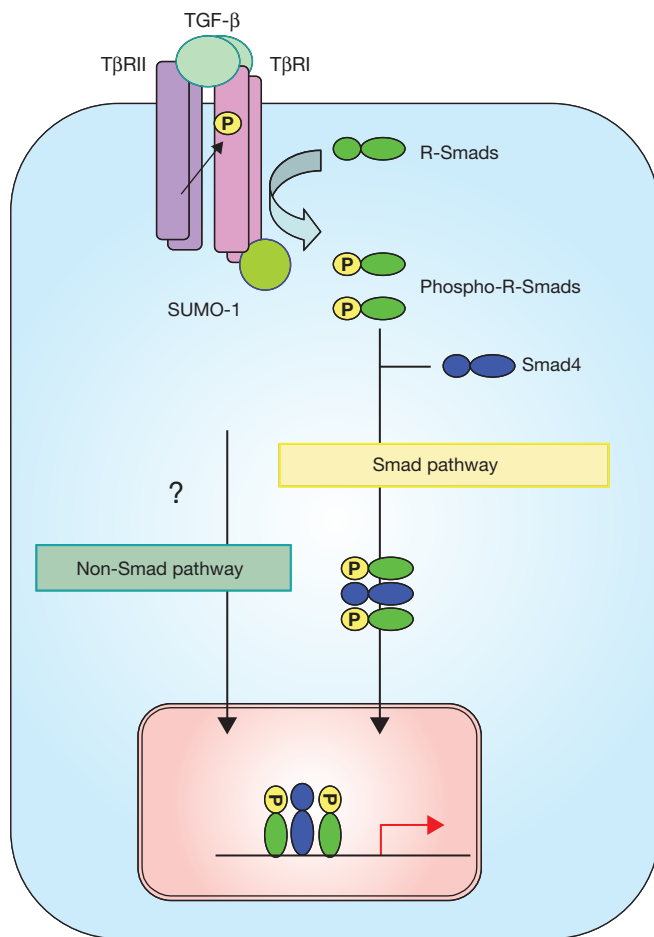


Figure 1 Proposed model of R-Smads phosphorylation by T β RI. TGF- β induces formation of a heterotetrameric receptor complex composed of T β RII and T β RI. T β RII kinase phosphorylates T β RI and conjugation of SUMO-1 occurs on T β RI. R-Smads are then phosphorylated by T β RI, form complexes with Smad4 and translocate into the nucleus where they regulate transcription of target genes. Smad signalling pathways, and possibly non-Smad pathways, are enhanced by sumoylation of T β RI.

GADD34-PP1 (protein phosphatase 1) complex to the receptors^{4,5,9}, inducing degradation and dephosphorylation of the receptors, respectively. An intriguing possibility is that when T β RI is sumoylated, R-Smads may be able to interact with T β RI with higher affinity than Smad7. Phosphorylation of R-Smads by T β RI is facilitated by some intracellular proteins, including Dab2 (ref. 10). Thus, another possibility is that binding of these Smad co-activators may be facilitated by sumoylation of T β RI.

A mis-sense mutation of human *TGFBR1* (encoding T β RI protein) mutating Ser 387 to Tyr has been found in breast and head-and-neck cancers^{11,12}. Ser 385 in rat T β RI (corresponding to Ser 387 in humans) localizes close to the Lys 389 sumoylation site. Although several loss-of-function mutations in T β RI have been reported, functional importance of these mutations has not been fully elucidated.

Interestingly, the authors found that the S385Y mutation does not significantly affect kinase activity of T β RI, but decreases its sumoylation. Thus, transcription by the S385Y mutant was attenuated, although less potently than the K389R mutant. Accordingly, Ras-transformed mouse embryonic fibroblasts (MEFs) expressing the S385Y mutant developed fewer metastases than those expressing wild-type T β RI in an animal model². This seems to contrast with the findings that the S387Y mutation in human *TGFBR1* was observed in metastatic tumours, but this may be due to bidirectional effects of TGF- β signalling on progression of cancer¹³.

The discovery of T β RI sumoylation may provide a better understanding of the unique action of the TGF- β signalling pathway. However, there also remain several important questions to be addressed. First, sumoylation of T β RI occurs on a Lys residue that is not in

a consensus sumoylation motif Ψ Kx(D/E), where Ψ is a large hydrophobic residue¹. Thus, identification of the E3 ligase will be important in understanding the mechanism of T β RI sumoylation. In the consensus Ψ Kx(D/E) motif, phosphorylation of Ser close to this motif contributes to sumoylation. It will therefore be interesting to study how phosphorylation of T β RI regulates the sumoylation of Lys 389. Second, sumoylation of T β RI results in accelerated activation of the T β RI kinase and downstream Smad signalling, but it is also possible that T β RI sumoylation may regulate non-Smad signalling pathways. Transcriptional activities were only partially suppressed, but metastasis of the Ras-transformed MEFs was markedly suppressed by the K389R and S385Y mutations. As such, whether non-Smad pathways are modulated by these mutants remains an important question. Third, the fate of T β RI protein may be altered by sumoylation. Kang *et al.*² have shown enhanced activation of R-Smads at the early phase of receptor activation. However, as sumoylation has been reported to induce endocytosis of the kinase receptor subunit GluR6 (ref. 14), it is possible that sumoylation may also regulate endocytosis and recycling of the TGF- β receptor complexes.

In advanced cancers, TGF- β is known to stimulate progression of cancer through induction of epithelial-mesenchymal transition (EMT), as well as its action on the tumour microenvironment¹³. TGF- β is also involved in the development of various fibrotic diseases. Monoclonal antibodies to TGF- β and small-molecule inhibitors for T β RI are currently being assessed in preclinical and clinical trials for use in the treatment of cancer and fibrotic diseases¹⁵. Although better tissue-penetration can be achieved by small-molecule inhibitors than by monoclonal antibodies, small-molecule inhibitors of T β RI that have been developed so far also inhibit the kinase activities of ALK-4 and ALK-7 (ref. 15). Thus, compounds that specifically inhibit TGF- β signalling may be more valuable for therapeutic purposes. Inhibition of ubiquitination by proteasome inhibitors is a recently developed, effective way to treat certain diseases, including multiple myeloma and rheumatoid arthritis. As the mechanism by which T β RI is sumoylated may differ from that of other proteins, it may be possible to specifically block TGF- β signalling by inhibition of T β RI sumoylation, providing a new strategy for treatment of diseases induced by aberrant TGF- β signalling.

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p53: The Janus of autophagy?

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The autophagy pathway functions in adaptation to nutrient stress and tumour suppression. The p53 tumour suppressor, previously thought to positively regulate autophagy, may also inhibit it. This dual interplay between p53 and autophagy regulation is enigmatic, but may underlie key aspects of metabolism and cancer biology.

p53, the ‘guardian of the cellular genome’, is the most commonly mutated gene in human cancers¹. In response to DNA damage, oncogenic activation, hypoxia or other forms of stress, p53 acts through both transcription-dependent and -independent mechanisms to coordinate cellular responses, which either prevent or repair genomic damage or eliminate potentially oncogenic cells. Although the best-studied functions of p53 relate to its control of cell-cycle arrest and cell death, increasing evidence suggests that this protein represents a central node in stress- and nutritional-response networks. These diverse activities of p53 are important not only in tumour suppression but also in metabolism, development, ageing and neurodegeneration^{1–3}. Another recently described p53-regulated cellular process is autophagy, a lysosomal pathway of cellular self-digestion, which represents an ancient mechanism used by eukaryotic cells to adapt to different forms of cellular stresses⁴. Previously, p53 activation was shown to induce autophagy^{5–10}; however, on page 676 of this issue, Tasdemir *et al.* show that basal levels of p53 inhibit autophagy¹¹.

Autophagy is induced in response to various stress stimuli, including starvation, trophic factor deprivation, hypoxia, endoplasmic reticulum (ER) stress and oxidative stress⁴. Under these conditions, autophagy is induced

through signalling events that commonly, but not invariably, involve activation of the nutrient energy sensor AMP kinase (AMPK), and inhibition of TOR (target of rapamycin). Formation of the autophagosome, a double-membraned vesicle that sequesters the cargo destined for degradation inside the lysosome, is mediated by a set of evolutionarily conserved proteins known as the Atg (autophagy-related) proteins. Through catabolism, autophagy supplies cells with amino acids and energy, allowing them to maintain vital functions and successfully adapt to environmental stress. Autophagy also has an essential role in cellular housekeeping, through routine protein and organelle turnover and the degradation of damaged organelles, toxic aggregate-prone mutant proteins and intracellular pathogens. Thus, autophagy has diverse physiological functions, including stress adaptation, development, lifespan extension, immunity and protection against neurodegeneration.

Autophagy can also function as a tumour suppressor or cell-survival pathway^{4,12}. Deletion of autophagy genes, such as *UVRAG* and *beclin 1*, are common in human cancer. Many of these genes, including *beclin 1*, *atg4C* and *atg5*, function as tumour suppressors in knockout or tumour xenograft mouse models. Loss of autophagy genes leads to increased DNA damage, chromosomal instability and deregulated control of cell growth, indicating a potential overlap in tumour suppressor-related autophagy effects and p53 actions. Paradoxically, elevated autophagy, often associated with the tumour microenvironment and/

or treatment with cytotoxic agents, can also increase tumour cell survival and in this sense, is pro-oncogenic.

The role of autophagy in tumour suppression is consistent with previous studies indicating that p53 positively regulates autophagy (Fig. 1a). For example, genotoxic stress caused by DNA-damaging agents induces p53-dependent autophagy^{5,6}. Similarly, oncogenic activation, simulated by forced expression of ARF or p53, induces autophagy in human cancer cells⁷. The mechanisms of p53-dependent induction of autophagy are still incompletely understood, but are thought to involve both transcription-independent functions (for example, AMPK activation), as well as transcription-dependent functions (for example, upregulation of mTOR inhibitors, PTEN and TSC1, or the p53-regulated autophagy and cell death gene, DRAM)^{5,8}. In some cases, p53-induced autophagy may lead to cell death and this can be blocked by DRAM siRNA⁸. However, in *cmyc*-driven lymphomas, p53-mediated autophagy increases cell survival, as blockade of autophagosomal maturation enhances p53-mediated tumour regression and tumour-cell death^{9,10}. These seemingly disparate effects of p53-mediated autophagy on life and death decisions of the cell may be cell-type or stimulus-specific, and/or reflect the activation of a different constellation of p53 signals.

The mysteries underlying p53 regulation of autophagy extend beyond the question of whether p53-mediated autophagy is pro-death or pro-survival. Tasdemir *et al.* directly challenge the notion that p53 is a positive regulator

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