Chapter 15
Deubiquitinating Enzymes as Novel Targets for Cancer Therapies

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Abstract Most ubiquitinated proteins can be recognized and degraded by the 26S proteasome. In the meantime, protein deubiquitination by various deubiquitinating enzymes (DUBs) regulates protein stability within cells, and it can counterbalance intracellular homeostasis mediated by ubiquitination. Numerous reports have demonstrated that an aberrant process of the ubiquitin-proteasome pathway (UPP) regulated by the ubiquitination and deubiquitination systems results in failure of balancing between protein stability and degradation, and this failure can lead to tumorigenesis in various organs and tissues of mammals. The identification of molecular properties for various DUBs is very critical to understand cancer development and tumorigenesis. Therefore, knowledge of DUBs and their association with cancer and diseases is indispensible for developing effective inhibitors for DUBs. This chapter describes various features and functions of cancer-related DUBs. In addition, we summarize several inhibitors that specifically target certain DUBs in cancer and suggest that DUBs may be one of the most ideal and attractive therapeutic targets.

Keywords Anticancer drug • Bortezomib • Deubiquitination • Oncogene • Proteasome • Tumor suppressor • Ubiquitin-specific protease • Ubiquitination

Abbreviations

CLL	Chronic lymphoid leukemia
CML	Chronic myeloid leukemia
DUB	Deubiquitinating enzyme
HR	Homologous recombination
ICL	Interstrand cross-link
JAMM	JAB1/MPN/MOV34 metalloenzyme
MCL	Mantle cell lymphoma

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MFT	Multiple familiar trichoepithelioma
MJD	Machado-Joseph disease
MM	Multiple myeloma
OTU	Ovarian-tumor protease
PDA	Pancreatic ductal adenocarcinoma
TLS	Translesion DNA synthesis
UCH	Ubiquitin carboxy-terminal hydrolase
UPP	Ubiquitin-proteasome pathway
USP	Ubiquitin-specific protease

15.1 Introduction

As a reverse process against ubiquitination, deubiquitination accomplished by deubiquitinating enzymes (DUBs) acts as counterbalancing regulation for the fate of proteins. The requirement of catalytic activity for DUBs in cellular processes has been shown in numerous studies. In terms of cellular homeostasis, controlled expression levels of proteins are essential, and abnormal expression of certain proteins can be directly linked to cancer and other diseases causing breakdown of the coordinated cellular system. A number of unregulated proteins are mediated by ubiquitination and deubiquitination. Therefore, it is necessary to understand and investigate in detail the cellular and molecular mechanisms underlining deubiquitinating activity. In addition, the targeting of DUBs as anticancer therapies is becoming an important field in cancer therapeutics. Knowledge of DUBs, their association with cancer and diseases, and use of DUB inhibitors in clinical and preclinical studies will be presented in this chapter.

15.2 Overview of DUBs

15.2.1 Classification of Proteases in Mammals

Proteases are essential enzymes that catalyze protein-peptide bonds in all species, and they have various cellular functions such as in food digestion, ovulation, fertilization, and inflammatory responses. Many studies have analyzed and revealed the roles of proteases, and the research results have been applied to the medical treatment of cancer and diverse diseases. Recent studies have suggested that the human genome encodes a total of 600 proteases [1]. Human proteases can be divided into five classes according to their catalytic characteristics: serine proteases, threonine proteases, cysteine proteases, aspartate proteases, and metalloproteases. The glutamic proteases are limited to fungal species. Of the 600 proteases, 176 are serine proteases (32 %), 74 are threonine proteases (4 %), 143 are cysteine proteases (26 %), 21 are aspartate proteases (4 %), and 186 are metalloproteases (34 %)

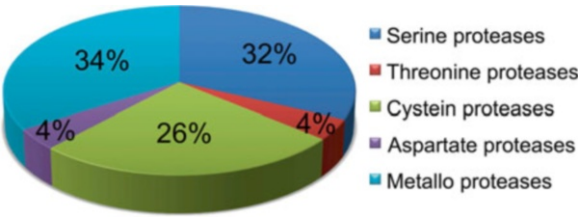

Fig. 15.1 Pie chart of mammalian protease classification. A pie chart representing the percentage of mammalian proteases classified by the expression pattern based on a genome encoding database

according to genomics and bioinformatics research on proteasomes (Fig. 15.1). To the cysteine proteases family, this chapter will focus on cysteine proteases to understand the biological functions of DUBs.

15.2.2 DUB and Its Family

DUBs are a subfamily of cysteine proteases and have reversible abilities against E3 ligases, in which they detach ubiquitin molecules from ubiquitinated substrates via their enzymatic activities (Fig. 15.2). This DUB-mediated process, which is the opposite of ubiquitination, is called deubiquitination. Like ubiquitination, deubiquitination can give signals to functional proteins to modulate their activities. Therefore, DUBs are involved in numerous cellular functions including cell cycle regulation, signal transduction, membrane trafficking, DNA damage response, immune response, and apoptosis or programmed cell death. The major known signal of ubiquitination guides ubiquitinated proteins heading to the 26S proteasome for protein degradation (the ubiquitin-proteasome pathway, UPP), while DUB-mediated deubiquitination can prevent the proteasomal degradation of the substrates. Thus, the orchestration of reversible posttranslational regulations by ubiquitination and deubiquitination affects cellular homeostasis and cell viability, based on not only protein levels, but also on protein functions. It is critical to systemically maintain expression levels and functions of cellular proteins for the healthy cells, tissues, organs, and individuals. Indeed, as we will discuss later in this chapter, the breakdown of coordinated regulation of functional proteins caused by altered activities or abnormal expression level of DUBs can induce severe diseases including cancer.

To date, almost 100 human genes encoding DUB enzymes have been identified; these can be grouped into the following five classes according to their properties: ubiquitin-specific proteases (USPs), ubiquitin carboxy-terminal hydrolases (UCHs), ovarian-tumor proteases (OTUs), Machado-Joseph diseases (MJDs), and JAB1/MPN/MOV34 metalloenzymes (JAMMs). This classification can be expanded to six categories, in order to include the recently identified monocyte chemotactic protein-induced protein (MCPIP) [2]. The DUBs which have been identified so far are listed in Table 15.1. Except for JAMMs, which are zinc metalloproteases, all DUBs have conserved domains including Cys, Asp/Asn, and His domains, which are associated with their catalytic activity [3].

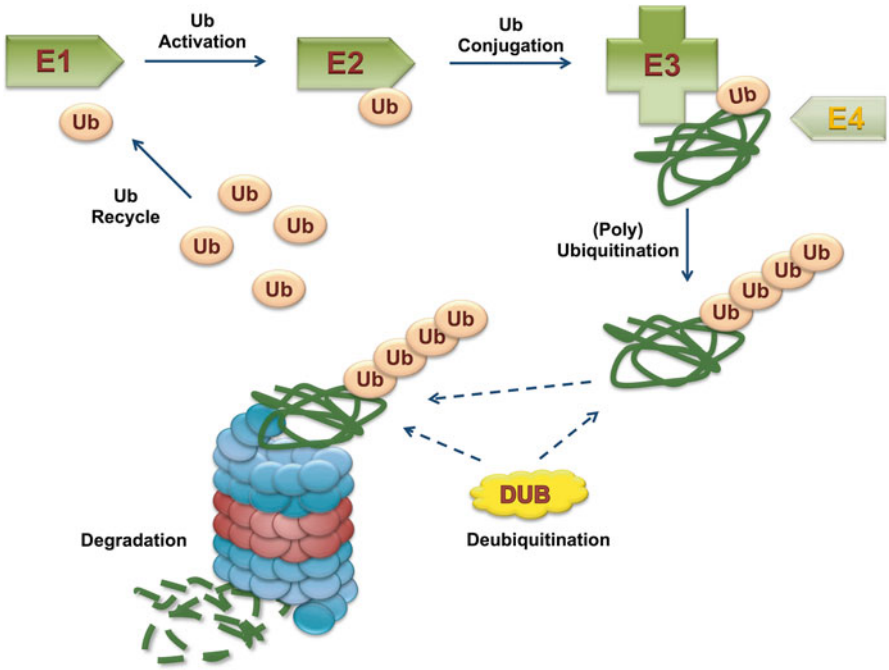

Fig. 15.2 Ubiquitination and deubiquitination. The proteasomal degradation of ubiquitinated proteins occurs via the ubiquitin-proteasome pathway (UPP). The coordinated ubiquitination and deubiquitination of target proteins are mediated by specific enzymes. For ubiquitination, successive action of several enzymes is required. For the first step, ubiquitin (Ub), which consists of 76 amino acids, is activated by a ubiquitin-activating enzyme (E1) in an ATP-dependent manner. This is followed by interaction with the ubiquitin-conjugating enzyme (E2). Lastly, the E2-bound ubiquitin is transferred to the E3 ubiquitin ligase. In some cases, E4 enzymes are needed for efficient ubiquitination. In addition to mono-ubiquitination, additional ubiquitins can be conjugated to the attached ubiquitin to form polyubiquitin chains. In a reversible process, deubiquitinating enzymes (DUBs) detach ubiquitin molecules from ubiquitinated substrates via their enzymatic activities. In this process, ubiquitin chains from proteasome-targeted proteins should be removed, thereby promoting protein degradation and recycling free ubiquitins. Deconjugating ubiquitin in proteasomal processing is mediated by certain DUBs including UCHL5, USP14, and POH1. Through these processes, DUBs generate free ubiquitin molecules, prevent proteasomal degradation of target proteins, and stabilize target proteins

15.2.3 Structure of DUBs

Structural analysis has been performed for diverse DUBs [4]. This is the most reliable way of gaining information about target protein activity, functions, and interaction motifs. The generalization of several 3D structures has emerged from diverse studies in which the molecular key features of DUBs as ubiquitin moieties have been established [4]. Each DUB subfamily shares similar sequences and structures.

Table 15.1 Subfamily types of deubiquitinating enzymes classified into six categories

Subfamily types	DUB names
USP family	USP1, USP2, USP3, USP4, USP5, USP6, USP7, USP8, USP9X, USP9Y, USP10, USP11, USP12, USP13, USP14, USP15, USP16, DUB3, USP18, USP19, USP20, USP21, USP22, USP24, USP25, USP26, USP27X, USP28, USP29, USP30, USP31, USP31, USP32, USP33, USP34, USP35, USP36, USP37, USP38, USP39, USP40, USP41, USP42, USP43, USP44, USP45, USP46, USP47, USP48, USP49, USP50, USP51, USP52, USP53, USP54, CYLD, USPL1
UCH family	UCH-L1, UCH-L3, UCH-L5, BAP1
JAMM family	BRCC36, CSN5, POH1, AMSH, AMSH-LP, MPND, MYSM1, PRPF8, EIF3
OTU family	OTUB1, OTUB2, OTUD1, OTUD3, OTUD4, OTUD5, OTUD6A, OTUD6B, OTU1, HIN1L, A20, Cezanne, Cezanne2, VCPIP, TRABID
MJD family	ATXN3, ATXN3L, JOSD1, JOSD2
MCPIP family[a]	MCPIP1, MCPIP2, MCPIP3, MCPIP4, MCPIP5, MCPIP6, MCPIP7

[a]MCPIP family, which is newly discovered, can be grouped as a subfamily of DUBs

In general, most USP family members have six homologue-conserved USP domains and consist of three domains organizing as a palm, a thumb, and fingers [4]. Among them, the finger domains interact with ubiquitin. In addition, a number of diverse motifs exist through USPs, and these specific domains and structures give unique functions to USPs. USP3, USP5, USP39, USP44, USP45, USP49, and USP51 have the zinc-finger USP domain, while the domain present in USP (DUSP) is located in USP4, USP11, USP15, USP20, USP33, and USP48. Moreover, other functional domains are present through different USPs [5]. It is known that UCHs are small in size and target only small peptides because of their structures, which include a confined loop [5]. The OTU family can be subdivided into three classes depending on their characteristics—otubains (OTUB1 and OTUB2), A20-like OTUs (A20/TNFα-induced protein 3 {TNFAIP3}; Cezanne, Cezanne2, TRABID, and VCPIP1), and OTUDs (OTUD1, OTUD2/YOD1, OTUD3, OTUD4, OTUD5, OTUD6A, OTUD6B, and ALG13). The Josephin family of DUBs also consists of four different subfamilies, including ataxin-3 (ATXN3), ATXN3L, JOSD1, and JOSD2. Unlike other DUBs, the JAMMs have zinc metalloprotease activity and contain an AMSH-LP structure and two conserved motifs that are related to the capacity for cleaving K63-linked polyubiquitin chains [5].

15.3 Various Roles of DUBs in Cancer

Most vertebrates have a balance that maintains cell birth and cell death through intracellular signaling from various stimuli. Cells disproportionated by abnormal protein expression or oncogene transcription from several stimuli can be transformed into cancer cells. For example, treatment of normal cells with viruses, carcinogenic compounds, UV, or IR can transform cell characterization, leading to a

cancerous state by deregulating gene or protein expression. DUBs widely participate in biological functions such as DNA repair, chromatin remodeling, transcription, the signal transduction cascade, protein localization, cell cycle progression, and apoptosis in cancer cells [6].

15.3.1 Oncogenic Functions of DUBs

The functions of USP2a (also known as USP2-69) were first found in prostate cancer. It exhibits oncogenic behavior and depletion of USP2a induces cancer cell apoptosis [7, 8]. USP2a mainly regulates and stabilizes the fatty acid synthase (FAS) which is frequently overexpressed in malignant tumors [7]. The deubiquitinating activity of USP2a in FAS regulation may lead to tumorigenesis. In addition, USP2a is associated with Mdm2 and MdmX [9]. Both Mdm2 and MdmX, known as oncogenic proteins, are negative regulators of p53. Depletion of USP2a enhances both Mdm2 and MdmX protein degradation [8, 9]. USP2a overexpression increases the c-MYC level and is able to inactivate p53 in prostate cancer cells [10]. With these findings, one might expect that USP2a would be strongly associated with tumorigenesis through the regulation of Mdm2 and MdmX and collaboration with c-Myc. A recent study has added the function of USP2a expression to cell death by targeting RIP and tumor necrosis factor receptor-associated factor 2 (TRAF2) degradation during tumor necrosis factor (TNF) response, and USP2a consequently promotes the activation of NF-κB [11].

Although USP4/UNP is associated with the TNF response and activates NF-κB as shown with USP2a, it has a different role, wherein USP4 regulates TAK-1 stability upon TNF response. Interestingly, USP4 deubiquitinates not only TRAF2 but also TRAF6 and leads to the regulation of cell migration [12]. Further, a genome-wide gain-of-function study revealed that AKT acts as a kinase for USP4 phosphorylation and phosphorylated USP4 moves into the cytoplasm from the nucleus. Molecular mechanism studies have shown that USP4 is strongly associated with the transforming growth factor-β (TGF-β) type I receptor (TβRI) and deubiquitinates and stabilizes TβRI at the plasma membrane. In addition, USP4 depletion inhibits breast cancer cell migration, which is induced by AKT [13]. ARF-BP1 is a p53-specific E3 ligase that binds to USP4. Through USP4 overexpression, stabilized ARF-BP1 reduces the stability of p53. The in vivo molecular mechanism study by which depleted USP4 in MEF cells showed resistance to tumorigenic transformation [14].

USP6 (also known as Tre17, Tre-2) was isolated as an oncogene in Ewing's sarcoma, and a further study revealed that the *Usp6* gene encodes a deubiquitinating enzyme and regulates mammalian cell growth [15, 16]. Moreover, the domain studies showed that *Usp6* is homologous to *Bub2* and *cdc16*, mitosis-regulating genes [17]. Aneurysmal bone cyst (ABC) can generate malignant bone tumor, and *Usp6* transcription is deregulated in ABCs [18]. The intracellular function of USP6 has

been identified in the regulation of Arf6 as a GTPase. USP6 interacts with Arf6 through its N-terminus Tre2/Bub2/Csc16 (TBC) domain, and depletion of USP6 decreases Arf6 activity [19].

USP7 (known as herpesvirus-associated ubiquitin-specific protease, HAUSP) is the most studied deubiquitinating enzyme in the USP family. Herpes simplex virus (HSV) protein ICP0 was initially identified as a USP7/HAUSP-associated protein, and interaction between these two proteins facilitates viral replication [20]. In addition, as a herpes virus regulatory protein, Vmw110 is also bound to and stabilized by USP7/HAUSP, and their interaction leads to the regulation of ND10 as a PML nuclear body [21]. Further, USP7/HAUSP interacts with EBNA1 as an Epstein-Barr virus (EBV) protein and regulates EBNA1 replication [22]. EBV infection is closely associated with nasopharyngeal carcinoma (NPC), and the EBNA1 protein disrupts ND10 [23]. Study of the mechanisms of cellular EBNA1 function showed that EBNA1 is required for binding of USP7/HAUSP to disrupt ND10 [23]. The tumor suppressor phosphatase and tensin homologue (PTEN) has been studied with cancer progression, and a recent study showed that USP7/HAUSP and PTEN interaction leads to the regulation of PTEN localization [24]. PTEN ubiquitinated by E3 ligase is translocated and accumulated in the nucleus. However, PTEN is deubiquitinated by USP7/HAUSP and released to the cytoplasm on the PML-RARα signaling network [24]. The tumor suppressor p53 has been identified as a USP7/HAUSP binding substrate in the nuclear extract of human lung carcinoma H1299 cells (known as p53 null cells) [25]. The expression of USP7/HAUSP prevents p53 ubiquitination from Mdm2 as a p53-specific E3 ligase and increases the p53 protein stability [25]. The overexpression of USP7/HAUSP induces cancer cell apoptosis, and this phenotype depends on p53 existence in the cells [25]. Further, USP7/HAUSP can make a complex with p53-Mdm2 and regulates the balancing of p53 expression between normal and stressed cell states [26]. USP7/HAUSP can elongate p53; however, depletion of USP7/HAUSP also induces upregulation of p53 protein expression [27]. USP7/HAUSP stability is regulated by phosphorylation and dephosphorylation via the ataxia-telangiectasia-mutated (ATM)-dependent pathway, and dephosphorylated USP7/HAUSP undergoes the proteasomal degradation [28]. For example, USP7/HAUSP is phosphorylated by CK2 as a serine/threonine kinase and leads to stabilization of USP7/HAUSP in a normal state [28]. The stabilized USP7/HAUSP can enhance Mdm2 and decrease p53 protein expression. In the DNA-damaged state, however, USP7/HAUSP is dephosphorylated by PPM1G as a phosphatase and then degraded, and it decreases the Mdm2 protein and accumulates the p53 protein [28]. In addition, approximately 60–80 % of phosphorylated USP7/HAUSP exists in human cells [28]. Thus, USP7/HAUSP expression is reduced to 45 % in adenocarcinoma [27]. Recently, one study identified a novel gene that is associated with oncogenesis in the breast, called *TSPYL5* [29]. TSPYL5 is frequently overexpressed in breast cancer, and the study showed that an increasing level of TSPYL5 decreased USP7/HAUSP expression and led to the accumulation of p53 ubiquitination [29].

USP9X/FAM is known as an X-linked deubiquitinating enzyme and the homologue of the *Drosophila fat facets* gene [30]. An oncogenic function of USP9X was

found in human lymphomas [31]. MCL1 is a substrate of USP9X that is abundantly expressed in mantle cell lymphoma (MCL), chronic myeloid leukemia (CML), and multiple myeloma (MM) [31]. The overexpression of USP9X stabilizes the MCL1 protein in human lymphomas, and the depletion of USP9X increases MCL1 ubiquitination, which leads to MM cell apoptosis [31]. A feature of this USP9X in cancer was confirmed by a further study on pancreatic ductal adenocarcinoma (PDA) [32]. More than 50 % of tumors exhibit inactive USP9X protein, and the deletion of *Usp9x* increases pancreatic tumorigenesis in mice [32].

Usp15 has sequence similarity with *Usp4*/*Unp* as a proto-oncogene [33]. The COP9 signalosome (CSN), as a conserved protein complex, is involved in the transformation of eukaryotic cells and is associated with the UPP [34]. USP15 is bound to the CSN complex, and a recent study showed that the Cullin-RING ubiquitin ligase (CRL), as a CSN-binding partner, is associated with USP15 [34, 35]. Under NF-κB degradation by CRL, USP15 is involved in Iκ-Bα as an NF-κB-inhibiting protein in the process of deubiquitination [35]. However, USP15 does not have deubiquitinating activity for other CSN-binding proteins, such as the microtubule end-binding protein 1 (EB1) [36]. This indicates that the enzyme activity of USP15 may work differently and selectively in CSN-mediated protein regulation.

Previously, the cancer cell marker was not fully defined, and several studies suggested that *Polycomb* genes could be markers for the identification of cancer stem cells [37]. An initial study of USP22 showed that USP22 is overexpressed in malignant tumors linked to the Polycomb group [38]. Furthermore, USP22 acts as an enzymatic component of the SAGA transcriptional cofactor complex and is activated by Myc as an oncogene [38]. Thus, it is considered that USP22 itself can be a positive marker of cancer stem cells [38, 39]. Further, several studies have shown that the level of USP22 in colorectal cancer tissues is highly expressed compared with that in noncancerous mucosa tissues, and colorectal cancer growth is significantly decreased by depletion of USP22 [40–42]. In addition, recent studies have demonstrated that USP22 is also increased in several cancer tissues such as breast cancer and oral squamous cell carcinoma [43, 44].

The function of USP44 was found in the duration of the mitotic spindle checkpoint. Anaphase-promoting complex (APC) as an E3 ubiquitin ligase is activated by Cdc20 to promote the progression of anaphase, and these two proteins' interaction regulates sister chromatin separation [45]. Several studies have indicated that the overexpression of Cdc20 and dysfunction of APC lead to genomic instability in various cancers [46]. USP44 does not affect the spindle checkpoint, but it exhibits deubiquitinating activity for Cdc20 regulation [46]. A further study has supported this result, in which non-transformed murine embryonic fibroblasts showed aneuploidy with the overexpression of USP44 [47]. In addition, the level of USP44 was increased in T-cell leukemia [47]. However, a recent study showed that USP44 expression is decreased in lung cancer [48].

Several studies have also shown the involvement of oncogenic functions of DUBs in various tumors. For instance, USP33 contributes to Slit-mediated breast cancer cell migration [49]. Tumor biopsy results have indicated that USP17 was

overexpressed in the lung, colon, esophagus, and cervix, and USP36 was overexpressed in ovarian cancer [50, 51].

15.3.2 DUBs Involved in Tumor Suppression

The tumor-suppressive functions of DUBs are mainly derived from their association with p53. Since p53, as a transcription factor, is a final gatekeeper between DNA damage repair and cell death in the case of untouchable DNA damage, stabilization and activation of p53 are essential requirements in tumor suppression. Therefore, the failure in defending p53 can be linked to cell survival signaling, and partially, to cancer development. Several DUBs are identified as p53 regulating and stabilizing DUBs including USP10, USP29, USP42, and Ub aldehyde-binding protein (Otub1, Otubain 1) [52]. In normal conditions (unstressed conditions), p53 is located in the cytosol and regulated by Mdm2 E3 ligase for its proteasomal degradation and nuclear export. However, under stress conditions, p53 is stabilized and translocated to the nucleus. USP10 is involved in the stress response of p53. USP10, upon ATM-dependent phosphorylation at threonine 42 and serine 337 residues, is stabilized and translocated to the nucleus to activate p53 through deubiquitinating activity, inducing tumor cell suppression [53]. USP29 is expressed by JTV1 and FBP transcriptional factors. Because these factors are activated by stressed condition or physiological signaling, USP29 can also be mediated through external stress signals. USP29, in turn, protects and upregulates p53 by directly deubiquitinating it [54]. USP42 has also been found to have deubiquitinating activity for p53. During the early phase of response to a stress signal, USP42 preferentially makes up a complex with and deubiquitinates p53, leading to the rapid activation of p53 for cell cycle arrest and p53-dependent transcription [55]. Otub1 has a somewhat different capacity from other DUBs toward p53. Unlike the catalytic activities of DUBs, the deubiquitinating activity of Otub1 for p53 rescue is weak. Instead, Otub1 has the ability to block the ubiquitin-conjugating activity of Mdm2. The Asp88 residue of Otub1 turns out to be essential for Mdm2 inhibition. Thus, Otub1-mediated stabilization and activation of p53 result from downregulated Mdm2 functions inducing p53-mediated apoptosis and inhibition of cell proliferation [56].

Another important pathway related to DUB-associated tumor suppression is NF-κB signaling. NF-κB is a transcription factor that induces several downstream genes for cell survival and inflammation. However, several oncogenic mutations lead to the abnormal activation of NF-κB in cases of many solid tumors as well as lymphoid malignancies [57]. In many cases, these affected factors are regulated by ubiquitination. Thus, as opposite processes, deubiquitination of NF-κB signaling factors has been considered as a vital mechanism for balancing systemic regulation and potential therapeutic targets. Many works to identify DUBs and substrates for these DUBs, involved in NF-κB signaling, have shown the relevance of several DUBs in NF-κB-associated tumor progression (described in a previous section) or suppression. A classic example of tumor-suppressive DUB is cylindromatosis

(CYLD). After the first identification of CYLD as a tumor suppressor, mutations in certain types of cancers, including familial cylindromatosis (FC) and multiple familiar trichoepithelioma (MFT), were found, and a number of studies underlining the molecular mechanisms of CYLD-mediated tumor-suppressive function have delineated the importance and pivotal roles of CYLD in the regulation of the NF-κB signaling pathway [58]. In addition, various studies using yeast two-hybrid, co-immunoprecipitation, and RNAi-based screening were performed to identify CYLD-regulated substrates of NF-κB signaling components. As a result, it was confirmed that the deubiquitinating activity of CYLD can regulate several factors of the NF-κB signaling pathway such as TRAF2, TRAF6, and NF-κB essential modulator (NEMO), resulting in negative regulation of NF-κB signaling and tumor-suppressive function [59, 60]. In contrast, deficiency in CYLD leads to increased ubiquitination of target proteins. Further studies using CYLD knockout mice have also supported critical functions of CYLD in tumor suppression, by showing enhanced susceptibility to tumor development [61, 62]. Indeed, recent studies involving clinical patients have revealed that the downregulation of CYLD is correlated with human colon and hepatocellular carcinoma and chronic lymphoid leukemia (CLL) [63, 64].

In addition to CYLD, A20 is another DUB that negatively regulates NF-κB signaling. Diverse components of the NF-κB signaling pathway are regulated by the deubiquitinating capacity of A20 [65]. RNAi and knockout model-based validation of A20-mediated deubiquitination has uncovered that receptor-interacting serine/threonine protein kinase 1 (RIPK1), RIPK2, TRAF2, TRAF6, and NEMO are substrates for A20 [66–71]. Overall, CYLD and A20 negatively regulate NF-κB pathway-mediated tumor progression by deubiquitinating and modulating upstream signal mediators.

USP46 is known to have a tumor-suppressive characteristic due to its activity for PH domain leucine-rich repeat protein phosphatase (PHLPP). PHLPP is a serine/threonine protein phosphatase and has a role in the negative regulation of AKT, a mediator of cell survival signaling. Li et al. showed that PHLPP is downregulated by UPP, and USP46 can protect PHLPP through deubiquitination and stabilization of PHLPP. Indeed, reduced expression of USP46 and PHLPP is often found in colon cancer patients. Thus, USP46 is possibly an important regulator that has antiproliferative roles via the stabilization of PHLLP and inhibition of Akt in colon cancer [72].

15.4 DUB Inhibitors for Cancer Therapy: Clinical and Preclinical Studies

In the previous section, we categorized DUBs as oncogenic or tumor suppressors depending on their major involvement in cellular functions such as cell proliferation or apoptosis. In accordance with the relevance of DUBs to cancer, it has been proposed that selective inhibition of the catalytic activity of DUBs can be efficient as anticancer therapeutics. Although there are many inhibitors of cysteine proteases,

the efficacy of these inhibitors is poor due to the difficulty of targeting these enzymes. The hardship of generating inhibitors specific for enzymes is derived from limited specificity and metabolic instability. Further, in the case of the USP family, only small numbers of inhibitors are reported. To date, however, there have been much effort in overcoming these difficulties, and several biological assays using high-throughput screening technology and fluorescence polarization assays have led to the development of small inhibitors for specific DUBs. Numerous endeavors have provided the possibility of using DUBs as therapeutic targets. It is considered as one of the leading therapeutic approaches to deal with such severe diseases including cancer [73, 74]. Here, we will describe specific DUB inhibitors, which have been generated and/or tested as effective drugs for cancer and neural disorders.

15.4.1 Targeting the 26S Proteasome

Bortezomib (Velcade®) is well known and is the most successful anticancer drug, which inhibits the 26S proteasome. After FDA approval, the use of bortezomib for the treatment of multiple myeloma and MCL patients showed remarkable therapeutic efficacy. The consequences of proteasome blockade are increased apoptosis and reduced cancer cell survival. Toward the goal of developing proteasome-targeting anticancer drugs and applying to subsequent preclinical and clinical studies,

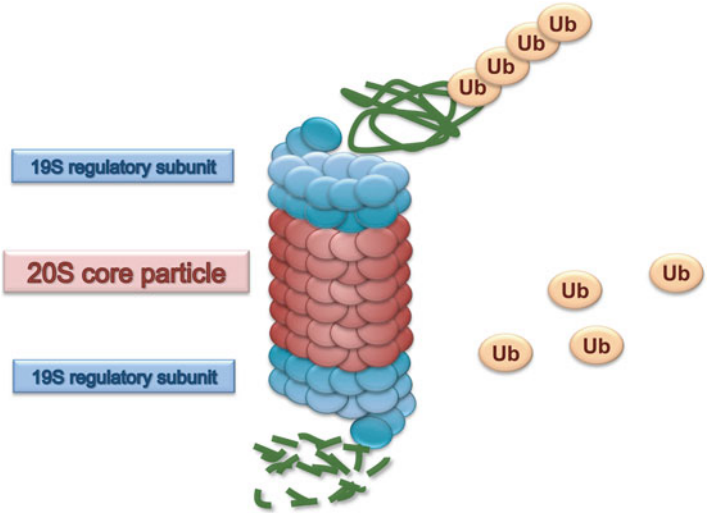

Fig. 15.3 The 26S proteasome. The 26S proteasome is a huge complex of 20,000 kDa in mass. This structure consists of two different large subunits. In the center of the 26S structure, there is a hollow with the 20S core particle, and each end of the core subunit is covered with the 19S regulatory subunits existing "cap"-like shape. The 19S regulatory subunit has ubiquitin-binding sites and ATPase active sites that allow entry of substrates into the catalytic core. The 20S core particle provides a chamber for protein degradation

numerous biochemical studies have been performed and newly developed proteasome inhibitors have been tested [75]. The 26S proteasome is composed of a 20S catalytic "core" particle forming pore inside and two 19S regulatory "cap" subunits located at each end of the core particle (Fig. 15.3). The 19S subunit recognizes ubiquitinated target proteins destined for proteasomal degradation through its ATPase active sites and ubiquitin-binding sites. For the next step, deubiquitinated targets are transferred to the catalytic core particle for degradation [76]. Besides direct blockade of proteasome function, there are also further efforts to target components of the 26S proteasome thereby preventing the transfer or degradation process. One example of this effort is b-AP15. This small molecule selectively blocks UCH-L5 and USP14 [77]. Both UCH-L5 and USP14 are DUBs, components of the 19S subunit of the 26S proteasome; they detach ubiquitin molecules from ubiquitinated proteins targeted to the 26S proteasome. Currently, b-AP15 is being used in preclinical trials; its effective inhibition of proteasomal activity gives a hope that it will be a strong proteasome inhibitory anticancer drug. IU1 is a newly developed USP14 inhibitor. Lee and his colleagues showed that IU1 specifically inhibits USP14 activity of the proteasome and thereby enhances proteasomal degradation of the substrates [78]. Another DUB associated with the 26S proteasome is POH1 (Rpn11). POH1 is localized in the "lid" region of the 19S regulatory subunit. Several studies have revealed that POH1 is pivotal for cell survival in certain cancers [76, 79, 80]. In addition, it affects drug resistance to the anticancer drugs in clinical use. There are some debates over whether b-AP15 can block not only UCH-L5 and USP14 but also inhibit POH1. Although the effect of b-AP15 on POH1 is uncertain, inhibitors targeting POH1 are also expected to be suitable anticancer drugs in certain types of cancers.

15.4.2 Specific DUB Inhibitors

One of the important proteins during cancer development is the p53 tumor suppressor; thus, p53 is often called a "guardian gene." More than 50 % of cancers are derived from p53 mutation or alteration in its function. In addition to the modulation of p53 function, the expression level of p53 also affects cellular viability and cancer progression. The p53 protein level is regulated by the ubiquitination system mediated by diverse enzymes, including E3 ligases and DUBs [52]. Mdm2 is an E3 ligase targeting p53. In its normal state, Mdm2 ubiquitinates p53 and leads to the proteasomal degradation. However, upon DNA damage, Mdm2 undergoes proteasomal degradation and, subsequently, p53 can be prevented from Mdm2-mediated degradation. The key regulating protein between p53 and Mdm2 is USP7/HAUSP [25]. USP7/HAUSP can deubiquitinate and stabilize both Mdm2 and p53 depending on cellular stress. Accordingly, targeting p53-regulating proteins including Mdm2 and USP7/HAUSP is attractive for cancer therapy.

In addition to the development of numerous inhibitors for Mdm2-p53 interaction [81], leading studies for the development of inhibitors targeting USP7/HAUSP have recently been conducted. HBX 19,818, P005091 and analogues such as P045204

and P022077, HBX 41,108, and others are found to be USP7/HAUSP inhibitors showing effective anticancer effects. HBX 19,818 has the ability to covalently bind with USP7/HAUSP, and thereby blocks USP7/HAUSP activity, leading to the possible activation of p53-mediated apoptosis in cancer cells [82]. P005091 has a great effect on reducing multiple myeloma growth and overcoming bortezomib resistance when combined with other drugs such as dexamethasone, lenalidomide, and/or suberoylanilide hydroxamic acid (SAHA). However, these treatments are currently under preclinical stage, and clinical trials are required to confirm their efficacy for cancer patients [83].

UCH-L1 is a well-known DUB due to its association with Parkinson's disease. The E3 ligase enzyme activity of UCH-L1 is linked to occurring of Parkinson's disease. UCH-L1 can be dimerized, and the UCH-L1 dimer has the ability to ligate ubiquitin molecules. In addition, UCH-L1 has shown different expression patterns in certain cancers including lung cancer. Based on a recent study on whether several lung cancers and lung cancer cell lines express more UCH-L1 than normal lung tissue, continual efforts to develop UCH-L1-specific inhibitors using high-throughput screening have been made. Isatin O-acyl oximes efficiently inhibit UCH-L1 and tumor growth in lung cancer cells. The importance of the enzymatic activity of UCH-L1 regarding association with diseases has also brought about the development of other specific inhibitors for UCH-L1. For instance, 3-amino-2-keto-7*H*-thieno[2,3-*b*]pyridin-6-one derivatives and other compounds discovered through in silico drug screening have been tested for inhibitory effects against UCH-L1 [84, 85]. UCH-L1 inhibitors showed a potential therapeutic activity for targeting neural disorders and cancers. HBX 90,397, another DUB-specific inhibitor, blocks USP8 activity. Small-molecule inhibitors targeting USP8 can prevent cell growth in several cancer cell lines including HCT116, colon cancer cells, and PC3, prostate cancer cells. USP1 is one of the well-characterized DUBs, and it plays an important role in the DNA repair processes [86]. USP1 combined with USP1-associated factor 1 (UAF1) deubiquitinates PCNA or FANCD2 during DNA repair process such as interstrand cross-link (ICL) repair, homologous recombination (HR) repair, and translesion DNA synthesis (TLS) [87]. Importantly, USP1 expression is deregulated in certain types of cancers, suggesting that USP1 may be an attractive target for cancer therapy. Indeed, treatment with pimozide, a USP1-specific inhibitor, showed synergistic effect in non-small cell lung cancer (NSCLC) when treated with the anticancer drug cisplatin [88].

15.4.3 DUB Inhibitors Targeting Multiple DUBs

The most important and unique feature of DUBs, as mentioned above, is its catalytic activity, whereby it can specifically recognize and target ubiquitinated substrates. Therefore, in many cases, DUBs share similar domains for their ability. This has brought about two advancements in developing inhibitors for DUBs. In general, it is thought to be difficult to generate inhibitors targeting the "hot spot" of a specific DUB, whereas it usefully generates inhibitors that block multiple DUBs at the same

time. Indeed, in addition to the specific DUB inhibitors described in Sect. 4.2, there are several inhibitors that could target two or more DUBs. Examples of such inhibitors will be introduced in this section.

WP1130 (degrasyn) was originally used to inhibit Janus-activated kinase2 (JAK2), thereby blocking the JAK-STAT pathway. This small molecule is also known to have an inhibitory effect toward the Bcr-Abl fusion protein, which is a major cause of several types of leukemias. However, many recent studies have demonstrated that the WP1130 treatment induces polyubiquitinated proteins, followed by the inhibition of several DUBs including USP5, USP9x, USP14, and UCH-L5. The effects of WP1130 have been further investigated, and it has been demonstrated that WP1130 can induce apoptosis by affecting anti- and proapoptotic factors, including MCL-1 and p53 [89, 90]. In addition, the effectiveness of WP1130 as a therapeutic drug is supported by the study of Bartholomeusz et al., which showed that WP1130 treatment combined with bortezomib had a synergistic effect as anticancer therapy concomitant with the inhibition of tumor cell growth, modulation of apoptosis, and prolonged survival period of animals [90].

PR619 is a well-known small molecule that inhibits a broad range of DUBs and other cysteine proteases. Activity-based chemical proteomics revealed that treatment with PR619 results in the accumulation of ubiquitinated proteins, suggesting it as an anticancer chemotherapeutic agent [91]. Chalcone-based derivatives such as AM114 and RA-1 were originally known to have an inhibitory effect on the 26S proteasome. However, further investigation of chalcone derivatives showed that AM146, RA-9, and RA-14 act as inhibitors for DUBs. These molecules induce a remarkable accumulation of polyubiquitinated proteins leading to an altered expression level of cell cycle regulating proteins, cell cycle arrest, and tumor cell death via apoptosis. In particular, they are able to block UCH-L1, UCH-L3, USP2, USP5, and USP8, which are known to have important functions in cell survival and proliferation [92]. These experimental results provide the rationale for and support the possibility of chalcone derivatives as anticancer drugs.

HBX 41,108 was originally identified as an USP7/HAUSP-specific inhibitor. Colland et al. showed that HBX 41,108 has a great effect in blocking USP7/HAUSP enzyme activity. The inactivity of USP7/HAUSP causes stabilization of p53 and an increase in p53-mediated apoptosis in cancer cells [93]. However, it was recently found that HBX 41,108 has an inhibitory effect on not only USP7/HAUSP but also other DUBs.

Cyclopentenone prostaglandins (cyPGs) are a type of prostaglandin (PG); they are biological metabolites found in animal bodies, and certain cyPGs are thought to increase apoptosis and ubiquitinated proteins. For example, PGD_2, a D series PG, can be modified to take a biologically active form, specifically as cyPGs of the J_2 series such as PGJ_2, Δ^{12}-PGJ_2, and 15-deoxy- $\Delta^{12,14}$-PGJ_2 (15d-PGJ_2). 15d-PGJ_2 has the ability to covalently modify and subsequently inhibit the hydrolase activity of UCH-L1 [94]. Treatment with Δ^{12}-PGJ_2 in cells also inhibited UCH-L1 and UCH-L3 without alteration of proteasomal activity, indicating that prostaglandins can be suitable for neural disorder therapy [95].

15.5 Therapeutic Prediction of DUB Inhibitors

Although there are only a few DUBs inhibitors, all the results discussed here raise the line of evidence for their possibility and importance as potential anticancer agents. Indeed, blocking DUBs, which are involved in abnormal regulation and cause cancer development, is one emerging anticancer therapeutic strategy. Moreover, the inhibition of DUBs is not limited to treating cancer, as shown in the case of targeting UCH-L1 and UCH-L3. There are also other classes of DUB inhibitors. Papain-like protease (PLpro) of coronavirus is a viral deubiquitinating enzyme that has a pivotal role in evading the immune system of human host cells inducing severe acute respiratory syndrome (SARS-CoV); it also has the ability to cleave viral polyprotein into functional derivatives. Thus, targeting PLpro can be considered as a primary target for antiviral drugs. Ratia et al. investigated efficient inhibitors specific for PLpro by screening around 50,000 library compounds. Among them, GRL0617 showed the most effective inhibition of PLpro and replication of the virus without cytotoxicity. More importantly, they uncovered a 3D binding structure between GRL0617 and PLpro. GRL0617 can dock with the catalytic active site of PLpro [96]. Their study suggests that GRL0617 can be developed as a promising antiviral drug with specificity that targets viral DUB but not host DUBs.

The functions and turnover of proteins are some of the most pivotal regulating mechanisms in a cellular process. These are followed by posttranslational modification by protein phosphorylation, methylation, or ubiquitination. In particular, for cellular homeostasis, proteins need to be degraded and newly synthesized. Proteins undergo two different degradation pathways through either the lysosome or the 26S proteasome. Over 80 % of cellular proteins are tagged with ubiquitin, followed by proteasomal degradation. For the well-organized UPP, several hundreds of E3 ligases help proteins to be conjugated with ubiquitin, whereas far fewer numbers of DUBs are responsible for reversely removing ubiquitin from ubiquitinated proteins. In this regard, each DUB has numerous substrates, and deregulation of a certain DUB can alter cellular processes via substrate-related functions, indicating that DUB is an important regulator in cells. Here, we have described the relevance of DUBs with cancer caused by deregulation of DUB expression, altered enzymatic activity, and complex effect on substrates' functions. In many cases, DUB inhibitors have shown anticancer effect mainly in preclinical levels. Their applications as anticancer drugs should be validated in clinical settings. Bortezomib is now used as an anticancer drug, but there are some problems associated with its use, including bortezomib resistance, severe toxicities, or a lower therapeutic effect in some individuals with solid tumors. Thus, treatments involving a combination of agents are recommended in current cancer therapy. As a result, we need more effective drugs to target not only cancer, but also other diseases. Through numerous studies and hypotheses that have been validated so far, DUBs may be one of the most ideal and attractive targets.

References

1. Puente XS, Sanchez LM, Overall CM, Lopez-Otin C (2003) Human and mouse proteases: a comparative genomic approach. Nat Rev Genet 4(7):544–558. doi:10.1038/nrg1111
2. Fraile JM, Quesada V, Rodriguez D, Freije JM, Lopez-Otin C (2012) Deubiquitinases in cancer: new functions and therapeutic options. Oncogene 31(19):2373–2388. doi:10.1038/onc.2011.443
3. Lim KH, Ramakrishna S, Baek KH (2013) Molecular mechanisms and functions of cytokine-inducible deubiquitinating enzymes. Cytokine Growth Factor Rev 24:427–431. doi:10.1016/j.cytogfr.2013.05.007
4. Lim KH, Baek KH (2013) Deubiquitinating enzymes as therapeutic targets in cancer. Curr Pharm Des 19(22):4039–4052
5. Komander D, Clague MJ, Urbe S (2009) Breaking the chains: structure and function of the deubiquitinases. Nat Rev Mol Cell Biol 10(8):550–563. doi:10.1038/nrm2731
6. Hussain S, Zhang Y, Galardy PJ (2009) DUBs and cancer: the role of deubiquitinating enzymes as oncogenes, non-oncogenes and tumor suppressors. Cell Cycle 8(11):1688–1697
7. Graner E, Tang D, Rossi S, Baron A, Migita T, Weinstein LJ, Lechpammer M, Huesken D, Zimmermann J, Signoretti S, Loda M (2004) The isopeptidase USP2a regulates the stability of fatty acid synthase in prostate cancer. Cancer Cell 5(3):253–261
8. Mahul-Mellier AL, Datler C, Pazarentzos E, Lin B, Chaisaklert W, Abuali G, Grimm S (2012) De-ubiquitinating proteases USP2a and USP2c cause apoptosis by stabilising RIP1. Biochim Biophys Acta 1823(8):1353–1365. doi:10.1016/j.bbamcr.2012.05.022
9. Allende-Vega N, Sparks A, Lane DP, Saville MK (2010) MdmX is a substrate for the deubiquitinating enzyme USP2a. Oncogene 29(3):432–441. doi:10.1038/onc.2009.330
10. Benassi B, Flavin R, Marchionni L, Zanata S, Pan Y, Chowdhury D, Marani M, Strano S, Muti P, Blandino G, Loda M (2012) MYC is activated by USP2a-mediated modulation of microRNAs in prostate cancer. Cancer Discov 2(3):236–247. doi:10.1158/2159-8290.CD-11-0219
11. Mahul-Mellier AL, Pazarentzos E, Datler C, Iwasawa R, AbuAli G, Lin B, Grimm S (2012) De-ubiquitinating protease USP2a targets RIP1 and TRAF2 to mediate cell death by TNF. Cell Death Differ 19(5):891–899. doi:10.1038/cdd.2011.185
12. Xiao N, Li H, Luo J, Wang R, Chen H, Chen J, Wang P (2012) Ubiquitin-specific protease 4 (USP4) targets TRAF2 and TRAF6 for deubiquitination and inhibits TNFalpha-induced cancer cell migration. Biochem J 441(3):979–986. doi:10.1042/BJ20111358
13. Zhang L, Zhou F, Drabsch Y, Gao R, Snaar-Jagalska BE, Mickanin C, Huang H, Sheppard KA, Porter JA, Lu CX, ten Dijke P (2012) USP4 is regulated by AKT phosphorylation and directly deubiquitylates TGF-beta type I receptor. Nat Cell Biol 14(7):717–726. doi:10.1038/ncb2522
14. Zhang X, Berger FG, Yang J, Lu X (2011) USP4 inhibits p53 through deubiquitinating and stabilizing ARF-BP1. EMBO J 30(11):2177–2189. doi:10.1038/emboj.2011.125
15. Onno M, Nakamura T, Mariage-Samson R, Hillova J, Hill M (1993) Human TRE17 oncogene is generated from a family of homologous polymorphic sequences by single-base changes. DNA Cell Biol 12(2):107–118
16. Papa FR, Hochstrasser M (1993) The yeast DOA4 gene encodes a deubiquitinating enzyme related to a product of the human tre-2 oncogene. Nature 366(6453):313–319. doi:10.1038/366313a0
17. Richardson PM, Zon LI (1995) Molecular cloning of a cDNA with a novel domain present in the tre-2 oncogene and the yeast cell cycle regulators BUB2 and cdc16. Oncogene 11(6):1139–1148
18. Oliveira AM, Perez-Atayde AR, Dal Cin P, Gebhardt MC, Chen CJ, Neff JR, Demetri GD, Rosenberg AE, Bridge JA, Fletcher JA (2005) Aneurysmal bone cyst variant translocations upregulate USP6 transcription by promoter swapping with the ZNF9, COL1A1, TRAP150, and OMD genes. Oncogene 24(21):3419–3426. doi:10.1038/sj.onc.1208506
19. Martinu L, Masuda-Robens JM, Robertson SE, Santy LC, Casanova JE, Chou MM (2004) The TBC (Tre-2/Bub2/Cdc16) domain protein TRE17 regulates plasma membrane-endosomal

trafficking through activation of Arf6. Mol Cell Biol 24(22):9752–9762. doi:10.1128/MCB.24.22.9752-9762.2004
20. Everett RD, Meredith M, Orr A, Cross A, Kathoria M, Parkinson J (1997) A novel ubiquitin-specific protease is dynamically associated with the PML nuclear domain and binds to a herpesvirus regulatory protein. EMBO J 16(7):1519–1530. doi:10.1093/emboj/16.7.1519
21. Everett RD, Freemont P, Saitoh H, Dasso M, Orr A, Kathoria M, Parkinson J (1998) The disruption of ND10 during herpes simplex virus infection correlates with the Vmw110- and proteasome-dependent loss of several PML isoforms. J Virol 72(8):6581–6591
22. Holowaty MN, Zeghouf M, Wu H, Tellam J, Athanasopoulos V, Greenblatt J, Frappier L (2003) Protein profiling with Epstein-Barr nuclear antigen-1 reveals an interaction with the herpesvirus-associated ubiquitin-specific protease HAUSP/USP7. J Biol Chem 278(32):29987–29994. doi:10.1074/jbc.M303977200
23. Sivachandran N, Sarkari F, Frappier L (2008) Epstein-Barr nuclear antigen 1 contributes to nasopharyngeal carcinoma through disruption of PML nuclear bodies. PLoS Pathog 4(10):e1000170. doi:10.1371/journal.ppat.1000170
24. Song MS, Salmena L, Carracedo A, Egia A, Lo-Coco F, Teruya-Feldstein J, Pandolfi PP (2008) The deubiquitinylation and localization of PTEN are regulated by a HAUSP-PML network. Nature 455(7214):813–817. doi:10.1038/nature07290
25. Li M, Chen D, Shiloh A, Luo J, Nikolaev AY, Qin J, Gu W (2002) Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. Nature 416(6881):648–653. doi:10.1038/nature737
26. Li M, Brooks CL, Kon N, Gu W (2004) A dynamic role of HAUSP in the p53-Mdm2 pathway. Mol Cell 13(6):879–886
27. Becker K, Marchenko ND, Palacios G, Moll UM (2008) A role of HAUSP in tumor suppression in a human colon carcinoma xenograft model. Cell Cycle 7(9):1205–1213
28. Khoronenkova SV, Dianova II, Ternette N, Kessler BM, Parsons JL, Dianov GL (2012) ATM-dependent downregulation of USP7/HAUSP by PPM1G activates p53 response to DNA damage. Mol Cell 45(6):801–813. doi:10.1016/j.molcel.2012.01.021
29. Epping MT, Meijer LA, Krijgsman O, Bos JL, Pandolfi PP, Bernards R (2011) TSPYL5 suppresses p53 levels and function by physical interaction with USP7. Nat Cell Biol 13(1):102–108. doi:10.1038/ncb2142
30. Wood SA, Pascoe WS, Ru K, Yamada T, Hirchenhain J, Kemler R, Mattick JS (1997) Cloning and expression analysis of a novel mouse gene with sequence similarity to the Drosophila fat facets gene. Mech Dev 63(1):29–38
31. Schwickart M, Huang X, Lill JR, Liu J, Ferrando R, French DM, Maecker H, O'Rourke K, Bazan F, Eastham-Anderson J, Yue P, Dornan D, Huang DC, Dixit VM (2010) Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. Nature 463(7277):103–107. doi:10.1038/nature08646
32. Perez-Mancera PA, Rust AG, van der Weyden L, Kristiansen G, Li A, Sarver AL, Silverstein KA, Grutzmann R, Aust D, Rummele P, Knosel T, Herd C, Stemple DL, Kettleborough R, Brosnan JA, Li A, Morgan R, Knight S, Yu J, Stegeman S, Collier LS, ten Hoeve JJ, de Ridder J, Klein AP, Goggins M, Hruban RH, Chang DK, Biankin AV, Grimmond SM, Wessels LF, Wood SA, Iacobuzio-Donahue CA, Pilarsky C, Largaespada DA, Adams DJ, Tuveson DA (2012) The deubiquitinase USP9X suppresses pancreatic ductal adenocarcinoma. Nature 486(7402):266–270. doi:10.1038/nature11114
33. Baker RT, Wang XW, Woollatt E, White JA, Sutherland GR (1999) Identification, functional characterization, and chromosomal localization of USP15, a novel human ubiquitin-specific protease related to the UNP oncoprotein, and a systematic nomenclature for human ubiquitin-specific proteases. Genomics 59(3):264–274. doi:10.1006/geno.1999.5879
34. Hetfeld BK, Helfrich A, Kapelari B, Scheel H, Hofmann K, Guterman A, Glickman M, Schade R, Kloetzel PM, Dubiel W (2005) The zinc finger of the CSN-associated deubiquitinating enzyme USP15 is essential to rescue the E3 ligase Rbx1. Curr Biol 15(13):1217–1221. doi:10.1016/j.cub.2005.05.059

35. Schweitzer K, Bozko PM, Dubiel W, Naumann M (2007) CSN controls NF-kappaB by deubiquitinylation of IkappaBalpha. EMBO J 26(6):1532–1541. doi:10.1038/sj.emboj.7601600
36. Peth A, Boettcher JP, Dubiel W (2007) Ubiquitin-dependent proteolysis of the microtubule end-binding protein 1, EB1, is controlled by the COP9 signalosome: possible consequences for microtubule filament stability. J Mol Biol 368(2):550–563. doi:10.1016/j.jmb.2007.02.052
37. Valk-Lingbeek ME, Bruggeman SW, van Lohuizen M (2004) Stem cells and cancer; the polycomb connection. Cell 118(4):409–418. doi:10.1016/j.cell.2004.08.005
38. Zhang XY, Varthi M, Sykes SM, Phillips C, Warzecha C, Zhu W, Wyce A, Thorne AW, Berger SL, McMahon SB (2008) The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. Mol Cell 29(1):102–111. doi:10.1016/j.molcel.2007.12.015
39. Goodliffe JM, Wieschaus E, Cole MD (2005) Polycomb mediates Myc autorepression and its transcriptional control of many loci in Drosophila. Genes Dev 19(24):2941–2946. doi:10.1101/gad.1352305
40. Liu YL, Yang YM, Xu H, Dong XS (2010) Increased expression of ubiquitin-specific protease 22 can promote cancer progression and predict therapy failure in human colorectal cancer. J Gastroenterol Hepatol 25(11):1800–1805. doi:10.1111/j.1440-1746.2010.06352.x
41. Liu Y, Yang Y, Xu H, Dong X (2010) Implication of USP22 in the regulation of BMI-1, c-Myc, p16INK4a, p14ARF, and cyclin D2 expression in primary colorectal carcinomas. Diagn Mol Pathol 19(4):194–200. doi:10.1097/PDM.0b013e3181e202f2
42. Xu H, Liu YL, Yang YM, Dong XS (2012) Knock-down of ubiquitin-specific protease 22 by micro-RNA interference inhibits colorectal cancer growth. Int J Colorectal Dis 27(1):21–30. doi:10.1007/s00384-011-1275-8
43. Zhang Y, Yao L, Zhang X, Ji H, Wang L, Sun S, Pang D (2011) Elevated expression of USP22 in correlation with poor prognosis in patients with invasive breast cancer. J Cancer Res Clin Oncol 137(8):1245–1253. doi:10.1007/s00432-011-0998-9
44. Piao S, Liu Y, Hu J, Guo F, Ma J, Sun Y, Zhang B (2012) USP22 is useful as a novel molecular marker for predicting disease progression and patient prognosis of oral squamous cell carcinoma. PLoS One 7(8):e42540. doi:10.1371/journal.pone.0042540
45. Wang Z, Wan L, Zhong J, Inuzuka H, Liu P, Sarkar FH, Wei W (2013) Cdc20: a potential novel therapeutic target for cancer treatment. Curr Pharm Des 19(18):3210–3214
46. Wasch R, Engelbert D (2005) Anaphase-promoting complex-dependent proteolysis of cell cycle regulators and genomic instability of cancer cells. Oncogene 24(1):1–10. doi:10.1038/sj.onc.1208017
47. Zhang Y, van Deursen J, Galardy PJ (2011) Overexpression of ubiquitin specific protease 44 (USP44) induces chromosomal instability and is frequently observed in human T-cell leukemia. PLoS One 6(8):e23389. doi:10.1371/journal.pone.0023389
48. Zhang Y, Foreman O, Wigle DA, Kosari F, Vasmatzis G, Salisbury JL, van Deursen J, Galardy PJ (2012) USP44 regulates centrosome positioning to prevent aneuploidy and suppress tumorigenesis. J Clin Invest 122(12):4362–4374. doi:10.1172/JCI63084
49. Yuasa-Kawada J, Kinoshita-Kawada M, Rao Y, Wu JY (2009) Deubiquitinating enzyme USP33/VDU1 is required for Slit signaling in inhibiting breast cancer cell migration. Proc Natl Acad Sci U S A 106(34):14530–14535. doi:10.1073/pnas.0801262106
50. Li J, Olson LM, Zhang Z, Li L, Bidder M, Nguyen L, Pfeifer J, Rader JS (2008) Differential display identifies overexpression of the USP36 gene, encoding a deubiquitinating enzyme, in ovarian cancer. Int J Med Sci 5(3):133–142
51. McFarlane C, Kelvin AA, de la Vega M, Govender U, Scott CJ, Burrows JF, Johnston JA (2010) The deubiquitinating enzyme USP17 is highly expressed in tumor biopsies, is cell cycle regulated, and is required for G1-S progression. Cancer Res 70(8):3329–3339. doi:10.1158/0008-5472.CAN-09-4152
52. Hock AK, Vousden KH (2014) The role of ubiquitin modification in the regulation of p53. Biochim Biophys Acta 1843:137–149. doi:10.1016/j.bbamcr.2013.05.022
53. Yuan J, Luo K, Zhang L, Cheville JC, Lou Z (2010) USP10 regulates p53 localization and stability by deubiquitinating p53. Cell 140(3):384–396. doi:10.1016/j.cell.2009.12.032

54. Liu J, Chung HJ, Vogt M, Jin Y, Malide D, He L, Dundr M, Levens D (2011) JTV1 co-activates FBP to induce USP29 transcription and stabilize p53 in response to oxidative stress. EMBO J 30(5):846–858. doi:10.1038/emboj.2011.11
55. Hock AK, Vigneron AM, Carter S, Ludwig RL, Vousden KH (2011) Regulation of p53 stability and function by the deubiquitinating enzyme USP42. EMBO J 30(24):4921–4930. doi:10.1038/emboj.2011.419
56. Sun XX, Challagundla KB, Dai MS (2012) Positive regulation of p53 stability and activity by the deubiquitinating enzyme Otubain 1. EMBO J 31(3):576–592. doi:10.1038/emboj.2011.434
57. Karin M (2009) NF-kappaB as a critical link between inflammation and cancer. Cold Spring Harb Perspect Biol 1(5):a000141. doi:10.1101/cshperspect.a000141
58. Sun SC (2010) CYLD: a tumor suppressor deubiquitinase regulating NF-kappaB activation and diverse biological processes. Cell Death Differ 17(1):25–34. doi:10.1038/cdd.2009.43
59. Kovalenko A, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Courtois G (2003) The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. Nature 424(6950):801–805. doi:10.1038/nature01802
60. Brummelkamp TR, Nijman SM, Dirac AM, Bernards R (2003) Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. Nature 424(6950):797–801. doi:10.1038/nature01811
61. Massoumi R, Chmielarska K, Hennecke K, Pfeifer A, Fassler R (2006) Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-kappaB signaling. Cell 125(4):665–677. doi:10.1016/j.cell.2006.03.041
62. Zhang J, Stirling B, Temmerman ST, Ma CA, Fuss IJ, Derry JM, Jain A (2006) Impaired regulation of NF-kappaB and increased susceptibility to colitis-associated tumorigenesis in CYLD-deficient mice. J Clin Invest 116(11):3042–3049. doi:10.1172/JCI28746
63. Wu W, Zhu H, Fu Y, Shen W, Xu J, Miao K, Hong M, Xu W, Liu P, Li J (2014) Clinical significance of down-regulated cylindromatosis gene in chronic lymphocytic leukemia. Leuk Lymphoma 55:588–594. doi:10.3109/10428194.2013.809077
64. Hellerbrand C, Bumes E, Bataille F, Dietmaier W, Massoumi R, Bosserhoff AK (2007) Reduced expression of CYLD in human colon and hepatocellular carcinomas. Carcinogenesis 28(1):21–27. doi:10.1093/carcin/bgl081
65. Hymowitz SG, Wertz IE (2010) A20: from ubiquitin editing to tumour suppression. Nat Rev Cancer 10(5):332–341. doi:10.1038/nrc2775
66. Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A (2000) Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. Science 289(5488):2350–2354
67. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, Wu P, Wiesmann C, Baker R, Boone DL, Ma A, Koonin EV, Dixit VM (2004) De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. Nature 430(7000):694–699. doi:10.1038/nature02794
68. Hitotsumatsu O, Ahmad RC, Tavares R, Wang M, Philpott D, Turer EE, Lee BL, Shiffin N, Advincula R, Malynn BA, Werts C, Ma A (2008) The ubiquitin-editing enzyme A20 restricts nucleotide-binding oligomerization domain containing 2-triggered signals. Immunity 28(3):381–390. doi:10.1016/j.immuni.2008.02.002
69. Turer EE, Tavares RM, Mortier E, Hitotsumatsu O, Advincula R, Lee B, Shifrin N, Malynn BA, Ma A (2008) Homeostatic MyD88-dependent signals cause lethal inflamMation in the absence of A20. J Exp Med 205(2):451–464. doi:10.1084/jem.20071108
70. Boone DL, Turer EE, Lee EG, Ahmad RC, Wheeler MT, Tsui C, Hurley P, Chien M, Chai S, Hitotsumatsu O, McNally E, Pickart C, Ma A (2004) The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. Nat Immunol 5(10):1052–1060. doi:10.1038/ni1110
71. Mauro C, Pacifico F, Lavorgna A, Mellone S, Iannetti A, Acquaviva R, Formisano S, Vito P, Leonardi A (2006) ABIN-1 binds to NEMO/IKKgamma and co-operates with A20 in inhibiting NF-kappaB. J Biol Chem 281(27):18482–18488. doi:10.1074/jbc.M601502200

72. Li X, Stevens PD, Yang H, Gulhati P, Wang W, Evers BM, Gao T (2013) The deubiquitination enzyme USP46 functions as a tumor suppressor by controlling PHLPP-dependent attenuation of Akt signaling in colon cancer. Oncogene 32(4):471–478. doi:10.1038/onc.2012.66
73. Eldridge AG, O'Brien T (2010) Therapeutic strategies within the ubiquitin proteasome system. Cell Death Differ 17(1):4–13. doi:10.1038/cdd.2009.82
74. Ernst A, Avvakumov G, Tong J, Fan Y, Zhao Y, Alberts P, Persaud A, Walker JR, Neculai AM, Neculai D, Vorobyov A, Garg P, Beatty L, Chan PK, Juang YC, Landry MC, Yeh C, Zeqiraj E, Karamboulas K, Allali-Hassani A, Vedadi M, Tyers M, Moffat J, Sicheri F, Pelletier L, Durocher D, Raught B, Rotin D, Yang J, Moran MF, Dhe-Paganon S, Sidhu SS (2013) A strategy for modulation of enzymes in the ubiquitin system. Science 339(6119):590–595. doi:10.1126/science.1230161
75. Mattern MR, Wu J, Nicholson B (2012) Ubiquitin-based anticancer therapy: carpet bombing with proteasome inhibitors vs surgical strikes with E1, E2, E3, or DUB inhibitors. Biochim Biophys Acta 1823(11):2014–2021. doi:10.1016/j.bbamcr.2012.05.005
76. D'Arcy P, Linder S (2012) Proteasome deubiquitinases as novel targets for cancer therapy. Int J Biochem Cell Biol 44(11):1729–1738. doi:10.1016/j.biocel.2012.07.011
77. D'Arcy P, Brnjic S, Olofsson MH, Fryknas M, Lindsten K, De Cesare M, Perego P, Sadeghi B, Hassan M, Larsson R, Linder S (2011) Inhibition of proteasome deubiquitinating activity as a new cancer therapy. Nat Med 17(12):1636–1640. doi:10.1038/nm.2536
78. Lee BH, Lee MJ, Park S, Oh DC, Elsasser S, Chen PC, Gartner C, Dimova N, Hanna J, Gygi SP, Wilson SM, King RW, Finley D (2010) Enhancement of proteasome activity by a small-molecule inhibitor of USP14. Nature 467(7312):179–184. doi:10.1038/nature09299
79. Gallery M, Blank JL, Lin Y, Gutierrez JA, Pulido JC, Rappoli D, Badola S, Rolfe M, Macbeth KJ (2007) The JAMM motif of human deubiquitinase Poh1 is essential for cell viability. Mol Cancer Ther 6(1):262–268. doi:10.1158/1535-7163.MCT-06-0542
80. Liu H, Buus R, Clague MJ, Urbe S (2009) Regulation of ErbB2 receptor status by the proteasomal DUB POH1. PLoS One 4(5):e5544. doi:10.1371/journal.pone.0005544
81. Guedat P, Colland F (2007) Patented small molecule inhibitors in the ubiquitin proteasome system. BMC Biochem 8(Suppl 1):S14. doi:10.1186/1471-2091-8-S1-S14
82. Reverdy C, Conrath S, Lopez R, Planquette C, Atmanene C, Collura V, Harpon J, Battaglia V, Vivat V, Sippl W, Colland F (2012) Discovery of specific inhibitors of human USP7/HAUSP deubiquitinating enzyme. Chem Biol 19(4):467–477. doi:10.1016/j.chembiol.2012.02.007
83. Nicholson B, Suresh Kumar KG (2011) The multifaceted roles of USP7: new therapeutic opportunities. Cell Biochem Biophys 60(1–2):61–68. doi:10.1007/s12013-011-9185-5
84. Mitsui T, Hirayama K, Aoki S, Nishikawa K, Uchida K, Matsumoto T, Kabuta T, Wada K (2010) Identification of a novel chemical potentiator and inhibitors of UCH-L1 by in silico drug screening. Neurochem Int 56(5):679–686. doi:10.1016/j.neuint.2010.01.016
85. Mermerian AH, Case A, Stein RL, Cuny GD (2007) Structure-activity relationship, kinetic mechanism, and selectivity for a new class of ubiquitin C-terminal hydrolase-L1 (UCH-L1) inhibitors. Bioorg Med Chem Lett 17(13):3729–3732. doi:10.1016/j.bmcl.2007.04.027
86. Colland F (2010) The therapeutic potential of deubiquitinating enzyme inhibitors. Biochem Soc Trans 38(Pt 1):137–143. doi:10.1042/BST0380137
87. Murai J, Yang K, Dejsuphong D, Hirota K, Takeda S, D'Andrea AD (2011) The USP1/UAF1 complex promotes double-strand break repair through homologous recombination. Mol Cell Biol 31(12):2462–2469. doi:10.1128/MCB.05058-11
88. Garcia-Santisteban I, Peters GJ, Giovannetti E, Rodriguez JA (2013) USP1 deubiquitinase: cellular functions, regulatory mechanisms and emerging potential as target in cancer therapy. Mol Cancer 12:91. doi:10.1186/1476-4598-12-91
89. Kapuria V, Peterson LF, Fang D, Bornmann WG, Talpaz M, Donato NJ (2010) Deubiquitinase inhibition by small-molecule WP1130 triggers aggresome formation and tumor cell apoptosis. Cancer Res 70(22):9265–9276. doi:10.1158/0008-5472.CAN-10-1530
90. Bartholomeusz GA, Talpaz M, Kapuria V, Kong LY, Wang S, Estrov Z, Priebe W, Wu J, Donato NJ (2007) Activation of a novel Bcr/Abl destruction pathway by WP1130 induces apoptosis of chronic myelogenous leukemia cells. Blood 109(8):3470–3478. doi:10.1182/blood-2006-02-005579

91. Altun M, Kramer HB, Willems LI, McDermott JL, Leach CA, Goldenberg SJ, Kumar KG, Konietzny R, Fischer R, Kogan E, Mackeen MM, McGouran J, Khoronenkova SV, Parsons JL, Dianov GL, Nicholson B, Kessler BM (2011) Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes. Chem Biol 18(11):1401–1412. doi:10.1016/j.chembiol.2011.08.018
92. Issaenko OA, Amerik AY (2012) Chalcone-based small-molecule inhibitors attenuate malignant phenotype via targeting deubiquitinating enzymes. Cell Cycle 11(9):1804–1817. doi:10.4161/cc.20174
93. Colland F, Formstecher E, Jacq X, Reverdy C, Planquette C, Conrath S, Trouplin V, Bianchi J, Aushev VN, Camonis J, Calabrese A, Borg-Capra C, Sippl W, Collura V, Boissy G, Rain JC, Guedat P, Delansorne R, Daviet L (2009) Small-molecule inhibitor of USP7/HAUSP ubiquitin protease stabilizes and activates p53 in cells. Mol Cancer Ther 8(8):2286–2295. doi:10.1158/1535-7163.MCT-09-0097
94. Liu H, Li W, Ahmad M, Miller TM, Rose ME, Poloyac SM, Uechi G, Balasubramani M, Hickey RW, Graham SH (2011) Modification of ubiquitin-C-terminal hydrolase-L1 by cyclopentenone prostaglandins exacerbates hypoxic injury. Neurobiol Dis 41(2):318–328. doi:10.1016/j.nbd.2010.09.020
95. Li Z, Melandri F, Berdo I, Jansen M, Hunter L, Wright S, Valbrun D, Figueiredo-Pereira ME (2004) Delta12-Prostaglandin J2 inhibits the ubiquitin hydrolase UCH-L1 and elicits ubiquitin-protein aggregation without proteasome inhibition. Biochem Biophys Res Commun 319(4):1171–1180. doi:10.1016/j.bbrc.2004.05.098
96. Ratia K, Pegan S, Takayama J, Sleeman K, Coughlin M, Baliji S, Chaudhuri R, Fu W, Prabhakar BS, Johnson ME, Baker SC, Ghosh AK, Mesecar AD (2008) A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. Proc Natl Acad Sci U S A 105(42):16119–16124. doi:10.1073/pnas.0805240105