The role of ubiquitin-specific peptidases in cancer progression

Ming-Jer Young 1, Kai-Cheng Hsu 2,3,4, Tony Eight Lin 3, Wen-Chang Chang 6 and Jan-Jong Hung 1,5*

Abstract

Protein ubiquitination is an important mechanism for regulating the activity and levels of proteins under physiological conditions. Loss of regulation by protein ubiquitination leads to various diseases, such as cancer. Two types of enzymes, namely, E1/E2/E3 ligases and deubiquitinases, are responsible for controlling protein ubiquitination. The ubiquitin-specific peptidases (USPs) are the main members of the deubiquitinase family. Many studies have addressed the roles of USPs in various diseases. An increasing number of studies have indicated that USPs are critical for cancer progression, and some USPs have been used as targets to develop inhibitors for cancer prevention. Herein we collect and organize most of the recent studies on the roles of USPs in cancer progression and discuss the development of USP inhibitors for cancer therapy in the future.

Keywords: Ubiquitination, Deubiquitinases, Ubiquitin-specific peptidases, Cancer

Background

After translation, most proteins can undergo various modifications, namely, phosphorylation, acetylation, methylation, sumoylation, glycosylation and ubiquitination, to modulate their activity. Posttranslational modification (PTM) of proteins is an important component of all physiological processes that functions by regulating various pathways, including protein degradation, DNA repair activity, gene regulation and signal transduction [1]. Evolutionarily higher plants and animals have more complex PTMs, indicating that the PTM process is beneficial to supporting the progression of life [2]. Ubiquitin is a small 76-amino-acids protein that can be conjugated to specific target proteins in various forms, namely, polyubiquitination and monoubiquitination. Three types of enzymes, namely, ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s), are responsible for adding the ubiquitin into target proteins [3]. Seven lysine residues in ubiquitin provide different types of linkages, including monoubiquitination, polyubiquitination and branched ubiquitination. Three types of enzymes, ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s), are responsible for adding the ubiquitin into target proteins [3]. Seven lysine residues in ubiquitin provide different types of linkages, including monoubiquitination, polyubiquitination and branched ubiquitination, to regulate the different functions of target proteins [4]. Protein monoubiquitination affects DNA repair activity, gene regulation, molecule trafficking and endocytosis [5]. Lys48-linked protein polyubiquitination affects protein degradation in a 26S proteasome-dependent manner. Lys63-linked protein polyubiquitination is involved in DNA repair activity, signal transduction, trafficking and endocytosis [6]. Branched ubiquitination of proteins, such as in the APC/C complex, is also associated with 26S proteasome-dependent degradation [4]. All types of ubiquitination as a protein modification are crucial to maintaining normal physiological conditions [7]. Dysregulation of protein ubiquitination leads to many diseases, including degenerative diseases and cancer [8, 9].

Deubiquitinases (DUBs) are a group of enzymes that are able to remove ubiquitin from ubiquitinated proteins, including monoubiquitinated, polyubiquitinated and branch polyubiquitinated proteins, leading to the regulation of the stability or activity of the target proteins [10, 11]. More than one hundred deubiquitinases that regulate all protein deubiquitination have been identified in humans. DUB members can be divided into five types: ubiquitin-specific proteases (USPs), ovarian tumor proteases (OTUs), ubiquitin C-terminal hydrolases (UCHs), Machado-Joseph disease protein domain proteases (MJDs) and JAMM motif proteases [12, 13]. USPs, OTUs, UCHs and MJDs are cysteine-dependent proteases [14, 15]. The JAMM motif is a metal-dependent protease...
Most of these enzymes exert their functions by reversing the polyubiquitination or monoubiquitination of target proteins. An increasing number of studies have indicated that dysregulation of the DUB causes malfunction of the ubiquitin system, which can either increase the effects of oncogenes or decrease the tumor suppressor gene activity. Herein we collected and organized all recent studies that address the roles of each USP in cancer progression.

The roles of USPs in tumorigenesis

Many studies indicate that USPs regulate tumor formation by modulating the proliferation and death of cancer cells. All USPs and their substrates are shown in Table 1.

**USPs are involved in cell cycle progression**
Protein ubiquitination is important for the regulation of cell cycle progression. Ubiquitinases, namely, E1/E2/E3, are well studied. Recently, several deubiquitinases have been reported to be involved in cell cycle progression. USP2 and USP22 can stabilize cyclin D1 to promote cell cycle progression [16, 17]. A recent study also revealed that a small molecule, ML364, can inhibit USP2 to promote degradation, leading to cell cycle arrest [18]. USP7 has been reported to promote the growth of non-small cell lung cancer cells by stabilizing Ki-67 protein [19]. However, metformin can inhibit esophageal cancer proliferation through the upregulation of USP7, suggesting that USP7 has different effects on tumorigenesis in the different cancer types [20–22]. USP24 stabilizes securin to block the cell cycle progression from metaphase to anaphase, leading to cell cycle arrest [23]. According to previous studies, APCC, as an E3-ligase in mitosis, regulates many factors, including securin, to promote cell cycle progression [24]. In addition to E3-ligases, deubiquitinases, such as USP24, may also be important for cell cycle progression [23]. More evidence is needed to support the hypothesis that downregulation of USP24 in mitosis is induced by APCC. USP37 also regulates the stability of oncogenic fusion protein PLZF/RARA [25]. USP37 links REST to the control of p27 stability and cell proliferation [26]. USP44 promotes prostate cancer tumorigenesis by stabilizing EZH2 [27]. USP44 also induces DNA aneuploidy in gastric cancer, which may induce cell cycle arrest and apoptosis [28]. Therefore, USP44 is a tumor suppressor against chromosome missegregation [29]. In addition, USP44 function as an integral component of N-CoR to regulate gene expression [30].

**USPs-stabilized c-Myc promotes cancer formation**
c-Myc is an oncoprotein that regulates gene expression and cell cycle progression. USP2 is reported to be involved in activating the c-Myc pathway to regulate prostate cancer formation [31]. USP10 can stabilize c-Myc expression [32]. USP22 promotes the proliferation, migration and invasion abilities of glioma, gastric cancer and colorectal cancer [33–35]. In addition, USP22 stabilizes c-Myc to promote tumor formation [36]. USP28 contributes to the proliferation and metastasis of gastric cancer [37]. The loss of USP28 enhances the radiosensitivity of esophageal cancer cells via the c-Myc pathway [38]. USP36 stabilizes c-Myc to promote ovarian cancer formation [39]. USP37 directly stabilizes c-Myc in lung cancer [40]. All the studies reveal that USPs are important in regulating c-Myc stability during tumorigenesis.

**USPs regulate apoptosis-related factors**
p53 is a tumor suppressor, and p53 degradation or mutations are critical factors in cancer formation [41]. Several E3 ligases, such as MDM2, have been well studied [42]. Recent studies have also indicated that several deubiquitinases are involved in the regulation of p53 degradation [43]. USP2 and USP7 stabilize MDM2 and MDM4 to degrade p53, leading to an anti-apoptosis phenotype [44–46]. USP4 and USP5 inhibit p53 expression, but the molecular mechanism has yet to be elucidated [47–49]. USP10 can interact with G3BP2 to block p53 signaling and subsequently contributes to a poor prostate cancer prognosis [50]. However, in lung cancer, USP10 can inhibit cell growth and invasion by stabilizing PTEN, suggesting that the roles of USP10 in the different cancer types are distinct [51]. USP15 stabilizes MDM2 to regulate p53 and NFATc2 in cancer cells and T cells, respectively, resulting in tumor cell apoptosis and antitumor T cell responses [52]. USP24 can stabilize p53 but not c-Myc to inhibit tumorigenesis. USP42 was reported to stabilize TP53, but USP42 knockdown inhibits cancer formation, implying that other unknown factors related to cancer formation may exist [53]. USP2 stabilizes MDM2 and MDM4 to inhibit the Ras/p53 pathway during tumorigenesis [46, 54]. USP5 inhibits the p53 pathway [55]. USP7, USP10 and USP24 can stabilize p53 to inhibit cancer formation [45, 56, 57]. Our previous studies indicated that USP24 is downregulated in patients with early stage lung cancer. Overexpression of USP24 induces apoptosis by stabilizing securin and Bax, respectively [23]. USP27X stabilizes BCL2L11 to increase the anti-apoptotic effects of MAPK activity [58]. USP30 also participates in inhibiting apoptosis by stabilizing Parkin [59].

**The roles of USPs in cancer malignancy**
Disrupted regulation of protein ubiquitination is a trigger of various diseases, including cancer. An increasing number of USPs have been shown to be involved in cancer malignancy. All USPs that are involved in cancer malignancy...
| Gene symbol | Cellular location | Substrate | Function and remarks in cancer | Inhibitor | References |
|-------------|------------------|-----------|-------------------------------|-----------|------------|
| USP1        | N                | FANCD2    | DNA repair; Oncogene          | Pimozone³, ML323, GW7647, CS27, 6-Amino-pyrimidines, SJ2-043, SJ3-019A, PR619 | [92, 110–114] |
| USP2        | C, N             | Fatty acid synthase, cyclin D1, MDM2 and 4 | Fas/p53, NF-κB, c-Myc; Oncogene | NSC632839, AM146, RA-9, RA-14, 2-cyano-pyrimidines and -triazines, ML364, PR619 | [18, 31, 44, 114–122] |
| USP3        | N                | H2A, H2B  | DDR, Oncogene                 | Vialinin A, PR619 | [123–125] |
| USP4        | C, N             | TRAF2, TRAF6 | TGFβ, NFκB, Wnt, p53; Oncogene | Vialinin A, PR619 | [81, 114, 126–128] |
| USP5        | L, V, C³        | p53, DDR, Oncogene | G9, Vialinin A, WP1130, EOAI3402143, AM146, RA-9, RA-14, PR619 | [49, 93, 106, 114, 118, 127, 129–132] |
| USP6        | Golgi, C         | NFκB activation; Oncogene or Suppressor | | | [133] |
| USP7        | N, C, PML body  | HDM2, p53, H2B, TP53, MDM2 & 4, FOXO4, PTEN | Oncogene | PS091, Cpd14, P22077, HBX41108, HBX 19818, HBX 28258, NSC632839, WO2013030218, P0050429, WO2013030218, PR619 | [114, 117, 121, 134–146] |
| USP8        | C, N             | NRDP1, RNF128, STAM2 | Oncogene | HBX90397, HBX41108, AM146, RA-9, RA-14, Ethyl5oxirimo-9H-indeno[1,2-b] pyrazine-2,3-dicarbonitrile, PR619 | [95, 114, 118, 147–150] |
| USP9X       | C, E, L, V       | β-catenin, epsins, AF-6, SMAD2 | TGFβ, Mcl-1, ERG, AGS-3, ITCH, Wnt, Notch; Oncogene or suppressor | G9, WP1130, PR619 | [106, 107, 114, 130–132, 151–154] |
| USP9Y       | C                | Spematogenesis | | | [155] |
| USP10       | C, N             | TP53, SNX3, CFTR | c-Myc, p53; Oncogene or suppressor | P22077, HBX19818, Spautin-1, PR619 | [32, 56, 114, 156–158] |
| USP11       | N, C             | BRCA2, NFκBIA | DDR, NFκB; Oncogene | Mitoxantrone⁶ | [70, 104, 159–161] |
| USP12       | Androgen receptor | Oncogene | GW7647 | Spautin-1 | [157, 165–167] |
| USP13       | L, V, C, N⁴     | MCL1, BECN1, USP10 | Oncogene | VLX1570⁰, IU1, WP1130, b-AP15, AC17, Auranofoo⁷, Tricyclic heterocyclics, Azepan-4-ones, PR619 | [106, 114, 132, 168–175] |
| USP14       | C, PM            | Wnt; Oncogene | | | |
| USP15       | C, N             | RBX1, SMAD1, 2, 3 & 7 | NFκB, Wnt; Oncogene | PR619 | [114, 176–179] |
| USP16       | N                | H2A       | Chromosome condensation; Oncogene | PR619 | [114, 180–183] |
| USP17       |                 | SUDS3     | Oncogene | | [184–186] |
| USP18       | C, N             | TAK1, TAB1, PTEN | JAK-STAT, NFκB; Oncogene | | [187, 188] |
| USP19       | ER               | RNF123    | ERAD | PR619 | [114, 189–191] |
| Gene symbol | Cellular location | Substrate | Function and remarks in cancer | Inhibitor | References |
|-------------|------------------|-----------|-------------------------------|-----------|------------|
| USP20       | C, N             | DIO2, ADRB2, TRAF6, Tax | Thyroid hormone, hypoxia, NFκB; Oncogene | PR619     | [114, 192, 193] |
| USP21       | C, N             | H2A, RPK1, DDXS8, GATA3, IL33 | NFκB, NEDD8; Oncogene |           | [72, 194–198] |
| USP22       | N                | H2A       | c-Myc; Oncogene                | PR619     | [114, 199–202] |
| USP24       | C                | TP53, DDB2, MCL1, Bax, p300, E2F4, securin, βTrCP | Cell growth repressor; Metastasis promoter; Overexpression in M2 macrophages | G9, PR619 | [23, 57, 75, 106, 114, 130, 131, 203] |
| USP25       | C, N             | DDXS8     | ERAD; Oncogene                 |           | [204–206] |
| USP26       | N (testis)       | AR        | Spermatogenesis                |           | [207–209] |
| USP27X      |                  | BCL2L11   | tumor suppressor               |           | [58, 210] |
| USP28       | N                |           | CLSPN, c-MYC; Oncogene or suppressor | PR619     | [114, 211, 212] |
| USP29       | N                |           | p53 pathway; Oncogene         |           | [213, 214] |
| USP30       | M                | MFN1, MFN2, DRP1, Parkin | Hepatocarcinogenesis          |           | [215–217] |
| USP31       | N, C             |           | Inhibition of NFκB            |           | [218] |
| USP32       | PM, Golgi        |           | Oncogene                      |           | [219] |
| USP33       | C, N, centrosome | HIF1α DIO2, ADRB2, CCP110, ARRB | Tumor suppressor               |           | [192, 220–223] |
| USP34       | C, N, PM, Extracellular | AXIN1, AXIN2 | Activation of Wnt; Inhibition of EMT and cancer stemness | | [102, 224] |
| USP35       | N                | ABIN-2, Aurora B | Tumor suppressor through inactivating NFκB | | [225, 226] |
| USP36       | N                | c-Myc    | Oncogene                      |           | |
| USP37       | N                | c-Myc    | Increase in DNA damage repair; Oncogene | | |
| USP38       | C, N, GA         |           | Oncogene                      |           | |
| USP39       | N                |           | Oncogene                      |           | |
| USP40       | C, N, PM         |           | Oncogene                      |           | |
| USP41       | N                |           | Oncogene                      |           | |
| USP42       | N                | TPS3      | p53; Oncogene                 |           | [53, 227, 228] |
| USP43       | N                | H2BK120   | Tumor suppressor               |           | [229] |
| USP44       | N                | CDC20, EZH2 | Oncogene                     |           | |
| USP45       | C, N             |           | Oncogene                      |           | |
| USP46       | L, V             | POLB      | Oncogene                      | Pimozideb | [113] |
| USP47       | C                | POLB      | Oncogene                      | P5091, Cpd14, P22077, PR619 | [114, 136, 137, 139, 230] |
| USP48       | C, N             | Gli1      | Oncogene                      | PR619     | [114] |
| USP49       | N                | H2B       | Tumor suppressor               |           | [231] |
| USP50       | N                | G2/M checkpoint | Oncogene                   |           | [232] |
| USP51       | N                |           | Oncogene                      |           | |
| PAN2        | C, N             |           | Oncogene                      |           | |
malignancy through the regulation of different pathways are then discussed.

**USPs are involved in EMT and the stemness of cancer**

USP11 stabilizes Snail to promote EMT in ovarian cancer [60]. USP24 also enhances TGFβ-induced EMT and metastasis of breast cancer [61]. Several previous studies have indicated that USP21 affects stem cells by stabilizing Nanog and IL8 [62]. Inhibition of USP34 induces EMT and stemness in mammary epithelial cells [63]. Previous reports indicated that USP47 promotes colorectal cancer EMT and malignancy by stabilizing Snail and activating the Wnt signaling pathway [64].

**USPs regulate related pathways to control cancer metastasis**

According to previous studies, several important cancer-related pathways are regulated by various USP members. The JNKs-STATs compose an important pathway for cancer malignancy. Recent studies indicated that STAT3 activation represses USP7, leading to colon cancer development [65]. Another recent study indicated that USP3 mRNA functioned as a sponge for miR-224 to increase the level of SMAD4, resulting in colorectal cancer metastasis [66]. However, the role of USP3, as a deubiquitinating enzyme, is still not known [67]. CYLD controls c-Myc expression through a JNK-dependent signaling pathway in hepatocellular carcinoma [68].

The NFκB pathway is important for physiological and pathological progression, including inflammation and cancer progression. Many recent studies have shown that ubiquitination regulates not only protein degradation but also protein activity by modulating the interaction between proteins. Several USPs have been reported to be involved in the NFκB pathway [69]. USP6 is involved in the activation of the NFκB pathway, thus positively regulating tumorigenesis; however, the molecular mechanism is not yet known. USP11 can negatively regulate the NFκB pathway by stabilizing IκB [70]. USP18 inhibits the NFκB pathway by targeting TAK1 and NEMO for deubiquitination [71]. USP21 stabilizes IL33 to increase the signal transduction of NFκB [72]. Many studies have revealed that CYLD can inhibit NFκB signal transduction by regulating various factors, such as TRAF2/6, NEMO and Tak1 [73]. The polyubiquitination of TRAFs can increase the recruitment of other related proteins to induce the NFκB signaling pathway. USP4 and USP20 can promote the cell migration and invasion activities in breast cancer by inhibiting NFκB activation via deubiquitination of TRAF2 and TRAF6 [74]. Our recent study also indicated that USP24 can induce the NFκB pathway by stabilizing the βTrCP, which is the E3-ligase of IκB and DNMT1, causing the degradation of IκB and DNMT1 [75]. Regulation of USP35 by the miR let-7a can inhibit NFκB activation via deubiquitination and stabilization of ABIN-2 protein to inhibit cancer progression [76].

The TGFβ pathway is involved in several aspects of cancer progression, including cancer malignancy [77]. Different USPs regulate the TGFβ pathway by stabilizing different factors in this pathway [78]. USP4 and USP15 can stabilize TGFβ receptor type 1 to increase TGFβ-mediated EMT, leading to metastasis of hepatocellular carcinoma and glioblastoma [79–81]. A recent study indicated that a long noncoding RNA, H19, can compete with the binding of miR-148a to USP4 mRNA to increase the signaling activity of TGFβ [82]. USP9X can control the monoubiquitination of SMAD4 to regulate TGFβ-mediated cancer metastasis [83]. According to previous studies, USPs are crucial for the regulation of the TGFβ-mediated pathway [84].

The Wnt pathway is important for cancer EMT and metastasis [85]. USP4 can positively regulate the Wnt signaling in colorectal cancer [86]. Previous studies indicated that USP9X increases adhesion by destabilizing β-catenin [87]. USP14 and USP34 are required for Wnt signaling, but the detailed molecular mechanism is not yet known [88].

| Gene symbol | Cellular location | Substrate | Function and remarks in cancer | Inhibitor | References |
|-------------|------------------|-----------|-------------------------------|-----------|------------|
| USP3        | Golgi, N        | H2AX      | Oncogene                      |           |            |
| USP4        | M                | TRAF2/6, NEMO, TRPA1, Tak1, Lck, Bc13, Dvl, DDXS8, K63polyUb-RIPK1, K63polyUb-β-IκB | G2/M checkpoint; Cancer associated | [233, 234] |
| USP11       | N, Cajal body   | TRAF2/6, NEMO, TRPA1, Tak1, Lck, Bc13, Dvl, DDXS8, K63polyUb-RIPK1, K63polyUb-β-IκB | NFκB and JNK-STAT; Familial tumor suppressor | [68, 235–239] |

The roles of USPs in the cancer progression. *Predicted; C: Cytoplasm; N: Nucleus; L: Lysosome; V: Vacuole; ER: Endoplasmic reticulum; M: Mitochondria; E: Endosome; 2Clinical trial on going (https://clinicaltrials.gov/ct2/home)
**USPs are involved in the tumor-associated microenvironment**

Our recent study found that USP24 is increased in M2 tumor-associated macrophages (TAMs), thereby promoting lung cancer malignancy through an increase in IL6 expression [75]. Increasing evidence indicates that TAMs are important for cancer malignancy and drug resistance [89-91]. Therefore, more USPs that are involved in regulating the tumor-associated microenvironment are expected to be identified in the future.

**The roles of USPs in DNA damage repair activity**

DNA damage repair activity is related to the genomic integrity. A decrease in the DNA damage repair activity causes drug resistance under drug treatment, such as chemotherapy. According to recent studies, many deubiquitinas are involved in DNA damage repair pathways, indicating that deubiquitinas may be important for the induction of drug resistance. USP1 participates in restoring sensitivity to cisplatin in drug-resistance lung cancer cells by stabilizing FANC D2 [92]. USP3, 5 and 11 have been reported to be involved in increasing DNA damage repair activity by activating the DDR pathway [67, 93, 94]. USP8 may participate in TKI-induced drug resistance by increasing the levels of several receptor tyrosine kinases, including EGFR, ERBB2, ERBB3, and MET [95]. However, no substrate has been found to date. A recent study indicated that USP14 may be involved in cisplatin resistance by modulating the Akt/ERK signaling pathway in gastric cancer [96]. USP21 increases DNA repair and tumor growth by stabilizing BRCA2 [97]. USP22 promotes resistance to EGFR-TKIs by stabilizing EGFR in EGFR-mutant lung adenocarcinoma [98]. A recent study also indicated that the loss of USP22 causes to myeloid leukemia upon Kras activation through a PU.1-dependent mechanism [99]. USP22 induces cisplatin resistance in lung cancer by regulating γH2AX-mediated DNA damage repair and Ku70/Bax-mediated apoptosis [100]. USP22 knockout increases the chemosensitivity of hepatocellular carcinoma cells to 5-FU by upregulating Smad4 and suppressing Akt [101]. USP26 is involved in the HR-dependent repair pathway. USP34 inhibits EMT and cancer stemness and may therefore induce more resistance to the drug treatment [102]. USP26 and USP37 participate in HR repair pathway by counteracting RAP80 [103]. USP47 promotes gastric cancer growth by regulating RelA. Many USPs discussed here are involved in DNA damage repair pathways, suggesting that USPs may be the potential targets for drug development of drug resistance in the future.

**USPs as targets for drug development in cancer prevention**

In the past ten years, an increasing number of studies have indicated that most of USPs positively regulate cancer progression, including cell growth and malignancy. Recently, more inhibitors of USPs have been identified (Table 1). Most of the inhibitors can block more than one USP. Thus, increasing the specificity and effect of the inhibitors should be important in the future development. Herein we discussed how to develop a specific inhibitor of USPs. The development of USP inhibitors has resulted in a range of small molecule inhibitors and has been summarized in previous reviews [104, 105]. Many identified USP inhibitors have been suggested to have paninhibitory activity [104, 105]. For example, compound WP1130 has a broad panenzymatic DUB profile and can directly inhibit USP9x, USP5 and USP14 [106, 107]. However, this paninhibition may produce unwanted side effects. Designing a drug targeting a specific USP has proven challenging. This is due to the similarity of the conserved catalytic domain of the USP family. Therefore, identifying nonconserved regions is useful for designing specific USP inhibitors. In addition, further research on the interactions between compounds and the USP catalytic site is needed.

Sequence conservation analysis can provide clues for designing a selective inhibitor against a target protein. Using the crystal structure of a target protein, researchers can infer interactions in the catalytic domain to identify and design selective inhibitors. A sequence conservation analysis of USP was performed for this review. USP domain sequences were obtained from the UniProt Consortium [108]. A multiple sequence alignment (MSA) was performed using T-Coffee (http://tcoffee.crg.cat). Next, the MSA was submitted to the Consurf server (http://consurf.tau.ac.il/2016/) to identify conserved and nonconserved sequences. Each residue position was assigned a conservation score from variable (1) to conserved (9). Finally, the conservation score was mapped to the structure of USP7. Conserved and nonconserved regions exist in the USP catalytic domain (Fig. 1). For example, USP7 residue F409 has a high conservation score of 9. Residue F409, when USP7 is in complex with an inhibitor, adopts a conformation that produces a hydrophobic region that can be exploited by an inhibitor [109]. With the absence of crystal structures in complex with an inhibitor for other USP family members, analyzing the catalytic domain sequence remains crucial for designing possible inhibitors.

The sequence conservation analysis of the catalytic domain produced two nonconserved regions, designated Site 1 and Site 2. These are unique regions that vary between the USP family members and may be used to design a selective compound (Fig. 1a). The side chains of USP7 residues Q297 and Q351 are angled toward the Site 1 region. This allows possible hydrogen bond formation between a compound and USP7. However, the sequence analysis revealed different types of amino acids in these positions for USP family members. For instance,
residue Q297 of USP7 is replaced by an alanine residue in USP18 and 54 (Fig. 1b). The alanine residue contains a shorter side chain than USP7 residue Q297. Furthermore, the alanine residue would not facilitate a hydrogen bond with its side chain. As a result, the catalytic region at Site 1 may be larger in other USP family members. This suggests that a compound with a larger nonpolar functional group would form additional van der Waals interactions with alanine. Such molecules may be more selective toward USP family members with alanine in this position. Many USP family members contain a serine at the 351 position (Fig. 1b). The serine side chain is shorter than the glutamine residue side chain. USP18 and USP41 contain an alanine and a threonine residue at the USP7 residue Q297 and Q351 position, respectively (Fig. 1b). This would suggest a larger Site 1 region. For example, the analysis suggests that USP18 and USP41 may have a larger Site 1 region. This region can accommodate a larger compound as well as a possible hydrogen bond with the threonine side chain to yield a selective USP18 or USP41 inhibitor. Finally, USP7 residue M410 occupies a region in the periphery of the USP7 catalytic site. Many USP family members contain residues at this position that are negatively charged. The presence of glutamate and aspartic acid residues at this position may form a salt bridge with a compound that has a positively charged functional group to make a specific interaction. Thus, the sequence conservation analysis suggests that a nonconserved pocket can be used to design selective USP inhibitors.

Site 2 is the other identified nonconserved region. This region consists of USP7 residues M292, N460, and H461 (Fig. 1a). According to the reference structure USP7, the side chains of residues at positions 292 and 460 face away from the catalytic region. This suggests that no direct interactions between compounds and the residue side chain occur with this region. However, the residue type at position 461 in USP7 is variable among the USP family (Fig. 1b). The side chain of residue USP7 H461 points inwards toward Site 2. This suggests that interactions at this position can greatly aid in USP selectivity. For example, USP12 contains an asparagine residue at position 461.
this position and can form a hydrogen bond with a compound in this region. Possible hydrogen bond formation is also observed at this position with a serine residue in USP37. USP37 may also have a larger catalytic region at Site 2 due to the shorter side chain of serine. As a result, USP37 may be able to accommodate a compound with a larger moiety at Site 2. In total, the sequence conservation analysis identified two nonconserved sites. Interactions with the nonconserved sites present the possibility of designing a selective UPS inhibitor.

Conclusion
Post-translational modification of protein is important for maintaining the physiological function. Dysregulation of protein ubiquitination will induce many diseases, such as cancer. E1/E2/E3-ligases and deubiquitinases regulate protein ubiquitination to control the function and stability of protein. Although many studies have addressed the importance of the USPs in cancer progression, several issues about USPs are still unknown. First, most of the substrates contain more than one deubiquitinates, why are more deubiquitinases needed to regulate the same protein? Second, according many previous studies, a lot of USPs are involved in the DNA damage repair activity, implying USPs may be related to drug resistance during cancer treatment. Therefore, more in-depth studies for clarifying the molecular mechanism are important. Finally, many USPs have been used as the target to develop the inhibitors. How to develop the inhibitors with more effective, low side effect and higher specificity is the most important issue in the future.

Acknowledgements
We will thank Dr. Wang Shao-An to give the suggestion for the manuscript writing.

Funding
This work was supported by the grants (106-2320-B-006-065-MY3, 106-2320-B-006-020-MY3, and 104-2923-B-038-002-MY3) obtained from the Ministry of Science and Technology, Taiwan.

Availability of data and materials
Not applicable.

Authors’ contributions
Hung JJ is contribution to conception and design, manuscript writing. Yang MJ is contribution to data collection, manuscript writing. Hsu KC is contribution to manuscript writing. Lin TE is contribution to data collection. Chang WC is contribution to manuscript writing and editing. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan 701, Taiwan. 2Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan. 3Ph.D. Program for Cancer Molecular Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan. 4Biomedical Commercialization Center, Taipei Medical University, Taipei, Taiwan. 5The Ph.D. Program for Neural Regenerative Medicine, Taipei Medical University, Taipei, Taiwan. 6Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan.

Received: 20 March 2019 Accepted: 16 April 2019
Published online: 27 May 2019

References
1. Millar AH, Heazlewood JL, Giglione C, Holdsworth MJ, Bachmair A, Schulze WX. The Scope, Functions, and Dynamics of Posttranslational Protein Modifications. Annu Rev Plant Biol. 2019;29(70):119-51.
2. Vu LD, Gevaert K, De Smet I. Protein Language: Post-Translational Modifications: Talking to Each Other. Trends Plant Sci. 2018;23(12):1086–80.
3. Scheffner M, Nuber U, Huibregtsje JM. Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. Nature. 1995;373(6509):81–3.
4. Swatek KN, Komander D. Ubiquitin modifications. Cell Res. 2016;26(4):399–422.
5. Haglund K, Dikic I. Ubiquitylation and cell signaling. EMBO J. 2005;24(19):3353–9.
6. Saeki Y, Kudo T, Sone T, Kikuchi Y, Yokosawa H, Tohe-A, Tanaka K, Lysine 63-linked polyubiquitin chain may serve as a targeting signal for the 26S proteasome. EMBO J. 2009;28(4):359–71.
7. Lecker SH, Goldberg AL, Mitch WE. Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. J Am Soc Nephrol. 2006;17(7):1807–19.
8. Ciechanover A, Kwon YT. Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. Exp Mol Med. 2015;47:a147.
9. Shi D, Grossman SR. Ubiquitin becomes ubiquitous in cancer: emerging roles of ubiquitin ligases and deubiquitinases in tumorigenesis and as therapeutic targets. Cancer Biol Ther. 2010;10(8):737–47.
10. Mevissen TET, Komander D. Mechanisms of Deubiquitinase Specificity and Regulation. Annu Rev Biochem. 2017;86:159–92.
11. Clague MJ, Urbe S, Komander D. Breaking the chains: deubiquitylating enzyme specificity begets function. Nat Rev Mol Cell Biol. 2019.
12. Komander D, Clague MJ, Urbe S. Breaking the chains: structure and function of the deubiquitinases. Nat Rev Mol Cell Biol. 2009;10(8):550–63.
13. Clague MJ, Barsukov I, Coulson JW, Liu H, Rigden DJ, Urbe S. Deubiquitylases from genes to organism. Physiol Rev. 2013;93(3):289–315.
14. Reyes-Turcu FE, Venti KH, Wilkinson KD. Regulation and Cellular Roles of Ubiquitin-Specific Deubiquitinating Enzymes. Annual Review of Biochemistry. 2009;78:363–97.
15. Nijman SMB, Luna-Vargas MPA, Velds A, Brummelkamp TR, Dirac AMG, Sixma TK, Bernards R. A genomic and functional inventory of deubiquitinating enzymes. Cell. 2005;123(5):773–86.
16. Shao J, Zhao WH, Gu W. Suppression of Cancer Cell Growth by Promoting Cyclin D1 Degradation. Mol Cell. 2009;36(3):469–76.
17. Gennaro VJ, Stanek TJ, Peck AR, Sun YG, Wang F, Qie S, Knudsen KE, Rui H, D’Andrea AD, Auld DS, DeLucas LJ, Li ZY, Boxer MB, Sinneonov A. Small Molecule Inhibition of the Ubiquitin-specific Protease USP2 Accelerates cyclin D1 Degradation and Leads to Cell Cycle Arrest in Colorectal Cancer and Mantle Cell Lymphoma Models. J Biological Chemistry. 2016;291(47):24628–40.
19. Zhang C, Lu J, Zhang QW, Zhao W, Guo JH, Liu SL, Wu YL, Jiang B, Gao FH. USP7 promotes cell proliferation through the stabilization of Ki-67 protein in non-small cell lung cancer cells. Int J Biochem Cell B. 2016;79:209–21.  
20. George A, Marcon E, Greenblatt J, Frapper L. Identification and Characterization of USP7 Targets in Cancer Cells. Sci Rep-Uk. 2018&.  
21. Zhang L, Wang H, Tian L, Li HK. Expression of USP7 and MARCH7 is Coordinated with Poor Prognosis in Epithelial Ovarian Cancer. Tohoku J Exp Med. 2016;239(3):165–75.  
22. Xu YH, Lu S. Metformin Inhibits Esophageal Cancer Proliferation by Upregulation of USP7. Cellular Physiology and Biochemistry. 2013;32(5):1178–86.  
23. Wang SA, Wang YC, Chuang YP, Huang YH, Su WC, Chang WC, Hung JJ. EGF-mediated inhibition of ubiquitin-specific peptidase 24 expression has a crucial role in tumorigenesis. Oncogene. 2017;36(21):2930–45.  
24. Vodermaier HC. APC/C and SCF: Controlling each other and the cell cycle. J Cell Biochem. 2016;115:1052–60.  
25. Yang WC, Shih HM. The deubiquitinating enzyme USP37 regulates the stability of p53 and c-Myc in breast cancer cells. Cell. 2010;140(3):384–92.  
26. Zhou KL, Liu YJ, Liu ZY, Ma XM, Wang XW, Jin H, Zhang XP, Fu D, Hou LJ, Lyu JY. Ubiquitin-specific protease 2a stabilizes MDM2 and regulates the p53-mediated intrinsic apoptotic pathway in glioblastoma. Carcinogenesis. 2014;35(7):1500–9.  
27. Wang CL, Wang YJ, Liu ZY, Xu H, Liu JL, Jin H, Deng LH, You T. USP22 links REST to the control of p27 stability and cell proliferation. Oncogene. 2013;32(13):1691–701.  
28. Park JM, Lee JE, Park CM, Kim JH. USP44 Promotes the Tumorigenesis of Prostate Cancer Cells through EZH2 Protein Stabilization. Mol Cells. 2019;42(1):17–27.  
29. Nishimura S, Oki E, Ando K, Iimori M, Nakaji Y, Nakashima Y, Saeki H, Oda Y, Maehara Y. High ubiquitin-specific protease 44 expression induces DNA aneuploidy and provides independent prognostic information in gastric cancer. Cancer Med. 2017;6(6):1453–64.  
30. Holland AJ, Cleveland DW. The deubiquitnase USP44 is a tumor suppressor that protects against chromosome missegregation. Journal of Clinical Investigation. 2012;122(12):4325–8.  
31. Lin ZH, Yang H, Tan C, Li JP, Liu ZJ, Quan Q, Kong SY, Ye JS, Gao BX, Fang JW. USP44 directly deubiquitinates and stabilizes USP10 with G3BP2 Inhibits p53 Signaling and Contributes to Poor Outcome in Prostate Cancer. Mol Cancer Res. 2018;16(6):596–66.  
32. Sun J, Li TX, Zhao YY, Huang LR, Sun H, Wu H, Jiang XF. USP10 inhibits lung cancer cell growth and invasion by upregulating PTEN. Mol Cell Biochem. 2018;441(1–2):1–7.  
33. Liu Y, Lu J, Chen Y, Wang P. USP10 Antagonizes c-Myc Transcriptional Activation through SIRT6 and Stability by deubiquitinating enzyme USP42. EMBO J. 2011;30(24):4921–30.  
34. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
35. Potu H, Peterson LF, Pal A, Verhaegem M, Cao J, Talpaz M, Donato N. USP5 links suppression of p53 and FAS localization and stability by deubiquitinating USP37 with G3BP2 Inhibits p53 Signaling and Contributes to Poor Outcome in Prostate Cancer. Mol Cancer Res. 2014;12(6):562–70.  
36. Hock AK, Vigneron AM, Carter S, Ludwig RL, Vousden KH. Regulation of p53 stability and function by the deubiquitinating enzyme USP42. EMBO J. 2011;30(24):4921–30.  
37. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
38. Potu H, Peterson LF, Pal A, Verhaegem M, Cao J, Talpaz M, Donato N. USP5 links suppression of p53 and FAS localization and stability by deubiquitinating USP37 with G3BP2 Inhibits p53 Signaling and Contributes to Poor Outcome in Prostate Cancer. Mol Cancer Res. 2014;12(6):562–70.  
39. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
40. Potu H, Peterson LF, Pal A, Verhaegem M, Cao J, Talpaz M, Donato N. USP5 links suppression of p53 and FAS localization and stability by deubiquitinating USP37 with G3BP2 Inhibits p53 Signaling and Contributes to Poor Outcome in Prostate Cancer. Mol Cancer Res. 2014;12(6):562–70.  
41. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
42. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
43. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
44. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
45. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
46. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
47. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
48. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
49. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
50. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.  
51. Koster R, Timmer-Bosscha H, Bischoff R, Gietema JA, de Jong J. Disruption of the MDM2-p53 interaction strongly potentiates p53-dependent apoptosis in cisplatin-resistant human testicular carcinoma cells via the Fas/FasL pathway. Cell Death Dis. 2011;2:e148.
87. Premarathne S, Murtaza M, Matigian N, Jolly LA, Wood SA. Loss of Usp9x
86. Yun SI, Kim HH, Yoon JH, Park WS, Hahn MJ, Kim HC, Chung CH, Kim KK.
84. Kim SY, Baek KH. TGF-beta signaling pathway mediated by deubiquitinating
78. Iyengar PV. Regulation of Ubiquitin Enzymes in the TGF-beta Pathway.
75. Wang YC, Wu YS, Hung CY, Wang SA, Young MJ, Hsu TI, Hung JJ. USP24
82. Zhu J, Luo Z, Pan Y, Zheng W, Li W, Zhang Z, Xiong P, Xu D, Du M, Wang B,
81. Zhang L, Zhou FF, Drabsch Y, Gao R, Snaar-Jagalska BE, Mickanin C, Huang
80. Eichhorn PJA, Rodon L, Gonzalez-Junca A, Dirac A, Gili M, Martinez-Saez E,
73. Sun SC. CYLD: a tumor suppressor deubiquitinase regulating NF-kappaB
36. Mitra B, Elnady FAEM, Ahn YH, Yang HJ, Liu JB, Dou QP. Discovering
24. Byun S, Lee SY, Lee J, Jeong CH, Farrand L, Sim D, Reddy K, Kim JY, Lee MH,
34. Zhang H, Han B, Lu HL, Zhou YB, Chen XS, Meng GW, Cao MR, Cai L, Hu J,
USP22 promotes resistance to EGFR-TKIs by preventing ubiquitination-
mediated EGFR degradation in EGFR-mutant lung adenocarcinoma.
Cancer Letters. 2018;433:186–98.
36. Melo-Cardenas J, Xu YM, Wei JC, Tan K, Cong KY, Gao BX, Montaui E,
Kissamper G, Lichte JD, Ji P, Crispino JD, Fang DY. USP22 deficiency leads to myeloid leukemia upon oncogenic Kras activation through a PU.1-
dependent mechanism. Blood. 2018;132(4):23–34.
30. Wang A, Ning Z, Lu C, Gao W, Liang JX, Yin Q, Tan G, Liu JW. USP22
Induces Cell Death in Lung Adenocarcinoma by Regulating gamma H2AX-Mediated DNA Damage Repair and Ku70/86-Mediated Apoptosis. Front Pharmacol. 2017;8.
34. Zhang HJ, Liu Q, Liu F, Yuan T, Ji J, Yu L, Qiao JP, Crispino JD, Fang DY. USP22 deficiency leads to myeloid leukemia upon oncogenic Kras activation through a PU.1-dependent mechanism. Blood. 2018;132(4):23–34.
30. Wang A, Ning Z, Lu C, Gao W, Liang JX, Yin Q, Tan G, Liu JW. USP22
Induces Cell Death in Lung Adenocarcinoma by Regulating gamma H2AX-Mediated DNA Damage Repair and Ku70/86-Mediated Apoptosis. Front Pharmacol. 2017;8.
34. Zhang HJ, Liu Q, Liu F, Yuan T, Ji J, Yu L, Qiao JP, Crispino JD, Fang DY. USP22 deficiency leads to myeloid leukemia upon oncogenic Kras activation through a PU.1-dependent mechanism. Blood. 2018;132(4):23–34.
30. Wang A, Ning Z, Lu C, Gao W, Liang JX, Yin Q, Tan G, Liu JW. USP22
Induces Cell Death in Lung Adenocarcinoma by Regulating gamma H2AX-Mediated DNA Damage Repair and Ku70/86-Mediated Apoptosis. Front Pharmacol. 2017;8.
34. Zhang HJ, Liu Q, Liu F, Yuan T, Ji J, Yu L, Qiao JP, Crispino JD, Fang DY. USP22 deficiency leads to myeloid leukemia upon oncogenic Kras activation through a PU.1-dependent mechanism. Blood. 2018;132(4):23–34.
30. Wang A, Ning Z, Lu C, Gao W, Liang JX, Yin Q, Tan G, Liu JW. USP22
Induces Cell Death in Lung Adenocarcinoma by Regulating gamma H2AX-Mediated DNA Damage Repair and Ku70/86-Mediated Apoptosis. Front Pharmacol. 2017;8.
34. Zhang HJ, Liu Q, Liu F, Yuan T, Ji J, Yu L, Qiao JP, Crispino JD, Fang DY. USP22 deficiency leads to myeloid leukemia upon oncogenic Kras activation through a PU.1-dependent mechanism. Blood. 2018;132(4):23–34.
Uniprot C. Uniprot: a worldwide hub of protein knowledge. Nucleic Acids Res. 2019;47(D1):D506–15.

Gavory G, O’Dowd CR, Helm MD, Flasz J, Arkoudis E, Dossang A, Hughes C, Cassidy E, McFieand L, Ouissewol E, Page N, Barker O, Milh H, Hamilton T. Discovery and characterization of highly potent and selective allostERIC USP7 inhibitors. Nat Chem Biol. 2018;14(2):118–24.

Williams SA, Maeker JL, French DM, Liu JF, Kong LY, Wang S, Estrov Z, Priebe W, Donato NJ. Activation of a novel Bcl/Abi destruction pathway by WP1130 induces apoptosis of chronic myelogenous leukemia cells. Blood. 2007;109(8):3470–8.

Kapuria V, Levitski A, Bornmann WG, Maxwell D, Priebe W, Sorenson RJ, Showalter HD, Talpaz M, Donato NJ. A novel small molecule deubiquitinating inhibitor blocks Jak2 signaling through Jak2 ubiquitination. Cellular Signalling. 2011;23(12):2076–85.

Perry JW, Ahmed M, Chang KO, Donato NJ, Showalter HD, Wobus CE. Antiviral activity of a small molecule deubiquitinating inhibitor occurs via induction of the unfolded protein response. PLoS pathogens. 2012;8(7):e1002783.

Pringle LM, Young R, Quick L, Riquelme DN, Oliveira AM, May MJ, Chou MM. Atypical mechanism of NF-kappa B activation by TREG1/ubiquitin-specific protease 6 (USP6) oncogene and its requirement in tumorigenesis. Oncogene. 2012;31(30):3525–35. 

Colland F, Formstecher E, Jacx X, Reversy C, Planquette C, Conrath S, Parpinell C, Kostelecky A, Fursev P, Gourse D, Varny V, Bossy G, Rain JC, Gueudt P, Delansone R, Davlet L. Small-molecule inhibitor of USP7/HAUSP ubiquitin protease stabilizes and activates p53 in cells. Mol Cancer Ther. 2009;8(9):2286–95.

Reversy C, Conrath S, Lopez R, Planquette C, Atmancene C, Collura V, Harpon J, Battaglia V, Vivat V, Sippel W, Colland F. Discovery of Specific Inhibitors of Human USP7/HAUSP Deubiquitinating Enzyme. Chem Biol. 2012;19(4):467–77.

Chauhan D, Tian Z, Nicholson B, Kumar GGS, Zhou B, Carrasco R, McDermott JL, Leach CA, Fulciniti M, Kodrasov MP, Weinstock J, Kingsbury WD, Zhu H, Shih D, Signorelli S, Altun M, Kessler B, Orlewski R, Richardson P, Murash N, Anderson K. A Small Molecule Inhibitor of Ubiquitin-Specific Protease-7 Induces Apoptosis in Multiple Myeloma Cells and Overcomes Bortezomib Resistance. Cancer Cell. 2012;22(3):345–58.

Weinstock J, Wu J, Cao P, Kingsbury WD, McDermott JL, Kodrasov MP, McEwkm D, Murash N, Siddiki MM, Matern MR, Nicholson B. Selective Dual Inhibitors of the Cancer-Related Deubiquitylating Proteases USP7 and USP47. ACS medicinal chemistry letters. 2012;3(10):769–92.

Kessler BM. Selective and reversible inhibitors of ubiquitin-specific protease 7: a patent evaluation (WO2013030218). Expert Opin Ther Pat. 2014;24(5):597–602.

Weinstock J, Wu J, Cao P, Kingsbury WD, McDermott JL, Kodrasov MP, McEwkm D, Murash N, Siddiki MM, Matern MR, Nicholson B. Selective Dual Inhibitors of the Cancer-Related Deubiquitylating Proteases USP7 and USP47. 2012;3(10):769–92.

Sacco JJ, Coulmont JM, Clayge MJ, Urbe S. Emerging Roles of Deubiquitases in Cancer-Associated Pathways. Jiblum Life. 2010;62(2):140–57.

Li MY, Chen DL, Shihlo A, Luo JY, Nikolaev A, Jin J, Wu G. Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. Nature. 2002;416(6881):648–53.

van der Horst A, de Vries-Smits AMM, Breukman AB, van Tiest MH, van den Broek N, Colland F, Maurice MM, Burger M. FOXP4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. Nat Cell Biol. 2006;8(10):1064–1040.

Song MS, Salmena L, Caracedo A, Egbia L, Lo-Coco F, Pandolfo M. The deubiquitylation and localization of PTEN are regulated by a HAUSP-PML network. Nature. 2008;455(7214):813–818.

Karni-Schmidt O, Lohikin D, Pives C. The Roles of MDM2 and MDMX in Cancer. Annu Rev Pathol-Mech. 2016;11:617–44.

Li MY, Brooks CL, Kor N, Gu W. A dynamic role of HAUSP in the p53-Mdm2 signalling. Cell Res. 2019;47(D1):D506–15.
150. Soares L, Senoogy C, Skrenta H, Anandasabapathy N, Lovelace P, Chung CD, Engleman E, Fathman CG. Two isoforms of otubain 1 regulate T cell energy via GRAIL. Nat Immunol. 2004;5(4):45–54.

151. Dupont S, Mamedii C, Cordemonisi M, Montagner T, Zacchigna L, Adorno M, Cordenonsi M, Montagner M, Zacchigna L, Adorno M, Soares L, Seroogy C, Skrenta H, Anandasabapathy N, Lovelace P, Chung CD, Young J. Deubiquitinating enzymes: Essential for TGF beta signaling. Controls Smad4 monoubiquitination. Cell. 2009;136(1):123–35.

152. Schwickart M, Huang XD, Lill JR, Liu JF, Ferrando R, French DM, Meecker H, O’Rourke K, Bazan F, Eastham-Anderson J, Yue P, Doman D, Huang DCS, Dikit VM. Deubiquitinating USP9X stabilizes MCL1 and promotes tumour cell survival. Nature. 2010;463(7277):103–11.

153. Cox JL, Wilder PJ, Gilmore JM, Wuebben EL, Washburn MP, Rizzino A. The SOX2-Inteearctome in Brain Cancer Cells Identifies the Requirement of MISO and USP9X for the Growth of Brain Tumor Cells. Plos One. 2013;8(5).

154. Peng J, Hu Q, Liu WP, He XL, Cui L, Chen XL, Yang M, Liu HQ, Wei W, Liu SL, Wang H. USP9X expression correlates with tumor progression and poor prognosis in esophageal squamous cell carcinoma. Diagn Pathol. 2013;8.

155. Sun C, Sääksijärvi H, Birren B, Devon K, Tang ZL, Silber S, Oates R, Page DC. Proliferation through the Accumulation of beta-Catenin. In: Proc Natl Acad Sci USA. 2013;110(1):7049–60.

156. Lee BH, Lee MJ, Park S, Oh D-C, Elsasser S, Chen P-C, Garner C, Dimova N, Hanna J, Gyp S, Wilson SM, King RW, Finley D. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. Nature. 2010;467:57.

157. D’Arcy P, Birnici S, Olfsson MH, Fryknas M, Lindsten K, De Cesare M, Perego P, Sadebghi B, Hassan M, Larsson R, Linder S. Inhibition of proteasome deubiquitinating activity as a new cancer therapy. Nature medicine. 2011;17(12):1636–40.

158. Zhou B, Zuo Y, Li B, Wang H, Liu H, Wang X, Qiu X, Hu Y, Wen S, Du J, Bu X. Deubiquitination inhibition of 195 regulatory particles by 4-arylidene curcumin analog AC17 causes NF-kappaB inhibition and p53 reactivation in human lung cancer cells. Molecular cancer therapeutics. 2013;12(8):1381–92.

159. Finley D. Recognition and Processing of Ubiquitin-Protein Conjugates by the Proteasome. Annual review of biochemistry. 2009;78:477–513.

160. Slivaca L, Nurrkala-Karssons M, Karlsson T, Ingelsten M, Nystrom J, Eriksson K, Nilsson L, Aschner M. Indoleamine 2,3-dioxygenase expression and functional activity in dendritic cells exposed to cholera toxin. Scandinavian journal of immunology. 2012;76(2):113–22.

161. Qu GZ, Sun W, Jin MZ, Lin J, Lu PG, Jin WL. The bad seed gardener: Deubiquitinas in the cancer stem-cell signaling network and therapeutic resistance. Pharmacol Therapeut. 2017;171:127–38.

162. Eichhorn PJ, Rodon L, Gonzalez-Junca A, Baselga J, Seoane J. USP15 stabilizes the TGF-beta receptor I and promotes oncogenesis through the inhibition of PI3K/AKT signaling. Cancer Research. 2012;72.

163. Inui M, Manfrini A, Mamedii C, Martello G, Morsut L, Soligo S, Enzo E, Moro S, Polo S, Dupont S, Cordemonisi M, Piccolo S. USP15 is a deubiquitylating enzyme for receptor-activated SMADs. Nat Cell Biol. 2011;13(11):1368–U1187.

164. Baker RT, Wang XW, Wolloott E, White JA, Sutherland GR. Identification, functional characterization, and chromosomal localization of USP15, a novel human ubiquitin-specific protease related to the UCP oncoprotein, and a potential tumor-specific nucelotide for human ubiquitin-specific proteases. Genomics. 1999;59(3):264–74.

165. Villeneuve NF, Tian W, Wu TD, Sun Z, Lau A, Chapman E, Fang DY, Zhang DD. USP15 Negatively Regulates NTR2 through Deubiquitination of Keap1. Mol Cell. 2013;51(1):618–79.

166. Gehler-Boyer V, Trouplin V, Adelida J, Aceto N, Remy P, Pinson S, Houdayer C, Aceto N, Remy V, Pinson S, Houdayer C, Argenton F, Newfeld SJ, Piccolo S. USP9X, a Deubiquitinating Enzyme Essential for the STING Pathway. Proc Natl Acad Sci USA. 2011;108(45):18360–5.

167. Lee RC, Fong IF, Wang X, Zhao Y, Xu JY, Liu L, Zhang Y, Li W, Wang X, Chen Y, Hwu W, et al. Induction of cell death and down-regulation of c-Myc by USP7 in human breast cancer cells. Cancer Res. 2013;73(9):2639–48.

168. Fortini ME, Bowerman B. Comparative Genomic Analysis of the Yeast DEG/UGA Pathway. Science. 2013;340(6139):1356–60.
190. Lu Y, Adegoke OAJ, Nepveu A, Nakayama KI, Bedard N, Cheng DM, Peng JMA and Wing S.S. USP19 Deubiquitinating Enzyme Supports Cell Proliferation by Stabilizing KPC1, a Ubiquitin Ligase for p27kip1 (vol 29, pg 547, 2009). Mol Cell Biol 29(11):3241-3241, 2009.

191. Hassink GC, Zhao B, Sompallae R, Altun M, Castaldello S, Zinin NV, Masucci MG, Lindsten K. The ER-resident ubiquitin-specific protease 19 participates in the UPR and rescues ERAD substrates. Embo Rep. 2009;10(7):755-61.

192. Lee JG, Kim W, Gyi S, Ye YH. Characterization of the deubiquitinating Activity of USP19 and Its Role in Endoplasmic Reticulum-associated Degradation. Journal of Biological Chemistry. 2014;289(6):3510-7.

193. Berthouze M, Venkataramanan V, Li Y, Shenoy SK. The deubiquitinases USP23 and USP20 coordinate beta(2) adrenergic receptor recycling and resensitization. Embo Journal. 2009;28(12):1684-96.

194. Yasunaga J, Lin FC, Lu XB, Jeang KT. Ubiquitin-Specific Peptidase 20 Targets TRAF6 and Human T Cell Leukemia Virus Type 1 Tax To Negatively Regulate NF-kappa B Signaling. J Virol. 2011;85(3):6122-9.

195. Nakagawa T, Kajitani T, Togo S, Masuko N, Ohdan H, Hishikawa Y, Koji T, Yasunaga J, Lin FC, Lu XB, Jeang KT. Ubiquitin-Specific Peptidase 20 Targets TRAF6 and Human T Cell Leukemia Virus Type 1 Tax To Negatively Regulate NF-kappa B Signaling. J Virol. 2011;85(3):6122-9.

196. Xu GF, Tan XJ, Wang HM, Sun WJ, Shi Y, Burlingame S, Gu X, Cao GW, Xu GF, Tan XJ, Wang HM, Sun WJ, Shi Y, Burlingame S, Gu X, Cao GW, Wang PJ, McCarrey JR, Yang F, Page DC. An abundance of X-linked genes drive lethal cancer progression. Cancer Research. 2014;74(1):272-82.

197. Dirac AMG, Bernards R. The Deubiquitinating Enzyme USP26 is a Regulator of Androgen Receptor Signaling. Mol Cancer Res. 2010(8):844-54.

198. Paduch DA, Mielnik A, Schlegel PN. Novel mutations in tests-specific ubiquitin protease 26 gene may cause male infertility and hypogonadism. Reprod Biomed Online. 2005;10(6):747-54.

199. Wang PJ, McCarey JR, Yang F, Page DC. An abundance of X-linked genes expressed in spermatogonia. Nat Genet. 2001;27(4):422-6.

200. Zhang Y, Fiorenli L, Byrum SD, Mackintosh SG, Calhoun-Davis T, Koutelou E, Wang L, Tang DG, Tackett AJ, Washburn MP, Workman JL, Dent SYR. ATXN1L and EN2 Coordinate Activity of Multiple H2B Deubiquitinases Important for Cellular Proliferation and Tumor Growth. Mol Cell. 2016;62(4):558-71.

201. Popov N, Herskov S, Llamazares M, Schulien C, Eilers M, Fisz7 and USP28 regulate Myc protein stability in response to DNA damage. Cell Cycle. 2007; 6(19):2327-31.

202. Zhang D, Zaug K, Mak TW, Eldedge S.J. A role for the deubiquitinating enzyme USP28 in control of the DNA-damage response. Cell. 2006;126(3): 529-42.
by Controlling Steady-State Levels of DNA Polymerase beta. Mol Cell. 2011;41(5):609–15.

231. Zhang Z, Jones A, Joo HY, Zhou DW, Cao Y, Chen SX, Erdjument-Bromage H, Renfrow M, He H, Tempst P, Townes TM, Giles KE, Ma LG, Wang HB. USP49 deubiquitinates histone H2B and regulates cotranscriptional premRNA splicing. Gene Dev. 2013;27(14):1581–95.

232. Aressy B, Jullien D, Cazales M, Marcellin M, Bugler B, Burlet-Schlitz O, Ducommun B. A screen for deubiquitinating enzymes involved in the G2/M checkpoint identifies USP50 as a regulator of HSP90-dependent Wee1 stability. Cell Cycle. 2010;9(18):3815–22.

233. Pereg Y, Liu BY, O'Rourke KM, Sagolla M, Dey A, Komuves L, French DM, Dixit VM. Ubiquitin hydrolase Dub3 promotes oncogenic transformation by stabilizing Cdc25A. Nat Cell Biol. 2010;12(4):400–U226.

234. Delgado-Diaz MR, In YM, Berg A, Freire R, and Smits V.A.J. Dub3 controls DNA damage signalling by direct deubiquitination of H2AX (vol 8, pg 884, 2014). Mol Oncol 11(8):1112-1112, 2017.

235. Ke HN, Augustine CK, Gandham VD, Jin JY, Deakyne SK, Hall RP, Zhang JY. CYLD Inhibits Melanoma Growth and Progression through Suppression of the JNK/AP-1 and beta 1-Integrin Signaling Pathways. J Invest Dermatol. 2013;133(1):221–9.

236. Friedman CS, O’Donnell MA, Legarda-Addison D, Ng A, Cardenas WB, Yount JS, Moran TM, Basler CF, Komuro A, Horvath CM, Xavier R, Ting AT. The tumour suppressor CYLD is a negative regulator of RIG-I-mediated antiviral response. Embo Rep. 2008;9(9):930–6.

237. Zhang MY, Wu XF, Lee AJ, Jin W, Chang M, Wright A, Imaizumi T, Sun SC. Regulation of I kappa B kinase-related kinases and antiviral responses by tumor suppressor CYLD. Journal of Biological Chemistry. 2008;283(27):18621–6.

238. Kovalenko A, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Cousto G. The tumour suppressor CYLD negatively regulates NF-kappa B signalling by deubiquitination. Nature. 2003;424(6950):801–5.

239. Wright A, Relley WW, Chang M, Jin W, Lee AJ, Zhang MY, Sun SC. Regulation of early wave of germ cell apoptosis and spermatogenesis by deubiquitinating enzyme CYLD. Dev Cell. 2007;13(5):705–16.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research; over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions