Chapter 4

The Role of Deubiquitinases in DNA Double-Strand Break Repair

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Abstract

DNA double-strand break (DSB) is a type of the most critical DNA lesions, and if not repaired promptly, it can result in cell death or a wide variety of genetic alterations including genome instability, large- or small-scale deletions, chromosome loss, loss of heterozygosity, and translocations. DSBs are repaired by double-strand break repair (DSBR), including nonhomologous end-joining (NHEJ) and homologous recombination (HR) pathway, and defects in these pathways cause genome instability and promote tumorigenesis. Accumulating evidence has demonstrated that the superfamily of deubiquitinases (DUBs) can regulate the action and stability of DNA repair enzymes involving in DSBR via modifying ubiquitination levels, a reversible posttranslational modification pathway. In this review, we will discuss ubiquitination/deubiquitination modification involving in DSBR genes, the role of DUBs in DSBR and corresponding mechanisms, and the potential effects of this modification on human diseases.

Keywords: double-strand break repair, deubiquitinase, ubiquitination, deubiquitination, double-strand break

1. Introduction

DNA double-strand break (DSB) is a fatal alteration in the chemical structure of DNA; if it has not been repaired in time, it may destroy the stability of genome and lead to a series of human diseases. Usually, they result from a variety of causes including abnormal metabolic process, ionizing radiation, ultraviolet radiation, and active oxygen damage factors [1, 2]. In organism, DNA double-strand break repair (DSBR), a complex reaction system consisting of nonhomologous end-joining (NHEJ) and homologous recombination (HR) pathway, can

repair DSBs [3, 4]. Accumulating evidence has demonstrated that ubiquitination and deubiquitination modification play a vital role in controlling the capacity of DSBR via regulating the action and stability of DNA repair enzymes involving in DSBR pathway. In the past decades, there has been great advance in the role of deubiquitinases (DUBs) in DNA damage repair. Here, we reviewed ubiquitination/deubiquitination modification of DSBR genes, the role of DUBs in DSBR and corresponding mechanisms, and the potential effects of this modification on human diseases.

2. Ubiquitination and deubiquitination

Ubiquitin is an important single-chain polypeptide consisting of 76 amino acid residues and ubiquitously exists in almost all eukaryotic cells and tissues [5, 6]. This polypeptide is characterized by highly conserved protein from yeast to human [6] and is invariant in higher plants and differs by only three residues from animals [7]. Structurally, ubiquitin polypeptide chain appears to be a highly compact β-grasp fold with an α-helix in the cavity formed by a five-strand mixed β-sheet and a marked hydrophobic core formed between the β-sheet and the α-helix [8]. A flexible six-residue tail in the C-terminal of ubiquitin protrudes from β-grasp fold and is requested for forming the bond between ubiquitin and its substrate [9].

Ubiquitination is defined as the process that ubiquitin attaches to its target proteins via catalysis of enzymes. This process is a reversible posttranslational modification that can regulate various processes including cell proliferation, apoptosis, transcription, protein stability and translocation, and DNA damage repair [10–12]. Ubiquitination process is an ATP-dependent enzymatic cascade reaction [13, 14]. During cascades reaction, C-terminal of ubiquitin is first adenylated by ubiquitin-activating enzyme (E1) via forming a bond between the adenosine monophosphate (AMP) and the C-terminal glycine carboxyl group of ubiquitin, and subsequently, the E1 cysteine side chain directly binds to C-terminal and results in the formation of a thiol-ester linkage. Then, the activated ubiquitin is presented to the active cysteine in a ubiquitin-conjugating enzyme (E2). The E2 delivers the ubiquitin to its substrate cooperating with ubiquitin ligases (E3), which plays a role in substrate recognition. Finally, the C-terminal glycine of the ubiquitin binds to a lysine residue of the substrate with an isopeptide bond. After multiple cycles of cascade reaction, substrate will bind one or more polyubiquitin chains that are formed between the lysine side of one ubiquitin and the C-terminal carboxyl group of another ubiquitin [13, 14]. The 26S proteasome can specifically recognize these target proteins with ubiquitination modification and lead them into ubiquitin-proteasome pathway (UPP) for inducting protein degradation, a key role of ubiquitination. However, UPP is not the only role of ubiquitination. Ubiquitination can also regulate protein activity and the interaction among proteins [13, 14].

Deubiquitination is the reverse process of ubiquitination and regulated by deubiquitinases (DUBs). DUBs, also known as deubiquitinating enzymes, can cleave the bonds between substrate and polyubiquitin chains and improve the stability of substrate. They can also remove single ubiquitin molecule from polyubiquitin chains. Until now, approximately 561 DUBs

have been identified in the human genome [15], and most of them are cysteine proteases. According to the difference in their structure and function, DUBs are divided into six classes: ubiquitin-specific proteases (USPs), ubiquitin carboxy-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Machado-Joseph disease protein domain proteases (MJDs), JAMM/MPN domain-associated metallopeptidases (JAMMs), and the monocyte chemotactic protein-induced protein (MCPIP) family. These enzymes can stabilize protein and play a crucial role in the life process [13, 14].

3. DSBs and DSBR pathways

3.1. DSBs and DSB response

DSBs are vital DNA damages caused by a variety of physiological or pathological factors. V(D)J recombination has been identified as the only physiological reason inducing DSBs that result from the recombination of variable (V), diversity (D), and joining (J) gene segments. It often appears in the early development process of the vertebrate immune system. Evidence has shown that diverse immunoglobulins and T-cell receptors are generated due to this special recombination pathway. During V(D)J recombination, DNA strands are cut by RAG-1 and RAG-2 protein between the recombination signal sequences (RSS) heptamer and the flanking sequence and result in the formation of DSBs [16, 17], whereas the ends of the broken strands are subsequently processed and connected through NHEJ pathway [18].

For pathological factors, reactive oxygen species (ROSs) resulting from cellular oxidation are one main source of pathological DSBs. Studies have shown that about one percent of the oxygen that we breathe is converted into oxidative free radicals and ultimately can cause DSBs in different degrees [19]. Pathological DSBs can also arise from DNA replication across a nick that is caused by exogenous or endogenous sources. Such ionizing radiation as X-rays and gamma rays may produce free radicals and induce the formation of DSBs [20]. This type of DSBs only occurs in the S phase and is repaired through HR pathway. Additionally, one unusual cause producing DSBs is the topoisomerase II poisons that can lead to DSBs formation, apoptotic cell death, and genomic instability via stabilizing the DNA topoisomerase II cleavable complexes [21]. Another unusual cause is physical stress on the DNA duplex, which may be from the mitotic spindle on chromosomal fusions or telomere failures [22].

Studies have shown that DSBs can induce DNA damage response, and such E3 ligases as ring finger protein (RNF8) subsequently accumulate around the lesions. After that, RNF8-recruiting RNF168 promotes histone H2A Lys13,15 mono-ubiquitination (H2AK13, 15ub). Therefore, the accumulation of DNA-repair regular factors, such as receptor-association protein (RAP80) and TP53 binding protein (53BP1), is allowed [23–26]. Finally, the ataxia telangiectasia mutated (ATM) and ATM/rad3-related (ATR) kinases, a central regulator of DSB response, are activated and induce the activation of Chk1 and Chk2 kinases and TP53 protein. The activated Chk1 and Chk2 kinases arrest cell cycle to obtain sufficient time for DNA repair, while activate TP53 induces cell death [27, 28].

3.2. DSBR pathways

Merely one DSB that triggers apoptosis or destroys a critical gene is enough to lead a cell to death [29], whereas losing ability to repair DSBs can also lead to genome rearrangement and cellular transformation [30]. In organism, the two primary pathways to correct DSBs are known as HR pathway and NHEJ pathway. For NHEJ pathway, it can repair DSBs with non-homological damaged ends and is the primary DSBR pathway in mammalian cells. This pathway consists of classical-NHEJ (C-NHEJ) and alternative-NHEJ (A-NHEJ). In C-NHEJ, Ku heterodimer (Ku70 and Ku80 subunits) recognizes and binds to the ends of a DSB to prevent the free ends from degradation. Subsequently, DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is recruited and then binds to Ku heterodimer to recruit XRCC4 and DNA ligase 4 (LIG4). XRCC4 and LIG4 form a complex with XLF to ligate the broken ends [31, 32]. Until now, although the detailed mechanism of NHEJ is poorly understood, a recent study has partly revealed the mechanism about how the complex of XRCC4, LIG4, and XLF connects the fragments of broken DNA [33]. It has shown that XRCC4-XLF complex first bridges the two DNA molecules generated by DSBs, and the bridge can slide along the DNA. Then, the ends of broken DNA are rapidly reconnected. Evidence from molecular epidemiological and genetical studies displays that low or losing capacity of NEEJ pathway is positively associated with the deficiency of immune reaction [34, 35]. For example, about 15% of human severe combined immune deficiency (SCID) has been observed to feature low NHEJ capacity caused by null mutations of Artemis gene [34, 35]. Patients carrying the mutations in the DNA ligase IV gene that is crucial in NHEJ pathway presented some NBS-like features; however, cancers were not observed on these patients [36].

For HR pathway, it was first illuminated in *Escherichia coli* and *Saccharomyces cerevisiae* [37], and the similar mechanisms of the key reaction in HR pathway are observed in bacteria, yeast and human cells. An intact double-strand DNA that has highly homologous sequence of the damaged molecule is needed to act as the template to direct repair [38]. HR pathway includes three main steps: termini procession, strand invasion and branch migration, and Holliday junction formation. The ends of DSB are first processed by a nuclease, such as Mre11-Rad50-NBS1 (MRN) complex, and produce a single-stranded region with a 3′ overhang. Replication protein A (RPA) subsequently binds to the single-strand region for stabilizing and protecting this single-strand status [39, 40]. The core procedure of HR pathway is RAD51-depended strand invasion and branch migration. RAD51 displaces the RPA from single-strand DNA to form a nucleoprotein filament and then directs the later to recognize homologous duplex DNA [41]. DNA strand exchange generates a Holliday junction between the homologous damaged and undamaged DNAs under the condition of cooperating RAD51 with RAD52, RAD54, and RAD55/57 protein. Finally, the MUS81/MMS4 can resolve Holliday junction to stop the process of HR pathway [3].

Except for above-mentioned directly regulated proteins, DSB response factors (including ATM/ATR and BRCA1/BRCA2) can indirectly regulate the capacity of HR pathway [42–46]. The defects of ATM may alter kinetics of radiation-induced RAD51 formation and the hallmark of RAD51 activation [42]. ATM/ATR can also mediate the phosphorylation of PALB2 to promote the formation of RAD51 nucleofilaments [43]. However, roles of ATM and ATR in

HR pathway are still poorly understood. BRCA1 is a protein with 1863 amine acids encoded by breast cancer susceptibility gene and can target DSB lesion through its N-terminal RING domain binding to BRCA1-associated RING domain 1 (BARD1) [44]. BRCA1 can also promote HR pathway via cooperating with RAD51 and forming the complex of BRCA1-PALB2-BRCA2-RAD51 (BRCC) [44]. Surprisingly, BRCA1 can also prevent HR pathway by its incorporating into the complex of BRCA1-Abraxas-RAP80-MERIT40 (BRCA1-A). This may be because BRCA1-A can limit DNA end-resection or sequester BRCA1 away from HR sites by binding to RNF8/RNF168-ubiquitylated chromatin [45, 46]. Studies have shown that low or lost capacity of HR pathway resulting from these causes may cause a series of cancer-prone diseases, including ataxia telangiectasia (AT), Nijmegen breakage syndrome (NBS), Bloom syndrome, Werner syndrome, and Fanconi anemia, reviewed by Thompson and Schild [47].

4. Deubiquitinases regulating DSBR

4.1. USPs

USPs, the largest subfamily of DUBs with approximately 100 members and the most divers structures, belong to cysteine protease family (clan CA, family C19) and were first identified in *Saccharomyces cerevisiae* [48]. The size of USPs is ranging between 330 and 3500 amino acids, with 800 and 1000 residues. These DUBs have three functional domains: a catalytic domain, a protein-protein interaction domain, and localization domain [48]. In the catalytic domain, USPs are marked with two short and well-conserved sequences, also called as the N-terminal Cys-box and the C-terminal His-box. These sequences are essential for catalytic activity of USPs [48, 49], while other domains provide the information of binding to their target protein. Interestingly, almost all the UBP deubiquitinases display a conserved three-domain architecture, comprising Fingers, Palm, and Thumb, and their C terminus are settled in the active site between the Palm and the Thumb, except for CYLD that has an obviously truncated Fingers subdomain [50, 51]. A later study has shown that the core catalytic domain of USPs contains six conserved boxes, and that boxes 1 and 2, boxes 3 and 4, and boxes 5 and 6 formed Thumb subdomain, Fingers subdomain, and Palm subdomain, respectively [52]. USPs have been found to involve in many diseases, such as cancer, inflammation and viral diseases [53]. At least 15 of USPs, including USP1, USP3, USP4, USP6, USP7, USP10, USP11, USP15, USP20, USP26, USP29, USP37, USP42, USP44, and USP51, can regulate DSBR.

USP1 contains 785 amino acids, and its catalytic domain is one of the largest among all USPs. Although two insertions between boxes 2 and 3 and between boxes 5 and 6 have been identified to locate away from the ubiquitin binding site of USP1, it is still not clear whether these insertions can reach the active site [52]. As USP1 has been reported to overexpress in osteosarcoma and non–small cell lung cancer, inhibitors of USP1 are supposed to have anticancer potential [54, 55]. Interestingly, USP1 can be stabilized by USP1-associated factor 1 (UAF1) that can increase the catalytic activity of USP1 [56]. This indicates that USP1 need to form a complex with UAF1 to carry out its functions. A recent study has further proved that three cell clones, $USP1^{-/-}$, $UAF1^{-/-/-}$, and $USP1^{-/-}$ $UAF1^{-/-/-}$ double-knockout cells, showed

hypersensitivity to both camptothecin and poly (ADP-ribose) polymerase (PARP), suggesting that the USP1/UAF1 complex can promote HR capacity. Moreover, the USP1/UAF1 complex promoting HR capacity is at least in part associated with the suppression of NHEJ, although corresponding mechanisms still need to be further researched [57].

USP3 is a nuclear protein that presents in the chromatin fraction and is also a chromatin-associated DUB [58]. In 1999, Sloper-Mold *et al.* firstly identified and analyzed USP3 and found that a human USP3 gene probe detected two different mRNA transcripts that were expressed at low levels in all examined tissues [59]. USP3 is required for the deubiquitination of H2A and H2B to revert corresponding mono-ubiquitination. It has been displayed that USP3 can also regulate the cellular levels of ubiquitinated H2A and H2B (uH2A and uH2B), as H2A and H2B are the two major mono-ubiquitinated chromosomal protein [13, 58]. In addition, uH2A and uH2B have been revealed to associate with transcriptional regulation, where USP3 potentially plays a vital role [14]. Furthermore, the results from a study on mice with the deficiency of USP3 have shown that these mice can develop tumor spontaneously, and cells with the deficiency of USP3 fail to preserve chromosomal integrity [60]. For DSBR pathway, USP3 plays a key role in regulate DSB response. Transient USP3 silencing will cause spontaneous DNA damage, and DNA damage response will be enhanced at the same time [60]. The ubiquitination of histone H2A and γH2AX initiated by RNF168 and RNF8 in DSB response generates a cascade reaction and results in the accumulation of DSBR enzymes, whereas USP3 can oppose RNF168 and RNF8 via deubiquitination modification for the ubiquitinated H2A and γH2AX. Moreover, ectopic expression of USP3 can also block the accumulation of downstream repair enzymes such as BRCA1 and 53BP1 [61].

Except for USP3, several other USPs (including USP6, USP51, USP29, and USP44) can also deubiquitinate H2A [26, 62, 63]. Among these USPs, USP51 acts as a DUB for histone H2B mono-ubiquitination (H2Bub1), and the depletion of USP51 will suppress DSB reaction and tumor growth [64].

USP4, also named as ubiquitous nuclear protein (UNP), was initially found to promote carcinogenesis of lung and act as an oncogene [65, 66]. The following studies showed that USP4 is overexpressed in several types of human cancers such as hepatocellular carcinoma and plays a crucial role in the progression of tumorigenesis [67, 68]. Growing evidence has exhibited that USP4 affecting tumorigenesis may be correlated with abnormal DSBR capacity [67]. During DSBR pathway, USP4 may display its regulation functions on DSBR in several different processes, including DSB response and HR capacity. It has been identified to act as an important TP53 regulator that can decrease TP53 by deubiquitinating and stabilizing ARF-BP1, a ubiquitin ligase for p53 degradation [67]. During HR pathway, USP4 is required for CtIP recruitment to DNA damage site. It also regulates the resection of DNA DSBs via interacting with CtIP and the MRE11-RAD50-NBS1 (MRN) complex. The depletion of USP4 may abolish DNA end resection [69]. In addition, USP4 is ubiquitinated on multiple sites, and auto-deubiquitination of USP4 can promote CtIP recruitment and affect HR capacity [70].

USP11 and USP15 are two paralogs of USP4, and all of them share a common functional domain consisting of two ubiquitin-like (UBL) and a motif with ubiquitin-specific protease (DUSP) activity [71, 72]. USP11 is identified as a component of HR pathway, but the molecular

mechanism is not clear [73], while USP15 is a DUB for murine double minute-2 (Mdm2), one of the E3 ligases that play a major role in regulating TP53 [74]. Thus, cell apoptosis induced by TP53 in DSB response may be inhibited by USP15 via deubiquitinating and stabilizing Mdm2. Except for USP15, USP26 can also deubiquitinate Mdm2 and play the same role as USP15 regulating TP53 [75]. Furthermore, USP26 and USP37 have been shown to inhibit the formation of BRCA1-A and promote the formation of BRCC. This function may involve in HR pathway [76]. However, further studies are needed to elucidate how USP26 and USP37 regulate HR pathway.

USP7, also called herpesvirus-associated ubiquitin-specific protease (HAUSP), is identified to act as a factor that promotes viral lytic growth, because it is associated with a herpesvirus protein ICP0 that is crucial for the viral lytic cycle [77, 78]. Substrates of USP7 are widespread, and a large part of them are tumor suppressors or oncogenes, such as TP53, PTEN, Chk1, Mdm2, and FOXO [79]. USP7 can regulate these tumor suppressors and play a key role in DSB response [80–82]. For example, USP7 directly deubiquitinates Chk1 *in vivo* and *in vitro* [83]; however, its family brother USP20 can only indirectly enhance the activity of ATR-Chk1 signaling by deubiquitinating Claspin [80]. Interestingly, deubiquitination of TP53 by USP7 prevents TP53 from degradation, whereas deubiquitination of Mdm2 by USP7 increases ubiquitination of TP53 and promotes the degradation of TP53 [81, 82]. This implies that the regulation of TP53 by USP7 is very complicated. Although USP7 displays its deubiquitination potential for both TP53 and Mdm2 that are substrates each other, this regulation potential is affected by different modificative status [81, 82]. Studies have shown that TP53 and Mdm2 bind to the same domain of USP7, but the binding capacity of Mdm2 is stronger except for phosphorylated status of Mdm2 induced by DNA damages [81, 82]. Additionally, USP10, USP29, and USP42 can deubiquitinate TP53, as well as USP7 [84–86]. However, they do not have the ability of deubiquitinating Mdm2. Thus, different USPs may exhibit different regulative potential for DSBR pathway via affecting different DNA repair factors such as Chk1, TP53, Mdm2, and so on [80–82] (**Table 1**).

4.2. OTUs

OTUs are divided into three subclasses: Otubians, A20-like OTUs, and other OTUs [91]. Otubians consist of OTUB 1 and OTUB 2 that are the first two proteins identified to display the DUB activity *in vitro* [92]. Structurally, OTUs are partly similar to USPs, exception for the incomplete catalytic triad [93, 94]. OTUs functionally involve in the regulation of diverse progresses, such as virus-triggered interferon induction, T cell anergy, and deubiquitination of p53 [87, 95, 96]. Interestingly, OTUB1 is a Lys48-specific DUB that can cleave ubiquitin from branched-polyubiquitin chains but not from ubiquitinated substrates. This DUB can bind to UBC13 (a cognate E2 enzyme for RNF168) and enhance DSB response potential via suppressing RNF168-dependent polyubiquitination but not via its catalytic ability [88]. OTUB1 also has the potential for directly deubiquitinating and stabilizing TP53 protein, which results in the decrease of cell death because of the increasing TP53 function [87]. Moreover, p53 is also the substrate of another OTU, OUTD5 [89]. OUTD5 has been shown to cleave the polyubiquitin chain from an essential type I interferon adaptor protein TRAF3 to interrupt the type I interferon signaling cascade [97]. As a DUB for p53, it can form a direct complex with p53 and is

DUB	Substrates	Process	Reference
USP1	Unclear	Promote HR and partly suppress NHEJ	[57]
USP3	H2A, γH2AX	Suppress DNA DSB response	[61]
USP4	ARF-BP1, USP4	Suppress p53-dependent apoptosis in DSB response	[67, 70]
USP6	H2A	Suppress DNA DSB response	[26]
USP7	Chk1, p53, Mdm2	Promote p53-dependent apoptosis in DSB response	[81–83]
USP10	p53	Promote p53-dependent apoptosis in DSB response	[84]
USP11	unclear	Promote HR	[73]
USP15	Mdm2	Suppress p53-dependent apoptosis in DSB response	[74]
USP20	Claspin	Promote DNA DSB response	[80]
USP26	Mdm2	Suppress p53-dependent apoptosis in DSB response and promote HR	[75, 76]
USP29	H2A, p53	Suppress DNA DSB response and promote p53-dependent apoptosis in DSB response	[63, 85]
USP37	Unclear	Promote HR	[76]
USP42	p53	Promote p53-dependent apoptosis in DSB response or promote DSB response	[86]
USP44	H2A	Suppress DNA DSB response	[63]
USP51	H2A, H2B	Suppress DNA DSB response	[62, 64]
OTUB1	p53	Promote p53-dependent apoptosis in DSB response not via its catalytic ability	[87, 88]
OTUD5	p53	Promote p53-dependent apoptosis in DSB response	[89]
POH1	K63	Promote HR but not via deubiquitinating K63	[90]

Table 1. DUBs regulate DNA DSBR.

required for the p53-dependent apoptosis in response to DSB. Recently, increasing evidence has exhibited that the dysregulation of this DUB may involve in the development of several types of cancer, such as lung, colorectal, and colon cancer [98–100]. Taken together, the regulation of OTUs may result in the defects of DSBR and ultimately promote damaged-cell carcinogenesis.

4.3. JAMM/MPN domain-associated metallopeptidases (JAMMs)

JAMMs, the important members of metalloproteinase (MMP), contain JAMM/MPN domain-associated metallopeptidases sequences. These sequences include three conserved residues (two His and one Asp) that make up of catalytic center with two zinc ions [101]. The 26S proteasome-associated PAD1 homolog 1 (POH1) is a representative member of JAMMs and plays a key role in DSBR pathway. POH1 has been shown to be required for HR, which was supposed to associate with its ability to restrict 53BP1 through cleaving ubiquitin from the polyubiquitin chains of K63 protein. However, the result from another study showed

that POH1-regulating HR process was independent of 53BP1 [90]. Thus, further studies are needed to elucidate detailed regulative mechanisms.

5. Summary and future directions

DSBR is a crucial DNA repair pathway and requests a series of DNA repair enzymes, whose activation is usually controlled via the post-translational modification regulation. In the regulation of DSBR capacity, DUBs play a vital role via deubiquitinating key proteins involving in DSBR pathway and/or enhance DSB response. However, there are several issues to be noted. First, although DUBs are a large posttranslational modification factor, only small part of them have functionally been identified. Second, despite DUBs that regulate DSBR capacity via increasing the stability and activation of DSBR enzymes, the detailed mechanisms are still unclear. Finally, some other signal pathways may affect DSBR, and it is not clear whether DUBs regulate these signal pathways. Thus, further studies are needed to solve more detailed molecular mechanisms of DUBs regulating DSBR.

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Abbreviations

AMP	adenosine monophosphate
DSB	DNA double-strand break
DSBR	double-strand break repair
DUB	deubiquitinase
HR	homologous recombination
MCPIP	the monocyte chemotactic protein-induced protein

MJD	Machado-Joseph disease protein domain protease
NHEJ	non-homologous end-joining
OUT	ovarian tumor protease
ROS	reactive oxygen species
RSS	recombination signal sequence
UCH	ubiquitin carboxy-terminal hydrolase
UPP	ubiquitin-proteasome pathway
USP	ubiquitin-specific protease

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