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Targeting the Ubiquitin Proteasome System in Cancer

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Abstract

The ubiquitin proteasome system is involved in a myriad of biological functions including cell cycle progression, intracellular signaling and protein degradation. As such, it is not surprising to find many components of the system misregulated in cancer. The clinical success of Bortezomib for treatment of multiple myeloma proves that targeting the ubiquitin proteasome system is valid and feasible. Here, a detailed examination of the strategies used to target the ubiquitin proteasome system in cancer is discussed. The inhibitors available, its targets, the cancer type and the developmental stage it is in are discussed.

Keywords: ubiquitin, proteasome, E1, E2, E3, ubiquitin proteasome system, cancer, deubiquitinase, DUBs inhibitors

1. Introduction

The function and activity of most proteins can be partially modulated by posttranslational modifications (PTMs). In particular, ubiquitination has emerged as one of the most versatile PTMs over the past few decades. Ubiquitination is a process that attaches ubiquitin, a short polypeptide of 76 amino acids, for its covalent link to proteins. It is a highly conserved process that mostly targets unwanted proteins for degradation either through proteasome-mediated or by directly sorting proteins to the lysosome and thus helps to maintain cellular homeostasis [1]. However, ubiquitination may also play a crucial role in other non-proteolytic regulatory functions such as protein activation, interaction, and translocation [2].

Ubiquitination is a multistep process and requires the sequential action of three enzymes, the E1 activating enzyme, E2 conjugating enzyme, and E3 ligase [3, 4] (**Figure 1**). The process of ubiquitin attachment commences when E1 recruits free ubiquitin in the cell through its active cysteine residue. The C-terminal glycine residue of ubiquitin is activated through ATP-dependent
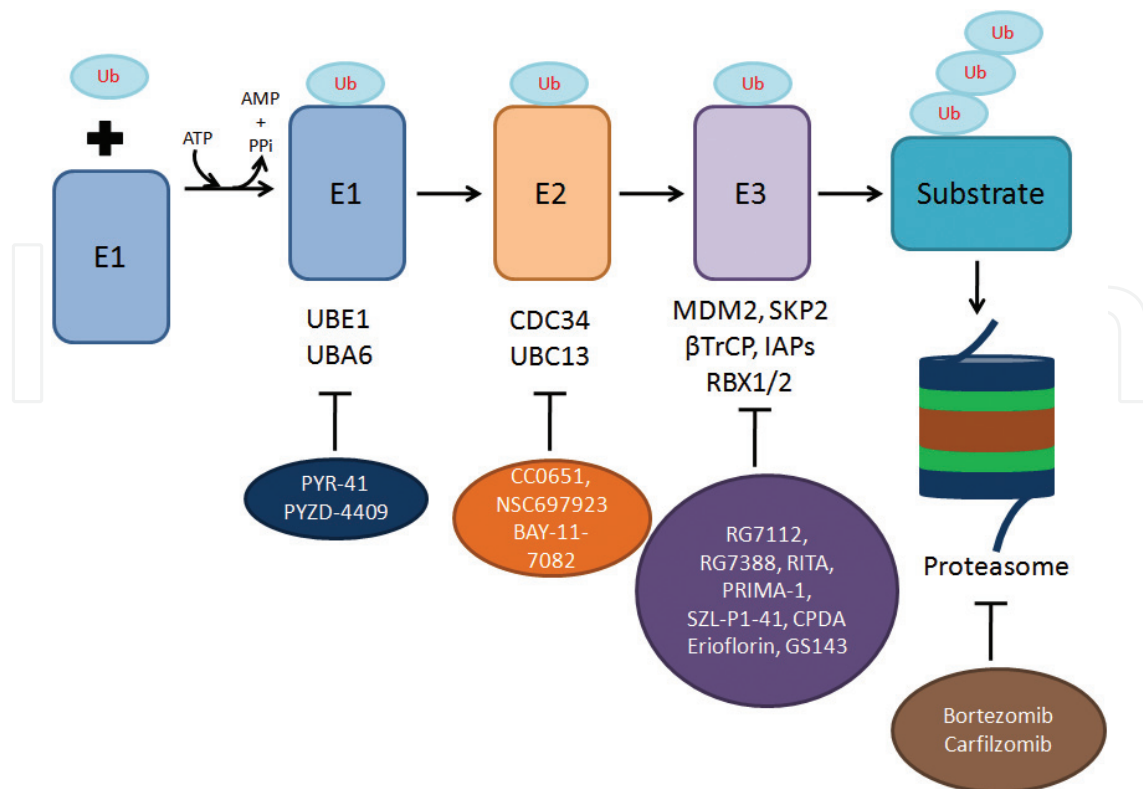
Figure 1. Schematic representation of ubiquitin conjugation cascade and inhibitors targeting specific cascade component.

adenylation and thioester bond formation catalysed by E1, resulting in attachment by non-covalent linkage to the E1 cysteine residue [5–7]. Activated ubiquitin is then transferred from E1 to a cysteine residue of the E2 conjugating enzyme linked through a thioester bond [4, 8].

The E3 ligases are responsible for substrate recognition and facilitates transfer of ubiquitin to the substrates from E2 resulting in covalent attachment of ubiquitin to the substrate's lysine residue [4, 9, 10]. The two major classes of E3s are the RING and HECT domain E3s which transfer ubiquitin through different mechanisms [9, 10]. HECT domain ligases accept ubiquitin from E2 through its catalytic cysteine residue and act as an intermediate entity capable of transferring ubiquitin to its recruited substrate [10]. RING domain ligases, instead of directly transferring ubiquitin, function as scaffolds and allow ubiquitin transfer from the E2 directly to the substrate [9]. In addition, other E3 classes such as ring-between-ring E3s are not discussed here [11].

Moreover, the ubiquitin molecule itself contains seven intrinsic lysine residues (K6, K11, K27, K29, K33, K48, and K63) and Met1 that can be further ubiquitinated allowing for the formation of various types of ubiquitin chains [12]. These come in the form of linear, branched, forked, homotypic, heterotypic kinds of monoubiquitin, multi-monoubiquitin, and polyubiquitin chain types. Each type could be associated with distinct cellular functions. For example, one of the best-known polyubiquitinations is K48-linked ubiquitination which acts as a degradation signal targeting substrate for proteasomal degradation [13].

The degradation of polyubiquitinated proteins is subsequently carried out in the 26S macro-molecular proteasome complex which is present in both the cytosol and nucleus of eukaryotic

cells [14]. These complexes keep the proteins under quality checks and help cells to degrade misfolded/unwanted proteins. The proteasome is an approximately 2.5 MDa proteinase complex containing the catalytic active 20S core particle and the regulatory 19S particles [15, 16]. The 20S core particle is a barrel-shaped structure containing four stacked rings with two outer α-rings and two inner β-rings [17]. Each ring is composed of seven distinct α (α1–α7) or β (β1–β7) subunits [17]. The outer α-ring serves as the "gate" for entry of substrates, while the β-rings contain the catalytic activity. Namely, β1, β2, and β5 subunits confer the peptidyl-glutamyl-hydrolysing or caspase-like, the trypsin-like, and the chymotrypsin-like activity, respectively [17].

The 19S subunit can be separated into the "base" and "lid." The base contains ATPase subunits (RPT1–6) and four non-ATPase subunits (Rpn1, 2, 10, 13) [17]. The non-ATPase subunits are ubiquitin receptors that identify ubiquitinated substrates [17]. The lid contains nine subunits (Rpn3, 5–9, 11, 12, 15) and two proteasome-associated deubiquitinating enzymes (UCHL5/Uch37, Ubp6/Usp14) [17, 18]. Together with Rpn11/PSMD14, UCHL5/Uch37 and Ubp6/Usp14 carry out the deubiquitination of substrates before it moves on to the 20S core for degradation [17]. Although it is generally assumed that ubiquitinated proteins end up degraded by the proteasome, a recent review highlighted the strict requirements needed for proteasomal degradation wherein certain ubiquitinated substrates which do not meet these requirements escape from the proteasome and survive degradation [18].
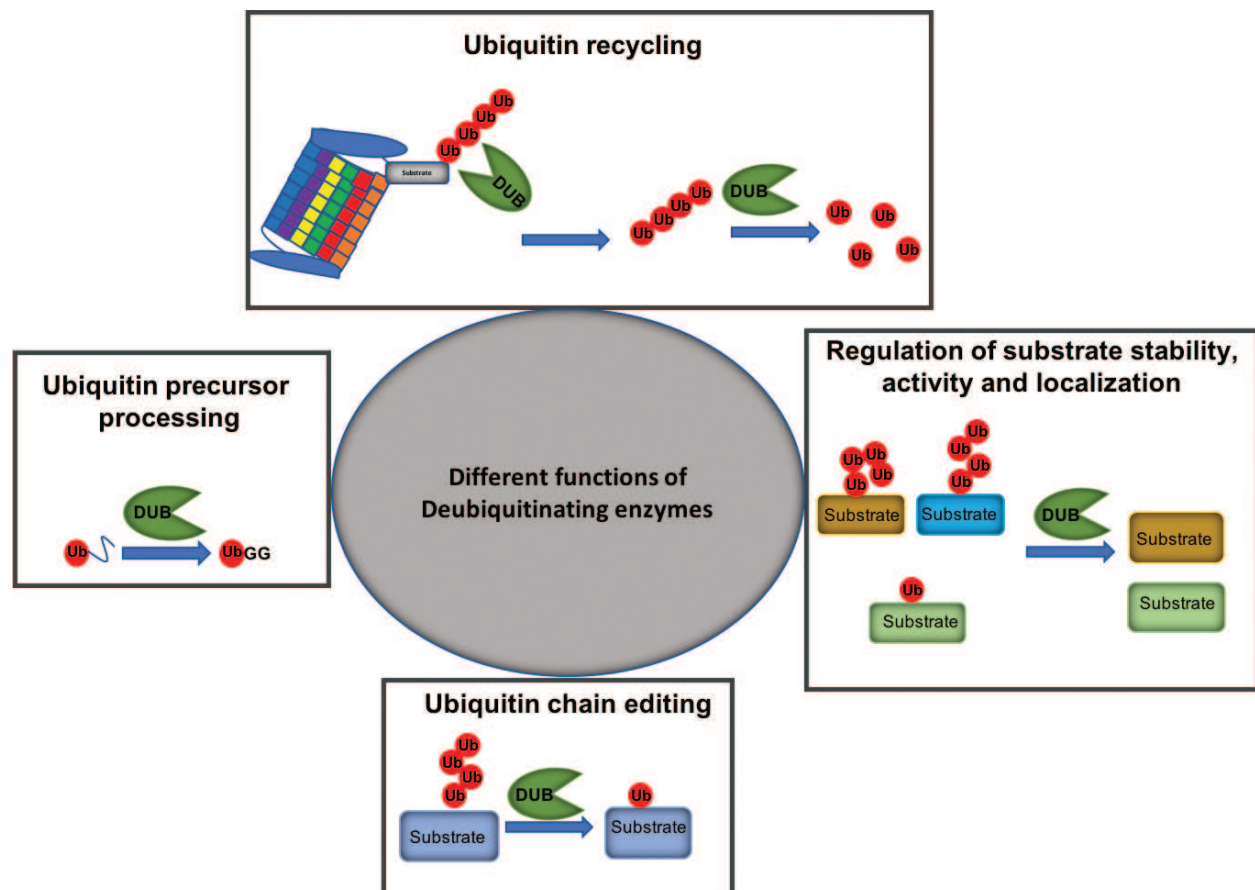

Figure 2. Pictorial representation for involvement of DUBs in different functions.

The ubiquitination process is antagonized by another set of enzymes that specifically removes ubiquitin moieties and counteracts ubiquitin-mediated function of a protein. These specific enzymes are called deubiquitinating enzymes (DUBs). As the name suggests, DUBs are responsible for cleaving the isopeptide bond between protein and ubiquitin. Other than regulating stability and function of its substrates, DUBs are also involved in ubiquitin precursor processing, ubiquitin recycling, and ubiquitin chain editing (**Figure 2**). By conducting the process of removing ubiquitin from its target, DUBs are mostly involved in opposing the effect of ubiquitination on substrates and thus leave a remarkable impact in the field of protein biology.

2. History of the ubiquitin proteasome system

The 2004 Nobel prize for Chemistry was awarded to Avram Hershko, Aaron Ciechanover, and Irwin Rose for the discovery of ubiquitin-mediated protein degradation [19]. Remarkably, the ubiquitin proteasome system (UPS) has been implicated in multiple cellular processes such as cell cycle, stress response, and DNA damage repair [20]. In 1978, Hershko and Ciechanover for the first time showed that ATP-dependent degradation required more than one component [21]. Using reticulocyte lysate and a DEAD cellulose column, they separated 2 fractions that individually do not catalyze ATP-dependent degradation but when combined, restored proteolysis [21]. Shortly after, the 2 fractions were identified. Fraction 1 contained ATP-dependent proteolysis factor 1 (APF-1) which was later identified to be ubiquitin [21–23]. Together with Irwin Rose, Aaron Ciechanover and Avram Hershko identified fraction 2 by further separating it into 2 other fractions containing a 450 kDa protein unknown at that time the proteasome, and the protease system containing E1, E2 and E3 enzymes [24]. It should be noted that prior to this, ubiquitin was first identified by Goldstein in 1975, as a universally present polypeptide, although its function was unknown at that time [25]. Prior to these findings, two reports in 1977 had characterized histone H2A covalently tagged with a single ubiquitin. Although not for degradation, the finding implied that ubiquitin could be used for tagging [26, 27]. Subsequently, a series of papers from the Nobel laureates characterized and defined the multi-step ubiquitin-tagging model for protein degradation through the E1, E2, and E3 enzymatic cascade [4–7, 28, 29]. Additionally, multiple ubiquitin could be tagged to a single molecule of lysozyme showcasing the first polyubiquitin chain [28].

Up till this point, the remaining piece of the puzzle was to identify the downstream protease(s) responsible for degradation of the tagged proteins. In order to characterize the protease(s) responsible, two large multi-subunit proteinase complexes were purified from reticulocytes [15, 16, 30]. One of which requires ATP to degrade the tagged protein (~1500 kDa), while the other is ATP independent (~700 kDa). It was later discovered that these were the 26S proteasome and the 20S core catalytic subunit of the proteasome, respectively [15, 31, 32]. Apart from the ATP-dependent E1 ubiquitin activation step, the process of degradation by the protease was also ATP dependent although the mechanism was unknown [33, 34]. This was resolved when it was found that the assembly of the 26S proteasome from the 20S catalytic core and 19S regulatory subcomplex is ATP dependent, explaining the reliance of energy for substrate degradation by the proteasome [31, 35].

The first papers to show a biological role for the ubiquitin cascade were in 1984 [36, 37]. In a mutant mouse cell line (ts85) that is conditionally lethal and temperature sensitive, monoubiqutinated H2A disappears at high temperatures, suggesting defects in the ubiquitin cascade [38, 39]. As it turns out, the E1 enzyme in ts85 was temperature sensitive, resulting in defects in ubiquitination at high temperatures [36, 37]. Additionally, the cells were arrested at G2 at higher temperature, indicating a role of the UPS in cell cycle regulation. These two papers set the stage for further discovery of biological roles played by the ubiquitin cascade in the coming years [20].

The first observation of deubiquitinating activity was in fact, in the very paper that the first scheme of ATP-dependent degradation was proposed [28]. Specifically, removing ATP from the ^{125}I-labeled ubiquitin-tagged lysozyme in the presence of endogenous proteins reversed the ubiquitin tagging, implying the presence of a DUB which the authors described as an amidase [28]. Subsequently, the first deubiquitination assay was developed and showed the deubiquitinating activity of mammalian ubiquitin C-terminal hydrolase L3 (UCHL3) and its yeast homolog Yuh1, which represented the first DUBs identified and characterized [40, 41]. The work of Varshavsky and colleagues ensued, identifying DUBs in yeast and up till today, there are a total of ~80 known DUBs [42, 43].

3. Ligases in cancer

Since ubiquitination occurs through a multi-step cascade, it can be inferred that multiple proteins along the cascade can be targeted. In particular, inhibitors targeting all three (E1, E2, and E3) classes of enzymes are utilized both in research and clinics. For an overview, the inhibitors that are about to be discussed in this section and the class of enzyme (E1, E2, and E3) which is targeted are summarized in **Figure 1**.

3.1. E1 enzymes

UBE1 and UBA6 are the only two E1 enzymes that are known in humans [44]. Till date, there are only two UBE1 inhibitors, PYR-41 and PYZD-4409 [45, 46]. Among the two, PYR-41 has been shown to inhibit the nuclear factor κB(NF-κB) pathway by regulating the stability of inhibitor of NF-κB (IκB). Additionally, it also prevents the degradation of the tumor suppressor p53 resulting in increased transcriptional activity of p53 [45]. On the other hand, PYZD-4409 was specifically shown to induce ER stress-induced apoptosis in cancer cells and, in a mouse model of leukemia, delayed tumor cell growth [46]. Although these results suggest the potential of targeting E1 in cancer treatment, none of these are currently in clinical trials, perhaps due to off-target effects or poor pharmacokinetic properties.

3.2. E2 enzymes

There are ~38 E2 enzymes in the human genome implying that they serve as more specific targets than E1 [8]. CC0651 is an allosteric inhibitor of CDC34, the common E2 enzyme for

Cullin ligase complexes. Treating cancer cells with CC0651 results in the accumulation of the tumor suppressor p27 and inhibition of proliferation, which suggests that CC0651 could be a potential inhibitor for clinical use [47]. However, development of this compound has met with great difficulties due to pharmacokinetic reasons [48]. Another potential target in cancer is the E2 enzyme UBC13-UEV1A, an important regulator of NF-κB pathway induction through the formation of ubiquitin K63-linked chains. The inhibitor NSC697923 has been shown to inhibit the formation of K63-linked chains by UBC13 *in vitro* and is effective in inhibiting the proliferation and survival of diffuse large B-cell lymphoma (DLBCL) [49]. BAY-11-7082 is a well-known inhibitor of the NF-κB pathway and has been thought to inhibit the IκB kinases [50]. However, it was found to inhibit the E2 UBC13 by preventing ubiquitin conjugation to it, thereby preventing K63-linked chain formation in the same way as NSC697923 [50]. Likewise, it was shown that BAY-11-7082 induces cell death to DLBCL HBL-1 cells [50]. Although E2 inhibitors show immense potential for cancer treatment, so far, E2 inhibitors are present only in preclinical stages.

3.3. E3 enzymes

Amongst enzymes in the ubiquitin conjugation cascade, E3s are the most abundant in number with ~700 ligases identified so far [48]. Due to the large number, targeting E3 will likely increase the specificity and decrease side effects. Due to space limitation, we will be discussing a few of the E3 ligases that are implicated in cancer and refer the readers to the following review about E3 ligases family [51].

3.3.1. Mouse double minute 2 homolog (MDM2)

Termed the guardian of the genome, p53 is frequently upregulated in stress conditions and functions to activate the expression of genes involved in apoptosis and cell cycle arrest to prevent cellular transformation [52]. MDM2 is an E3 ligase of p53 responsible for its degradation and is frequently upregulated in cancer [53, 54]. Thus, targeting MDM2 could be useful for cancer treatment. To this end, several MDM2 inhibitors are available. In particular, the Nutlin family of cis-imidazoline inhibitors shows the greatest potential [55]. One of the latest developed Nutlin inhibitors, RG7112, has been tested in phase I clinical trials and shows activity against relapsed leukemia [56]. Although it showed good clinical outcomes, a high dose was required and it caused gastrointestinal side effects [56, 57]. A more potent pyrrolidine-based MDM2 inhibitor, RG7388 is currently in clinical trial and might be able to overcome these issues [58]. In preclinical setting, RG7388 showed potent tumor inhibition specifically in p53 wild-type xenograft neuroblastoma indicating its possible use in neuroblastoma treatment where majority of tumors are p53 wild-type at diagnosis [59].

A majority of MDM2 inhibitors bind to MDM2 itself to prevent it from binding to p53. RITA (reactivation of p53 and induction of tumor cell apoptosis), however, binds to p53 and prevents MDM2 from interacting [60]. In this case, the mechanism of stabilization might be MDM2 independent and could possibly be used to treat MDM2-independent p53-destabilized cancers. Thus far, the mentioned MDM2 inhibitors aim to restore p53 levels. Given that p53 is known to be mutated in ~50% of all cancers, these therapies are severely limited to a subpopulation of p53 wild-type tumors [48, 61]. An ingenious way to overcome this is through the use

of drugs, which restore mutant p53 function. One example of such an approach is the drug PRIMA-1 which alkylates the thiol groups of mutant p53, correcting protein folding and enabling p53 to carry out its tumor suppressive function [62].

3.3.2. S-phase kinase associated protein 2 (SKP2)

SKP2 is a F-box protein which functions as the substrate recognition subunit of the SCF (SKP1/Cullin/F-box) RING E3 ligase complex [63]. In particular, its role in ubiquitinating and degrading cell cycle regulators, p27 and p21 makes it a potential target in cancer [64, 65]. Additionally, SKP2 is upregulated in several different cancers and serves as a prognostic marker for cancer patient survival [66–68]. Particularly, a structural pocket formed by SKP2 and its neighboring subunit CKS1 within the SCF complex is important for binding and degradation of p27. This outlines a potential vulnerability which could be targeted in cancer therapy. As such, using *in silico* screening to identify inhibitors for this structural pocket, four compounds were shown to increase p27 levels and arrest cells at G1 [69]. Another mechanism that could be utilized to inhibit SKP2 could be by targeting its association with the SCF complex through inhibiting SKP1-SKP2 binding. SZL-P1-41was identified to block SKP1-SKP2 interaction and shows strong antitumor effects against lung and prostate tumor xenograft in mouse models with concomitant increase of p27 [70]. Lastly, CPDA is another compound identified due to its ability to inhibit *in vitro* ubiquitination of p27 by SCF complex [71]. Although its mechanism is unknown, it has been shown to induce cell cycle arrest specifically in leukemic cells but not marrow components [71].

3.3.3. Beta-transducin repeat containing E3 ubiquitin protein ligase (βTrCP)

Like SKP2, βTrCP is a component of the SCF-Cullin E3 ligase complex. It utilizes its N-terminal F-box domain to bind to SKP1 and its C-terminal WD40 domain to bind to substrates including pro-caspase-3, IκB, p53, CDC25, and WEE1. In most cancers, it is upregulated and acts as an oncogene [72]. Erioflorin and GS143 are two βTrCP inhibitors that block the interaction of βTrCP with its targets, PDCD4 and IκB, respectively, leading to their stabilizations [73].

3.3.4. RING box protein 1/RING box protein 2 (RBX1/RBX2)

Both RBX1 and RBX2 are important subunits of the SCF complex and function to physically bring the activated E2 closer to the substrate for ubiquitination [74]. Increased expression of RBX1 is seen in breast, liver, kidney, and lung cancer indicating an oncogenic function [75]. An exception is in melanoma where RBX1 is higher in nevi than in melanomas [76]. Likewise, RBX2 is overexpressed in many human cancers and targets IκB, c-Jun, HIF-1α, and NF1 for degradation [75]. Although its precise mechanism in cancer progression is not well studied, depletion of RBX2 induces apoptosis, decreases tumor growth, and sensitizes cells to DNA damage [77, 78].

3.3.5. Inhibitor-of-apoptosis proteins (IAPs)

The IAPs are RING E3 ligases that inhibit caspases and thereby block apoptosis which makes them putative targets in cancer [79]. During apoptosis, the second mitochondria-derived activator of caspase (SMAC) is released from the mitochondria and binds IAPs which releases

caspases to perform their pro-apoptotic function. IAPs such as c-IAP1 and c-IAP2 are reported to have genomic amplifications in a variety of cancers like hepatocellular carcinoma, cervical, pancreatic and esophageal cancers. SMAC on the other hand gave a better prognosis in breast, colorectal, and bladder carcinomas [80]. Mimicking the SMAC binding region, a few inhibitors were shown to bind IAPs and activate apoptosis in cancer. These are currently in phase I clinical trials [80].

4. Ubiquitin proteasome system in virus-induced cancers

The idea of viral oncoproteins hijacking the cellular degradation system to degrade potential tumor suppressors is exemplified by the early papers showing human papillomavirus (HPV) oncoproteins E6 and E7 utilizing E6-associated protein (E6-AP) and Cullin 2 RING ligase to target p53 and retinoblastoma for degradation, respectively [81–85]. By developing small molecule inhibitors, targeting these ligases in virus-induced cancers holds great potential for cancer therapy. Additionally, depleting E6-AP expression has been shown to increase p53 protein levels and inhibit growth in HPV-positive cells [86]. So far, inhibitors identified that abalte E6-AP and E6 binding using binding assays have been disappointing as they show low efficacy in inducing cell death in culture [86–88]. It is suggested that more structural data are required in order to design better inhibitors [86]. On the other hand, the small molecule RITA mentioned earlier was shown to block the binding of E6 to p53 thereby preventing E6-mediated degradation degradation of p53 [89]. Cervical carcinoma xenografts showed substantial growth suppression when treated with RITA, suggesting its potential use in cervical cancer [89].

Another important tumor suppressor targeted by the HPV E6 protein is Tat-interactive protein 60 kDa (TIP60) [90]. In addition to HPV E6, adenovirus oncoproteins were also reported to target TIP60, implying an important tumor suppressive role played by TIP60 in virus-induced cancer [91]. The mechanism of degradation in HPV-positive cells involves the use of E3 identified by Differential Display (EDD1) to ubiquitinate and target TIP60 to the proteasome [92]. Importantly, overexpression of TIP60 or depletion of EDD1 in cervical cancer mouse xenografts inhibited tumor growth implying that EDD1 could be a novel target in cervical cancer therapy [92]. In addition, EDD1 is also upregulated in ovarian, breast, and pancreatic adenocarcinoma, as such an EDD1 inhibitor could be extended to these cancers [93].

Latent membrane protein 2A (LMP2A) is one of the 9 proteins expressed from Epstein-Barr virus transformed genome and is involved in viral latency and persistence [94]. In order to perform its function, it recruits neural precursor cell-expressed developmentally down-regulated 4-like (NEDD4-like) ligase to facilitate degradation of Lyn, a tyrosine kinase [94, 95]. This in turn blocks signal transduction of B-cell receptor. Although the mechanism is not completely understood, it increases our understanding of how cellular ligases are utilized at different stages of viral-induced cancers [95].

Apart from individual ligases utilized by viral proteins to degrade cellular substrates, there have been many cases reported where viral oncoproteins interact with the proteasome and hijacks it for their own purposes [95]. For example, through binding to the 20S proteasome and the NF-κB precursor p105, Tax, which is the human T-cell leukemia virus (HTLV) oncoprotein,

enhances the proteolytic activation of NF-κB, which sustains T-cell proliferation [95–97]. In particular, this represents a potential susceptibility using proteasome inhibitors in HTLV-infected T-cell leukemia treatment. Indeed, treatment with proteasome inhibitor Bortezomib was investigated in mouse models with mixed results [98, 99]. In HTLV-1-associated xenograft models, Bortezomib inhibited tumor growth and the mice showed prolonged survival [98, 99]. However, heterogeneity in response was seen between tumors treated with vehicle or Bortezomib derived from Tax transgenic mice [98]. More studies need to be conducted before proteasome inhibitors could be used for HTLV infected T-cell leukemia.

Hepatitis B virus X-antigen (HBX) is another oncoprotein known to interact with proteasome subunits PSMA7 and PSMC1 [100]. In the presence of HBX, two well-defined proteasomal substrates had increased half-lives suggesting that HBX can block proteasomal activity [100]. The importance of proteasome inhibition by HBX is shown in its ability to regulate HBV virus replication. Particularly in cells infected with mutated HBV not expressing HBX, proteasomal inhibitors MG132 and Epoxomicin were able to rescue virus replication back to wild-type levels supporting HBX's role in inhibiting proteasome [101, 102].

Apart from the proteasome, HBX also binds to cellular DDB1, a subunit of the Cullin 4 RING ligase (CRL4) complex [101]. Rather than being degraded by the CRL4 complex, it is stabilized and has been suggested to alter CRL4 specificity by displacing DDB1-CUL4-associated factors (DCAFs), which are proteins that confer substrate specificity to the CRL4 complex [103, 104]. Indeed, two recent papers identified structural maintenance of chromosomes 5/6 (SMC5/6) as novel degradation targets of the CRL4-HBX complex [105, 106]. Since SMC5/6 complex is essential for inhibiting the extrachromosomal HBV gene expression, identification of SMC5/6 as CRL4-HBX targets solves the long-standing question of how HBX-DDB1 interaction is important in HBV virus replication [101, 107, 108]. From these data, one plausible strategy would be to design inhibitors to block the interaction between HBX and DDB1 or the proteasome.

5. Families of deubiquitinating enzymes

There are approximately 80 functional DUBs known in humans [109]. These DUBs are mainly divided into six different classes based on their structure and active site homology: ubiquitin specific proteases (USPs), ubiquitin carboxyl-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Machado-Joseph disease protein domain proteases (MJDs), JAMM/MPN (JAB1/MPN/MOV34 metalloenzyme) domain associated metallopeptidases (JAMMs), and monocyte chemotactic protein-induced protein (MCPIP) [110] (**Figure 3**). All DUB families belong to cysteine proteases with the exception of JAMMs family of DUBs, which are zinc-dependent metalloproteases. The mechanism of action for cysteine-dependent DUBs is through nucleophilic attack on the isopeptide linkage of an ubiquitinated lysine residue by the catalytic cysteine, which is facilitated by a nearby histidine side chain that helps to decrease the pKa of the cysteine. A third residue, aspartic acid or asparagine, helps in this whole process. This residue aligns and polarizes the catalytic histidine. Some enzymes which do not have this third residue use other means to polarize histidine [111, 112]. On the other hand, the mechanism of action for JAMMs which are metalloproteases is facilitated by two zinc ions which are present
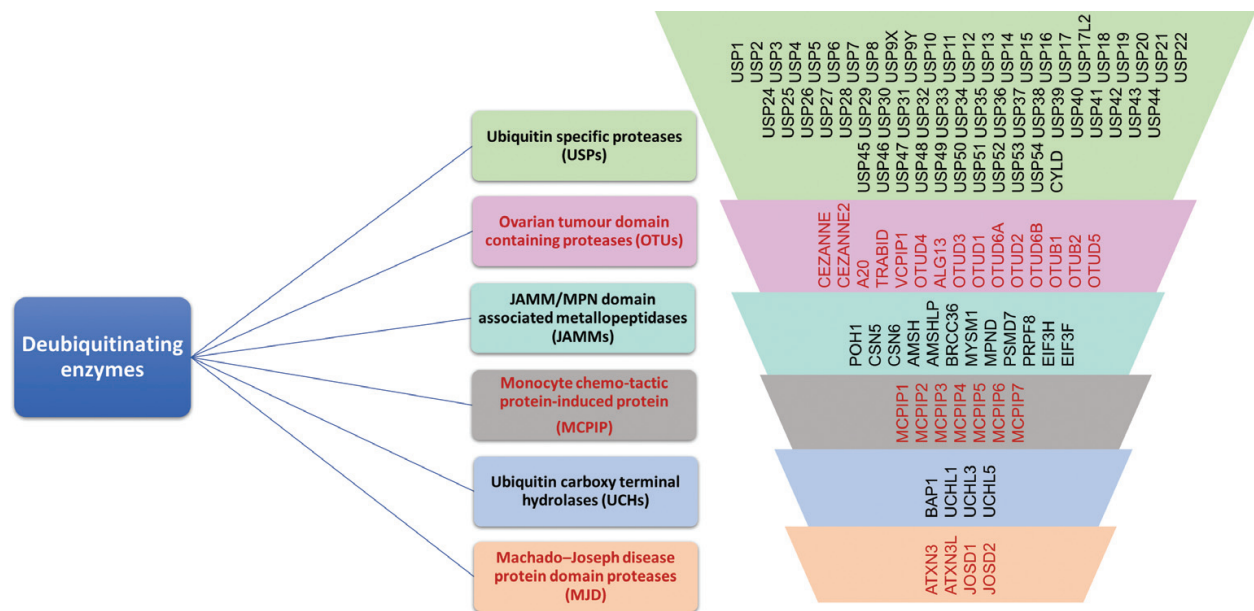
Figure 3. Schematic representation of different families of deubiquitinating enzymes and their members.

within its catalytic site and coordinated by invariant histidine, aspartic acid, and serine side chains [113]. This zinc ion activates a water molecule to form a hydroxide ion which in turn attacks the carboxyl carbon in the isopeptide link [114].

Out of the six classes of DUB families, the USP family is the largest with more than 50 members. These proteins belong to cysteine protease family (clan CA, family C19) [115]. USPs are characterized by the presence of a catalytic core involving histidine and cysteine boxes [116]. DUBs from the USP family contain a highly conserved USP domain characterized by three subdomains which form the palm, thumb, and fingers of a right hand [117]. The active site cysteine is present between the palm and thumb while the finger is used for interaction with ubiquitin. CYLD (cylindromatosis D) is the only USP which does not have the finger domain but possesses an additional domain known as B-box domain [118]. The presence of additional domain and terminal extensions has also been seen in several other USPs, which plays critical roles in conferring specificity to DUBs. For example, USP3, USP5, USP39, USP44, USP45, USP49, and USP51 have zinc finger USP domain, USP25 and USP37 contains ubiquitin-interacting motif, USP5 and USP13 possess ubiquitin-associated domain, USP4, USP11, USP15, USP20, USP33, and USP48 have the domain in USPs (DUSP), and USP52 has the exonuclease III domain. Moreover, several USPs such as USP4, USP7, USP14, USP32, USP47, and USP48 have the ubiquitin-like domain which can be found within and outside of the catalytic domain [115, 119].

UCHs are another family of DUBs, which contain four members in humans, UCHL-1, UCHL-3, UCHL-5, and BAP1. This class of DUBs was the first to be structurally characterized. In particular, UCHs have a short catalytic domain of approximately 200–300 amino acids [109] and can only target short peptide from the C-terminus of ubiquitin because of the presence of a confined loop which prevents polyubiquitin chain recognition and large protein processing. A well-studied member of the UCH class of DUBs is UCHL-1, which is one of the shortest DUBs, having only 223 amino acids [120]. UCHL-1 was initially known to be involved in ubiquitin

maturation by cleaving single amino acids or short peptides from the C-terminus of ubiquitin precursors to generate mono-ubiquitin rather than cleaving ubiquitin from proteins [121].

In UCHL-5 and BAP1, there is the presence of additional C-terminal extension of about 100 and 500 amino acids, respectively. The additional extension at the C-terminus of UCHL-5 directs it to proteasome and helps in trimming polyubiquitin chain from conjugated protein as they are degraded [122]. However, the additional extension of BAP1 contains a nuclear localization signal and helps it to interact with the N-terminal ring finger of BRCA1 (a ubiquitin ligase) [122, 123].

Due to space limitation, we have summarized the targets of different USPs and UCHs in cancer in **Table 1**.

DUBs family	DUBs	Important targets (direct/indirect)	Mechanism/pathway	Relevance to neoplasm
Ubiquitin specific proteases (USPs)	Cylindromatosis (CYLD)	TNFR-associated factor 2 (TRAF2) and TRAF6 [197]	Promotes apoptosis [198]; negatively regulates NFκB signaling [197]	Downregulated in lung cancer [199], liver cancer [200], colon cancer [200] and multiple myeloma [201]
	USP1	Fanconi anaemia complementation group D2 (FANCD2) [202]; proliferating cell nuclear antigen (PCNA) [203]	Involved in DNA repair and DNA-damage response pathways [202]	Overexpressed in hydatidiform mole [204]
	USP2	MDM2 [205], MDMX [206], Cyclin D1 [207]	Indirect regulation of tumor suppressor p53; increase cell proliferation [208]	Associated with bladder cancer and increase in proliferation, invasion and migration in bladder epithelial cells [209]; overexpressed in prostate cancer [210]
	USP4	TGFβRI [211]	Regulates TGFβ signaling pathway [211]; important player mediating crosstalk between TGFβ and PI3K signaling pathway [211]	Upregulated in human hepatocellular-carcinoma samples and has been suggested to induce aggressive phenotype [212]; downregulated in small cell lung cancer cell lines [213]
	USP5	p53 [214]	Inhibits accumulation of free unanchored polyubiquitin chains	
	USP7	p53 [187], PTEN [188], IRS1/2 [215], Chk1 [216], Claspin [217]	Regulates stability of p53 and MDM2 [218]; reported to induce IGF signaling [215]; modulates ATR-Chk1 pathway [216, 217]	Overexpressed in prostate cancer [188]
	USP8	EGFR [219]; ERBB2, ERBB3 and MET [220]	Regulates endosomal ubiquitin dynamics and required for RTK downregulation following internalization [221, 222]	Gain-of-function mutation in Cushing disease [223]; depletion of USP8 leads to selective death of Gefitinib resistant non-small cell lung carcinoma (NSCLs) cells [220, 224]
	USP9X	SMAD4 [225], β-catenin [226]	Regulates signaling pathway such as TGFβ [225] and MAPK pathway [227]	Overexpressed in breast cancer [228], ERG-positive prostate tumors [229] and osteosarcoma cell line SaOS2 [230] and its

DUBs family	DUBs	Important targets (direct/indirect)	Mechanism/pathway	Relevance to neoplasm
				increased expression has been correlated with ill prognosis outcomes in multiple myeloma patients [231] and esophageal squamous cell carcinoma [232]
	USP10	p53 [233], T-box transcriptional factor (T-bet) [234]	Regulates ATM-p53 and mismatch repair (MMR) [235, 236]	Overexpressed in breast cancer [228], glioblastoma [237] and in metastatic melanoma [147]
	USP11	TGFβRII [238], p53 [239]	Regulates TGFβ signaling pathway [240] and BRCA2 mediated damage response [241]	High expression of USP11 has been observed in murine lung tissue [238]
	USP12	Androgen receptor (AR) [242]		Suggested as a putative regulator of progression and metastasis in prostate cancer [242]
	USP13	PTEN [243]	Implicated in PI3K signaling	Important for melanoma growth in soft agar assay and nude mice [244]
	USP15	TGFβRI [245], E6 (human papilloma virus (HPV) protein) [246]	Enhances TGFβ signaling pathway [245]	Overexpressed in glioblastoma, ovarian and breast cancer [245, 247]
	USP16	Histone H2A	Regulates progression of cell cycle and gene expression [248]	Regulates stem cell self-renewal and pathologies associated with Down syndrome [249]
	USP17	Ras-converting enzyme 1 (RCE1) [250]	Important for chemotaxis and chemokinesis, and have a crucial role in cell migration [251]	USP17 is amplified in tumors and found to regulate G1/S cell cycle advancement and proliferation [252]
	USP18	EGFR [253]	Involved in interferon signaling [254]	Implicated in regulation of viral disease and malignancies; identified as anticancer target in acute promyelocytic leukemia (APL) [254, 255]
	USP19	KPC (Kip1 ubiquitination-promoting complex) [256]	Regulates cell growth [256]	Putative target for inhibiting proliferation [256]
	USP20	HIF1α [257], Claspin [258]	Promotes transcription of hypoxic response genes [257]	Decreased expression in gastric cancer cells and negative correlation with tumor size and tumor invasion [258]
	USP21	GATA3 [259], Histone H2A [260], EZH2 [261]	Activates transcription [260]	Upregulated in bladder carcinoma [261], breast carcinoma [262, 263] and cancer stem-like cells (CSCs) of renal cell carcinoma cell lines [263] and its expression was correlated with tumorigenic behavior of cells such as tumor size, proliferation, metastasis and invasion
	USP22	Histones H2A and H2B [264]; c-MYC [265]	Regulates epigenetic modulations that support neoplastic change [264, 266]; involved in regulation of various	Elevated expression of USP22 has been reported to be associated with ill prognosis of several cancer like breast [268],

DUBs family	DUBs	Important targets (direct/indirect)	Mechanism/pathway	Relevance to neoplasm
			tumor associated processes such as cell cycle, proliferation, and apoptosis [267]	colorectal [269] and esophageal squamous cell carcinoma [270]
	USP25	Tankyrases (TNKS1 and TNKS2) [271]	Regulates Wnt signaling pathway [271]	The upregulated mRNA and protein level of USP25 was observed in NSCLC patients which was linked to metastasis [272]
	USP28	c-MYC [273, 274], Chk2 [275], LSD1 (lysine-specificdemethylase1) [276]	Involved in DNA damage response [274, 275]	Somatic mutation has been observed in case of lobular breast cancer [277]; overexpressed in colon [278] and breast cancer [273]
	USP29	p53 [279], Claspin [280]	Involved in regulation of p53 and ATR-Chk1 pathway	
	USP30			
	USP33	Interact with Robol [281]; CP110 (centriolar protein) [169]	Required for Slit signaling [281]; involved in regulation of centrosome duplication and genomic stability [169]	Overexpressed in pediatric acute lymphoblastic leukemia [282]
	USP34	RNF168 [283], AXIN [284]	Regulate genome stability [283]; positively regulates Wnt signaling pathway [284]	
	USP42	p53 [285]	Supports "protect and repair function" of p53 without altering its basal level [285]	
	USP44	Mad2-Cdc20 [286], H2B [287]	Regulates mitotic spindle checkpoint [286]	Overexpression in human T-cell-leukemia [288]; defects in chromatin segregation has been observed with USP44 depletion [286]
	USP47	Polβ [289]	Regulates base excision repair (BER) [289]	USP47 is suggested to be possible therapeutic target as USP47 depletion upregulated level of Cdc25A and decreased cell survival [290]
	USP50	Cdc25B Wee1 [291]	DNA damage response signaling pathway [292]	
Ubiquitin C-terminal hydrolases (UCHs)	UCHL-1	p53 [293]	Involved in ubiquitin maturation [294] and activation of AKT signaling pathway [295]	Linked to several types of cancer including Breast [296], lung [297], colorectal [298] and pancreatic [299]
	BRCA1-associated protein1 (BAP1)	Host cell factor 1 (HCF-1) [300]	Participate in epigenetic regulation in tumor and regulation of histone stability [301]	Mutated in melanoma [302] and implicated in lung and breast cancer [123]
	UCHL-5	Smad2 and Smad3 [303]; NFRKB [304]	Promotes TGFβ signaling [303]; regulates DNA double-strand breaks (DSBs) resection and repair by homologous recombination [304]	Overexpressed in epithelial ovarian cancer [63] and hepatocellular carcinoma [175]

Table 1. USP and UCH family of DUBs, their targets and relevance in cancer.

Ovarian tumor (OTU) represents a superfamily of proteins which are characterized by the presence of an ovarian tumor domain (OTUD) [124]. This domain was first described in the ovarian tumor gene in fruit flies which is involved in the development of ovaries [125]. In 2003, some members of the OTU superfamily were identified to have active cysteine protease site and were described as deubiquitinating enzymes [126]. Based on its characteristics, this class is further subdivided into four groups: Otubains, A20-like OTUs, OTUDs, and OTULIN like OTUs [127]. According to recent studies, the OTU core domain is suggested to consist of five β-strands placed between two αhelical domains. The helical domains vary in sizes among OTU DUBs [128–130]. Like USPs, OTU members also possess additional domains. For example, A20 has A20-type Zn fingers, TRABID has NP14-type Zn fingers, OTUD1 and OTUD5 have ubiquitin-interacting motif, and CEZANNE contains ubiquitin-associated domain [118]. In humans, there are 14 DUBs which belong to the OTU family of DUBs [124]. These DUBs are able to cleave different linkages of ubiquitin chains. For example, OTUB1 and A20 specifically remove K48-linked ubiquitin chains, CEZANNE is specific for K11-linked chains, and TRABID cleaves K29- and K33-linked chains [131].

OTUB1 has a crucial role in DNA damage repair through regulating the RNF8/168 pathway. Recently, OTUB1 is reported to be overexpressed in non-small-cell lung carcinoma (NSCLC) and promotes RAS activation by inhibiting RAS monoubiquitination [132]. Moreover, high expression of OTUD1 is also seen in thyroid carcinoma signifying its oncogenic nature [133]. On the other hand, OTUD5 is linked to apoptosis and is involved in stabilization and activation of p53, suggesting a possible tumor suppressive role [134].

A20 and CEZZANE take part in the negative regulation of NFκB signaling, whereas TRABID positively regulates Wnt signaling pathway [135–137]. A20 is unique and known to have activity of both an E3 ligase and a DUB [138]. A20 cleaves K63-linked ubiquitin chains from RIP1 (receptor interacting protein 1) and negatively regulates the NFκB pathway [137]. A20 genes have been reported to be mutated/deleted in lymphoma suggesting it to be a tumor suppressor [139]. On the other hand, increased A20 is associated with poor outcome in glioma patients [140] and Tamoxifen resistance in breast cancer [141]. Overall, the widespread involvement of these DUBs in a variety of tumorigenic processes makes them potential targets for cancer treatment.

The Josephin family of DUBs is named after a neurodegenerative disease known as Machado-Joseph disease. Particularly, genetic mutations of ATXN3, a member of MJD class of DUBs, are linked to the cause of Machado-Joseph disease [142]. There are four DUBs in humans that form the MJD class: Josephin domain-containing protein 1 (JOSD1), JOSD2, ATXN3-like and ATXN3. ATXN3 can cleave both K48- and K63-linked chains with a higher preference for K63 chains. ATXN3 controls protein folding and stability by editing polyubiquitin chains [143]. The other three members of the Josephin family (JOSD1, JOSD2, and ATXN3L) have highly conserved catalytic triad formed by one cysteine and two histidine residues. An additional domain such as ubiquitin-interacting motif has been identified in ATXN3 and ATXN3L, indicating probable interaction between two distal ubiquitins in a polymer [144]. It has been reported that all Josephin family DUBs especially ATXN3 inhibits PTEN transcription in lung cancer and inhibition of these DUBs induces PTEN expression [145]. In light of these observations, ATXN3 could be a putative target for PTEN repressed tumors. JOSD1 is a membranous DUB and is involved in

regulating membrane dynamics and endocytosis [146]. Moreover, JOSD1 was found to be significantly overexpressed in NSCLCs but its function remains to be elucidated [147].

As mentioned earlier, the JAMM family of DUBs has zinc metalloprotease activity. The crystal structure of AMSH-LP (associated molecule with SH3 domain-like proteases), a DUB from the JAMM family, bound to K63-linked diubiquitin, assisted the understanding of catalytic mechanism of JAMM family [148]. The members of the AMSH family are involved in specific removal of K63-linked polyubiquitin chains and regulate vesicle trafficking and receptor recycling. The domain of AMSH-LP consists of a JAMM core and two conserved insertions. JAMM proteases which do not have AMSH-specific inserts show no specificity for K63-linked polyubiquitin.

There are 12 JAMM proteins along with AMSH-LP that are encoded by human genome. Seven out of the 12 JAMM proteins have isopeptidase activity for ubiquitin or ubiquitin-like proteins while the rest are catalytically inactive. The JAMM proteins with isopeptidase activities are: AMSH-LP, AMSH/STAMBP, BRCC36 (BRCA1/BRCA2-containing complex subunit 36), POH1/PSMD14 (26S proteasome-associated PAD1 homolog 1), MYSM1 (Myb-like with SWIRM and MPN domains 1), MPND (MPN domain-containing protein), and CSN5/JAB1 (COP9 signalosome subunit 5). The high degree of similarity between POH1, AMSH, and AMSH-LP sequences indicates a common mechanism for ubiquitin recognition and catalysis for these JAMMs [148].

BRCC36 belongs to the JAMM class of DUBs whose overexpression has been observed in breast cancer cell lines and tumors [149, 150]. EIF3H and COP6S are other examples of DUBs that belong to JAMM class. COP6S is amplified in breast cancer [151] and EIF3H is amplified in breast and prostate cancer [152].

MCPIP1 proteins possess a domain with deubiquitinating activity which suggests the presence of a sixth family of DUBs in the human genome [153]. This family is suggested to have seven members according to bioinformatics analysis of a recent study [110]. The interaction of MCPIP1, which is the founding member of this family with ubiquitinated proteins, is carried out by ubiquitin-associated domain placed at the N-terminus. However, this domain is not essential for its DUB activity. The other domains of MCPIP1 proteins include N-terminal conserved region, a conserved CCCH-type zinc-finger domain in the middle region of the protein, and a proline-rich domain at its C terminus. The domains that are required for activity of the MCPIP1 proteins are the N-terminal conserved region and zinc finger. In addition, similar to cysteine proteases, the catalytic domain of MCPIP1 also consists of cysteine and aspartic acid boxes but lacks histidine in the catalytic core. However, possibility of histidine outside the core cannot be ruled out [153].

6. Targeting proteasome in cancer

The 26S proteasome is a 2.4 MDa multi-subunit complex responsible for the degradation of intracellular proteins [154]. Currently, there are two FDA-approved proteasome inhibitors

namely Bortezomib (Velcade) and the more potent Carfilzomib (Kyprolis) (**Figure 1**). The FDA initially approved Bortezomib in 2003 for relapsed multiple myeloma (MM) patients [155]. Now, its use has been extended to new MM patients as well as for the treatment of mantle cell lymphoma [156]. Generally, there are three well-accepted models. These are NF-κB inhibition through stabilization of IκB, activation of the unfolded protein response by proteasome inhibition due to high endoplasmic reticulum (ER) stress, and stabilization of pro-apoptotic proteins such as BAX and NOXA [48, 155, 157, 158]. Carfilzomib was approved by FDA in 2012 for relapsed and refractory MM patients, who had previously been treated with Bortezomib [156, 157]. It binds irreversibly to proteasome and inhibits its function by up to 80% resulting in nonfunctional proteasomes as such, it is used for Bortezomib-resistant MM patients [48].

6.1. Proteasome associated DUBs as therapeutic target

Although Bortezomib and Carfilzomib have shown great promise in the clinic [159, 160], it also exhibits side effects [161]. As such, targeting proteasome-associated DUBs might present better specificity by minimizing off-target toxicity attributed to inhibiting the entire proteasome complex. These DUBs play two critical roles in the UPS system. First, by cleaving the attached ubiquitin molecules, it promotes the entry of polyubiquitinated substrate to the 20S catalytic portion of the proteasome. Second, the cleaved ubiquitin would then be available to be recycled as free ubiquitin [162]. Considering the fact that DUBs are intrinsic part of ubiquitin-proteasome system and majority of cancers demonstrate altered expression of DUBs which might drive a number of cancer-associated pathways, targeting DUBs may be considered as a reasonable approach for regulating UPS and is current area of research. There are three DUBs which are associated with the proteasome: PSMD14 (or POH1), USP14, and UCHL5.

6.1.1. PSMD14

PSMD14 is a JAMM metalloprotease. Other than recycling ubiquitin, it is also essential for the structure and function of the 26S proteasome [163]. The importance of PSMD14 in cancer is seen in MM, where its level has been shown to be negatively correlated with the overall patient survival [164]. Depletion of PSMD14 showed decrease in cell viability in multiple myeloma cells. Moreover, upregulation of nuclear PSMD14 is reported in hepatocellular carcinoma and correlates with E2F transcription factor 1 (E2F1) expression and cancer prognosis [165]. PSMD14 is also known to deubiquitinate and modulate the stability of ERBB2 [166]. In addition, PSMD14 has been reported to promote cellular responses to DNA double-strand breaks through homologous recombination. In light of these observations, targeting PSMD14 could lead to better therapeutics in cancer patients.

6.1.2. USP14

Another DUB which is important for ubiquitin recycling is USP14 and has been shown to be involved in delaying protein breakdown by the proteasome and thus, inhibits proteasome activity [167]. USP14 perhaps does so by preventing deubiquitination of proteasome substrate by PSMD14. Although USP14 depletion in mammalian cells has no detectable effect on the accumulation of polyubiquitin [122], it has been shown to inhibit proteasome through its deubiquitinating

activity. USP14 also assists substrate degradation by increasing 20S gate opening [168]. USP14 is overexpressed in NSCLC [169] and in ovarian cancer cells [170]. The expression of USP14 in NSCLC is associated with poor overall survival of patients and tumor cell proliferation, which further strengthens the evidence of USP14 as a tumor-promoting factor in NSCLCs, and a promising therapeutic target. Moreover, USP14 expression in colorectal cancer has been found to be associated with liver and lymph node metastases [171]. It is also implicated in several important signaling pathways [172, 173]. The small molecule inhibitor of USP14, IU1 was shown to stimulate proteasome degradation, further proving its role in proteasome inhibition. This inhibitor specifically binds and inhibits proteasome-bound USP14 [167].

6.1.3. UCHL5

Similar to USP14, UCHL5 is involved in removing ubiquitin from the distal tip of polyubiquitin chains. However, in contrast to USP14, UCHL5 can only release mono-ubiquitin [174]. Clinically, UCHL5 is overexpressed in epithelial ovarian cancer [63] and hepatocellular carcinoma [175]. It has been shown to be associated with poor clinical outcomes in epithelial ovarian cancer [63] and promotes cell migration and invasion in hepatocellular carcinoma [175], implying that it could be a novel predictor of hepatocellular carcinoma reoccurrence. A small molecule compound WP1130 has been shown to inhibit UCHL5 and is expected to functionally block proteasome [176].

Another small molecule compound b-AP15, which was initially identified in cell-based screen, was found to increase the accumulation of polyubiquitin in the cells. Later b-AP15 was identified as an inhibitor of USP14 and UCHL5 [177]. Utilized in solid tumor and MM, b-AP15 showed considerable anti-cancerous effect in animal models. Thus, inhibiting these DUBs by b-AP15, IU1 or related inhibitor may be of therapeutic benefits.

7. DUBs inhibitors

By regulating ubiquitin homeostasis, DUBs have been implicated in tumorigenesis as both its overexpression or loss may drive oncogenesis. Hence, it is not surprising that deregulation of DUBs can lead to severe pathological conditions. To target DUBs, a number of inhibitors either specific for a single DUB or pan-enzyme inhibitors have been identified and are currently explored for its risk-free use in patients. Another approach to target DUBs would be to identify an antagonist that can bind to the DUB's substrate for cancer therapy. DUBs show a great degree of substrate specificity and have a well-defined active site such as the catalytic cysteine which makes DUBs attractive targets for small molecule drug discovery. The active site catalytic cysteine of DUBs is very reactive toward electrophiles. A majority of the DUBs inhibitors are compounds with Michael acceptors such as α, β-unsaturated ketones which are capable of forming covalent adducts with free thiols of nucleophilic cysteine which in turn blocks the DUBs activity [178]. A diverse number of compounds ranging from synthetic small molecules to natural compounds with inhibitory properties for DUBs have been identified and studied. Several strategies can be used to target DUBs and we have summarized them in **Figure 4** and **Table 2**.
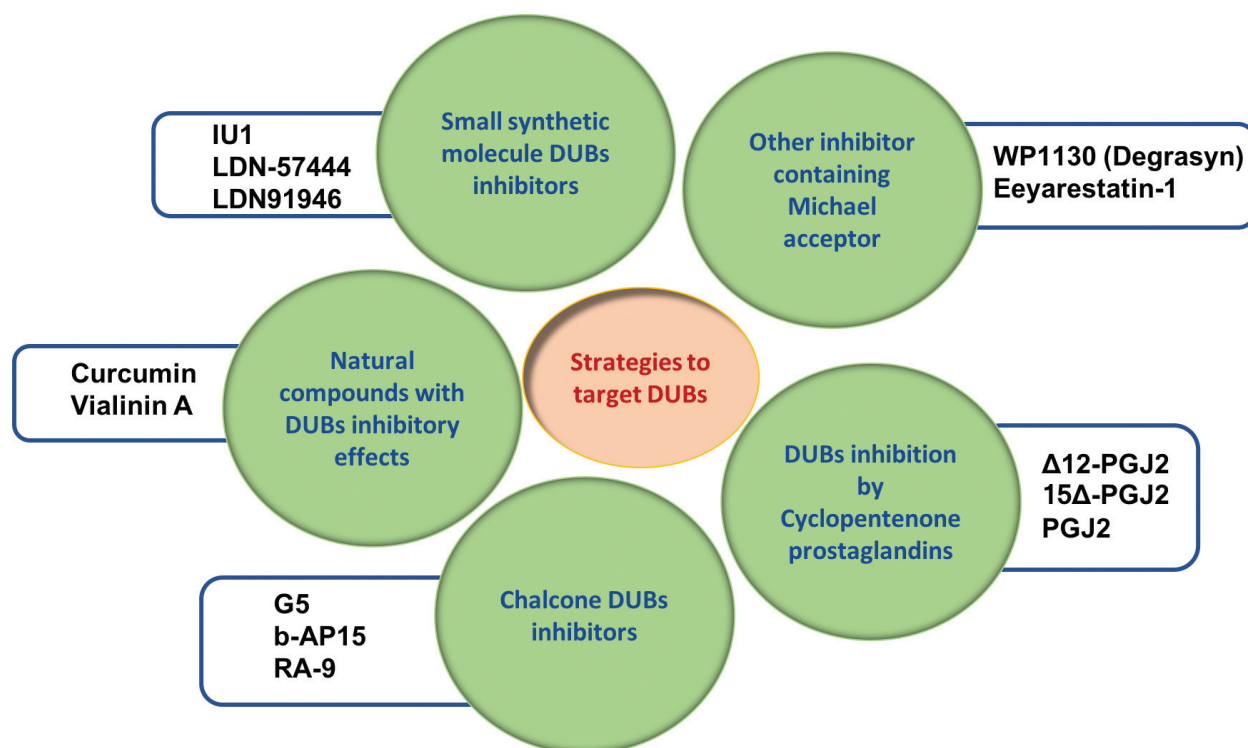
Figure 4. Pictorial representation of different strategies to target DUBs.

7.1. Cyclopentenone prostaglandins

The induction of polyubiquitinated proteins in cells by prostaglandins of the PGJ2 class was first reported by Fitzpatrick and coworkers [179]. DUB activity was shown to be inhibited by prostaglandin PGJ2 which contains α, β-unsaturated ketones. PGJ2 is then further metabolized to $\Delta12$-PGJ2 and 15Δ-PGJ2 [179, 180] which show inhibitory effect toward UCHL3 and UCHL1, respectively [167].

7.2. Chalcone compound with DUB inhibitory effect

A chalcone is an aromatic ketone and an enone that is centrally essential for a broad range of biological compounds. These compounds have cross-conjugated α, β-unsaturated ketones and accessible β-carbons that are important for inhibiting DUBs [181]. These compounds act as either relatively specific or broad-spectrum inhibitors. For example, b-AP15 and its analogue VLX1570 are relatively specific to USP14 and UCH37, whereas another chalcone compound G5 possesses broad inhibitory effect [182, 183].

7.3. Other DUB inhibitors containing Michael acceptors

A small molecule, WP1130, which was derived from a compound with inhibitory activity for Janus-activated kinase 2 (JAK2) kinase was reported to selectively inhibit the activity of USP5 along with USP9X, USP14, and UCH37 [176].

S. no.	Inhibitor	Target (DUB)	Major attributes	Developmental stage
1.	LDN-57444 [195]	UCHL-1	A potent active site directed inhibitor for UCHL-1 [195]; cell permeable inhibitor; decreases proteasome activity	Preclinical
2.	LDN-91946 [196]	UCHL-1	Is able to inhibit UCHL-1 in a noncompetitive manner [196]	Preclinical
3.	15Δ-PGJ2	UCHL-1	Is a metabolite of prostaglandin, PGJ2 that was identified to retain inhibitory effects towards UCHL-1 by affecting overall structure and thus activity [305, 306]	
4.	AM146, RA-9, and RA-14 [307]	UCHL-1	Are chalcones which act as partially selective DUBs inhibitor and can inhibit UCHL-1 activity [307]	
5.	LS1 [308]	UCHL-3	Inhibits UCHL-3, identified in FRET-based screen [309]	
6.	NSC112200 and NSC267309 [310]	TRABID	Inhibited the growth of colorectal tumor cell lines HCT-116 and SW480 [310]	
7.	b-AP15 [177]	UCHL-5 and USP14	Anti-cancerous effect against solid tumor and multiple myeloma *in vivo*	
8.	WP-1130 [176]	USP9X, USP5, USP14, UCH37, UCHL-5	A small molecule, WP1130 serves as a pan DUBs inhibitor which was derived from AG490 (JAK2 inhibitor) and reported to inhibit activity of several DUBs [176]; elicits apoptosis of tumor cells	Preclinical
9.	Pimozide [311] and ML323 [312]	USP1	Works by blocking complex formation between USP1-UAF1, which in turn inhibits USP1activity. ML323 and related N-benzyl1-2-phenylpyrimidine-4-amine derivatives shows higher selectivity and inhibitory potency towards USP1/UAF1 than Pimozide [312]	Preclinical
10.	ML364 [313]	USP2	Is a small molecule inhibitor, which has been identified to enhance Cyclin D1 degradation in colorectal cancer and lymphoma model [313]	
11.	Vialinin A [185]	USP4 and USP5	A natural compound isolated from Chinese mushroom *Thelephoravialis* and has been shown to inhibit enzymatic activity of USP4 and USP5 [185, 186]	Preclinical
12.	P5091 [191]	USP7	Selective inhibitor of USP7, triggers apoptosis in multiple myeloma cells [191]	Preclinical
13.	P22077 [192]	USP7 and USP47	A specific inhibitor of USP7 identified by Progenra [190]	Preclinical
14.	Cpd14 [192]	USP7 and USP47	Resulted in increase in p53 and induction of p21 protein in HCT-116 cells upon treatment [192]	
15.	HBX41, 108 [193]	USP7	HBX 41,108 is an noncompititive reversible inhibitor and it allosterically modulates the catalytic reaction of USP7 [193]	Preclinical
16.	HBX19, 818 [194]	USP7	Binds selectively to the active site of USP7 [194]	Preclinical
17.	HBX28, 258 [194]	USP7	Selective inhibitor for USP7	
18.	HBX90397 [314]	USP8	Specifically target USP8 [116, 314]; inhibited cancer cell growth	
19.	Spautin1 [315]	USP10 and USP13	Induce Vps34 PI3K complex degradation [315]	Preclinical

S. no.	Inhibitor	Target (DUB)	Major attributes	Developmental stage
20.	Mitoxantrone [316]	USP11		Preclinical
21.	IU1 [167]	USP14	Cell permeable; reversible; encourages ubiquitin dependent protein degradation *in vitro*	Preclinical
22.	GSK2643943A [317]	USP20	Identified by GSK from a screen involving compounds targeting USP20/Ub-Rho. It has an IC_{50} of 160 nM [317]	Preclinical
23.	15-oxospiramilacetone [318]	USP30	15-oxospiramilacetone is the only inhibitor for USP30 defined so far which can be used in case of some mitochondrial dysfunctions [318]; natural compound from spiramine A; induce mitochondrial fusion	Preclinical
24.	PR619 [319]	Broad range DUBs inhibitor	Nonselective, noncompetitive, reversible; results in accumulation of ubiquitinated proteins	
25.	1,10-phenanthroline [320, 321]	JAMM type isopeptidase	Chelates active site Zn^{2+}	

Table 2. Different inhibitors and their target DUBs.

7.4. Natural products with DUB inhibitory effect

A number of natural compounds have been identified to have DUB inhibitory effect. One of such is Curcumin, which is a yellow pigment isolated from the *Curcuma longa.* Curcumin possesses two α, β-unsaturated ketones moieties and has been linked with suppression of tumorigenesis and various other diseases. It was reported that Curcumin accelerates polyubiquitinated protein accumulation at concentrations of 40 μM [184]. USP4 has been reported to be targeted by a small natural compound known as Vialinin A. Vialinin A is isolated from the Chinese mushroom *Thelephoravialis* and has been shown to inhibit the enzymatic activity of USP4 and USP5 [185, 186].

7.5. Synthetic small molecule DUB inhibitors

Several inhibitors have been developed to target the multifunctional deubiquitinating enzyme USP7. USP7, also known as HAUSP, is probably the most attractive DUB in the field of cancer biology. USP7 has been reported to regulate the function and stability of at least three important tumor suppressor p53 [187], PTEN [188] and TIP60 [189]. Progenra identified P022077 as a specific inhibitor for USP7 [190]. Other inhibitors of USP7 are P5091 and Cpd14, which triggers apoptosis in MM cells and inhibits tumor growth [191, 192]. Other Hybrigenics compounds which could inhibit USP7 function are HBX41108 [193], HBX19818 [194], and HBX28258 [194]. An isatin O-acyl oxime, LDN-57444 is a most potent active site directed inhibitor for UCHL-1 [195]. LDN-91946 is another compound which was identified as a hit in an *in vitro* screen for identifying blockers of Ub-AMC activity and was able to inhibit UCHL-1 in a noncompetitive manner [196].

Despite the multitude of inhibitors identified to target DUBs, so far, no DUB inhibitors are approved for clinical use. Only a few of these inhibitors, such as VLX1570, are in clinical trial for

cancer therapy. Out of 98 DUBs, only several DUBs have been explored structurally providing a platform for understanding, identifying, and validating various DUB inhibitors for clinical usage.

8. Conclusion

The UPS is implicated in several human diseases such as neurodegenerative disease, inflammation, bacterial and viral infection and most importantly, in cancer. The type of ubiquitin linkages formed/cleaved with the help of a cascade of enzymes (E1, E2, E3/DUBs) intensifies biological complexities. Hence, it is important to discover and identify the targets for therapeutic intervention. One of the strategies that can be used is targeting components of the UPS. Over the past 35 years, our knowledge and understanding of the UPS has significantly increased and it is evident that the UPS plays critical roles in various important cellular functions and can regulate both structural and functional behavior of cells. The success of Bortezomib provides a proof-of-concept to expand the use of other inhibitors targeting different components of UPS system in cancer. However, the results were not satisfying due to challenges in bringing these inhibitors to clinic. This is mostly because E3 ligases and DUBs have multiple substrates which makes it complicated. Therefore, it is critical to find the right target(s) for a specific cancer, to understand how the target functions and eventually find the finest way to effectively manipulate these targets for treatment intervention.

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References

[1] Ross JM, Olson L, Coppotelli G. Mitochondrial and ubiquitin proteasome system dysfunction in ageing and disease: Two sides of the same coin? International Journal of Molecular Sciences. 2015;**16**(8):19458-19476

[2] Chen ZJ, Sun LJ. Nonproteolytic functions of ubiquitin in cell signaling. Molecular Cell. 2009;**33**(3):275-286

[3] Pickart CM. Mechanisms underlying ubiquitination. Annual Review of Biochemistry. 2001;**70**:503-533

[4] Hershko A et al. Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. The Journal of Biological Chemistry. 1983; **258**(13):8206-8214

[5] Haas AL et al. Ubiquitin-activating enzyme. Mechanism and role in protein-ubiquitin conjugation. The Journal of Biological Chemistry. 1982;**257**(5):2543-2548

[6] Ciechanover A et al. Activation of the heat-stable polypeptide of the ATP-dependent proteolytic system. Proceedings of the National Academy of Sciences of the United States of America. 1981;**78**(2):761-765

[7] Ciechanover A et al. "Covalent affinity" purification of ubiquitin-activating enzyme. The Journal of Biological Chemistry. 1982;**257**(5):2537-2542

[8] Ye Y, Rape M. Building ubiquitin chains: E2 enzymes at work. Nature Reviews. Molecular Cell Biology. 2009;**10**(11):755-764

[9] Deshaies RJ, Joazeiro CA. RING domain E3 ubiquitin ligases. Annual Review of Biochemistry. 2009;**78**:399-434

[10] Rotin D, Kumar S. Physiological functions of the HECT family of ubiquitin ligases. Nature Reviews. Molecular Cell Biology. 2009;**10**(6):398-409

[11] Morreale FE, Walden H. Types of ubiquitin ligases. Cell. 2016;**165**(1):248-248.e1

[12] Komander D. The emerging complexity of protein ubiquitination. Biochemical Society Transactions. 2009;**37**(Pt 5):937-953

[13] Chau V et al. A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. Science. 1989;**243**(4898):1576-1583

[14] Chen D, Dou QP. The ubiquitin-proteasome system as a prospective molecular target for cancer treatment and prevention. Current Protein & Peptide Science. 2010;**11**(6):459-470

[15] Hough R, Pratt G, Rechsteiner M. Purification of two high molecular weight proteases from rabbit reticulocyte lysate. The Journal of Biological Chemistry. 1987;**262**(17):8303-8313

[16] Waxman L, Fagan JM, Goldberg AL. Demonstration of two distinct high molecular weight proteases in rabbit reticulocytes, one of which degrades ubiquitin conjugates. The Journal of Biological Chemistry. 1987;**262**(6):2451-2457

[17] Livneh I et al. The life cycle of the 26S proteasome: From birth, through regulation and function, and onto its death. Cell Research. 2016;**26**(8):869-885

[18] Collins GA, Goldberg AL. The logic of the 26S proteasome. Cell. 2017;**169**(5):792-806

[19] A prize for protein degradation. Nature Cell Biology. 2004;**6**(11):1011-1011

[20] Varshavsky A. The early history of the ubiquitin field. Protein Science: A Publication of the Protein Society. 2006;**15**(3):647-654

[21] Ciehanover A, Hod Y, Hershko A. A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes. Biochemical and Biophysical Research Communications. 1978;**81**(4):1100-1105

[22] Wilkinson KD, Urban MK, Haas AL. Ubiquitin is the ATP-dependent proteolysis factor I of rabbit reticulocytes. The Journal of Biological Chemistry. 1980;**255**(16):7529-7532

[23] Ciechanover A et al. Characterization of the heat-stable polypeptide of the ATP-dependent proteolytic system from reticulocytes. The Journal of Biological Chemistry. 1980;**255**(16):7525-7528

[24] Hershko A, Ciechanover A, Rose IA. Resolution of the ATP-dependent proteolytic system from reticulocytes: A component that interacts with ATP. Proceedings of the National Academy of Sciences of the United States of America. 1979;**76**(7):3107-3110

[25] Goldstein G et al. Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. Proceedings of the National Academy of Sciences of the United States of America. 1975;**72**(1):11-15

[26] Goldknopf IL, Busch H. Isopeptide linkage between nonhistone and histone 2A polypeptides of chromosomal conjugate-protein A24. Proceedings of the National Academy of Sciences of the United States of America. 1977;**74**(3):864-868

[27] Hunt LT, Dayhoff MO. Amino-terminal sequence identity of ubiquitin and the nonhistone component of nuclear protein A24. Biochemical and Biophysical Research Communications. 1977;**74**(2):650-655

[28] Hershko A et al. Proposed role of ATP in protein breakdown: Conjugation of protein with multiple chains of the polypeptide of ATP-dependent proteolysis. Proceedings of the National Academy of Sciences of the United States of America. 1980;**77**(4):1783-1786

[29] Ciechanover A et al. ATP-dependent conjugation of reticulocyte proteins with the polypeptide required for protein degradation. Proceedings of the National Academy of Sciences of the United States of America. 1980;**77**(3):1365-1368

[30] Hough R, Pratt G, Rechsteiner M. Ubiquitin-lysozyme conjugates. Identification and characterization of an ATP-dependent protease from rabbit reticulocyte lysates. The Journal of Biological Chemistry. 1986;**261**(5):2400-2408

[31] Eytan E et al. ATP-dependent incorporation of 20S protease into the 26S complex that degrades proteins conjugated to ubiquitin. Proceedings of the National Academy of Sciences of the United States of America. 1989;**86**(20):7751-7755

[32] Driscoll J, Goldberg AL. The proteasome (multicatalytic protease) is a component of the 1500-kDa proteolytic complex which degrades ubiquitin-conjugated proteins. The Journal of Biological Chemistry. 1990;**265**(9):4789-4792

[33] Hershko A et al. ATP-dependent degradation of ubiquitin-protein conjugates. Proceedings of the National Academy of Sciences of the United States of America. 1984;**81**(6):1619-1623

[34] Tanaka K, Waxman L, Goldberg AL. ATP serves two distinct roles in protein degradation in reticulocytes, one requiring and one independent of ubiquitin. The Journal of Cell Biology. 1983;**96**(6):1580-1585

[35] Hoffman L, Pratt G, Rechsteiner M. Multiple forms of the 20 S multicatalytic and the 26 S ubiquitin/ATP-dependent proteases from rabbit reticulocyte lysate. The Journal of Biological Chemistry. 1992;**267**(31):22362-22368

[36] Ciechanover A, Finley D, Varshavsky A. Ubiquitin dependence of selective protein degradation demonstrated in the mammalian cell cycle mutant ts85. Cell. 1984;**37**(1):57-66

[37] Finley D, Ciechanover A, Varshavsky A. Thermolability of ubiquitin-activating enzyme from the mammalian cell cycle mutant ts85. Cell. 1984;**37**(1):43-55

[38] Marunouchi T et al. Disappearance of a basic chromosomal protein from cells of a mouse temperature-sensitive mutant defective in histone phosphorylation. Biochemical and Biophysical Research Communications. 1980;**95**(1):126-131

[39] Matsumoto Y et al. Decrease in uH2A (protein A24) of a mouse temperature-sensitive mutant. FEBS Letters. 1983;**151**(1):139-142

[40] Wilkinson KD et al. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. Science. 1989;**246**(4930):670

[41] Miller HI et al. Cloning and expression of a yeast ubiquitin-protein cleaving activity in *Escherichia coli*. Nature Biotechnology. 1989;**7**(7):698-704

[42] Baker RT, Tobias JW, Varshavsky A. Ubiquitin-specific proteases of *Saccharomyces cerevisiae*. Cloning of UBP2 and UBP3, and functional analysis of the UBP gene family. Journal of Biological Chemistry. 1992;**267**(32):23364-23375

[43] Wilkinson KD. The discovery of ubiquitin-dependent proteolysis. Proceedings of the National Academy of Sciences. 2005;**102**(43):15280-15282

[44] Jin J et al. Dual E1 activation systems for ubiquitin differentially regulate E2 enzyme charging. Nature. 2007;**447**(7148):1135-1138

[45] Yang Y et al. Inhibitors of ubiquitin-activating enzyme (E1), a new class of potential cancer therapeutics. Cancer Research. 2007;**67**(19):9472-9481

[46] Xu GW et al. The ubiquitin-activating enzyme E1 as a therapeutic target for the treatment of leukemia and multiple myeloma. Blood. 2010;**115**(11):2251-2259

[47] Ceccarelli DF et al. An allosteric inhibitor of the human Cdc34 ubiquitin-conjugating enzyme. Cell. 2011;**145**(7):1075-1087

[48] Huang X, Dixit VM. Drugging the undruggables: Exploring the ubiquitin system for drug development. Cell Research. 2016;**26**(4):484-498

[49] Pulvino M et al. Inhibition of proliferation and survival of diffuse large B-cell lymphoma cells by a small-molecule inhibitor of the ubiquitin-conjugating enzyme Ubc13-Uev1A. Blood. 2012;**120**(8):1668-1677

[50] Strickson S et al. The anti-inflammatory drug BAY 11-7082 suppresses the MyD88-dependent signalling network by targeting the ubiquitin system. The Biochemical Journal. 2013;**451**(3):427-437

[51] Metzger MB, Hristova VA, Weissman AM. HECT and RING finger families of E3 ubiquitin ligases at a glance. Journal of Cell Science. 2012;**125**(Pt 3):531-537

[52] Levine AJ. p53, the cellular gatekeeper for growth and division. Cell. 1997;**88**(3):323-331

[53] Haupt Y et al. Mdm2 promotes the rapid degradation of p53. Nature. 1997;**387**(6630): 296-299

[54] Ladanyi M et al. MDM2 gene amplification in metastatic osteosarcoma. Cancer Research. 1993;**53**(1):16-18

[55] Vassilev LT et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science. 2004;**303**(5659):844

[56] Andreeff M et al. Results of the phase I trial of RG7112, a small-molecule MDM2 antagonist in leukemia. Clinical Cancer Research. 2016;**22**(4):868

[57] Burgess A et al. Clinical overview of MDM2/X-targeted therapies. Frontiers in Oncology. 2016;**6**:7

[58] Ding Q et al. Discovery of RG7388, a potent and selective p53-MDM2 inhibitor in clinical development. Journal of Medicinal Chemistry. 2013;**56**(14):5979-5983

[59] Lakoma A et al. The MDM2 small-molecule inhibitor RG7388 leads to potent tumor inhibition in p53 wild-type neuroblastoma. Cell Death Discovery. 2015;**1**:15026

[60] Issaeva N et al. Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. Nature Medicine. 2004;**10**(12):1321-1328

[61] Soussi T, Wiman KG. Shaping genetic alterations in human cancer: The p53 mutation paradigm. Cancer Cell. 2007;**12**(4):303-312

[62] Lambert JM et al. PRIMA-1 reactivates mutant p53 by covalent binding to the core domain. Cancer Cell. 2009;**15**(5):376-388

[63] Wang Z et al. Roles of F-box proteins in cancer. Nature Reviews. Cancer. 2014;**14**(4):233-247

[64] Carrano AC et al. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. Nature Cell Biology. 1999;**1**(4):193-199

[65] Yu ZK, Gervais JL, Zhang H. Human CUL-1 associates with the SKP1/SKP2 complex and regulates p21(CIP1/WAF1) and cyclin D proteins. Proceedings of the National Academy of Sciences of the United States of America. 1998;**95**(19):11324-11329

[66] Hershko DD. Oncogenic properties and prognostic implications of the ubiquitin ligase Skp2 in cancer. Cancer. 2008;**112**(7):1415-1424

[67] Li JQ et al. Correlation of Skp2 with carcinogenesis, invasion, metastasis, and prognosis in colorectal tumors. International Journal of Oncology. 2004;**25**(1):87-95

[68] Gstaiger M et al. Skp2 is oncogenic and overexpressed in human cancers. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**(9):5043-5048

[69] Wu L et al. Specific small molecule inhibitors of Skp2-mediated p27 degradation. Chemistry & Biology. 2012;**19**(12):1515-1524

[70] Chan CH et al. Pharmacological inactivation of Skp2 SCF ubiquitin ligase restricts cancer stem cell traits and cancer progression. Cell. 2013;**154**(3):556-568

[71] Chen Q et al. Targeting the p27 E3 ligase SCF(Skp2) results in p27- and Skp2-mediated cell-cycle arrest and activation of autophagy. Blood. 2008;**111**(9):4690-4699

[72] Frescas D, Pagano M. Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: Tipping the scales of cancer. Nature Reviews. Cancer. 2008;**8**(6):438-449

[73] Blees JS et al. Erioflorin stabilizes the tumor suppressor Pdcd4 by inhibiting its interaction with the E3-ligase beta-TrCP1. PLoS One. 2012;**7**(10):e46567

[74] Petroski MD, Deshaies RJ. Function and regulation of cullin-RING ubiquitin ligases. Nature Reviews. Molecular Cell Biology. 2005;**6**(1):9-20

[75] Bielskiene K et al. E3 ubiquitin ligases as drug targets and prognostic biomarkers in melanoma. Medicina (Kaunas, Lithuania). 2015;**51**(1):1-9

[76] Nai G, Marques M. Role of ROC1 protein in the control of cyclin D1 protein expression in skin melanomas. Pathology—Research and Practice. 2011;**207**(3):174-181

[77] Jia L, Sun Y. SCF E3 ubiquitin ligases as anticancer targets. Current Cancer Drug Targets. 2011;**11**(3):347-356

[78] Jia L et al. Validation of SAG/RBX2/ROC2 E3 ubiquitin ligase as an anticancer and radiosensitizing target. Clinical Cancer Research. 2010;**16**(3):814

[79] Deveraux QL, Reed JC. IAP family proteins-suppressors of apoptosis. Genes & Development. 1999;**13**(3):239-252

[80] Fulda S, Vucic D. Targeting IAP proteins for therapeutic intervention in cancer. Nature Reviews. Drug Discovery. 2012;**11**(2):109-124

[81] Munger K et al. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. The EMBO Journal. 1989;**8**(13):4099-4105

[82] Scheffner M et al. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell. 1993;**75**(3):495-505

[83] Lou Z, Wang S. E3 ubiquitin ligases and human papillomavirus-induced carcinogenesis. The Journal of International Medical Research. 2014;**42**(2):247-260

[84] Scheffner M et al. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell. 1990;**63**(6):1129-1136

[85] Huh K et al. Human papillomavirus type 16 E7 oncoprotein associates with the cullin 2 ubiquitin ligase complex, which contributes to degradation of the retinoblastoma tumor suppressor. Journal of Virology. 2007;**81**(18):9737-9747

[86] D'Abramo CM, Archambault J. Small molecule inhibitors of human papillomavirus protein-protein interactions. The Open Virology Journal. 2011;**5**:80-95

[87] Baleja JD et al. Identification of inhibitors to papillomavirus type 16 E6 protein based on three-dimensional structures of interacting proteins. Antiviral Research. 2006;**72**(1):49-59

[88] Malecka KA et al. Identification and characterization of small molecule human papillomavirus E6 inhibitors. ACS Chemical Biology. 2014;**9**(7):1603-1612

[89] Zhao CY et al. Rescue of p53 function by small-molecule RITA in cervical carcinoma by blocking E6-mediated degradation. Cancer Research. 2010;**70**(8):3372-3381

[90] Jha S et al. Destabilization of TIP60 by human papillomavirus E6 results in attenuation of TIP60-dependent transcriptional regulation and apoptotic pathway. Molecular Cell. 2010;**38**(5):700-711

[91] Gupta A et al. Tip60 degradation by adenovirus relieves transcriptional repression of viral transcriptional activator EIA. Oncogene. 2013;**32**(42):5017-5025

[92] Subbaiah VK et al. E3 ligase EDD1/UBR5 is utilized by the HPV E6 oncogene to destabilize tumor suppressor TIP60. Oncogene. 2016;**35**(16):2062-2074

[93] Shearer RF et al. Functional roles of the E3 ubiquitin ligase UBR5 in cancer. Molecular Cancer Research. 2015;**13**(12):1523-1532

[94] Ikeda M et al. The Epstein-Barr virus latent membrane protein 2A PY motif recruits WW domain-containing ubiquitin-protein ligases. Virology. 2000;**268**(1):178-191

[95] Masucci MG. Epstein-Barr virus oncogenesis and the ubiquitin-proteasome system. Oncogene. 2004;**23**(11):2107-2115

[96] Rousset R et al. Effects on NF-kappa B1/p105 processing of the interaction between the HTLV-1 transactivator Tax and the proteasome. Nature. 1996;**381**(6580):328-331

[97] Hemelaar J et al. Human T-cell leukemia virus type 1 Tax protein binds to assembled nuclear proteasomes and enhances their proteolytic activity. Journal of Virology. 2001;**75**(22):11106-11115

[98] Mitra-Kaushik S et al. Effects of the proteasome inhibitor PS-341 on tumor growth in HTLV-1 Tax transgenic mice and Tax tumor transplants. Blood. 2004;**104**(3):802

[99] Satou Y et al. Proteasome inhibitor, bortezomib, potently inhibits the growth of adult T-cell leukemia cells both in vivo and in vitro. Leukemia. 2004;**18**(8):1357-1363

[100] Zhang Z et al. Structural and functional characterization of interaction between hepatitis B virus X protein and the proteasome complex. The Journal of Biological Chemistry. 2000;**275**(20):15157-15165

[101] Minor MM, Slagle BL. Hepatitis B virus HBx protein interactions with the ubiquitin proteasome system. Viruses. 2014;**6**(11):4683-4702

[102] Zhang Z et al. Inhibition of cellular proteasome activities mediates HBX-independent hepatitis B virus replication in vivo. Journal of Virology. 2010;**84**(18):9326-9331

[103] Bontron S, Lin-Marq N, Strubin M. Hepatitis B virus X protein associated with UV-DDB1 induces cell death in the nucleus and is functionally antagonized by UV-DDB2. The Journal of Biological Chemistry. 2002;**277**(41):38847-38854

[104] Li T et al. A promiscuous alpha-helical motif anchors viral hijackers and substrate receptors to the CUL4-DDB1 ubiquitin ligase machinery. Nature Structural & Molecular Biology. 2010;**17**(1):105-111

[105] Decorsiere A et al. Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. Nature. 2016;**531**(7594):386-389

[106] Murphy CM et al. Hepatitis B virus X protein promotes degradation of SMC5/6 to enhance HBV replication. Cell Reports. 2016;**16**(11):2846-2854

[107] Hodgson AJ et al. Hepatitis B virus regulatory HBx protein binding to DDB1 is required but is not sufficient for maximal HBV replication. Virology. 2012;**426**(1):73-82

[108] Leupin O et al. Hepatitis B virus X protein stimulates viral genome replication via a DDB1-dependent pathway distinct from that leading to cell death. Journal of Virology. 2005;**79**(7):4238-4245

[109] Komander D, Clague MJ, Urbé S. Breaking the chains: Structure and function of the deubiquitinases. Nature Reviews. Molecular Cell Biology. 2009;**10**(8):550

[110] Fraile J et al. Deubiquitinases in cancer: New functions and therapeutic options. Oncogene. 2012;**31**(19):2373

[111] Johnston SC et al. Crystal structure of a deubiquitinating enzyme (human UCH-L3) at 1.8 Å resolution. The EMBO Journal. 1997;**16**(13):3787-3796

[112] Cstorer A, Ménard R. Catalytic mechanism in papain family of cysteine peptidases. Methods in Enzymology. 1994;**244**:486-500

[113] Maytal-Kivity V et al. MPN+, a putative catalytic motif found in a subset of MPN domain proteins from eukaryotes and prokaryotes, is critical for Rpn11 function. BMC Biochemistry. 2002;**3**(1):28

[114] Tran HJ et al. Structure of the Jab1/MPN domain and its implications for proteasome function. Biochemistry. 2003;**42**(39):11460-11465

[115] Quesada V et al. Cloning and enzymatic analysis of 22 novel human ubiquitin-specific proteases. Biochemical and Biophysical Research Communications. 2004;**314**(1):54-62

[116] Daviet L, Colland F. Targeting ubiquitin specific proteases for drug discovery. Biochimie. 2008;**90**(2):270-283

[117] Hu M et al. Crystal structure of a UBP-family deubiquitinating enzyme in isolation and in complex with ubiquitin aldehyde. Cell. 2002;**111**(7):1041-1054

[118] Komander D et al. The structure of the CYLD USP domain explains its specificity for Lys63-linked polyubiquitin and reveals a B box module. Molecular Cell. 2008;**29**(4):451-464

[119] Zhu X, Ménard R, Sulea T. High incidence of ubiquitin-like domains in human ubiquitin-specific proteases. Proteins: Structure, Function, and Bioinformatics. 2007;**69**(1):1-7

[120] Das C et al. Structural basis for conformational plasticity of the Parkinson's disease-associated ubiquitin hydrolase UCH-L1. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(12):4675-4680

[121] Larsen CN, Krantz BA, Wilkinson KD. Substrate specificity of deubiquitinating enzymes: Ubiquitin C-terminal hydrolases. Biochemistry. 1998;**37**(10):3358-3368

[122] Koulich E, Li X, DeMartino GN. Relative structural and functional roles of multiple deubiquitylating proteins associated with mammalian 26S proteasome. Molecular Biology of the Cell. 2008;**19**(3):1072-1082

[123] Jensen DE et al. BAP1: A novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. Oncogene. 1998;**16**(9):1097-1112

[124] Borodovsky A et al. Chemistry-based functional proteomics reveals novel members of the deubiquitinating enzyme family. Chemistry & Biology. 2002;**9**(10):1149-1159

[125] Steinhauer W, Walsh R, Kalfayan L. Sequence and structure of the *Drosophila melanogaster* ovarian tumor gene and generation of an antibody specific for the ovarian tumor protein. Molecular and Cellular Biology. 1989;**9**(12):5726-5732

[126] Balakirev MY et al. Otubains: A new family of cysteine proteases in the ubiquitin pathway. EMBO Reports. 2003;**4**(5):517-522

[127] Mevissen TE et al. OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis. Cell. 2013;**154**(1):169-184

[128] Messick TE et al. Structural basis for ubiquitin recognition by the Otu1 ovarian tumor domain protein. Journal of Biological Chemistry. 2008;**283**(16):11038-11049

[129] Reyes-Turcu FE, Ventii KH, Wilkinson KD. Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. Annual Review of Biochemistry. 2009;**78**:363-397

[130] Nanao MH et al. Crystal structure of human otubain 2. EMBO Reports. 2004;**5**(8):783-788

[131] Eletr ZM, Wilkinson KD. Regulation of proteolysis by human deubiquitinating enzymes. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2014;**1843**(1):114-128

[132] Baietti MF et al. OTUB1 triggers lung cancer development by inhibiting RAS monoubiquitination. EMBO Molecular Medicine. 2016;**8**(3):288-303

[133] Carneiro A et al. A putative OTU domain-containing protein 1 deubiquitinating enzyme is differentially expressed in thyroid cancer and identifies less-aggressive tumours. British Journal of Cancer. 2014;**111**(3):551

[134] Luo J et al. OTUD5 regulates p53 stability by deubiquitinating p53. PLoS One. 2013;**8**(10): e77682

[135] Tran H et al. Trabid, a new positive regulator of Wnt-induced transcription with preference for binding and cleaving K63-linked ubiquitin chains. Genes & Development. 2008;**22**(4):528-542

[136] Enesa K et al. NF-κB suppression by the deubiquitinating enzyme cezanne a novel negative feedback loop in pro-inflammatory signaling. Journal of Biological Chemistry. 2008;**283**(11):7036-7045

[137] Wertz IE et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. Nature. 2004;**430**(7000):694

[138] Ma A, Malynn BA. A20: Linking a complex regulator of ubiquitylation to immunity and human disease. Nature Reviews. Immunology. 2012;**12**(11):774

[139] Honma K et al. TNFAIP3/A20 functions as a novel tumor suppressor gene in several subtypes of non-Hodgkin lymphomas. Blood. 2009;**114**(12):2467-2475

[140] Hjelmeland AB et al. Targeting A20 decreases glioma stem cell survival and tumor growth. PLoS Biology. 2010;**8**(2):e1000319

[141] Vendrell J et al. A20/TNFAIP3, a new estrogen-regulated gene that confers tamoxifen resistance in breast cancer cells. Oncogene. 2007;**26**(32):4656

[142] Kawaguchi Y et al. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32. 1. Nature Genetics. 1994;**8**(3):221-228

[143] Mao Y et al. Deubiquitinating function of ataxin-3: Insights from the solution structure of the Josephin domain. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(36):12700-12705

[144] Burnett B, Li F, Pittman RN. The polyglutamine neurodegenerative protein ataxin-3 binds polyubiquitylated proteins and has ubiquitin protease activity. Human Molecular Genetics. 2003;**12**(23):3195-3205

[145] Sacco J et al. The deubiquitylase Ataxin-3 restricts PTEN transcription in lung cancer cells. Oncogene. 2014;**33**(33):4265

[146] Seki T et al. JosD1, a membrane-targeted deubiquitinating enzyme, is activated by ubiquitination and regulates membrane dynamics, cell motility, and endocytosis. Journal of Biological Chemistry. 2013;**288**(24):17145-17155

[147] Luise C et al. An atlas of altered expression of deubiquitinating enzymes in human cancer. PLoS One. 2011;**6**(1):e15891

[148] Sato Y et al. Structural basis for specific cleavage of Lys 63-linked polyubiquitin chains. Nature. 2008;**455**(7211):358-362

[149] Dong Y et al. Regulation of BRCC, a holoenzyme complex containing BRCA1 and BRCA2, by a signalosome-like subunit and its role in DNA repair. Molecular Cell. 2003;**12**(5):1087-1099

[150] Chen X et al. Aberrant expression of BRCC36, a novel subunit of a BRCA1 E3 ubiquitin ligase complex, in sporadic breast cancer. AACR. Vol 64, Issue 7 Supplement, pp. 977. 2004

[151] Zhao R et al. Subunit 6 of the COP9 signalosome promotes tumorigenesis in mice through stabilization of MDM2 and is upregulated in human cancers. The Journal of Clinical Investigation. 2011;**121**(3):851

[152] Nupponen NN, Isola J, Visakorpi T. Mapping the amplification of EIF3S3 in breast and prostate cancer. Genes, Chromosomes and Cancer. 2000;**28**(2):203-210

[153] Liang J et al. MCP-induced protein 1 deubiquitinates TRAF proteins and negatively regulates JNK and NF-κB signaling. Journal of Experimental Medicine. 2010;**207**(13): 2959-2973

[154] Kisselev AF, Goldberg AL. Proteasome inhibitors: From research tools to drug candidates. Chemistry & Biology. 2001;**8**(8):739-758

[155] Kisselev AF, van der Linden WA, Overkleeft HS. Proteasome inhibitors: An expanding army attacking a unique target. Chemistry & Biology. 2012;**19**(1):99-115

[156] Morrow JK et al. Targeting ubiquitination for cancer therapies. Future Medicinal Chemistry. 2015;**7**(17):2333-2350

[157] Grigoreva TA et al. The 26S proteasome is a multifaceted target for anti-cancer therapies. Oncotarget. 2015;**6**(28):24733-24749

[158] Goldberg AL. Development of proteasome inhibitors as research tools and cancer drugs. The Journal of Cell Biology. 2012;**199**(4):583-588

[159] Chen D et al. Bortezomib as the first proteasome inhibitor anticancer drug: Current status and future perspectives. Current Cancer Drug Targets. 2011;**11**(3):239-253

[160] Richardson PG, Hideshima T, Anderson KC. Bortezomib (PS-341): A novel, first-in-class proteasome inhibitor for the treatment of multiple myeloma and other cancers. Cancer Control. 2003;**10**(5):361-369

[161] Zhang W, Sidhu SS. Development of inhibitors in the ubiquitination cascade. FEBS Letters. 2014;**588**(2):356-367

[162] Yao T, Cohen RE. A cryptic protease couples deubiquitination and degradation by the proteasome. Nature. 2002;**419**(6905):403

[163] Gallery M et al. The JAMM motif of human deubiquitinase Poh1 is essential for cell viability. Molecular Cancer Therapeutics. 2007;**6**(1):262-268

[164] Song Y et al. Deubiquitylating enzyme Rpn11/POH1/PSMD14 as therapeutic target in multiple myeloma. American Society of Hematology. 2016;**128**(22):4469

[165] Wang B et al. POH1 deubiquitylates and stabilizes E2F1 to promote tumour formation. Nature Communications. 2015;**6**:8704

[166] Liu H et al. Regulation of ErbB2 receptor status by the proteasomal DUB POH1. PLoS One. 2009;**4**(5):e5544

[167] Lee B-H et al. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. Nature. 2010;**467**(7312):179-184

[168] Peth A, Besche HC, Goldberg AL. Ubiquitinated proteins activate the proteasome by binding to Usp14/Ubp6, which causes 20S gate opening. Molecular Cell. 2009;**36**(5):794-804

[169] Wu N et al. Over-expression of deubiquitinating enzyme USP14 in lung adenocarcinoma promotes proliferation through the accumulation of β-catenin. International Journal of Molecular Sciences. 2013;**14**(6):10749-10760

[170] Wang Y et al. Ubiquitin-specific protease 14 (USP14) regulates cellular proliferation and apoptosis in epithelial ovarian cancer. Medical Oncology. 2015;**32**(1):379

[171] Shinji S et al. Ubiquitin-specific protease 14 expression in colorectal cancer is associated with liver and lymph node metastases. Oncology Reports. 2006;**15**(3):539-543

[172] Xu D et al. Phosphorylation and activation of ubiquitin-specific protease-14 by Akt regulates the ubiquitin-proteasome system. eLife. 2015;**4**:e10510

[173] Jung H et al. Deubiquitination of dishevelled by Usp14 is required for Wnt signaling. Oncogene. 2013;**2**(8):e64

[174] Lam YA et al. Editing of ubiquitin conjugates by an isopeptidase in the 26S proteasome. Nature. 1997;**385**(6618):737

[175] Fang Y et al. Ubiquitin C-terminal hydrolase 37, a novel predictor for hepatocellular carcinoma recurrence, promotes cell migration and invasion via interacting and deubiquitinating PRP19. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2013;**1833**(3):559-572

[176] Kapuria V et al. Deubiquitinase inhibition by small-molecule WP1130 triggers aggresome formation and tumor cell apoptosis. Cancer Research. 2010;**70**(22):9265-9276

[177] D'arcy P et al. Inhibition of proteasome deubiquitinating activity as a new cancer therapy. Nature Medicine. 2011;**17**(12):1636-1640

[178] Santos MM, Moreira R. Michael acceptors as cysteine protease inhibitors. Mini Reviews in Medicinal Chemistry. 2007;**7**(10):1040-1050

[179] Mullally JE et al. Cyclopentenone prostaglandins of the J series inhibit the ubiquitin isopeptidase activity of the proteasome pathway. Journal of Biological Chemistry. 2001; **276**(32):30366-30373

[180] Fitzpatrick F, Wynalda MA. Albumin-catalyzed metabolism of prostaglandin D2. Identification of products formed in vitro. Journal of Biological Chemistry. 1983;**258**(19): 11713-11718

[181] D'arcy P, Wang X, Linder S. Deubiquitinase inhibition as a cancer therapeutic strategy. Pharmacology & Therapeutics. 2015;**147**:32-54

[182] Tian Z et al. A novel small molecule inhibitor of deubiquitylating enzyme USP14 and UCHL5 induces apoptosis in multiple myeloma and overcomes bortezomib resistance. Blood. 2014;**123**(5):706-716

[183] Aleo E et al. Identification of new compounds that trigger apoptosome-independent caspase activation and apoptosis. Cancer Research. 2006;**66**(18):9235-9244

[184] D'Aguanno S et al. Shotgun proteomics and network analysis of neuroblastoma cell lines treated with curcumin. Molecular BioSystems. 2012;**8**(4):1068-1077

[185] Okada K et al. Vialinin A is a ubiquitin-specific peptidase inhibitor. Bioorganic & Medicinal Chemistry Letters. 2013;**23**(15):4328-4331

[186] Yang J et al. Cutting edge: Ubiquitin-specific protease 4 promotes Th17 cell function under inflammation by deubiquitinating and stabilizing RORγt. The Journal of Immunology. 2015;**194**(9):4094-4097

[187] Li Z et al. Ubiquitination of a novel deubiquitinating enzyme requires direct binding to von Hippel-Lindau tumor suppressor protein. Journal of Biological Chemistry. 2002;**277**(7):4656-4662

[188] Song MS et al. The deubiquitinylation and localization of PTEN are regulated by a HAUSP–PML network. Nature. 2008;**455**(7214):813

[189] Dar A, Shibata E, Dutta A. Deubiquitination of Tip60 by USP7 determines the activity of the p53-dependent apoptotic pathway. Molecular and Cellular Biology. 2013;**33**(16):3309-3320

[190] Tian X et al. Characterization of selective ubiquitin and ubiquitin-like protease inhibitors using a fluorescence-based multiplex assay format. Assay and Drug Development Technologies. 2011;**9**(2):165-173

[191] Chauhan D et al. A small molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance. Cancer Cell. 2012;**22**(3):345-358

[192] Weinstock J et al. Selective dual inhibitors of the cancer-related deubiquitylating proteases USP7 and USP47. ACS Medicinal Chemistry Letters. 2012;**3**(10):789-792

[193] Colland F et al. Small-molecule inhibitor of USP7/HAUSP ubiquitin protease stabilizes and activates p53 in cells. Molecular Cancer Therapeutics. 2009;**8**(8):2286-2295

[194] Reverdy C et al. Discovery of specific inhibitors of human USP7/HAUSP deubiquitinating enzyme. Chemistry & Biology. 2012;**19**(4):467-477

[195] Liu Y et al. Discovery of inhibitors that elucidate the role of UCH-L1 activity in the H1299 lung cancer cell line. Chemistry & Biology. 2003;**10**(9):837-846

[196] Mermerian AH et al. Structure–activity relationship, kinetic mechanism, and selectivity for a new class of ubiquitin C-terminal hydrolase-L1 (UCH-L1) inhibitors. Bioorganic & Medicinal Chemistry Letters. 2007;**17**(13):3729-3732

[197] Trompouki E et al. CYLD is a deubiquitinating enzyme that negatively regulates NF-kappaB activation by TNFR family members. Nature. 2003;**424**(6950):793

[198] Ramakrishna S, Suresh B, Baek K-H. The role of deubiquitinating enzymes in apoptosis. Cellular and Molecular Life Sciences. 2011;**68**(1):15-26

[199] Zhong S et al. Pharmacologic inhibition of epigenetic modifications, coupled with gene expression profiling, reveals novel targets of aberrant DNA methylation and histone deacetylation in lung cancer. Oncogene. 2007;**26**(18):2621-2634

[200] Hellerbrand C et al. Reduced expression of CYLD in human colon and hepatocellular carcinomas. Carcinogenesis. 2007;**28**(1):21-27

[201] Jenner MW et al. Gene mapping and expression analysis of 16q loss of heterozygosity identifies WWOX and CYLD as being important in determining clinical outcome in multiple myeloma. Blood. 2007;**110**(9):3291-3300

[202] Nijman SM et al. The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. Molecular Cell. 2005;**17**(3):331-339

[203] Huang TT et al. Regulation of monoubiquitinated PCNA by DUB autocleavage. Nature Cell Biology. 2006;**8**(3):341-347

[204] Kim SJ et al. Differential expression profiling of genes in a complete hydatidiform mole using cDNA microarray analysis. Gynecologic Oncology. 2006;**103**(2):654-660

[205] Stevenson LF et al. The deubiquitinating enzyme USP2a regulates the p53 pathway by targeting Mdm2. The EMBO Journal. 2007;**26**(4):976-986

[206] Allende-Vega N et al. MdmX is a substrate for the deubiquitinating enzyme USP2a. Oncogene. 2010;**29**(3):432

[207] Shan J, Zhao W, Gu W. Suppression of cancer cell growth by promoting cyclin D1 degradation. Molecular Cell. 2009;**36**(3):469-476

[208] Bingol B et al. The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. Nature. 2014;**510**(7505):370

[209] Kim J et al. The ubiquitin-specific protease USP2a enhances tumor progression by targeting cyclin A1 in bladder cancer. Cell Cycle. 2012;**11**(6):1123-1130

[210] Graner E et al. The isopeptidase USP2a regulates the stability of fatty acid synthase in prostate cancer. Cancer Cell. 2004;**5**(3):253-261

[211] Zhang L et al. USP4 is regulated by AKT phosphorylation and directly deubiquitylates TGF-[beta] type I receptor. Nature Cell Biology. 2012;**14**(7):717

[212] Heo MJ et al. microRNA-148a dysregulation discriminates poor prognosis of hepatocellular carcinoma in association with USP4 overexpression. Oncotarget. 2014;**5**(9):2792

[213] Frederick A, Rolfe M, Chiu MI. The human UNP locus at 3p21.31 encodes two tissue-selective, cytoplasmic isoforms with deubiquitinating activity that have reduced expression in small cell lung carcinoma cell lines. Oncogene. 1998;**16**(2):153-165

[214] Dayal S et al. Suppression of the deubiquitinating enzyme USP5 causes the accumulation of unanchored polyubiquitin and the activation of p53. Journal of Biological Chemistry. 2009;**284**(8):5030-5041

[215] Yoshihara H et al. Insulin/insulin-like growth factor (IGF) stimulation abrogates an association between a deubiquitinating enzyme USP7 and insulin receptor substrates (IRSs) followed by proteasomal degradation of IRSs. Biochemical and Biophysical Research Communications. 2012;**423**(1):122-127

[216] Alonso-de Vega I, Martín Y, Smits VA. USP7 controls Chk1 protein stability by direct deubiquitination. Cell Cycle. 2014;**13**(24):3921-3926

[217] Faustrup H et al. USP7 counteracts SCFβTrCP-but not APCCdh1-mediated proteolysis of Claspin. The Journal of Cell Biology. 2009;**184**(1):13-19

[218] Cummins JM et al. Tumour suppression: Disruption of HAUSP gene stabilizes p53. Nature. 2004:**428**(6982):1 p following 486

[219] Alwan HA, van Leeuwen JE. UBPY-mediated epidermal growth factor receptor (EGFR) de-ubiquitination promotes EGFR degradation. Journal of Biological Chemistry. 2007; **282**(3):1658-1669

[220] Byun S et al. USP8 is a novel target for overcoming gefitinib resistance in lung cancer. Clinical Cancer Research. 2013

[221] Row PE et al. The ubiquitin isopeptidase UBPY regulates endosomal ubiquitin dynamics and is essential for receptor down-regulation. Journal of Biological Chemistry. 2006; **281**(18):12618-12624

[222] Niendorf S et al. Essential role of ubiquitin-specific protease 8 for receptor tyrosine kinase stability and endocytic trafficking in vivo. Molecular and Cellular Biology. 2007;**27**(13): 5029-5039

[223] Ma Z-Y et al. Recurrent gain-of-function USP8 mutations in Cushing's disease. Cell Research. 2015;**25**(3):306

[224] Jeong C-H. Inhibition of ubiquitin-specific peptidase 8 suppresses growth of gefitinib-resistant non-small cell lung cancer cells by inducing apoptosis. Journal of Cancer Prevention. 2015;**20**(1):57

[225] Dupont S et al. FAM/USP9x, a deubiquitinating enzyme essential for TGFβ signaling, controls Smad4 monoubiquitination. Cell. 2009;**136**(1):123-135

[226] Murray RZ, Jolly LA, Wood SA. The FAM deubiquitylating enzyme localizes to multiple points of protein trafficking in epithelia, where it associates with E-cadherin and β-catenin. Molecular Biology of the Cell. 2004;**15**(4):1591-1599

[227] Nagai H et al. Ubiquitin-like sequence in ASK1 plays critical roles in the recognition and stabilization by USP9X and oxidative stress-induced cell death. Molecular Cell. 2009; **36**(5):805-818

[228] Deng S et al. Over-expression of genes and proteins of ubiquitin specific peptidases (USPs) and proteasome subunits (PSs) in breast cancer tissue observed by the methods of RFDD-PCR and proteomics. Breast Cancer Research and Treatment. 2007;**104**(1):21-30

[229] Wang S et al. Ablation of the oncogenic transcription factor ERG by deubiquitinase inhibition in prostate cancer. Proceedings of the National Academy of Sciences. 2014; **111**(11):4251-4256

[230] Nadiminty N et al. Prostate-specific antigen modulates genes involved in bone remodeling and induces osteoblast differentiation of human osteosarcoma cell line SaOS-2. Clinical Cancer Research. 2006;**12**(5):1420-1430

[231] Schwickart M et al. Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. Nature. 2010;**463**(7277):103

[232] Peng J et al. USP9X expression correlates with tumor progression and poor prognosis in esophageal squamous cell carcinoma. Diagnostic Pathology. 2013;**8**(1):177

[233] Jochemsen AG, Shiloh Y. USP10: Friend and foe. Cell. 2010;**140**(3):308-310

[234] Pan L et al. Deubiquitination and stabilization of T-bet by USP10. Biochemical and Biophysical Research Communications. 2014;**449**(3):289-294

[235] Yuan J et al. USP10 regulates p53 localization and stability by deubiquitinating p53. Cell. 2010;**140**(3):384-396

[236] Zhang M et al. Ubiquitin-specific peptidase 10 (USP10) deubiquitinates and stabilizes MutS homolog 2 (MSH2) to regulate cellular sensitivity to DNA damage. Journal of Biological Chemistry. 2016;**291**(20):10783-10791

[237] Grunda JM et al. Increased expression of thymidylate synthetase (TS), ubiquitin specific protease 10 (USP10) and survivin is associated with poor survival in glioblastoma multiforme (GBM). Journal of Neuro-Oncology. 2006;**80**(3):261-274

[238] Jacko A et al. De-ubiquitinating enzyme, USP11, promotes transforming growth factor β-1 signaling through stabilization of transforming growth factor β receptor II. Cell Death & Disease. 2016;**7**(11):e2474

[239] Ke J-Y et al. USP11 regulates p53 stability by deubiquitinating p53. Journal of Zhejiang University. Science. B. 2014;**15**(12):1032

[240] Al-Salihi MA et al. USP11 augments TGFβ signalling by deubiquitylating ALK5. Open Biology. 2012;**2**(6):120063

[241] Schoenfeld AR et al. BRCA2 is ubiquitinated in vivo and interacts with USP11, a deubiquitinating enzyme that exhibits prosurvival function in the cellular response to DNA damage. Molecular and Cellular Biology. 2004;**24**(17):7444-7455

[242] Burska UL et al. Deubiquitinating enzyme Usp12 is a novel co-activator of the androgen receptor. Journal of Biological Chemistry. 2013;**288**(45):32641-32650

[243] Zhang J et al. Deubiquitination and stabilization of PTEN by USP13. Nature Cell Biology. 2013;**15**(12):1486

[244] Zhao X et al. Regulation of MITF stability by the USP13 deubiquitinase. Nature Communications. 2011;**2**:414

[245] Eichhorn PJ et al. USP15 stabilizes TGF-[beta] receptor I and promotes oncogenesis through the activation of TGF-[beta] signaling in glioblastoma. Nature Medicine. 2012;**18**(3):429-435

[246] Ovaa H et al. Activity-based ubiquitin-specific protease (USP) profiling of virus-infected and malignant human cells. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(8):2253-2258

[247] Inui M et al. USP15 is a deubiquitylating enzyme for receptor-activated SMADs. Nature Cell Biology. 2011;**13**(11):1368

[248] Joo H-Y et al. Regulation of cell cycle progression and gene expression by H2A deubiquitination. Nature. 2007;**449**(7165):1068

[249] Adorno M et al. Usp16 contributes to somatic stem cell defects in Down syndrome. Nature. 2013;**501**(7467):380

[250] Burrows JF et al. USP17 regulates Ras activation and cell proliferation by blocking RCE1 activity. Journal of Biological Chemistry. 2009;**284**(14):9587-9595

[251] de La Vega M et al. The deubiquitinating enzyme USP17 is essential for GTPase subcellular localization and cell motility. Nature Communications. 2011;**2**:259

[252] McFarlane C et al. The deubiquitinating enzyme USP17 is highly expressed in tumor biopsies, is cell cycle regulated, and is required for G1-S progression. Cancer Research. 2010;**70**(8):3329-3339

[253] Duex JE, Sorkin A. RNA interference screen identifies Usp18 as a regulator of epidermal growth factor receptor synthesis. Molecular Biology of the Cell. 2009;**20**(6):1833-1844

[254] Yan M et al. Ubp43 regulates BCR-ABL leukemogenesis via the type 1 interferon receptor signaling. Blood. 2007;**110**(1):305-312

[255] Guo Y et al. Blockade of the ubiquitin protease UBP43 destabilizes transcription factor PML/RARα and inhibits the growth of acute promyelocytic leukemia. Cancer Research. 2010

[256] Lu Y et al. USP19 deubiquitinating enzyme supports cell proliferation by stabilizing KPC1, a ubiquitin ligase for p27Kip1. Molecular and Cellular Biology. 2009;**29**(2):547-558

[257] Li Z et al. VHL protein-interacting deubiquitinating enzyme 2 deubiquitinates and stabilizes HIF-1α. EMBO Reports. 2005;**6**(4):373-378

[258] Wang C et al. Deubiquitinating enzyme USP20 is a positive regulator of Claspin and suppresses the malignant characteristics of gastric cancer cells. International Journal of Oncology. 2017;**50**(4):1136-1146

[259] Zhang J et al. Identification of the E3 deubiquitinase ubiquitin-specific peptidase 21 (USP21) as a positive regulator of the transcription factor GATA3. Journal of Biological Chemistry. 2013;**288**(13):9373-9382

[260] Nakagawa T et al. Deubiquitylation of histone H2A activates transcriptional initiation via trans-histone cross-talk with H3K4 di-and trimethylation. Genes & Development. 2008;**22**(1):37-49

[261] Chen Y, Zhou B, Chen D. USP21 promotes cell proliferation and metastasis through suppressing EZH2 ubiquitination in bladder carcinoma. OncoTargets and Therapy. 2017;**10**:681

[262] Peng L et al. Ubiquitin specific protease 21 upregulation in breast cancer promotes cell tumorigenic capability and is associated with the NOD-like receptor signaling pathway. Oncology Letters. 2016;**12**(6):4531-4537

[263] Peng L et al. Ubiquitin specific peptidase 21 regulates interleukin-8 expression, stem-cell like property of human renal cell carcinoma. Oncotarget. 2016;**7**(27):42007

[264] Zhao Y et al. A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Molecular Cell. 2008;**29**(1):92-101

[265] Kim D et al. Deubiquitinating enzyme USP22 positively regulates c-Myc stability and tumorigenic activity in mammalian and breast cancer cells. Journal of Cellular Physiology. 2017;**232**(12):3664-3676

[266] Sacco JJ et al. Emerging roles of deubiquitinases in cancer-associated pathways. IUBMB Life. 2010;**62**(2):140-157

[267] Schrecengost RS et al. USP22 regulates oncogenic signaling pathways to drive lethal cancer progression. Cancer Research. 2014;**74**(1):272-286

[268] Zhang Y et al. Elevated expression of USP22 in correlation with poor prognosis in patients with invasive breast cancer. Journal of Cancer Research and Clinical Oncology. 2011;**137**(8):1245-1253

[269] Liu YL et al. Aberrant expression of USP22 is associated with liver metastasis and poor prognosis of colorectal cancer. Journal of Surgical Oncology. 2011;**103**(3):283-289

[270] Li J, Wang Z, Li Y. USP22 nuclear expression is significantly associated with progression and unfavorable clinical outcome in human esophageal squamous cell carcinoma. Journal of Cancer Research and Clinical Oncology. 2012;**138**(8):1291-1297

[271] Xu D et al. USP25 regulates Wnt signaling by controlling the stability of tankyrases. Genes & Development. 2017;**31**(10):1024-1035

[272] Li J et al. miRNA-200c inhibits invasion and metastasis of human non-small cell lung cancer by directly targeting ubiquitin specific peptidase 25. Molecular Cancer. 2014;**13**(1):166

[273] Popov N et al. The ubiquitin-specific protease USP28 is required for MYC stability. Nature Cell Biology. 2007;**9**(7):765-774

[274] Popov N et al. Fbw7 and Usp28 regulate myc protein stability in response to DNA damage. Cell Cycle. 2007;**6**(19):2327-2331

[275] Zhang D et al. A role for the deubiquitinating enzyme USP28 in control of the DNA-damage response. Cell. 2006;**126**(3):529-542

[276] Wu Y et al. The deubiquitinase USP28 stabilizes LSD1 and confers stem-cell-like traits to breast cancer cells. Cell Reports. 2013;**5**(1):224-236

[277] Shah SP et al. Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature. 2009;**461**(7265):809-813

[278] Diefenbacher ME et al. The deubiquitinase USP28 controls intestinal homeostasis and promotes colorectal cancer. The Journal of Clinical Investigation. 2014;**124**(8):3407

[279] Liu J et al. JTV1 co-activates FBP to induce USP29 transcription and stabilize p53 in response to oxidative stress. The EMBO Journal. 2011;**30**(5):846-858

[280] Martin Y et al. USP29 controls the stability of checkpoint adaptor Claspin by deubiquitination. Oncogene. 2015;**34**(8):1058

[281] Yuasa-Kawada J et al. Deubiquitinating enzyme USP33/VDU1 is required for Slit signaling in inhibiting breast cancer cell migration. Proceedings of the National Academy of Sciences. 2009;**106**(34):14530-14535

[282] De Pittà C et al. A leukemia-enriched cDNA microarray platform identifies new transcripts with relevance to the biology of pediatric acute lymphoblastic leukemia. Haematologica. 2005;**90**(7):890-898

[283] Sy SM et al. The ubiquitin specific protease USP34 promotes ubiquitin signaling at DNA double-strand breaks. Nucleic Acids Research. 2013;**41**(18):8572-8580

[284] Lui TT et al. The ubiquitin specific protease USP34 regulates Axin stability and Wnt/β-catenin signaling. Molecular and Cellular Biology. 2011;**31**(10):2053-2065

[285] Hock AK et al. Regulation of p53 stability and function by the deubiquitinating enzyme USP42. The EMBO Journal. 2011;**30**(24):4921-4930

[286] Stegmeier F et al. Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. Nature. 2007;**446**(7138):876

[287] Fuchs G et al. RNF20 and USP44 regulate stem cell differentiation by modulating H2B monoubiquitylation. Molecular Cell. 2012;**46**(5):662-673

[288] Zhang Y, Van Deursen J, Galardy PJ. Overexpression of ubiquitin specific protease 44 (USP44) induces chromosomal instability and is frequently observed in human T-cell leukemia. PLoS One. 2011;**6**(8):e23389

[289] Parsons JL et al. USP47 is a deubiquitylating enzyme that regulates base excision repair by controlling steady-state levels of DNA polymerase β. Molecular Cell. 2011;**41**(5):609-615

[290] Peschiaroli A et al. The ubiquitin-specific protease USP47 is a novel β-TRCP interactor regulating cell survival. Oncogene. 2010;**29**(9):1384-1393

[291] Aressy B et al. 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): 3839-3846

[292] Boutros R, Dozier C, Ducommun B. The when and wheres of CDC25 phosphatases. Current Opinion in Cell Biology. 2006;**18**(2):185-191

[293] Yu J et al. Epigenetic identification of ubiquitin carboxyl-terminal hydrolase L1 as a functional tumor suppressor and biomarker for hepatocellular carcinoma and other digestive tumors. Hepatology. 2008;**48**(2):508-518

[294] Liu Y et al. The UCH-L1 gene encodes two opposing enzymatic activities that affect α-synuclein degradation and Parkinson's disease susceptibility. Cell. 2002;**111**(2):209-218

[295] Luo Y et al. UCHL1 promotes invasion of breast cancer cells through activating Akt signaling pathway. Journal of Cellular Biochemistry. 2018;**119**(1):691-700

[296] Xiang T et al. The ubiquitin peptidase UCHL1 induces G0/G1 cell cycle arrest and apoptosis through stabilizing p53 and is frequently silenced in breast cancer. PLoS One. 2012;**7**(1):e29783

[297] Hibi K et al. Serial analysis of gene expression in non-small cell lung cancer. Cancer Research. 1998;**58**(24):5690-5694

[298] Yamazaki T et al. PGP9.5 as a marker for invasive colorectal cancer. Clinical Cancer Research. 2002;**8**(1):192-195

[299] Leiblich A et al. Human prostate cancer cells express neuroendocrine cell markers PGP 9.5 and chromogranin A. The Prostate. 2007;**67**(16):1761-1769

[300] Machida YJ et al. The deubiquitinating enzyme BAP1 regulates cell growth via interaction with HCF-1. Journal of Biological Chemistry. 2009;**284**(49):34179-34188

[301] Scheuermann JC et al. Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. Nature. 2010;**465**(7295):243

[302] Murali R, Wiesner T, Scolyer RA. Tumours associated with BAP1 mutations. Pathology. 2013;**45**(2):116-126

[303] Nan L et al. Ubiquitin carboxyl-terminal hydrolase-L5 promotes TGFβ-1 signaling by deubiquitinating and stabilizing Smad2/Smad3 in pulmonary fibrosis. Scientific Reports. 2016;**6**:33116

[304] Nishi R et al. Systematic characterization of deubiquitylating enzymes for roles in maintaining genome integrity. Nature Cell Biology. 2014;**16**(10):1016-1026

[305] Liu H et al. Modification of ubiquitin-C-terminal hydrolase-L1 by cyclopentenone prostaglandins exacerbates hypoxic injury. Neurobiology of Disease. 2011;**41**(2):318-328

[306] Koharudin LM et al. Cyclopentenone prostaglandin-induced unfolding and aggregation of the Parkinson disease-associated UCH-L1. Proceedings of the National Academy of Sciences. 2010;**107**(15):6835-6840

[307] Issaenko OA, Amerik AY. Chalcone-based small-molecule inhibitors attenuate malignant phenotype via targeting deubiquitinating enzymes. Cell Cycle. 2012;**11**(9):1804-1817

[308] Fraile JM, López-Otín C, Freije JM. Functional relevance of deubiquitinases in life and disease. In: Proteases in Physiology and Pathology. Springer; 2017. pp. 355-382

[309] Ohayon S et al. Targeting deubiquitinases enabled by chemical synthesis of proteins. Journal of the American Chemical Society. 2012;**134**(6):3281-3289

[310] Shi T et al. Identification of small molecule TRABID deubiquitinase inhibitors by computation-based virtual screen. BMC Chemical Biology. 2012;**12**(1):4

[311] Chen J et al. Selective and cell-active inhibitors of the USP1/UAF1 deubiquitinase complex reverse cisplatin resistance in non-small cell lung cancer cells. Chemistry & Biology. 2011;**18**(11):1390-1400

[312] Dexheimer TS et al. Synthesis and structure–activity relationship studies of N-benzyl-2-phenylpyrimidin-4-amine derivatives as potent USP1/UAF1 deubiquitinase inhibitors with anticancer activity against nonsmall cell lung cancer. Journal of Medicinal Chemistry. 2014;**57**(19):8099-8110

[313] Davis MI et al. 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. Journal of Biological Chemistry. 2016;**291**(47):24628-24640

[314] Edelmann MJ, Nicholson B, Kessler BM. Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases. Expert Reviews in Molecular Medicine. 2011;**13**

[315] Liu J et al. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. Cell. 2011;**147**(1):223-234:e35

[316] Burkhart RA et al. Mitoxantrone targets human ubiquitin-specific peptidase 11 (USP11) and is a potent inhibitor of pancreatic cancer cell survival. Molecular Cancer Research. 2013;**11**(8):901-911

[317] Kemp M. Chapter three-recent advances in the discovery of deubiquitinating enzyme inhibitors. Progress in Medicinal Chemistry. 2016;**55**:149-192

[318] Yue W et al. A small natural molecule promotes mitochondrial fusion through inhibition of the deubiquitinase USP30. Cell Research. 2014;**24**(4):482

[319] Seiberlich V et al. The small molecule inhibitor PR-619 of deubiquitinating enzymes affects the microtubule network and causes protein aggregate formation in neural cells: Implications for neurodegenerative diseases. Biochimica Et Biophysica Acta (BBA)-Molecular Cell Research. 2012;**1823**(11):2057-2068

[320] Cooper EM et al. K63-specific deubiquitination by two JAMM/MPN+ complexes: BRISC-associated Brcc36 and proteasomal Poh1. The EMBO Journal. 2009;**28**(6):621-631

[321] Sigman DS et al. Nuclease activity of 1, 10-phenanthroline-copper in study of protein—DNA interactions. Methods in Enzymology. 1991;**208**:414-433