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The emerging roles of deubiquitylating enzymes (DUBs) in the TGF and BMP pathways

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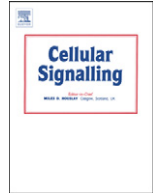
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Review

The emerging roles of deubiquitylating enzymes (DUBs) in the TGF β and BMP pathways



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ABSTRACT

The members of the transforming growth factor beta (TGF β) family of cytokines, including bone morphogenetic proteins (BMP), play fundamental roles in development and tissue homeostasis. Hence, aberrant TGF β /BMP signalling is associated with several human diseases such as fibrosis, bone and immune disorders, cancer progression and metastasis. Consequently, targeting TGF β signalling for intervention potentially offers therapeutic opportunities against these diseases. Many investigations have focussed on understanding the molecular mechanisms underpinning the regulation of TGF β signalling. One of the key areas has been to investigate the regulation of the protein components of the TGF β /BMP signal transduction pathways by ubiquitylation and deubiquitylation. In the last 15 years, extensive research has led to the discovery and characterisation of several E3 ubiquitin ligases that influence the TGF β pathway. However, the research on DUBs regulating the TGF β pathway has received prominence only recently and is still an emerging field. This review will provide a concise summary of our current understanding of how DUBs regulate TGF β signalling.

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1. Introduction

The TGF β family of cytokines comprises some 40 members including the bone morphogenetic protein (BMP) and controls a plethora of context-dependent cellular processes including proliferation,

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differentiation, extracellular matrix production, motility, survival and fate. Abnormal TGF β signalling is linked to the manifestation of multiple human diseases, including fibrosis, immune disorders and cancer [1–4]. TGF β signalling is initiated when the ligands bind to a pair of cognate receptor serine/threonine protein kinases (termed type I and type II) on the cell surface. This triggers the phosphorylation of intracellular receptor regulated SMAD transcription factors (R-SMADs) by type I receptor kinases [5]. TGF β ligands are divided into two subfamilies: the TGF β subfamily, which primarily signals through the phosphorylation of SMADs 2 and 3, and the BMP subfamily, which signals through SMADs 1/5/8 [5] (Fig. 1). In the canonical pathway, the phosphorylated R-SMADs interact with SMAD4 and translocate to the nucleus, where together with other co-factors they regulate the transcription of over 500 target genes [5–7]. The context-dependent transcriptional programme driven by the TGF β signals modulates cell behaviour [8–10].

The powerful action of TGF β cytokines in cells and tissues is tightly regulated. Complex biochemical mechanisms have evolved to intricately control the extent, duration and potency of signalling in response to TGF β cytokines. From secreted molecules that act as ligand traps to eventual transcriptional events that provide positive or negative feedback, complex regulatory inputs establish a dynamic fine-tuning of the TGF β pathway [11,12]. Regulation is also achieved through the addition or removal of post-translational modifications, such as phosphorylation and ubiquitylation, on core protein components of the TGF β signalling pathway that alters their activity or stability [13,14].

The regulatory inputs collectively shape and define the nature of cellular responses to TGF β /BMP signals in diverse biological processes and contexts. Understanding the molecular details of how TGF β signalling is regulated in cells could be key to unravelling new opportunities for therapeutic intervention against diseases associated with abnormal signalling.

Reversible ubiquitylation of key components of the TGF β pathway, including the type I TGF β /BMP receptor kinases and SMADs, is known to play a critical role in regulating the outcome of TGF β signalling [14–19]. Much is known about the roles of various E3 ubiquitin ligases in regulating their stability. However, investigations into the regulation of the TGF β /BMP pathways by DUBs primarily constitute recent progress and are still emerging.

2. Ubiquitylation of the TGF β /BMP pathway components

Ubiquitylation is a reversible post-translational modification that is essential in many cellular regulatory mechanisms [20,21]. During the ubiquitylation cascade, ubiquitin is attached to target proteins through the concerted actions of an E1-ubiquitin activating enzyme, an E2-ubiquitin conjugating enzyme and an E3-ubiquitin ligase. This cascade is initiated by the ATP dependent activation of ubiquitin by the E1. The E1 links the C-terminal glycine residue of ubiquitin via a thioester bond to a cysteine residue within its active site. The activated ubiquitin intermediate is then transferred to a cysteine residue

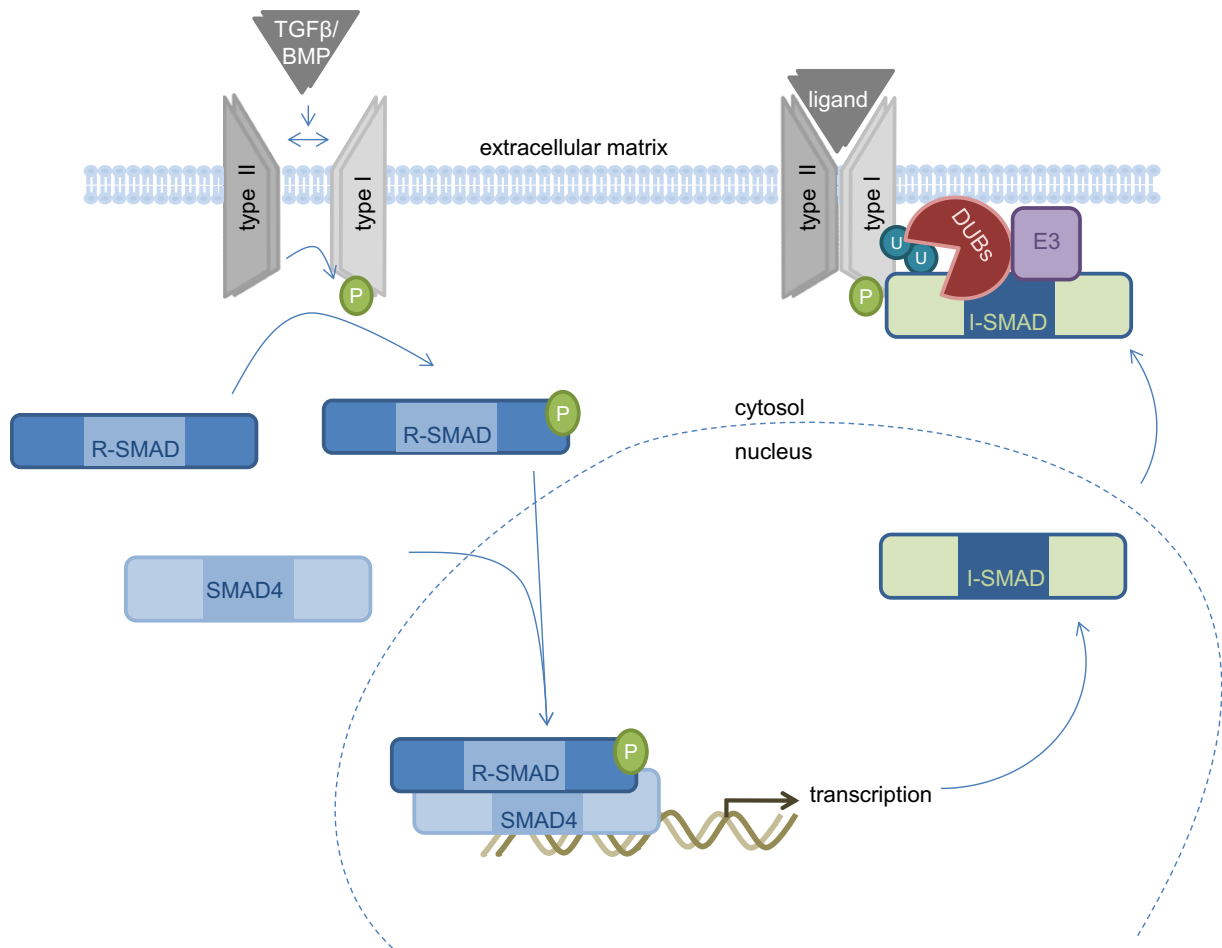


Fig. 1. An overview of the core-components of TGF β /BMP signalling. Ligand binding to serine/threonine receptor kinases induces TGF β /BMP signalling and leads to quaternary complex formation of type I and type II receptors. When in close proximity, the type II receptor kinase phosphorylates and activates the type I receptor kinase. The activated type I receptor kinase can then phosphorylate R-SMADs at their conserved C-termini. TGF β ligands induce the phosphorylation of SMAD2/3, whereas BMP ligands promote SMAD1/5/8 phosphorylation. Phosphorylated R-SMADs are then able to form a complex with SMAD4. The R-SMAD–SMAD4 complex travels to the nucleus where TGF β /BMP-mediated target gene transcription is initiated. Inhibitory SMADs (SMAD6/7) are also transcribed and act in a feedback loop, as SMAD6/7 compete with R-SMADs for receptor binding and bind to E3 ligases and/or DUBs for the regulation of receptor ubiquitylation.

of the E2 enzyme. In the human genome, there are ~45 different E2 enzymes, which determine the type of the ubiquitin chain linkage that is assembled on the substrate. The ubiquitin-loaded E2 then pairs with specific E3-ubiquitin ligases, which facilitates the conjugation of C-terminal glycine of ubiquitin via an isopeptide bond to the ϵ -amino group of the target lysine on the substrate protein, which can be ubiquitin itself. More than 600 E3 ubiquitin ligases are encoded in the human genome, and in certain cases their associated substrate adaptor proteins often recruit substrates, thereby providing specificity in the ubiquitylation process [22–26]. Target proteins can be monoubiquitylated, multi-monoubiquitylated or by repeated action of the E1, E2 and E3 ligases ubiquitin can be added onto one of several lysine residues or the α -amino group of the attached ubiquitin to form unique polyubiquitin chains. The nature of ubiquitylation on the target protein defines its fate, from altered sub-cellular localisation or activity to destruction through the proteasome or lysosome [27–29].

The E3 ubiquitin ligases involved in the ubiquitylation of TGF β pathway components have been reviewed extensively [14,15,17,30–32] (Fig. 1). However, very little is known about the E2 enzymes that are involved in the ubiquitylation of TGF β pathway components, with UBE2L3 and UBE2O the only members implicated [33,34]. Furthermore, the precise ubiquitylation sites on most of the TGF β pathway components known to be ubiquitylated have not been established at the endogenous level. Other than the K48-linked ubiquitin chains that mostly confer destructive fate on target proteins, there has been a lack of comprehensive linkage-type analyses on polyubiquitin chains on the TGF β -pathway components. Similarly, whether and how these chains are recognised by potential ubiquitin-binding proteins, that then modulate the fate of the target protein, remain to be defined.

3. Regulation of the TGF β /BMP pathway components by deubiquitylating enzymes

The functional consequences of ubiquitylation of the TGF β pathway components can be reversed by the action of deubiquitylating enzymes that remove the ubiquitin or ubiquitin chains attached to the target protein. Similarly, the prevention of ubiquitylation of the target proteins through inhibition of the ubiquitylation cascade components can also counter the effects of ubiquitylation to achieve pathway fine-tuning. In the TGF β pathway, both deubiquitylation and prevention of ubiquitylation of the target proteins by deubiquitylating enzymes have been reported as mechanisms to counter the effects of ubiquitylation. We provide a thorough overview of the current knowledge on the roles of deubiquitylating enzymes in the regulation of the TGF β /BMP pathways.

3.1. Deubiquitylating enzymes (DUBs)

DUBs are isopeptidases that remove ubiquitin chains or individual ubiquitin molecules attached to their target proteins. Around 100 DUBs are encoded in the human genome [35,36], which are classified into five distinct structural groups based on their catalytic domains: i. ubiquitin-specific proteases (USPs); ii. ubiquitin C-terminal hydrolases (UCHs); iii. ovarian tumour proteases (OTUs); iv. Josephins; and v. JAB1/MPN/MOV34 metalloenzymes (JAMM/MPN+) [35,36]. Members belonging to the UCH, USP, OTU and Josephin families are cysteine proteases, while those that belong to JAMM/MPN+ family are zinc metalloproteases [35,36].

The limited number of DUBs encoded by the human genome in comparison to the number of E3 ligases implies that catalytically DUBs are potentially promiscuous. Therefore, the substrate specificity and activity of DUBs have to be tightly regulated in order to ensure proper ubiquitin processing and achieve normal homeostasis in cells. One could envisage that this is achieved through conformational/post-translational modifications on the DUBs or target proteins, specific interactions with

regulatory partners or regulated sub-cellular location of the DUBs. Indeed, enzymatic activity of DUBs can be concealed by occluding the substrate-binding sites of certain DUBs and regulated by inducing conformational changes that activate the catalytic site [35–40]. Apart from their catalytic core, DUBs contain multiple domains that mediate protein–protein interactions including ubiquitin-interacting motifs, ubiquitin associated domains, ubiquitin binding domains or ubiquitin-like folds and/or zinc finger ubiquitin-specific protease domains. These domains contribute to the binding and recognition of different ubiquitin chain linkages, although some DUBs also display direct affinity for their ubiquitylated target proteins [35–40]. Furthermore, substrates can be ubiquitylated by different E3 ligases resulting in distinct ubiquitin chains. Hence, DUBs also distinguish between ubiquitin-like molecules, linear peptides, isopeptides and different types of ubiquitin linkage as well as *exo*- versus *endo*-deubiquitylation [35–40].

The research on cellular roles and regulation of DUBs is an emerging field. The modes of action, physiological substrates and roles of most DUBs are still poorly understood. The investigations of their roles in the regulation of the TGF β and BMP pathways have picked up pace in the last five years and have uncovered several insightful mechanisms, which are discussed below. A summary of all the DUBs reported so far to regulate the components of the TGF β and BMP pathways are summarised in Table 1.

The USP family, which represents the most abundant group within DUBs, is prominently reported to be involved in regulating the TGF β and BMP pathways, with USP4, USP11, USP15, USP18, USP9X and CYLD all implicated. The UCH member UCH37, OTU members A20 and OTUB1 and JAMM/JPN+ member AMSH, are also reported to regulate the TGF β pathway.

3.2. USP4, USP11 and USP15 target type I TGF β receptors for deubiquitylation in the TGF β pathway

Among the USP family of DUBs, USP4, USP11 and USP15 are highly similar and display conserved structural domains and protein sequences. They harbour a conserved DUSP (domain in USPs of unknown function) at the N-terminus and two UBLs (ubiquitin like domains), one preceding the USP catalytic domain and one within [35]. Intriguingly all three of them were independently discovered as DUBs for the type I TGF β receptors through contrasting approaches (Fig. 2A). A gain-of-function screen looking for activators of TGF β signalling identified USP4 [41], a proteomic approach looking at interactors of SMAD7 identified USP11 [42] and a siRNA loss-of-function screening looking for DUBs affecting the TGF β -induced luciferase reporter activity identified USP15 [43].

USP4 has been reported to enhance TGF β signalling by directly interacting with and deubiquitylating type I TGF β receptor, ALK5 [41]. In this study, it was reported that upon phosphorylation by

Table 1
DUBs in the TGF β /BMP signalling pathway.

DUB	Mode of action
A20	Inhibits non-canonical TGF β signalling via the TRAF6–TAK1–p38 MAPK/JNK pathway [74].
AMSH	AMSH prolongs BMP signalling by sequestering SMAD6 [67].
AMSH-LP	AMSH-LP prolongs TGF β signalling by sequestering SMAD7 [68].
CYLD	CYLD deubiquitylates SMAD7 [52] and AKT [53].
OTUB1	OTUB1 inhibits the ubiquitylation of active pSMAD2/3 [54].
UCH37	UCH37 associates with SMAD7 and ALK5 and influences TGF β -mediated transcription [65,66].
USP4	USP4 deubiquitylates ALK5 [41] and TAK1 [71].
USP9X	USP9X reverses monoubiquitylation of SMAD4 [48,49].
USP11	USP11 deubiquitylates ALK5 [42].
USP15	USP15 deubiquitylates ALK3 [45], ALK5 [43] and monoubiquitylated R-SMADs [47].
USP18	USP18 deubiquitylates the TAK1–TAB1 complex [73].

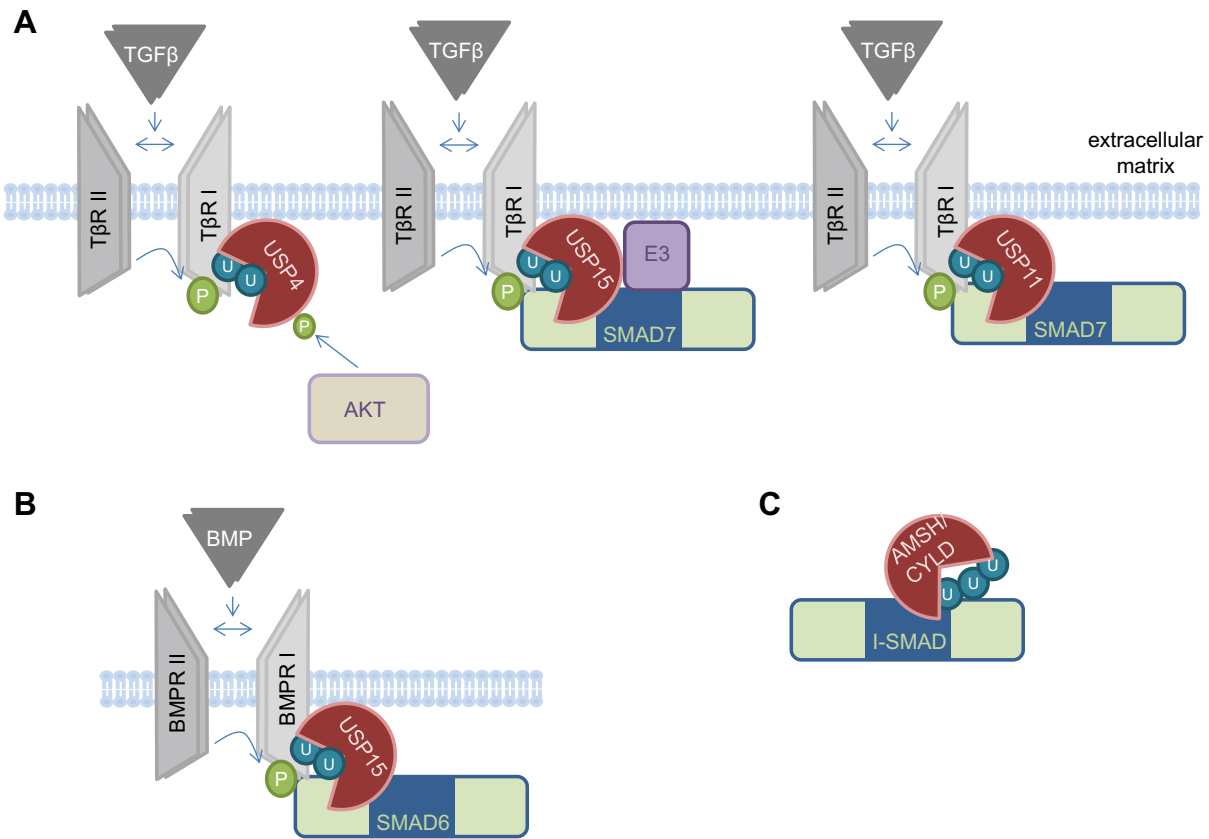


Fig. 2. TGF β /BMP receptor deubiquitylation. A) USP4, USP15, and USP11 have been identified to reverse the TGF β receptor ubiquitylation, through distinct molecular mechanisms (see text). It is not known whether these DUBs target the ligand-activated receptor complexes or just the type I receptors. B) USP15 binds to and targets the BMP type I receptor for deubiquitylation. C) CYLD and AMSH/AMSH-LP are DUBs that reverse the ubiquitylation of I-SMADs, which in turn direct E3 ligases and DUBs to the receptors.

AKT, USP4 translocates to the membrane, where it associates with ALK5, deubiquitylates it and protects it from destruction (Fig. 2A). The AKT-mediated phosphorylation of USP4 also affects its stability and DUB activity. Moreover, USP4 depletion inhibits TGF β -induced epithelial to mesenchymal transition (EMT) and AKT-induced breast cancer cell migration [41].

USP11 was identified as an interactor of SMAD7 and ALK5. When bound to ALK5, USP11 deubiquitylates and protects ALK5 from proteasomal degradation resulting in enhanced TGF β signalling. Consequently, TGF β -induced levels of phosphorylated SMAD2/3 and transcription were augmented (Fig. 2A). SMAD7, a transcriptional target of TGF β signals, negatively regulates the TGF β pathway by recruiting HECT E3 ubiquitin ligases and targeting TGF β receptors for ubiquitin-mediated degradation. USP11 could override the negative effects of SMAD7 on the TGF β pathway, demonstrating that a dynamic balance between ubiquitylation and deubiquitylation could determine the fate of ALK5. It was shown that depletion of USP11 resulted in inhibition of TGF β -induced transcription as well as EMT [42].

USP15 was reported to enhance TGF β signalling by binding to the SMAD7–SMURF2 complex and deubiquitylating ALK5 in the process [43] (Fig. 2A). Moreover, USP15 amplification was observed in glioblastoma, breast and ovarian cancers, which highly correlated with enhanced TGF β signalling activity and poor prognostic outcomes in individuals with glioblastoma [43]. Depletion of USP15 reduced the oncogenic capacity of patient-derived glioma-initiating cells through the inhibition of TGF- β signalling, suggesting a direct role for USP15 and TGF β signalling in glioblastoma pathogenesis [43].

The roles of three very closely related DUBs on type I TGF β receptors pose interesting questions. Do these DUBs work in conjunction with each other? Or could they act independently in the absence of others?

The evidence from published reports suggests that both possibilities are likely. The study that identified USP4 as a DUB for ALK5 also demonstrated that phosphorylation of USP4 on S445 triggers homomeric and heteromeric complex formation with USP11, USP15 and USP19, suggesting that these DUBs could act as a complex [41]. Similarly, a size exclusion analysis showing that USP11 elutes at a very high molecular weight fraction also suggests its existence as part of a macromolecular complex [42]. On the other hand, a recent study suggested that TGF β signalling was normal in T-cells derived from USP15-knockout mice and in A375 and HCT116 cells upon USP15 depletion [44]. Similar experiments in USP4 and USP11 knockout cells (or knockin of catalytically inactive mutants) will be important to determine whether these DUBs play critical roles in deubiquitylating ALK5. Collectively, it is likely that USP4, USP11 and USP15 act either as monomeric or heteromeric complexes and in the prolonged absence of one or two members, the third one could compensate for the loss of other two. Furthermore, the cell type or context-dependent selectivity of one of these DUBs over the others (e.g. the AKT phosphorylation site in USP4 is not conserved in USP11 or USP15) is a possibility and needs to be investigated further.

3.3. USP15 targets type I BMP receptors for deubiquitylation in the BMP pathway

In addition to its regulation of type I TGF β receptors, USP15 has been reported to act as a deubiquitylase for type I BMP receptors. USP15 was shown to interact with SMAD6, a negative regulator of BMP signalling (Fig. 2B). Further characterisation of the role of USP15 in the BMP pathway established that USP15 enhances BMP-induced phosphorylation of SMAD1 and transcription. It was shown that USP15 interacts with type I BMP receptors, including ALK3, co-localises with ALK3 at the membrane and deubiquitylates ALK3, thereby

countering the inhibition of the BMP pathway caused by SMAD6 [45]. This study further demonstrated that USP15 was critical for BMP signalling in mammalian cells, mouse osteoblastic differentiation and *Xenopus* embryogenesis. Interestingly, related DUBs USP4 and USP11 have been shown to have no effects on BMP signalling [41,42,45]. A possible role for USP15 in BMP signalling was also proposed by a genome-wide loss-of-function study of DUBs that impact dorsoventral patterning during zebrafish development [46].

3.4. USP15 targets monoubiquitylated R-SMADs

Prior to its reported roles in deubiquitylating the type I TGF β /BMP receptors, USP15 was reported to interact with and deubiquitylate monoubiquitylated R-SMADs, resulting in enhanced TGF β and BMP signalling [47]. Monoubiquitylation of R-SMADs reportedly occurs at the DNA-binding domain of R-SMADs, thereby preventing their association with DNA at the promoters (Fig. 3). This report demonstrated that USP15 was required for TGF β and BMP responses in both mammalian cells and *Xenopus* embryos [47]. Although the mechanisms by which USP15 acts on TGF β and BMP signalling proposed in this study differ from those described above, the fundamental observations that USP15 enhances both TGF β and BMP signalling are consistent with studies described above [43,45]. As DUBs are promiscuous, it is possible that USP15 acts by targeting multiple substrates to regulate TGF β and BMP signalling.

3.5. USP9X targets SMAD4 for deubiquitylation in the TGF β pathway

A siRNA screening of DUBs identified USP9X/FAM as a DUB that affected TGF β -induced transcription of p21 without affecting phosphorylation of SMAD3. Subsequently it was shown that USP9X deubiquitylates SMAD4 that is monoubiquitylated at K519 (Fig. 3). It was shown that

monoubiquitylation of SMAD4 inhibits transcription by impeding its association with phospho-SMAD2/3. Thus, USP9X enhances TGF β signalling by countering SMAD4 monoubiquitylation [48]. The study further demonstrated that USP9X is required for TGF β -induced growth arrest and cellular migration. Further evidence for the role of USP9X on TGF β signalling comes from two recent studies in *Drosophila* and mouse knockout models. In *Drosophila*, the absence of *fat facets* (USP9X homologue) inhibits the activity of Medea (SMAD4 homologue) below the threshold necessary for adequate decapentaplegic (BMP homologue) signalling due in part to excessive ubiquitylation of Medea on K738 (equivalent of human SMAD4 K519). The study further showed that the USP9X-mediated stabilisation of SMAD4 was critical for regulation of the zygotic BMP2/4 (decapentaplegic) morphogen gradient that determines dorsal-ventral axis formation [49]. Similarly, in mice when USP9X was deleted from neural progenitors, TGF β -dependent axon genesis was inhibited [50]. The levels of SMAD4 ubiquitylation in USP9X-null axons were not tested in this study [50].

3.6. CYLD modulates SMAD7 and SMAD3 levels to inhibit TGF β signalling

CYLD is a member of the USP DUBs that selectively hydrolyses K63-linked polyubiquitin chains [51]. Enhanced levels of regulatory T cells (Tregs), whose development is promoted by TGF β signalling, were observed in CYLD-knockout mice. Further characterisation showed that CYLD controls the development of Tregs by deubiquitylating SMAD7, thereby inhibiting TGF β signalling (Fig. 2C). CYLD was shown to target K63-linked ubiquitylation of SMAD7 at K360 and K374, which are ubiquitylation sites required for the activation of TAK1 and p38 MAP kinases. Knockdown of SMAD7 or inhibition of p38 activity in primary T cells hindered Treg differentiation, demonstrating that TGF β signalling in T cells and the development of Tregs are regulated by K63-linked ubiquitylation of SMAD7 [52]. An alternative mechanism of how TGF β signalling is regulated by CYLD has also been proposed, again using

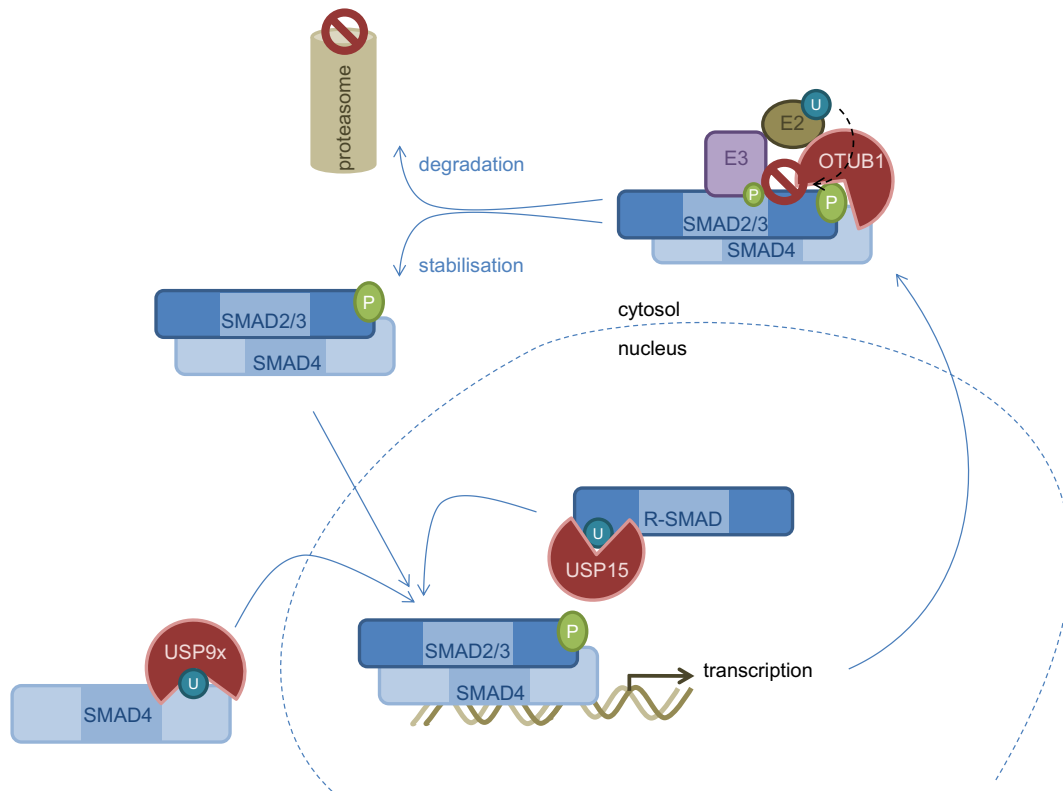


Fig. 3. SMAD deubiquitylation: USP9X and USP15 reverse the monoubiquitylation of SMAD4 and R-SMADs respectively, which enables the formation of transcription factor complex and efficient transcription of TGF β /BMP-mediated target genes. OTUB1 recognises TGF β -activated SMAD2/3 and inhibits their ubiquitylation, thereby stabilising the complex and enhancing TGF β signalling.

CYLD knockout mice [53]. This study proposes that CYLD inhibits TGF β signalling by decreasing the stability of SMAD3 via the AKT–GSK3–CHIP pathway. CYLD deubiquitylates K63-polyubiquitylated AKT, resulting in the inhibition of AKT. This leads to activation of GSK3 β , which promotes CHIP-mediated SMAD3 degradation, thereby inhibiting TGF β signalling and fibrotic responses [53]. Based on this study, it would be interesting to investigate whether USP4 function, which is also modulated by AKT as described above, is also affected in CYLD knockout mice [41,53].

3.7. OTUB1 interacts with active SMAD2/3 and impacts TGF β signalling

OTUB1, a member of the OTU family of DUBs, has been shown to inhibit the ubiquitylation of only TGF β -activated SMAD2/3 [54]. While OTUB1 cleaves K48-linked polyubiquitin chains, it has also been shown to act in a non-canonical mode. The non-canonical mode of action, where the catalytic activity of OTUB1 is dispensable, relies on its interaction with ubiquitin charged-E2 and inhibition mediated by the ubiquitin-binding motif in the N-terminus of OTUB1 [55–62]. Binding to uncharged E2s on the other hand can increase the catalytic activity of OTUB1 [63]. OTUB1 was identified as an interactor of SMAD3 only under conditions where cells were treated with TGF β [54]. Further characterisation revealed that endogenous OTUB1 is recruited to the active phospho-SMAD2/3 complex only upon TGF β induction. The phosphorylation of SMAD2/3 at the C-terminus was necessary and sufficient for the interaction with OTUB1. It was shown that OTUB1 is critical for TGF β -mediated gene transcription and cellular migration. Interestingly, it was demonstrated that OTUB1 stabilises the active SMAD2/3 complex by preventing the ubiquitylation of phospho-SMAD2/3 through inhibition of the E2 ubiquitin-conjugating enzymes, independently of its catalytic activity (Fig. 3). The findings from this study highlight the dynamic interplay between phosphorylation and ubiquitylation processes in the regulation of the TGF β pathway [54].

3.8. UCH37 (UCHL5) targets ALK5 in the TGF β pathway

The UCH family of DUBs consists of 4 members that are primarily implicated in polyubiquitin chain trimming/editing [64]. One of its members, UCH37, has been reported to deubiquitylate the type I receptor (ALK5) and sustain early TGF β pathway activation [65,66]. UCH37 has its catalytic domain in the N-terminus and harbours a coiled-coil domain at its C-terminus [35]. It has been shown that UCH37 is directed to ALK5 via its interaction with SMAD7. UCH37 also weakly binds to SMAD2 and 3, however it only deubiquitylates ALK5 and hence modifies TGF β -induced transcription [65]. UCH37 especially influences TGF β -mediated transcription during the early phase of TGF β receptor activation. UCH37 knockdown decreases transcription of TGF β -dependent target genes and also slows lateral cell migration [66].

3.9. The role of zinc metalloproteases AMSH and AMSH-LP in TGF β and BMP signalling

AMSH has been shown to interact with SMAD6 upon BMP receptor activation [67]. It has been proposed that AMSH sequesters SMAD6, thus impeding its inhibitory role on the BMP pathway and thereby resulting in a prolonged BMP signalling. Similarly, AMSH-LP has been reported to sequester SMAD7, thereby exerting a positive impact on the TGF β pathway (Fig. 2C) [68]. However, the roles of AMSH and AMSH-LP DUB activity on BMP and TGF β signalling were not investigated at the time of their discoveries, partly because they had not been established as DUBs at the time. AMSH and AMSH-LP have since been reported to selectively deubiquitylate K63-ubiquitin chains [69].

3.10. DUBs implicated in the regulation of non-canonical TGF β signalling

Most DUBs implicated in the non-canonical TGF β pathway have focussed primarily on the TRAF6–TAK1–p38 MAPK signalling axis. While TGF β induced p38 MAPK activation plays an important role in immune signalling, the role of TAK1 on TGF β -induced p38 MAPK has recently been questioned [70]. USP4 has been reported to deubiquitylate TAK1 [71]. It was shown that TNF α induces the association of USP4 with TAK1 and deubiquitylation of K63-linked ubiquitin chains from TAK1, leading to the inhibition of NF- κ B production. Similarly, USP4 inhibited IL-1 β -, LPS- and TGF β -induced NF- κ B production [71].

TGF β together with IL-6 initiates T helper 17 (Th17) cell differentiation [72]. Recently, USP18 has been shown to regulate T cell activation and Th17 cell differentiation by associating with and deubiquitylating the TAK1–TAB1 complex, thereby restricting IL-2 expression. USP18 knockout mice were found to be defective in Th17 generation and resistant to experimental autoimmune encephalomyelitis. The negative regulation of TAK1 activity during Th17 differentiation by USP18, led the authors to suggest USP18 as a target to treat autoimmune diseases [73].

A20 has been shown to be recruited to TRAF6 by SMAD6 and abolish K63-linked polyubiquitylation of TRAF6, resulting in inhibition of the TRAF6–TAK1–p38 MAPK/JNK pathway. Knockdown of the deubiquitylating enzyme A20 or its transporter SMAD6, resulted in increased apoptosis, while maintaining TAK1 and p38 MAPK/JNK phosphorylation, indicating that SMAD6 and A20 are essential for the negative regulation of non-canonical TGF β signalling [74].

4. Perspective

The past five years have seen a huge surge on research reports and interest on DUBs regulating the BMP and TGF β pathways. While many DUBs purported to play essential roles on the TGF β and BMP pathways have been discovered, the precise molecular details of their function, substrate selectivity and regulation in the TGF β and BMP pathways remain to be defined in some cases. Several DUBs are known to regulate the deubiquitylation of TGF β and BMP type I receptors, R-SMADs, SMAD4 and SMAD7. For the most part, we still do not know the precise lysine residues on which the initial ubiquitin is attached and the type of subsequent ubiquitylation chains, if any, that are formed. Furthermore, precisely how selective DUBs are recruited to specific targets in the TGF β pathway is not completely understood. Different extra-cellular signals and post-transcriptional modifications within the DUBs or targets are likely to tweak substrate recognition and catalytic activity of DUBs. Further research needs to focus on identifying such signals that modify DUB activity, their subcellular localisation and their selection of substrates and investigate the importance of potential interplay between PTMs (such as phosphorylation or acetylation) and ubiquitylation.

As we have discussed above, various diseases are linked to the malfunction of the TGF β pathway. The findings that several DUBs target components of the TGF β and BMP pathways for deubiquitylation to drive normal signalling imply that DUBs could be suitable candidate targets for the development of small molecule inhibitors of the TGF β and BMP pathways. Selective inhibitors of these DUBs could be potentially useful therapeutically against pathologies associated with abnormal TGF β and BMP signalling. Furthermore, there are recent reports that imply certain DUBs are themselves modified or mis-expressed in cancers [43] thereby affecting TGF β signalling. While research projects aiming to develop selective inhibitors of DUBs are underway, caution needs to be taken, as each DUB would be predicted to target many substrates beyond TGF β /BMP signalling. A better strategy would therefore be to understand the molecular mechanisms by which a DUB is recruited to its target in the TGF β and BMP pathways. This would allow for the development of small molecules that selectively inhibit the interaction between the DUB and its target in the TGF β pathway but not other

targets. Indeed, the tail phosphorylation-dependent recruitment of OTUB1 to SMAD2/3 is a case for which interaction determinants are known [54].

Many studies reporting on discoveries of DUBs in the TGF β and BMP pathways have relied on RNAi strategies to investigate loss-of-function impact. While RNAi strategies are useful tools, there are limitations ranging from limited knockdowns of targets to potential off-target effects. New and better technologies now exist that could and should be harnessed to definitively establish the roles of selective DUBs in the TGF β and BMP pathways. Targeted DUB knockouts in cells (either derived from knockout mice or using CRISPR/CAS9 technology [75,76] to knockout genes in somatic cells) could allow for restoration of DUB mutants lacking catalytic activity or substrate-interaction determinant. Further issues of potential redundancy could be addressed in such systems. The next decade is sure to address the molecular mechanisms by which DUBs regulate the TGF β and BMP pathways.

Conflict of interest

The authors declare no conflict of interests.

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