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REVIEW

Ubiquitination and deubiquitination emerge as players in idiopathic pulmonary fibrosis pathogenesis and treatment

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Idiopathic pulmonary fibrosis (IPF) is a fatal fibrotic lung disease that is associated with aberrant activation of TGF-β, myofibroblast differentiation, and abnormal extracellular matrix (ECM) production. Proper regulation of protein stability is important for maintenance of intracellular protein homeostasis and signaling. Ubiquitin E3 ligases mediate protein ubiquitination, and deubiquitinating enzymes (DUBs) reverse the process. The role of ubiquitin E3 ligases and DUBs in the pathogenesis of IPF is relatively unexplored. In this review, we provide an overview of how ubiquitin E3 ligases and DUBs modulate pulmonary fibrosis through regulation of both TGF-β–dependent and –independent pathways. We also summarize currently available small-molecule inhibitors of ubiquitin E3 ligases and DUBs as potential therapeutic strategies for the treatment of IPF.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and devastating lung disease characterized by fibroblast differentiation and extracellular matrix (ECM) accumulation, which ultimately results in irreversible destruction of alveolar architecture (1). IPF is the most common of the so-called idiopathic interstitial pneumonias, and patients with IPF exhibit a median survival of only 3–4 years, a prognosis that is worse than that for many cancers (2). A conservative estimate of incidence ranges from 3–9 cases per 100,000 people per year in Europe and North America (3). While pirfenidone and nintedanib can slow the rate of deterioration of pulmonary function and are currently available for IPF therapy worldwide, neither drug has been shown to improve survival or quality of life (4). Currently, the only cure for IPF is lung transplantation (2). Although the causes of the disease remain unknown, the rapidly expanding fields of cellular and molecular biology continue to explore the fundamental process involved in its initiation and progression. A better understanding of IPF etiology has potential to identify molecular targets for therapeutic intervention.

TGF-β is believed to play a vital role in the progression of fibrotic diseases (5). Among the three isoforms of TGF-β (6), TGF-β1 has been implicated as a critical factor in the development of IPF. In the lungs, TGF-β1 is made by a variety of cell types, such as alveolar macrophages and alveolar epithelial cells. TGF-β1 is thought to drive pulmonary fibrosis, at least in part, by promoting the differentiation of quiescent resident fibroblasts into myofibroblasts, which results in aberrant ECM deposition (7). In animal models of pulmonary fibrosis, TGF-β blockade reduces fibroblast activation, differentiation, and collagen deposition (8, 9). TGF-β transduces signals via membrane serine/threonine kinase receptors (TβRI/TβRII) to activate SMAD protein–dependent and –independent pathways. The effects of TGF-β on ECM protein expression are thought to be mediated primarily at the level of transcription. While numerous studies have focused on the expression of RNA species in IPF pathogenesis (10–14), other studies have shown that protein posttranslational modification plays an important role in IPF pathogenesis (15). Accumulating evidence suggests that ubiquitination — a posttranslational modification that is central to maintenance of intracellular protein levels, protein-protein interactions, protein localization, and enzyme activity — contributes to pathologic tissue remodeling in IPF. Several review articles have also highlighted the role of ubiquitination in TGF-β signal transduction (16, 17). Here, we review recent findings regarding the importance of ubiquitination and deubiquitination in IPF.

Conflict of interest: The authors have declared that no conflict of interest exists.

Published: May 17, 2018

Reference information:
JCI Insight. 2018;3(10):e120362.
https://doi.org/10.1172/jci.insight.120362.

Ubiquitination and deubiquitination

Ubiquitin is an evolutionarily conserved 8.5-kDa protein that posttranslationally modulates substrate protein stability and localization, thereby regulating a wide range of cellular functions. The ubiquitination process is carried out through a cascade of enzymatic reactions catalyzed by ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). These three types of enzymes sequentially mediate the linkage of ubiquitin molecules to lysine residues on the target proteins (18). Ubiquitin conjugation is initiated with the activation of a ubiquitin molecule by the E1 enzyme, followed by transfer of ubiquitin to the E2 enzyme. Subsequently, the E3 enzyme links with the targeted substrate protein and the E2-ubiquitin complex, and mediates the transfer of ubiquitin to lysine residues on the targeted protein (19). Target proteins can be mono-, multimono-, and polyubiquitinated, and ubiquitination can be further characterized as linear, Lys63, Lys48, Lys11, and other Lys links based on the ubiquitin linkage types. The type of ubiquitin modification can differentially affect their substrates, such as modulating signaling, protein-protein interactions, and localization, as well as lysosomal and proteasomal degradation (Figure 1).

Deubiquitination is mediated by deubiquitinating enzymes (DUBs), which are cysteine proteases that cleave ubiquitin molecules from ubiquitin-conjugated protein substrates (20). Because of their ability to reverse ubiquitination, DUBs can regulate diverse cellular functions. The dynamic balance between ubiquitination and deubiquitination is required for protein turnover and function (Figure 1).

TGF-β–targeting ubiquitin E3 ligases in lung fibrosis

Ubiquitin E3 ligases can contribute to lung fibrosis by regulation of TGF-β–dependent pathways. Below, we discuss E3 ligases that have been shown to influence TGF-β signaling.

FIEL1. Fibrosis-inducing E3 ligase 1 (FIEL1, encoded by *KIAA031*) promotes polyubiquitination and proteasomal degradation of protein inhibitor of activated STAT4 (PIAS4) (21), which mediates the sumoylation of SMAD3, leading to inhibition of SMAD3/4–dependent profibrotic transcription (22, 23). Thus, FIEL1 exhibits a profibrotic property by enhancing signaling via SMAD3/4 in lung fibroblasts. FIEL1 has also been shown to be highly expressed in lung tissues from IPF patients and bleomycin-challenged mice. In the murine model of bleomycin-induced lung fibrosis, overexpression of FIEL1 activates TGF-β signaling and enhances lung fibrosis, whereas knockdown of FIEL1 attenuates fibrotic responses. Further, Lear et al. showed that a small-molecule inhibitor of FIEL1 (BC-1485) increased the half-life of PIAS4 and ameliorated lung fibrosis in murine models (21). FIEL1 was also shown to affect protein levels, total cell accounts, and CXCL1 levels in bronchoalveolar lavage fluid within three days after bleomycin challenge, suggesting that FIEL1 may also contribute to the lung injury phase of IPF. Further exploration of the role of FIEL1 in the pathogenesis of acute lung injury may provide insight into mechanisms by which FIEL1 regulates lung fibrosis.

Arkadia. Arkadia is a ubiquitin E3 ligase originally identified as a key player in amplification of nodal signaling, which may play a critical role in early embryonic development (24, 25). Koinuma et al. demonstrated that arkadia is widely expressed in mammalian tissues and promotes TGF-β signaling (26). Arkadia was recently shown to enhance TGF-β signaling by physically interacting with TGF-β–inhibiting SMAD7, inducing its polyubiquitination and proteasome-dependent degradation (26). Consistent with in vitro experiments, Elkouris et al. found that in bleomycin-treated and TGF-β–injured mice, arkadia interacts with methylated SMAD7 and potentiates TGF-β–mediated ECM production (27). Arkadia has also been associated with progression of renal fibrosis through catalyzing the ubiquitination and degradation of SMAD7 (28, 29). Levy et al. and others established that arkadia is required for TGF-β–induced proteasomal degradation of SnoN and Ski, which directly suppress transcription of TGF-β–responsive genes and play important roles in regulating the fibrotic response (30–32). These findings indicate that arkadia targets multiple components of TGF-β signaling and exhibits profibrotic properties. In addition to regulating TGF-β signaling, arkadia facilitates the DNA-damage response through ubiquitination and degradation of sumoylated xeroderma pigmentosum C (XPC) (33), which is involved in DNA repair. Though XPC has not been evaluated in lung fibrosis, alteration of DNA-damage repair in lung epithelial cells has been shown to contribute to the pathogenesis of lung fibrosis (34). It is possible that arkadia also contributes to the progression of lung fibrosis through targeting DNA repair signaling. Developments of therapeutic strategies that target arkadia have potential for treating pulmonary fibrosis or other fibrotic diseases.

TIF1γ. Transcriptional intermediary factor 1 γ (TIF1γ, also known as tripartite motif 33 [TRIM33] and ectodermin) belongs to the evolutionarily conserved TIF1 family of nuclear factors. TIF1γ is also

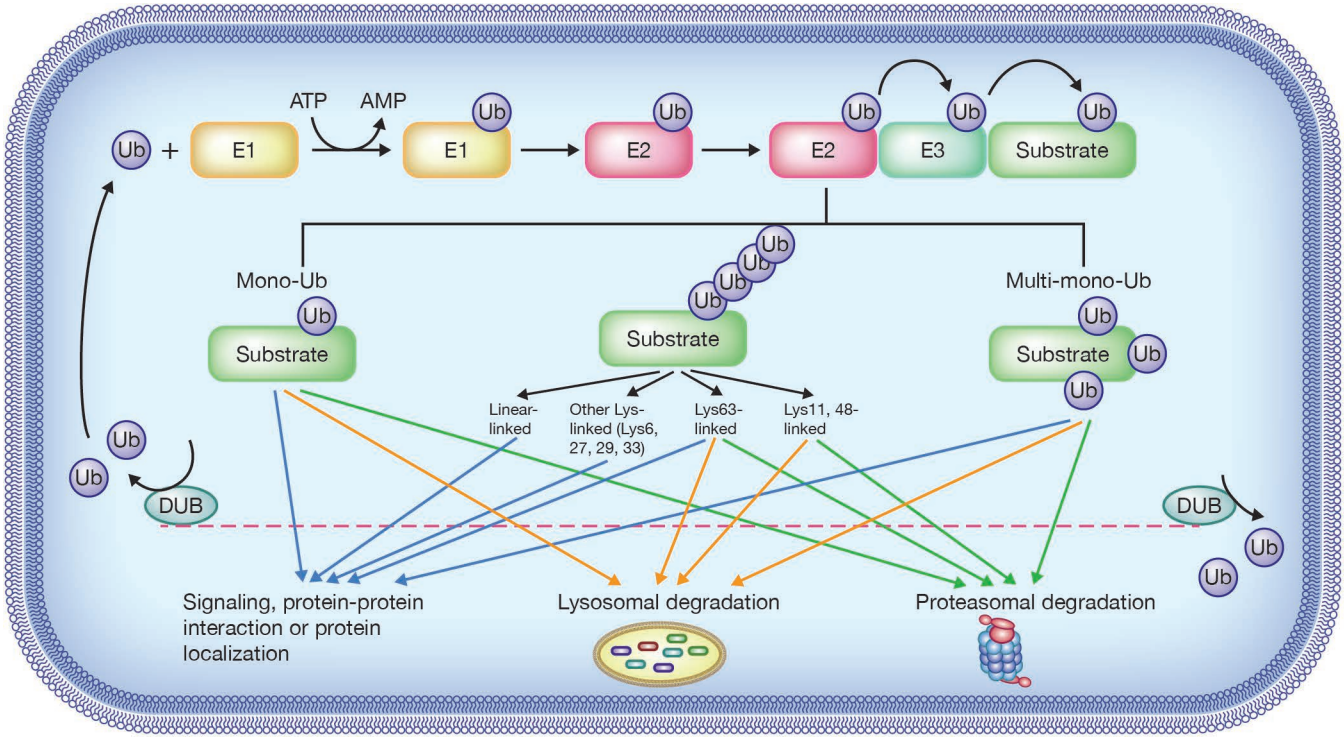

Figure 1. Protein ubiquitination and deubiquitination. E1, E2, and E3 enzymes mediate protein ubiquitination, which is reversed by DUBs. The balance between the actions of ubiquitin E3 ligases and DUBs controls ubiquitin pool dynamics, protein homeostasis, and cellular functions. Illustrated by Rachel Davidowitz.

recognized as a ubiquitin E3 ligase (35) and is involved in several biologic processes, such as embryonic development, cell differentiation, transcription elongation, and cell mitosis (36–38), as well as tumor growth (39, 40). TIF1γ has also been reported to have a role in TGF-β signaling and the pathogenesis of pulmonary fibrosis. Specifically, TIF1γ mediates SMAD4 monoubiquitination, thereby promoting its nuclear export and inhibiting the formation of SMAD2/3/4 nuclear complexes (41). Additionally, TIF1γ interacts with phosphorylated SMAD2/3 to form an alternative SMAD2/3/TIF1γ complex, which inhibits interaction of SMAD2/3 with SMAD4, resulting in dampened TGF-β signaling (42). Bellaye et al. demonstrated that the small heat-shock protein αB-crystallin disrupts TIF1γ interaction with SMAD4, preventing its monoubiquitination, limiting its nuclear export, and promoting the TGF-β1/SMAD4–mediated profibrotic pathway in experimental pulmonary fibrosis (43). These studies demonstrate that TIF1γ is an antifibrotic ubiquitin E3 ligase. While downregulation of *TIF1G* gene expression has been observed in hematopoietic cells from patients with chronic myelomonocytic leukemia (CMML) (44), the molecular regulation of TIF1γ expression in lung fibrosis has not been reported. The generation of a TIF1γ-deficient murine model of lung fibrosis using mice may reveal a contribution of TIF1γ in IPF pathogenesis. Future studies may also focus on the identification of small-molecule inhibitors for αB-crystallin to promote TIF1γ-SMAD4 interaction, thus reducing fibrotic responses.

STUB1. STIP1 homology and U box–containing protein 1 (STUB1, previously known as carboxyl terminus of HSC70-interacting protein [CHIP]) is a chaperone-dependent ubiquitin E3 ligase (45). STUB1 has been reported to modulate degradation of multiple substrates, including receptor tyrosine kinase ERBB2, p53, protein phosphatase CIP2A, SRC-3, and transcription factor EB (TFEB), thus regulating cell proliferation, metastasis, autophagy, and tumor progression (46, 47). Xin et al. found that STUB1 reduces the basal level of SMAD3 through ubiquitin-proteasome–mediated degradation (48), indicating that STUB1 inhibits TGF-β signaling; however, there is no direct evidence showing the effect of STUB1 on TGF-β–induced ECM accumulation. NOX4/NADPH oxidase expression is increased in pulmonary fibroblasts from patients with IPF (49), and NOX4 regulates TGF-β1–induced fibroblast differentiation by modulating activation of SMAD2/3 (49). Recently, Tsubouchi et al. demonstrated that azithromycin (AZM) increases NOX4 degradation in the proteasome and exhibits an

antifibrotic effect in bleomycin-injured mice (50). Interestingly, AZM increased STUB1 protein levels and proteasome activity, and AZM-mediated NOX4 degradation was dependent on STUB1 (50), indicating an antifibrotic effect of STUB1. These studies demonstrate that AZM may be beneficial for IPF by increasing protein levels of STUB1 and promoting NOX4 degradation. AZM is an FDA-approved antibiotic drug that has been shown to be beneficial for treating cystic fibrosis (51). A retrospective study from Kawamura et al. showed that AZM significantly improved survival in patients with acute exacerbation of IPF (52).

Ubiquitin E3 ligases that regulate TGF-β–independent pathways in lung fibrosis

MDM2. The ubiquitin E3 ligase mouse double minute 2 homolog (MDM2) is one of the most important cellular regulators of p53, as it mediates p53 mono- and polyubiquitination and degradation of the protein (53–55) and inhibits *p53* transcriptional activation (56). DNA damage and apoptosis are associated with p53 upregulation in bronchiolar and alveolar epithelial cells in the setting of IPF (57). In unstressed cells, MDM2 targets and ubiquitinates p53, leading to p53 nuclear exportation. After DNA damage, phosphorylation of p53 at Ser15 reduces the interaction of p53 with MDM2 and accumulation in the nuclei (58). Moreover, compared with those from healthy controls, epithelial cells from IPF patients have significantly higher levels of phosphorylated p53 (59). Kusko et al. reported that both *MDM2* gene expression and protein levels are increased in lungs of IPF patients (60). Phosphorylation of p53 may prevent MDM2 binding, suggesting that MDM2 may have important roles in IPF (59). Genetic abrogation of *Mdm2* in hepatocytes leads to p53 activation, thereby increasing connective tissue growth factor (CTGF), hepatocyte apoptosis, and spontaneous liver fibrosis (61). Nutlin-3a (patent: WO2014145389 A1) is a small-molecular inhibitor of the MDM2-p53 interaction (62) and is a potential agent for treating ovarian carcinomas expressing WT p53 (63). Based on the role of MDM2-p53 in fibrotic responses, nutlin-3a may be a potential therapy for IPF; however, Chen et al. found that nutlin-3a could not ameliorate fibroblast activation in tubulointerstitial fibrosis (64). In tubulointerstitial fibrosis, MDM2 participates in the process of fibroblast activation through MDM2/NOTCH1 signaling, but not the classic MDM2/p53 pathway (64). The effect of nutlin-3a in lung fibrosis needs further investigation.

BARD1. BRCA1-associated RING domain 1 (BARD1) is a ubiquitin E3 ligase that is aberrantly expressed and correlated with poor prognosis in lung cancer (65). Recently, BARD1 expression was shown to be upregulated in TGF-β–treated lung fibroblasts and in bleomycin-challenged mouse lungs, indicating that BARD1 may contribute to lung fibrosis. BARD1 binds BRCA1, and the BRCA1-BARD1 heterodimer exhibits E3 ubiquitin ligase activity (66, 67). Several proteins are ubiquitinated and degraded by BRCA1-BARD1 (68), including aurora kinases (69, 70), which regulate the cell cycle and are related to cell proliferation. BARD1-BRCA1 abrogates fibroblast proliferation by reducing the stability of aurora kinases (71); therefore, BARD1 could be involved in lung fibrosis owing to its ability to regulate both fibroblast proliferation and ECM deposition (71).

HRD1. E3 ubiquitin ligase HRD1 (also known as synoviolin) is localized to the ER and mediates proteasomal degradation of ER-related proteins (72). HRD1 has reported roles in fibrotic diseases. HRD1 is significantly upregulated in fibrotic kidneys and participates in the regulation of collagen I synthesis (73). Hasegawa et al. confirmed similar effects of HRD1 in liver fibrogenesis (74) and demonstrated markedly less liver fibrosis in heterozygous *Hrd1*$^{+/-}$ mice compared with control animals (74). The effects of HRD1 downregulation in murine lung fibrosis models have not been investigated. Recent lines of evidence indicate that ER stress causes alveolar epithelial cell dysfunction and subsequent lung fibrosis (75, 76), supporting a potential role of HRD1 in the pathogenesis of lung fibrosis. A HRD1 inhibitor (LS-102) reportedly reduces collagen secretion in lung epithelial cells under ER stress conditions (77), supporting HRD1 as a profibrotic factor in the lung. Expression of HRD1 in lung fibrosis and the effect of LS-102 in experimental models of fibrosis are unknown. Further study is needed to identify the targets of HRD1.

In summary, there is extensive evidence that ubiquitin E3 ligases contribute to the pathogenesis of fibrotic diseases through targeting TGF-β–dependent and –independent pathways (Figure 2). FIFL1, arkadia, HRD1, and MDM2 are profibrotic ubiquitin E3 ligases, while TIFγ, STUB1, and BARD1 are antifibrotic ubiquitin E3 ligases in the setting of lung fibrosis. There are still critical knowledge gaps that require attention; however, targeting these enzymes may provide beneficial for treating organ fibrosis.

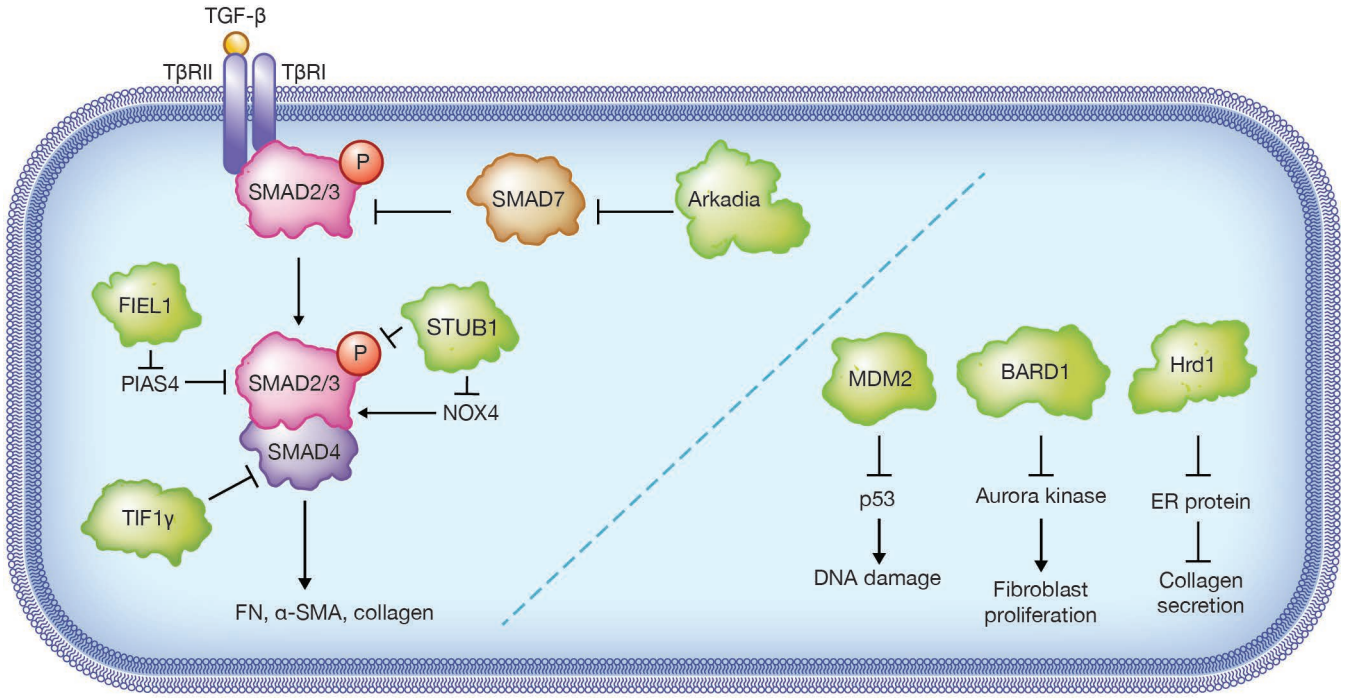

Figure 2. Ubiquitin E3 ligases regulate TGF-β–dependent and –independent pathways. Both TGF-β–dependent and –independent pathways contribute to the pathogenesis of IPF. Ubiquitin E3 ligases, such as FIEL1, arkadia, TIF1γ, and STUB1 regulate TGF-β/TβR/SMAD pathways, while ubiquitin E3 ligases, MDM2, BARD1, and HRD1 regulate SMAD-independent pathways. Illustrated by Rachel Davidowitz.

DUBs contribute to lung fibrosis through TGF-β–dependent pathway regulation

UCHL5. Ubiquitin carboxyl-terminal hydrolase-L5 (UCHL5, also known as UCH37) is a DUB that has been reported to interact with SMAD7 and potentially reverse ubiquitination of TβRI. UCGL5 stabilizes TβRI and upregulates TGF-β–dependent transcription in HEK293 and mesangial cells (78, 79). Nan et al. demonstrated that UCHL5 levels are elevated in lung tissues from IPF patients and bleomycin-challenged mice (80). UCHL5 has also been shown to deubiquitinate and stabilize SMAD2/3, subsequently promoting TGF-β1 signaling and contributing to pulmonary fibrosis pathogenesis (80). The UCHL5 inhibitor b-AP15 induces polyubiquitination and proteasomal degradation of SMAD2/3 (80). Nan et al. also found that UCHL5 has no effects on SMAD7 and TβRI levels in lung fibroblasts. The discrepant effects of UCHL5 observed in this study compared with others might be due to differences in the cell types utilized. Most importantly, administration of b-AP15 reduced the protein levels of fibronectin, type I collagen, and SMAD2/3 in lung tissues of bleomycin-challenged mice (80), thereby indicating that targeting UCHL5 has potential as a pulmonary fibrosis treatment. b-AP15 triggers apoptosis in a variety of cell types (81, 82) and it inhibits another DUB, USP14, raising concerns about possible side effects. A UCHL5-specific inhibitor or approaches to block the interaction between UCHL5 and SMAD2/3 are attractive as possible antifibrotic approaches for treating IPF.

USP11. USP11 has also been shown to stabilize the TGF-β receptors TβRI and TβRII, thus augmenting TGFβ-1 signaling (83, 84). Al-Salihi et al. demonstrated that USP11 cleaves Lys48-linked polyubiquitin chains and stabilizes TβRI through interaction with SMAD7 leading to TGF-β–induced SMAD2/3 phosphorylation and epithelial-to-mesenchymal transition (84). However, most of the study was performed using HEK293 cells transfected with TβRI or SMAD7; thus, the relationship between endogenous USP11 and TβRI was not evaluated. Recently, Jacko et al. revealed that USP11 may contribute to the pathogenesis of pulmonary fibrosis by stabilizing TβRII and promoting TGF-β signaling in lung fibroblasts. Specifically, they found that USP11 and TβRII levels are increased in lung tissues from bleomycin-challenged mice and IPF patients and that inhibition or downregulation of USP11 increases TβRII ubiquitination and reduces TβRII stability. Finally, mitoxantrone (MTX), which inhibits USP11, was shown to attenuate TGF-β signaling (83), supporting targeting USP11 as a potential antifibrotic strategy for pulmonary fibrosis. Unfortunately MTX, the only known inhibitor of USP11,

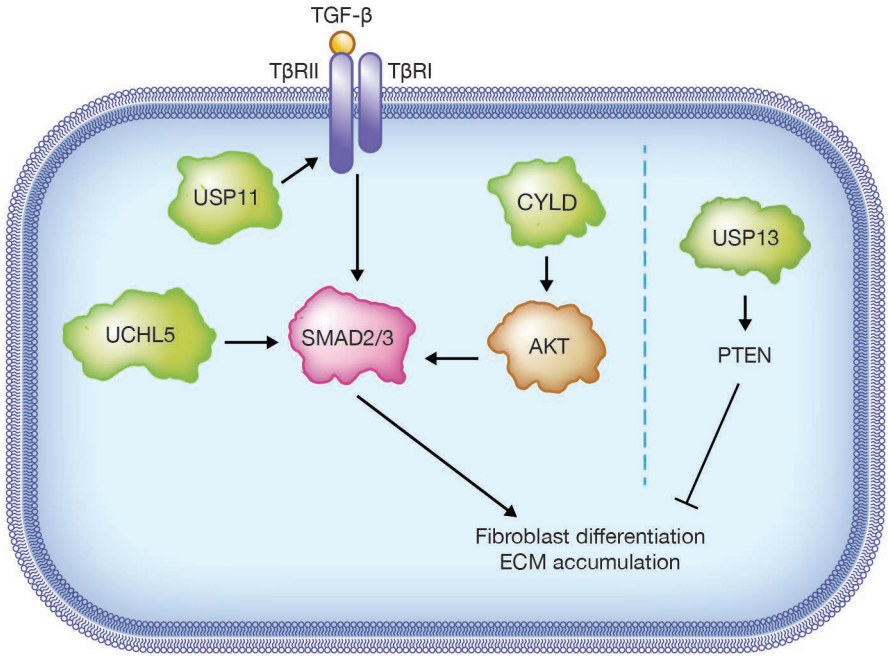

Figure 3. DUBs regulate TGF-β–dependent and –independent pathways. UCHL5, USP11, and CYLD regulate TβR/SMAD–induced fibroblast differentiation and ECM accumulation. USP13 contributes to the pathogenesis of IPF through targeting PTEN. Illustrated by Rachel Davidowitz.

also inhibits type II topoisomerase and induces apoptosis (85); therefore, development of USP11-specific inhibitors will be essential for exploring USP11 as a therapeutic target for IPF.

CYLD. The DUB cylindromatosis (CYLD) is a crucial regulator of diverse cellular processes, such as cell proliferation and survival (86), and acts as a tumor suppressor in different types of cancer (87). CYLD knockdown reportedly promotes lung fibrosis after *Streptococcus pneumoniae* infection (88). In this study, CYLD was shown to inhibit TGF-β signaling and prevent lung fibrosis by decreasing SMAD3 stability via deubiquitinating Lys63-ubiquitinated AKT (88). Additionally, CYLD expression was inhibited by TGF-β (88), further suggesting that CYLD downregulation contributes to the pathogenesis of lung fibrosis. In experimental hepatic injury, CYLD ameliorates hepatocellular damage and liver fibrogenesis through regulation of hepatocyte growth factor (HGF) levels, but this interaction is independent of CYLD DUB activity (89). HGF regulates TGF-β signaling through induction of SMAD7 (90). Taken together, the results show that CYLD regulates the TGF-β pathway through modulating SMAD3 stability and SMAD7 expression.

DUBs that regulate IPF-associated TGF-β–independent pathways

USP13. USP13 regulates polyubiquitination and protein stability of the tumor suppressor PTEN (91), which is involved in a variety of cellular responses, including cell survival, apoptosis, adhesion, migration, and invasion (92). PTEN-deficient mice exhibit increased fibroproliferation in response to bleomycin-induced lung fibrosis (93). A recent study from Geng et al. showed that loss of PTEN enhances aggressive phenotypes in fibroblasts, resulting in lung fibrosis (94). Interestingly, USP13 was decreased in IPF fibroblasts (94), suggesting a role of USP13 in the pathogenesis of lung fibrosis. USP13 has been shown to regulate energetic metabolism by stabilizing ATP citrate lyase and oxoglutarate dehydrogenase (95). Zhao et al. reported that dysregulated energy consumption during lung remodeling may contribute to the pathogenesis of IPF (96). It is not clear, however, whether USP13-mediated regulation of energetic metabolism contributes to IPF pathogenesis. Future studies of USP13-deficient mice could clarify the effect of USP13 on PTEN and energetic metabolism in lung fibrosis.

In summary, DUBs contribute to IPF pathogenesis through regulation of both TGF-β–dependent and –independent pathways (Figure 3). Based on the available knowledge, USP11 and UCHL5 exhibit profibrotic properties, while CYLD and USP13 have antifibrotic effects in lung fibrosis. Studies of the role of

Table 1. Small-molecule inhibitors targeting ubiquitin E3 and DUBs in IPF

Compound names	Molecular targets	Mechanisms	References
BC-1485	FIEL1	Inhibits FIEL1-mediated degradation of PIAS4	21
Azithromycin (AZM)	STUB1	Increases STUB1-mediated degradation of NOX4	50, 52
Nutlin-3a	MDM2	Inhibits MDM2-mediated degradation of p53	63
LS-102	HRD1	Reduces ER stress–induced collagen secretion	77
b-AP15	UCHL5	Attenuates TGFβ-1 signaling through reduction of SMAD2 and SMAD3	80
Mitoxantrone (MTX)	USP11	Attenuates TGFβ-1 signaling through reduction of TβRII	83

DUBs in cancer may provide useful information for studying these enzymes in IPF; however, the effects of DUBs on specific cell types will need to be carefully considered, as DUBs reverse the effects of ubiquitin E3 ligase and improve protein stability. The balance between the action of DUBs and ubiquitin E3 ligases needs to be further studied in IPF.

Conclusions and perspectives

Despite recent studies demonstrating ubiquitination and deubiquitination in the regulation of TGF-β signaling, the ubiquitin E3 ligases and DUBs responsible for turnover of most key proteins in pulmonary fibrosis have been relatively understudied. The identification of specific E3 ligases and DUBs involved in fibrosis is critical because these enzymes, and by extension their downstream targets, represent druggable targets, as was clearly shown by Lear et al. (21). Here, we have summarized a subset of ubiquitin E3 ligases and DUBs that have been implicated in lung fibrosis through regulating TGF-β–dependent pathways, DNA damage response–related proteins, and ER stress–related proteins. Additionally, ubiquitin E3 ligases and DUBs have been shown to correlate with abnormal expression of ECM proteins and fibroblast proliferation. Several SNPs of genes, such as mucin 5B (*MUC5B*), telomerase reverse transcriptase (*TERT*), Toll interacting protein (*TOLLIP*), and signal peptide peptidase–like 2C (*SPPL2C*), have been associated with IPF (13, 97, 98); however, little is known about the regulation of the stability of these proteins. Future studies should focus on investigating the stability of MUC5B, TERT, TOLLIP, and SPPL2C in lung cells, including epithelial cells, endothelial cells, macrophages, and fibroblasts. As lung transplant remains the only option for extending and improving quality of life for IPF patients, the development of antifibrotic therapeutics is a major unmet need. Small molecules that target specific E3 ubiquitin ligases and DUBs have great potential for the treatment of pulmonary fibrosis (Table 1); therefore, a more detailed and integrated understanding of ubiquitination and deubiquitination in regulation of pulmonary fibrosis could help identify promising targets. Furthermore, targeting E3 ligase and DUBs in combination with currently FDA-approved therapies to treat IPF, such as the drugs pirfenidone and nintedanib, have potential for improved treatment of IPF.

Acknowledgments

This study was supported by the US NIH (R01 HL112791 and HL131665 to Y. Zhao; R01GM115389 to J. Zhao), and the American Heart Association (16GRNT30660001 to Y. Zhao).

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1. Todd NW, Luzina IG, Atamas SP. Molecular and cellular mechanisms of pulmonary fibrosis. *Fibrogenesis Tissue Repair*. 2012;5(1):11.
2. Raghu G, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med*. 2011;183(6):788–824.
3. Hutchinson J, Fogarty A, Hubbard R, McKeever T. Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. *Eur Respir J*. 2015;46(3):795–806.
4. Bahudhanapati H, Kass DJ. Unwinding the collagen fibrils: Elucidating the mechanism of pirfenidone and nintedanib in pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2017;57(1):10–11.
5. Sheppard D. Epithelial-mesenchymal interactions in fibrosis and repair. Transforming growth factor-β activation by epithelial

cells and fibroblasts. *Ann Am Thorac Soc*. 2015;12 Suppl 1:S21–S23.
6. Gorelik L, Flavell RA. Transforming growth factor-beta in T-cell biology. *Nat Rev Immunol*. 2002;2(1):46–53.
7. Fernandez IE, Eickelberg O. The impact of TGF-β on lung fibrosis: from targeting to biomarkers. *Proc Am Thorac Soc*. 2012;9(3):111–116.
8. Bonniaud P, et al. Progressive transforming growth factor beta1-induced lung fibrosis is blocked by an orally active ALK5 kinase inhibitor. *Am J Respir Crit Care Med*. 2005;171(8):889–898.
9. Nakao A, et al. Transient gene transfer and expression of Smad7 prevents bleomycin-induced lung fibrosis in mice. *J Clin Invest*. 1999;104(1):5–11.
10. Rosas IO, et al. MMP1 and MMP7 as potential peripheral blood biomarkers in idiopathic pulmonary fibrosis. *PLoS Med*. 2008;5(4):e93.
11. Bauer Y, et al. A novel genomic signature with translational significance for human idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2015;52(2):217–231.
12. Tan J, et al. Expression of RXFP1 is decreased in idiopathic pulmonary fibrosis. Implications for relaxin-based therapies. *Am J Respir Crit Care Med*. 2016;194(11):1392–1402.
13. Noth I, et al. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. *Lancet Respir Med*. 2013;1(4):309–317.
14. Zhang Y, Noth I, Garcia JG, Kaminski N. A variant in the promoter of MUC5B and idiopathic pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1576–1577.
15. Schiller HB, et al. Time- and compartment-resolved proteome profiling of the extracellular niche in lung injury and repair. *Mol Syst Biol*. 2015;11(7):819.
16. De Boeck M, ten Dijke P. Key role for ubiquitin protein modification in TGFβ signal transduction. *Ups J Med Sci*. 2012;117(2):153–165.
17. Izzi L, Attisano L. Regulation of the TGFbeta signalling pathway by ubiquitin-mediated degradation. *Oncogene*. 2004;23(11):2071–2078.
18. Popovic D, Vucic D, Dikic I. Ubiquitination in disease pathogenesis and treatment. *Nat Med*. 2014;20(11):1242–1253.
19. Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem*. 1998;67:425–479.
20. Reyes-Turcu FE, Ventii KH, Wilkinson KD. Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu Rev Biochem*. 2009;78:363–397.
21. Lear T, et al. Ubiquitin E3 ligase FIEL1 regulates fibrotic lung injury through SUMO-E3 ligase PIAS4. *J Exp Med*. 2016;213(6):1029–1046.
22. Lee PS, Chang C, Liu D, Derynck R. Sumoylation of Smad4, the common Smad mediator of transforming growth factor-beta family signaling. *J Biol Chem*. 2003;278(30):27853–27863.
23. Imoto S, et al. Sumoylation of Smad3 stimulates its nuclear export during PIASy-mediated suppression of TGF-beta signaling. *Biochem Biophys Res Commun*. 2008;370(2):359–365.
24. Episkopou V, Arkell R, Timmons PM, Walsh JJ, Andrew RL, Swan D. Induction of the mammalian node requires Arkadia function in the extraembryonic lineages. *Nature*. 2001;410(6830):825–830.
25. Niederländer C, Walsh JJ, Episkopou V, Jones CM. Arkadia enhances nodal-related signalling to induce mesendoderm. *Nature*. 2001;410(6830):830–834.
26. Koinuma D, et al. Arkadia amplifies TGF-beta superfamily signalling through degradation of Smad7. *EMBO J*. 2003;22(24):6458–6470.
27. Elkouris M, et al. SET9-mediated regulation of TGF-β signaling links protein methylation to pulmonary fibrosis. *Cell Rep*. 2016;15(12):2733–2744.
28. Liu FY, Li XZ, Peng YM, Liu H, Liu YH. Arkadia-Smad7-mediated positive regulation of TGF-beta signaling in a rat model of tubulointerstitial fibrosis. *Am J Nephrol*. 2007;27(2):176–183.
29. Gai Z, et al. Trps1 haploinsufficiency promotes renal fibrosis by increasing Arkadia expression. *J Am Soc Nephrol*. 2010;21(9):1468–1476.
30. Levy L, Howell M, Das D, Harkin S, Episkopou V, Hill CS. Arkadia activates Smad3/Smad4-dependent transcription by triggering signal-induced SnoN degradation. *Mol Cell Biol*. 2007;27(17):6068–6083.
31. Nagano Y, et al. Arkadia induces degradation of SnoN and c-Ski to enhance transforming growth factor-beta signaling. *J Biol Chem*. 2007;282(28):20492–20501.
32. Le Scolan E, et al. Transforming growth factor-beta suppresses the ability of Ski to inhibit tumor metastasis by inducing its degradation. *Cancer Res*. 2008;68(9):3277–3285.
33. Poulsen SL, et al. RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. *J Cell Biol*. 2013;201(6):797–807.
34. Garcia CK. Insights from human genetic studies of lung and organ fibrosis. *J Clin Invest*. 2018;128(1):36–44.
35. Hatakeyama S. TRIM proteins and cancer. *Nat Rev Cancer*. 2011;11(11):792–804.
36. Dupont S, et al. Germ-layer specification and control of cell growth by Ectodermin, a Smad4 ubiquitin ligase. *Cell*. 2005;121(1):87–99.
37. Bai X, et al. TIF1gamma controls erythroid cell fate by regulating transcription elongation. *Cell*. 2010;142(1):133–143.
38. Sedgwick GG, et al. Transcriptional intermediary factor 1γ binds to the anaphase-promoting complex/cyclosome and promotes mitosis. *Oncogene*. 2013;32(39):4622–4633.
39. Ding ZY, et al. Reduced expression of transcriptional intermediary factor 1 gamma promotes metastasis and indicates poor prognosis of hepatocellular carcinoma. *Hepatology*. 2014;60(5):1620–1636.
40. Hesling C, et al. Antagonistic regulation of EMT by TIF1γ and Smad4 in mammary epithelial cells. *EMBO Rep*. 2011;12(7):665–672.
41. Dupont S, et al. FAM/USP9x, a deubiquitinating enzyme essential for TGFbeta signaling, controls Smad4 monoubiquitination. *Cell*. 2009;136(1):123–135.
42. He W, Dorn DC, Erdjument-Bromage H, Tempst P, Moore MA, Massagué J. Hematopoiesis controlled by distinct TIF1gamma

and Smad4 branches of the TGFbeta pathway. *Cell*. 2006;125(5):929–941.
43. Bellaye PS, et al. The small heat-shock protein αB-crystallin is essential for the nuclear localization of Smad4: impact on pulmonary fibrosis. *J Pathol*. 2014;232(4):458–472.
44. Aucagne R, et al. Transcription intermediary factor 1γ is a tumor suppressor in mouse and human chronic myelomonocytic leukemia. *J Clin Invest*. 2011;121(6):2361–2370.
45. Meacham GC, Patterson C, Zhang W, Younger JM, Cyr DM. The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. *Nat Cell Biol*. 2001;3(1):100–105.
46. Shang Y, et al. Hsp70 and Hsp90 oppositely regulate TGF-β signaling through CHIP/Stub1. *Biochem Biophys Res Commun*. 2014;446(1):387–392.
47. Sha Y, Rao L, Settembre C, Ballabio A, Eissa NT. STUB1 regulates TFEB-induced autophagy-lysosome pathway. *EMBO J*. 2017;36(17):2544–2552.
48. Xin H, et al. CHIP controls the sensitivity of transforming growth factor-beta signaling by modulating the basal level of Smad3 through ubiquitin-mediated degradation. *J Biol Chem*. 2005;280(21):20842–20850.
49. Amara N, Goven D, Prost F, Muloway R, Crestani B, Boczkowski J. NOX4/NADPH oxidase expression is increased in pulmonary fibroblasts from patients with idiopathic pulmonary fibrosis and mediates TGFbeta1-induced fibroblast differentiation into myofibroblasts. *Thorax*. 2010;65(8):733–738.
50. Chuang CT, Guh JY, Lu CY, Chen HC, Chuang LY. S100B is required for high glucose-induced pro-fibrotic gene expression and hypertrophy in mesangial cells. *Int J Mol Med*. 2015;35(2):546–552.
51. Cogen JD, et al. Chronic azithromycin use in cystic fibrosis risk of treatment-emergent respiratory pathogens [published online ahead of print February 23, 2018]. *Ann Am Thorac Soc*. https://doi.org/10.1513/AnnalsATS.201801-012OC.
52. Kawamura K, Ichikado K, Yasuda Y, Anan K, Suga M. Azithromycin for idiopathic acute exacerbation of idiopathic pulmonary fibrosis: a retrospective single-center study. *BMC Pulm Med*. 2017;17(1):94.
53. Honda R, Tanaka H, Yasuda H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett*. 1997;420(1):25–27.
54. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature*. 1997;387(6630):296–299.
55. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. *Nature*. 1997;387(6630):299–303.
56. Chène P. Inhibiting the p53-MDM2 interaction: an important target for cancer therapy. *Nat Rev Cancer*. 2003;3(2):102–109.
57. Kuwano K, et al. P21Waf1/Cip1/Sdi1 and p53 expression in association with DNA strand breaks in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 1996;154(2 Pt 1):477–483.
58. Shieh SY, Ikeda M, Taya Y, Prives C. DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell*. 1997;91(3):325–334.
59. Nakashima N, et al. The p53-Mdm2 association in epithelial cells in idiopathic pulmonary fibrosis and non-specific interstitial pneumonia. *J Clin Pathol*. 2005;58(6):583–589.
60. Kusko RL, et al. Integrated genomics reveals convergent transcriptomic networks underlying chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2016;194(8):948–960.
61. Kodama T, et al. Increases in p53 expression induce CTGF synthesis by mouse and human hepatocytes and result in liver fibrosis in mice. *J Clin Invest*. 2011;121(8):3343–3356.
62. Shen H, Maki CG. Pharmacologic activation of p53 by small-molecule MDM2 antagonists. *Curr Pharm Des*. 2011;17(6):560–568.
63. Crane EK, et al. Nutlin-3a: A potential therapeutic opportunity for TP53 wild-type ovarian carcinomas. *PLoS ONE*. 2015;10(8):e0135101.
64. Ye C, et al. MDM2 mediates fibroblast activation and renal tubulointerstitial fibrosis via a p53-independent pathway. *Am J Physiol Renal Physiol*. 2017;312(4):F760–F768.
65. Zhang YQ, et al. BARD1: an independent predictor of survival in non-small cell lung cancer. *Int J Cancer*. 2012;131(1):83–94.
66. Baer R, Ludwig T. The BRCA1/BARD1 heterodimer, a tumor suppressor complex with ubiquitin E3 ligase activity. *Curr Opin Genet Dev*. 2002;12(1):86–91.
67. Irminger-Finger I, Jefford CE. Is there more to BARD1 than BRCA1? *Nat Rev Cancer*. 2006;6(5):382–391.
68. Irminger-Finger I, Ratajska M, Pilyugin M. New concepts on BARD1: Regulator of BRCA pathways and beyond. *Int J Biochem Cell Biol*. 2016;72:1–17.
69. Joukov V, et al. The BRCA1/BARD1 heterodimer modulates ran-dependent mitotic spindle assembly. *Cell*. 2006;127(3):539–552.
70. Ryser S, et al. Distinct roles of BARD1 isoforms in mitosis: full-length BARD1 mediates Aurora B degradation, cancer-associated BARD1beta scaffolds Aurora B and BRCA2. *Cancer Res*. 2009;69(3):1125–1134.
71. André PA, et al. BARD1 mediates TGF-β signaling in pulmonary fibrosis. *Respir Res*. 2015;16:118.
72. Kikkert M, et al. Human HRD1 is an E3 ubiquitin ligase involved in degradation of proteins from the endoplasmic reticulum. *J Biol Chem*. 2004;279(5):3525–3534.
73. Li L, Shen Y, Ding Y, Liu Y, Su D, Liang X. Hrd1 participates in the regulation of collagen I synthesis in renal fibrosis. *Mol Cell Biochem*. 2014;386(1–2):35–44.
74. Hasegawa D, et al. E3 ubiquitin ligase synoviolin is involved in liver fibrogenesis. *PLoS ONE*. 2010;5(10):e13590.
75. Heindryckx F, et al. Endoplasmic reticulum stress enhances fibrosis through IRE1α-mediated degradation of miR-150 and XBP-1 splicing. *EMBO Mol Med*. 2016;8(7):729–744.
76. Zhang L, et al. Endoplasmic reticulum stress, a new wrestler, in the pathogenesis of idiopathic pulmonary fibrosis. *Am J Transl Res*. 2017;9(2):722–735.
77. Nakajima F, et al. Synoviolin inhibitor LS-102 reduces endoplasmic reticulum stress-induced collagen secretion in an in vitro model of stress-related interstitial pneumonia. *Int J Mol Med*. 2015;35(1):110–116.
78. Wicks SJ, et al. The deubiquitinating enzyme UCH37 interacts with Smads and regulates TGF-beta signalling. *Oncogene*. 2005;24(54):8080–8084.
79. Ko YM, et al. Ubiquitin C-terminal hydrolase-L5 is required for high glucose-induced transforming growth factor-β receptor I expression and hypertrophy in mesangial cells. *Arch Biochem Biophys*. 2013;535(2):177–186.

80. Nan L, et al. Ubiquitin carboxyl-terminal hydrolase-L5 promotes TGFβ-1 signaling by de-ubiquitinating and stabilizing Smad2/Smad3 in pulmonary fibrosis. *Sci Rep*. 2016;6:33116.
81. Oh YT, Deng L, Deng J, Sun SY. The proteasome deubiquitinase inhibitor b-AP15 enhances DR5 activation-induced apoptosis through stabilizing DR5. *Sci Rep*. 2017;7(1):8027.
82. Wang X, et al. The 19S deubiquitinase inhibitor b-AP15 is enriched in cells and elicits rapid commitment to cell death. *Mol Pharmacol*. 2014;85(6):932–945.
83. Jacko AM, et al. De-ubiquitinating enzyme, USP11, promotes transforming growth factor β-1 signaling through stabilization of transforming growth factor β receptor II. *Cell Death Dis*. 2016;7(11):e2474.
84. Al-Salihi MA, Herhaus L, Macartney T, Sapkota GP. USP11 augments TGFβ signalling by deubiquitylating ALK5. *Open Biol*. 2012;2(6):120063.
85. Koceva-Chyła A, Jedrzejczak M, Skierski J, Kania K, Jóźwiak Z. Mechanisms of induction of apoptosis by anthraquinone anticancer drugs aclarubicin and mitoxantrone in comparison with doxorubicin: relation to drug cytotoxicity and caspase-3 activation. *Apoptosis*. 2005;10(6):1497–1514.
86. Sun SC. CYLD: a tumor suppressor deubiquitinase regulating NF-kappaB activation and diverse biological processes. *Cell Death Differ*. 2010;17(1):25–34.
87. Massoumi R. CYLD: a deubiquitination enzyme with multiple roles in cancer. *Future Oncol*. 2011;7(2):285–297.
88. Lim JH, et al. CYLD negatively regulates transforming growth factor-β-signalling via deubiquitinating Akt. *Nat Commun*. 2012;3:771.
89. Pannem RR, Dorn C, Hellerbrand C, Massoumi R. Cylindromatosis gene CYLD regulates hepatocyte growth factor expression in hepatic stellate cells through interaction with histone deacetylase 7. *Hepatology*. 2014;60(3):1066–1081.
90. Shukla MN, Rose JL, Ray R, Lathrop KL, Ray A, Ray P. Hepatocyte growth factor inhibits epithelial to myofibroblast transition in lung cells via Smad7. *Am J Respir Cell Mol Biol*. 2009;40(6):643–653.
91. Zhang J, et al. Deubiquitylation and stabilization of PTEN by USP13. *Nat Cell Biol*. 2013;15(12):1486–1494.
92. Yamada KM, Araki M. Tumor suppressor PTEN: modulator of cell signaling, growth, migration and apoptosis. *J Cell Sci*. 2001;114(Pt 13):2375–2382.
93. Xia H, et al. Pathological integrin signaling enhances proliferation of primary lung fibroblasts from patients with idiopathic pulmonary fibrosis. *J Exp Med*. 2008;205(7):1659–1672.
94. Geng J, et al. Down-regulation of USP13 mediates phenotype transformation of fibroblasts in idiopathic pulmonary fibrosis. *Respir Res*. 2015;16:124.
95. Han C, et al. Amplification of USP13 drives ovarian cancer metabolism. *Nat Commun*. 2016;7:13525.
96. Zhao YD, et al. Metabolic heterogeneity of idiopathic pulmonary fibrosis: a metabolomic study. *BMJ Open Respir Res*. 2017;4(1):e000183.
97. Seibold MA, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1503–1512.
98. Mushiroda T, et al. A genome-wide association study identifies an association of a common variant in TERT with susceptibility to idiopathic pulmonary fibrosis. *J Med Genet*. 2008;45(10):654–656.