Chemistry-Based Functional Proteomics to Identify Novel Deubiquitylating Enzymes Involved in Viral Infection

Yunlong Lei[1,§], Ke Xie[2,§], Kai Huang[1], Hong Wu[3] and Canhua Huang[*,1]

[1]*The State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, P.R. China*

[2]*Department of Oncology, Sichuan Provincial People's Hospital, Chengdu 610072, P.R. China*

[3]*Department of Hepatobiliary Pancreatic Surgery, West China Hospital, Sichuan University, Chengdu 610041, P.R. China*

Abstract: Ubiquitylation is a reversible post-translational modification pathway that regulates a variety of cellular processes including protein degradation and trafficking, intracellular localization, DNA repair, immune response and cell-cycle progression. Deubiquitylating enzymes (DUBs) can remove the ubiquitin from the modified proteins and reverse the ubiquitylation-induced biological processes; hence it isn't hard to understand that viral pathogens take advantage of the host cell ubiquitin system through disturbing DUBs, for infection and replication. Although accumulated virus-related DUBs have been defined, but how viruses regulate their expression and activities is poor understand because of limitation of technologies. Recently, chemistry-based functional proteomics, which can not only monitor the alteration of abundance but also changes in activity of enzymes, was used to study the function of DUBs involved in virus infection and held much promise. Theses works suggest that chemistry-based functional proteomics is a potent strategy for high throughput screening of virus-related DUBs and exploring their roles in virus infection.

Keywords: Activity-based protein profiling, chemistry-based functional proteomics, deubiquitylating enzymes, HAUb-derived probes, virus infection.

INTRODUCTION

Ubiquitin (Ub) is 76-amino-acid polypeptide (about 8.3 kDa) that becomes covalently attached to other proteins through an isopeptide between its carboxy-terminal glycine and the lysine of the target proteins [1-8]. It is a highly conserved protein with only three amino-acid different from yeast to human [1, 2]. The types of ubiquitin modification on the target protein are diverse, leading to different function of ubiquitylation [1-8]. First, ubiquitylation can happen on single lysine residue (monoubiquitylation) or on several lysine residues (multiple monoubiquitylation) in a substrate, these modifications are involved in endocytosis, endosomal sorting, sub cellular localization, DNA repair and virus budding [2-5]. Further more, ubiquitin contains seven Lys residues itself (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63) and can function as an acceptor to form polyubiquitin chain, most of identified which forms through Lys48 (K48) or Lys63 (K63) [2]. Polyubiquitylation *via* Lys48 is implicated in proteasome-mediated degradation independent lysosome, and this discovery was awarded the Nobel Prize in Chemistry for 2004 [2, 6]. Chains linked *via* Lys63 (K63) could participate in DNA repair, activate protein kinases or provide a scaffold for the nucleation [1-3, 6, 7] (Fig. **1**).

Ubiquitylation is a reversible process. Free ubiquitin is first activated by E1 ubiquitin-activating enzyme in an ATP-dependent manner, resulting in the formation of a high energy thiol-ester linkage between the glycine residue of ubiquitin and the cysteine residue of E1. The activated ubiquitin is then transferred to the active site Cys of the ubiquitin conjugating enzyme, E2. Finally, E2 catalyzes the transfer of ubiquitin onto the protein substrate in the presence of an ubiquitin ligase (E3), which specifically binds to a protein substrate and recruits ubiquitin–E2 complex [4-8] (Fig. **1**).

The ubiquitin and polyubiquitin chains can be removed from the substrates by deubiquitylating enzymes (DUBs) [9-16]. Deubiquitylation process can not only reverse the function of ubiquitinated substrate proteins, but is also essential for the maintenance of a sufficient pool of free ubiquitin molecules in cell [9-12]. Besides, ubiquitin may be encoded as inactive precursors because of forming fusion protein either to itself or to a ribosomal subunit, and some DUBs can cleave them into the monomeric protein [9, 11]. Thus, DUBs are important in ubiquitylation pathway, and the deregulation of them has been linked with many diseases such as cancer, immunological disorders and viral infections [9-16].

All viral pathogens have developed a series of means to exploit the host cell biochemical pathways for promoting their infection, proliferation and survival. Ubiquitylation is a ubiquitous mechanism for post-translational modification of proteins. Over a thousand proteins have been linked to this pathway, which have been associated with most of cell biochemical pathways such as protein degradation, cell-cycle progression, signaling and immune response [1-8]. The ability of viruses to utilize the ubiquitin pathway has been appreciated at many levels [5, 17, 18]. DUBs play an essential role in ubiquitylation pathway, thus it is not

*Address correspondence to this author at The State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, P.R. China; Tel: + 86-13258370346; Fax: + 86-28-85164060;
E-mail: hcanhua@hotmail.com

§These authors contributed equally to this work.

1875-5402/12 $58.00+.00

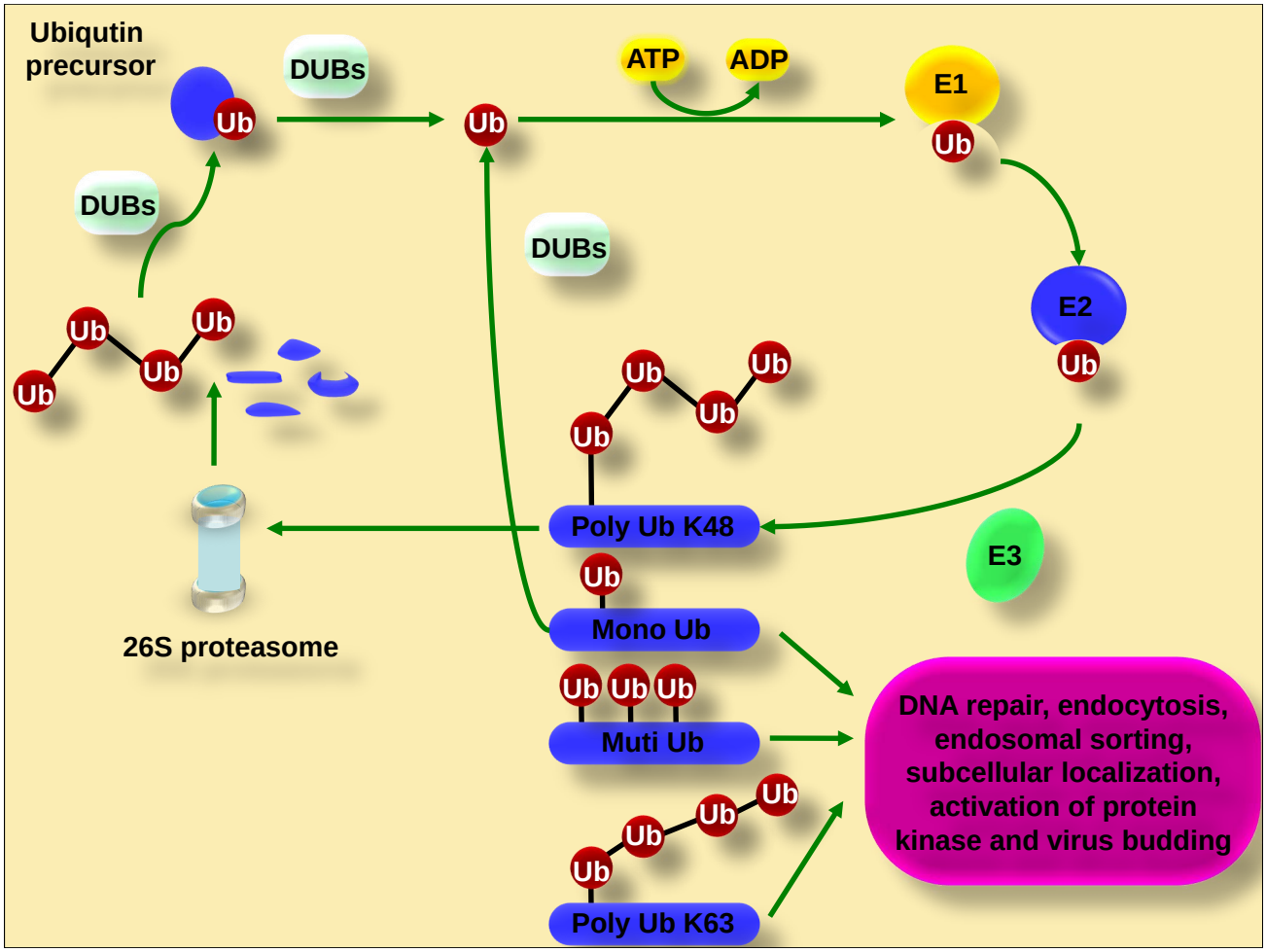

Fig. (1). Ubiquitylation is a reversible process. Free ubiquitin is activated by E1 ubiquitin- activating enzyme in an ATP-dependent manner, resulting in formation a high energy thiol-ester linkage between the glycine residue of ubiquitin and the cysteine residue of E1. The activated ubiquitin is then transferred to the active site Cys of the ubiquitin conjugating enzyme, E2. Finally, E2 catalyses the transfer of ubiquitin onto the protein substrate in the presence of an ubiquitin ligase (E3), which specifically binds a protein substrate and recruits ubiquitin–E2 complex. The function of ubiqutiylation can roughly be classified two aspects: proteasome-mediated protein degradation and other functions. Proteasome-mediated protein degradation can only be induced by polyubiquitylation *via* Lys48 (K48). Other types of ubiquitylation, such as monoubiquitylation, multiple monoubiquitylation and polyubiquitylation *via* Lys63 (K63), have been implicated in diverse biochemical pathways, including endocytosis, endosomal sorting, sub cellular localization, DNA repair and virus budding. Deubiquitylating enzymes (DUBs) can either reverse the function of ubiquitinated substrate proteins, and is also essential for maintaining a sufficient pool of free ubiquitin molecules in the cell.

surprising that viruses interfere with DUBs pool of host cells to benefit of their propagation [17-25]. Viruses disturb the deubiquitylation system of host cells in two ways: first, viruses can hijack the host's DUBs machinery, for example, herpes simplex virus infected cell protein 0 (ICP0) can interact with USP7 to inactivate p53 [26]; second, many viruses such as coronaviruses and adenoviruses, can encode their own deubiquitylating enzymes to manipulate the host's ubiquitylation system [17-25].

Increasing evidences suggest involvement of deregulation of DUBs in viral pathogenesis, but there is only a limited body of information on the overall virus-related DUBs and DUBs-related activities that relate to host-virus interactions [17-25]. Because some DUBs are often synthesized as inactive form that requires further activation while some proteins have no deubiquitylating activity which are similar to identified DUBs in structure and sequence [9-16], traditional global genomic and proteomic methods are limited in identifying virus-related DUBs, thus more efficacious technologies for global analysis of DUBs function are needed. Activity-based protein profiling which can directly monitor the state of enzymes has been successfully used to identify DUBs and explore their role in

pathological process [13, 27-31]. Activity-based probes (ABPs) for screening DUBs containing an epitope-tagged Ub (HAUb) with a C-terminal thiol-reactive group, which acts as suicide substrate, can be used to analyze complex examples such as cell lysates, intact cells and so on. The probes covalently attach the active enzymes, retrieved by immunoprecipitation, followed by their isolation and identification by tandem mass spectrometry (MS/MS) [27, 28]. Using this approach, some new deubiquitylating enzymes have been identified and the activity of many DUBs, such as USP7, is characterized [27]. In this review, we will focus on the interaction between DUBs and viral infections and the identification of virus-related DUBs through activity-based protein profiling.

THE DUBS FAMILY

Corresponding with their divers and special functions, the member of DUBs families is huge. Nearly 100 putative human DUBs are found through bioinformatics analysis and activity-based profiling [9-16]. Based on similarity of sequence and structure and likely mechanisms of action, DUBs can be divided into five classic subclasses. Four of the subclasses belong to papain-like cysteine proteases: the ubiquitin C-terminal hydrolase (UCH), the ubiquitin-specific protease (USP/UBP), the ovarian tumor domain (OTU) and the Modified Josephin domain (MJD) DUBs; while the fifth subclass is the JAB1/PAB1/MPN-domain containing zinc-dependent metallo-enzymes (JAMMs) [9-16]. In addition, some large tegument protein-like and adenain-like proteases encoded by some bacteria and viruses are recently found capable of deubiquitylation, with no sequence homology to any known DUBs [10, 32].

Despite the sequential and structural similarity in the cysteine protease DUBs families is low, they share a conserved catalytic core domain which contain a classical papain active-site structure consisting of the catalytic triad of cysteine, histidine and aspartate (asparagine or rarely serine in sometimes) residue. The cysteine is the active site which performs a nucleophilic attack to the carbonyl of substrates; the histidine plays a role in the process of deprotonation of cysteine, and the aspartate residue can polarize the histidine [15, 16]. In contrast to cysteine proteases, the catalytic core of metalloproteases family consists of a Zn^{2+} atom, which can noncovalently attach to the substrates, an aspartate and two histidine residues serving as stabilizers for the Zn^{2+} atom [15, 16] (Fig. **2A**).

Apart for the catalytic domains, DUBs have additional ubiquitin-binding domains or various protein-protein interaction domains that make DUBs have a certain degree of substrate specificity [9, 11, 14, 16]. In addition, some DUBs may undergo a post protein modification such as phosphorylation, ubiquitylation and sumoylation, which can affect their physiological function through modulating activity, localization or half-life [9]. In the following section we will briefly summarize the structural and functional features of each DUBs subclass.

UCHS SUBCLASS

UCHs and USP/UBP are the best characterized DUBs. UCHs are the first identified DUBs and have few members; four UCH domain-containing DUBs were found in human and only one in *Saccharomyces* [9-16]. UCHs preferentially cleave small leaving groups or substrates (about 20–30 amino acids), hence they act predominantly in maintaining a sufficient pool of free ubiquitin molecules through following ways: first, UCHs can cleave ubiquitin precursor fused to itself or one of two ribosome subunit into mature ubiquitin monomer; second, UCHs can remove the additional residue of uniquitin to activate it; third, activated free ubiquitin is susceptible to attack by small cellular nucleophiles such as glutathione and polyamines, and UCHs are essential for preventing the inappropriately conjugation [9, 15, 16].

Of course, the process of mature and stabilization of ubiquitin is performed by multiple DUBs, and this is not the only role of UCHs [9, 15, 16]. Some recent studies showed that UCHs could accommodate certain large substrates and were also actively involved in the regulation of ubiquitylation. For example, Drosophila UCH can remove the polyubiquitin from poly-ubiquitinated proteins [33]; when bound to proteasome, UCH37 releases the polyubiquitin chain for recycling ubiquitin [34]; and UCH-L3 is associated with deubiquitylation of the epithelial sodium channel [35].

USP/UBP SUBCLASS

The USP (UBP in yeast) subclass own the most members in DUBs family and there are over 50 USP family DUBs encoded by the human or mouse genome and 16 USP family DUBs in yeast [9, 13-15]. In contrast to the UCH family, USPs usually process larger leaving groups from the C-terminus of ubiquitin, forasmuch, USPs play a key role in reversing the ubiquitylation and maintaining ubiquitin recycling, and is the most diverse DUBs [9, 15, 16, 36]. USP family DUBs disassemble polyubiquitin- and ubiquitin-protein conjugates in three-step: USP firstly binds to the substrate; then the cysteine in the core domain performs a nucleophilic attack on the scissile bond of ubiquitin, which leads to the generation an acyl-enzyme intermediate; finally the intermediate is hydrolyzed to release the ubiquitin or polyubiquitin [36] (Fig. **2B**).

Despite the low sequence similarity, all USPs contain two well-conserved sequences — the cysteine and histidine boxes. The two boxes contain the active site residues of catalytic triad, and are not adjacent to each other but with about 300 to 800 amino acids insertion, which may act as a regulatory element [9, 15, 16, 36]. Besides, some USPs contain extensions on one or both sides, which may play a role in recognition of substrate, binding to adapters and scaffolds or the subcellular localization [11, 36], For instance, the N-terminal extension of UBP6 binding to the RPN11 subunit is necessary for the localization of UBP6 to the regulatory particle of the proteasome [37]; and USP7 also contains a N-terminal TRAF-like MATH domain functions in the localization of USP7 to a subset of PML bodies [36].

THE OVARIAN TUMOR (OTU) SUBCLASS

OTU is the second largest DUB family in mammalian, and over 24 OTU family DUBs encoded by the human have been identified [14]. The first OTU gene is discovered in

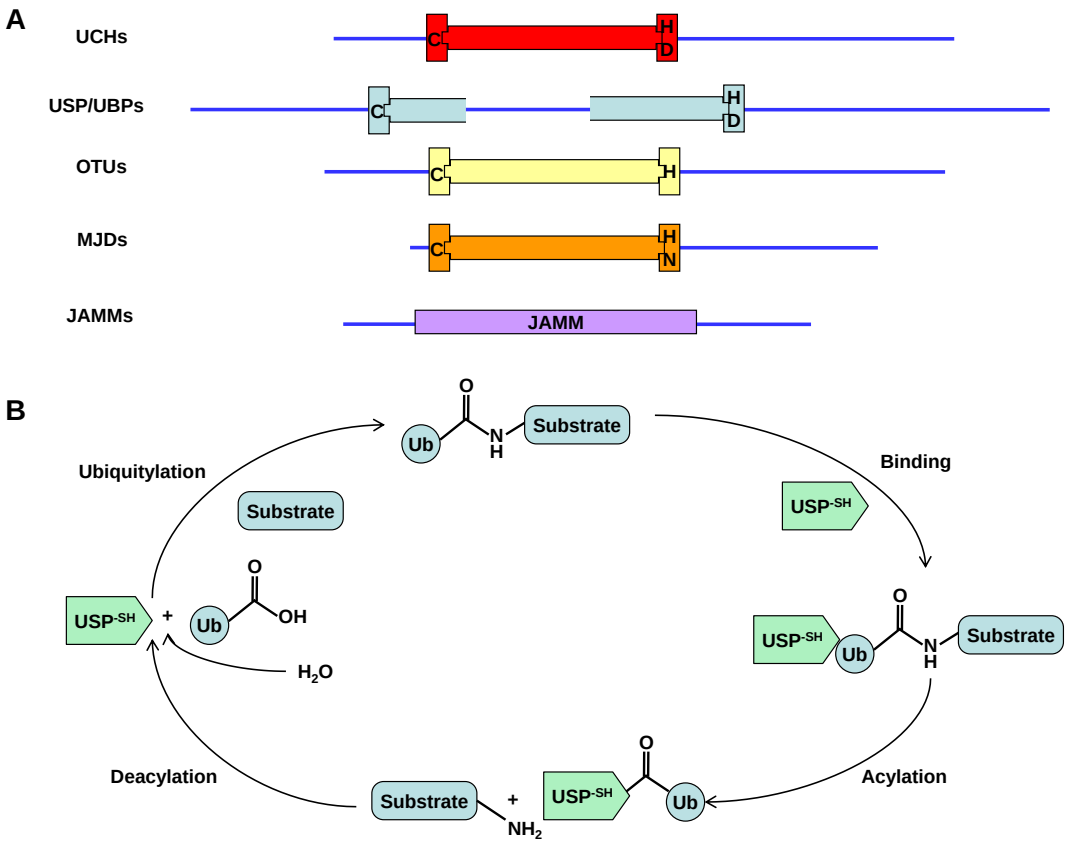

Fig. (2). (**A**) The domain structures of DUBs. DUBs can be divided into five classic subclasses. Four subclasses belong to cysteine proteases: the ubiquitin C-terminal hydrolase (UCH), the ubiquitin-specific proteaseficarin (USP/UBP), the ovarian tumor domain (OTU), and the Modified Josephin domain (MJD) DUBs; while the fifth subclass is the JAB1/PAB1/MPN-domain containing zinc-dependent metallo-enzymes (JAMMs). These cysteine proteases contain a well-conserved catalytic triad which is made up by a cysteine, a histidine and an aspartate residue. The OTU domain of OTU2 lacks of the conserved Asp in the catalytic center, while the Asp is replaced by asparagines residue in MJDs. In addition to the catalytic domains, DUBs may have N- and C-terminal extensions that make DUBs have a certain degree of substrate specificity. Further more, between the catalytic triad in USP/UBPs, there are about 300 to 800 amino acids insertion which may act as a regulatory function. C: cysteine; H: histidine; D: arginine; N: asparagines. (**B**) USP family DUBs disassemble polyubiquitin- and ubiquitin-protein conjugates in three-step: USP firstly binds to the substrate; then the cysteine in the core domain performs a nucleophilic attack on the scissile bond of ubiquitin leading to generation of an acyl-enzyme intermediate; finally the intermediate is hydrolyzed to release the ubiquitin or polyubiquitin [36].

Drosophila melanogaster, which is involved in the development of ovary *via* regulating the localization and translation of certain RNA transcripts [15, 16]. On the basis of their homology to the OTU-domain, subsequent studies have identified a series of genes encoding OTU family DUBs in virus, eukaryote and pathogenic bacteria through bioinformatics analysis [10, 15, 16]. Through resolving the structure of DUBs containing OTU domains, Nanao *et al.* found that the catalytic triad of OTU domain was incomplete and stabilized by a new method involving a hydrogen bonding network [38].

Although the physiological role and deubiquitylating activity of most OTU proteases remain unclear, the DUB activity of some OTU family DUBs has been validated [9, 10, 14-16]. A20 (also known as TNFAIP3) acts as an OTU family DUB and an ubiquitin ligase, which can negatively regulate the NF-κB by removing the K63-linked chains from the TRAF2 [tumor necrosis factor receptor (TNFR)-associated factor 2], TRAF6, and NEMO (NF-κB essential modulator), or mediate K48-linked ubiquitylation and degradation to RIP1 [9, 10, 14]. And more interestingly, the activity of A20 can be further reinforced *via* phosphorylation by IκB kinase β [39]. Cezanne, another OTU-family member, can also suppress NF-κB signaling through deubiquitylating RIP1 [40]. Also, Otubain-1 and Otubain-2 are the first manifested OTU proteins which can cleave ubiquitin from either an ubiquitin–GFP fusion protein or a tetraubiquitin fusion *in vitro* [10, 41].

JOSEPHIN DOMAIN (MJD) SUBCLASS

The fourth DUB family, the Josephin domain family, is also identified using a bioinformatics approach [15]. At least 30 proteins are characterized by a domain called the Josephin domain which may act as a deubiquitylating enzyme, but only the ataxin-3 has been confirmed with DUB activity to date [10, 15]. Ataxin-3 is implicated in the neurodegenerative disorder spinocerebellar ataxia type 3 (also known as Machado-Joseph disease) due to the expansion of the CAG repeat in it which leads to protein misfolding, aggregation and cellular toxicity [15, 16].

Although Josephin family proteins show low sequence similarity with the catalytic domain of other DUBs, the structure of the Josephin domain of ataxin-3 resembles that of the UCHs [10, 15, 16]. Besides, accompanying a mutation in the active site cysteine, Ataxin-3 is deprived the DUBs activity [42]. Ataxin-3 binds both K48- and K63-linked chains mediated by the N-terminal UIM domains, but can only cleave the K63-linked chains [9, 16].

THE JAMMs SUBCLASS

The JAMM domain has been found in bacteria, archaea and eukaryote, but those found in bacteria do not contain Ub protease activity, and archaea lack ubiquitin or ubiquitin-like conjugation system [9-16]. The JAMMs subclass of eukaryote contains at least 14 members which can be further divided into three families: POH1, CSN5, and AMSH [9-10, 15, 16]. The JAMM protease POH1 (Rpn11 in yeast) is a non-ATPase subunit of the 19S regulatory particle of the proteasome [43]. As part of proteasome, POH1 has a high degree of specificity to substrates and only cleave the ubiquitin from mistakenly ubiquitinated proteins [9-10, 15, 16]. CSN5, a subunit of the COP9 signalosome complex which mainly cleaves Nedd8 (Neural Precursor Cell Expressed, Developmentally Down-regulated 8, an ubiquitin-like protein) from the NEDDylated substrate [44], may contain Ub protease activity in some condition [9, 10, 15, 16]. AMSH (Associated molecule with the SH3 domain of STAM) has also been demonstrated to have deubiquitylating activity and is involved in endocytosis [45]. More importantly, the structure of K63-linked polyubiquitin bounding to AMSH-LP (AMSH-like protein), which modulates vesicle trafficking and specifically disassembles K63-linked polyubiquitin, has been solved and this structure represents the first time a molecular description of a DUB bound to polyubiquitin [9, 16].

DUBs IN VIRAL PATHOGENESIS

Like ubiquitin, DUBs have been implicated in various cell physiological processes such as signal transduction, immune response, gene transcription, DNA damage repair, apoptosis, cell cycle progression, kinase activation and chromosome segregation [9-15]. Deregulation of DUBs has been linked to many diseases such as cancer, neurological disorders and microbial infection [36]. For example, the promoter of the osteoblast cadherin 11 (CDH11) is fused with the full-length USP6 gene by clonal chromosomal translocations, which results in the upregulation of USP6 transcription and the development of most aneurysmal bone cysts (ABCs) [46]. Moreover, deregulation of A20, which negatively regulates the NF-κB pathway, is observed in diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma and T-cell lymphomas [47]. In neurological disorders, in addition to the mutation of ataxin-3 could induce the development of Machado-Joseph disease (which has been exhaustively described in the section of Joseph Domain (MJD) subclass), a point mutation in UCH-L1 (a UCH specifically expressed in neurons), I93M, has been found in two siblings with family history Parkinson's Disease (PD) [48], and a polymorphism in this gene has also been linked to reduce PD risk [15].

In microbial infection diseases, three major infectious pathogens (virus, bacteria and protozoa) have been reported to manipulate deubiquitylating enzymes encoded by host or derive DUBs themselves for promoting infection, proliferation and survival [19, 20]. For example, Yersinia T3S effector YopJ/P, the first identified bacterial DUB, could interfere with the NF-κB pathway through removing the ubiquitin from the ubiquitinated IκBα [21], resulting in the inhibition of the inflammatory response and the induction of apoptosis of macrophages [19, 20]. Some protozoa, including *Plasmodium falciparum*, *P. falciparum* and *toxoplasma gondii*, could also express DUBs [19, 20]. For instance, *Plasmodium falciparum* can encode two deubiquitylating enzymes, PfUCH54 and PfUCHL3, which function remain unclear [22].

Viruses can evolve a series of molecular strategies to subvert the ubiquitylation and deubiquitylation system of host cells [17-25]. Studying and researching the interactions between viruses and DUBs, not only deepens our knowledge of how various viruses manipulate DUBs to their advantage, but also makes us a further understanding of the underlying molecular mechanisms of ubiquitylation and deubiquitylation system [17, 25]. An abundance of virus-related DUBs have been characterized and the functions of some of them have also been illuminated [17-25]. The goal of viruses is obvious: first, to utilize the DUB system to overcome host cell defense mechanisms, including apoptosis and the type 1 interferon (IFN) response; second, to promote virus life cycle progression, including viral replication and maturation, and viral progeny release [18]. Next, we will summarize the role of virus-related DUBs in these aspects (Table **1**).

THE VIRUS-RELATED DUBs INTERFERENCE WITH HOST CELL DEFENSE MECHANISMS

The list of virus-related DUBs continues to grow, especially those encoded by viruses themselves. Given the parsimonious use of genetic capacity available to viruses, it suggests that DUBs should play an indispensable role in viral infection [5]. Although the function of most of virus-related DUBs remains elusive, more and more evidences reveal that DUBs are responsible for the interference with host cell defense mechanisms, including apoptosis and IFN response [14, 17-25].

Since premature apoptosis of host cell will terminate the life cycle of virus, viruses have evolved pleiotropic strategies to suppress or delay apoptosis of host cells [18]. Several virus-related DUBs such as DUB2, USP18 and USP7, have been reported to be associated with apoptosis of host cells. For example, in human T-lymphotropic virus 1 (HTLV-1)-transformed cells, constitutively expressed DUB2 induced by interleukin-2 could suppress apoptosis [49]. USP18 can negatively regulate the JAK-STAT pathway which plays an important role in IFN-triggered apoptosis [50, 51].

Besides USP18, the papain-like protease (PLpro) encoded by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and papain-like protease domain 2 (PLP2) (a catalytic domain of the nonstructural protein 3 (nsp3) of MHV-A59) encoded by mouse hepatitis virus A59

Table 1. The Role of Virus-Related DUBs in Virus Infection

DUBs	Origin	Roles	References
USP7	Host	Interact with EBNA1 of EBV to inhibit the deubiquitylation of P53 and reduce the level of intracellular P53 for promoting the survival of host cells	[5, 23, 73]
		Interact with ICP0 of HSV-1 to induce initiation of lytic infection by stimulating the reactivation of quiescent viral genomes	[5, 23, 26]
DUB-2	Host	Suppress apoptosis of human T-lymphotropic virus 1 (HTLV-1)-trans-formed cells	[49]
USP18	Host	Negatively regulate the JAK-STAT pathway, which play an important role in the apoptosis stimulated by IFN	[50,51]
USP9X UCH-L1	Host	Bind to and stabilize beta-catenin, which is the central effector of the Wnt pathway and further known to be stabilized in latency type III EBV-transformed B cells	[73-75]
SARS-CoV PLpro	Severe acute respiratory syndrome coronavirus	Block IRF3-dependent antiviral responses through deubquitylating IRF3 and preventing its nuclear translocation	[17,53]
PLP2	Mouse hepatitis virus A59	Disturb activation of the type I interferon (IFN) responses in host cells	[52]
pUL48	Human cytomegalovirus	Mutation of either the putative catalytic cysteine or histidine residue in pUL48 lead to a lower virus yields and delay the development of cytopathic effects	[57]
PLP2	Human coronavirus NL63	Process viral RNA replication complex	[58]
BPLF1	Epstein-Barr virus	Bind to the small subunit of the viral ribonucleotide reductase (RR) resulting in abolishing ubiquitylation of the large subunit (RR1) of the RR	[59]
Adenain (Avp, L323K proteinase) 3	Adenovirus	Cytoplasmic adenain can cleave cytoskeletal proteins such as cytokeratin 18 and actin, which contribute to cell lysis and release of the virions	[60, 85]
$UL36^{USP}$	Herpes simplex virus 1	May play a role in the interactions between virus particles and membrane trafficking through the trans-Golgi network	[23, 77-78]
M48	Murine cytomegalovirus	Unknown	[78,79]
ORF64	Murine gammaherpesvirus 68	Be beneficial to the virus during acute infection, particularly *in vivo*	[80]
MDV^{USP}	Marek's disease virus	Be involved in lymphoma formation and metastasis in chicken	[81]
pUL36	Pseudorabies virus	Decrease the viral neuroinvasion	[82]

(MHV-A59), could also disturb the activation of IFN responses in host cells [17, 52, 53]. The papain-like protease has been characterized by its activity of DUBs, which has been shown to block IRF3-dependent antiviral responses through deubiquitylating IRF3 to prevent its nuclear translocation [53].

THE FUNCTION OF DUBs IN THE VIRAL CYCLE LIFE

Virus can recruit the host's DUBs or encode DUBs itself to promote virus life cycle progression for their own needs, including viral replication and maturation as well as viral progeny release.

Virus can utilize the ubiquitin system for its replication on many levels. For example, the immediate–early HSV-1 gene product ICP0, which function as an E3 ligases, could regulate the replication of DNA viral genomes [54, 55]. Also, other virus-related DUBs have also been involved in viral replication. Roger *et al.* found that herpes simplex virus type 1 immediate-early protein Vmw110 could activate gene expression and stimulate virus replication, depending on its strong and specific affinity to an ubiquitin-specific protease known as herpesvirus associated ubiquitin specific protease (HAUSP/USP7) [56]. Furthermore, UL36 and pUL48 USP are important for human cytomegalovirus replication in cell or tissue culture [57]. In human coronavirus NL63, the papain-like proteases (PLPs), PLP2, which belongs to the USP family of DUBs, is indispensable for RNA synthesis and viral replication by processing viral RNA replication complex [58]. A recent study showed that Epstein-Barr virus (EBV) BPLF1, a newly discovered virally encoded deubiquitinating enzyme, could bind to the small subunit of viral ribonucleotide reductase (RR) resulting in the abolishment ubiquitylation of the large subunit (RR1) of the RR. This is the first verified protein target of the EBV deubiquitylating enzyme which plays an important role in viral maturation [59].

Viral progeny release (budding) is a critical step in the virus lifecycle, which ultimately liberates virion from host cell, and several virus-related DUBs have been evidenced to be engaged in this process. Adenain-like proteases are a novel type of DUBs, which are encoded by certain bacteria and viruses such as adenovirus. Adenain-like proteases are found both in the nucleus and the cytosol, and the cytoplasmic adenain can cleave cytoskeletal proteins such as cytokeratin 18 and actin, which contribute to cell lysis and release of the virions [60]. The herpesvirus $UL36^{USP}$ can directly bind to UL37, which may participate in the interactions between virus particles and membrane

trafficking through the trans-Golgi network [23]. In retrovirus-infected cells, proteasome inhibition could interfere with viral progeny release through preventing monoubiquitylation of $p6^{Gag}$. These results suggest that DUBs may play an important role in viral budding [18].

CHEMISTRY-BASED FUNCTIONAL PROTEOMICS

As mentioned above, most DUBs are identified by bioinformatics analysis according to the similarity of sequences and structure of core catalytic domain. But some proteins, which are similar with DUBs in structure or sequence, lack deubiquitylating activity. Moreover, deubiquitylating activity of most human DUBs is far from completely proved [9-16]. In addition, some proteins, different from the classic DUBs, have been reported to own the activity of deubiquitylation such as adenain-like proteases [10], therefore the activity and function of DUBs need be confirmed by other experimental methods. Most DUB activity is cryptic for preventing adventitious cleavage of inappropriate substrates, and they can be activated with the help of the energy of associating with the substrate or a scaffolding protein. More interestingly, the activity of DUBs is also regulated by some post-translocational modification mechanisms such as phosphorylation, ubiquitylation and sumoylation. In identifying the activity and function of DUBs [9], traditional global genomic and proteomic methods are limited, which mainly monitor the change of abundance. The activity and function of DUBs is not only depending on protein abundance, but is also necessarily correlated with the state of DUBs, thus more efficacious technologies for global analysis of DUBs function will be needed.

Activity-based protein profiling is an ideally suited technology for the global analysis of protein function, which can directly monitor functional regulation in complex proteomes [61]. In general, activity-based protein profiling can adopt two strategies in monitoring the activity of proteins. The first strategy is to identify a class of specific enzymes through designing probes based on the knowledge of catalytic mechanism. The second strategy is to explore regulatory patterns of some proteins through design of generally reactive probes [62]. The activity-based probe (ABP) is the most important tool in activity-based protein profiling. Structurally, all activity-based probes are designed in a similar construction: a reactive group links a tag by a linker. The reactive group is the most crucial component of an ABP, which can covalently attach to the active catalytic residues of the target proteins. The tag is for direct detection and purification of the covalently bound target, and the most common tags are biotin and various types of fluorophores. The main function of linker includes preventing steric hindrance, improving the probe accessibility and ensuring specificity [31,61-64]. In some cases, ABP contains a binding group, which can act as recognition site to enhance specificity for a certain enzyme class [61]. Based on an irreversible inhibition of the target protein by ABP, one or two-dimensional polyacrylamide gel electrophoresis coupled with ESI (or MALDI) MS/MS analysis could be used to isolate and identify the labeled target proteins [31, 61-64]. Using this approach, activity based probes (ABPs) have been designed to target different types of protease classes, such as threonine proteases; metalloproteases; serine hydrolases including serine proteases, lipases and PAF-acetylhydrolases; and cysteine proteases including caspases, papains and ubiquitin- and ubiquitin-like specific proteases [61-64].

Activity-based protein profiling has been successfully applied to identify DUBs and associated components, first by Borodovsky and coworkers [27]. In the process of deubiquitylation, the cysteine, which is the active site of DUBs, can perform a nucleophilic attack on the carbonyl of the scissile peptide bond between the target and ubiquitin to form a transition-state intermediate [36]. Further more, with utilization of the reversible inhibitors and substrates of USPs, Hu *et al.* found that a significant portion of the ubiquitin sequence could interact with DUBs in addition to recognizing the cleavage site [28, 65]. Based on this information, Borodovsky *et al.* designed a panel of site-directed probes that contained an epitope-tagged ubiquitin (HAUb) with a C-terminal thiol-reactive group that could act as suicide substrate. These HAUb–derived probes are also composed of three parts: the Ub moiety which confers specificity for DUBs, a thiol-reactive group that allows covalent mechanism-based trapping of the active site cysteine, and an N-terminal epitope tag that allows nonradioactive detection of modified DUBs as well as their isolation and subsequent identification [27]. For the synthesis of the HAUb–derived probes, the intein-based chemical ligation method was used to introduce N-terminal epitope tags and generate C-terminal electrophilic derivatives of Ub molecule. A fusion protein consisting of an N-terminal influenza hemagglutinin (HA)-tagged Ub (HAUb) lacking Gly76 fused with an intein domain and a chitin-binding domain (CBD) was firstly expressed in *E. coli*. Because a thioester could be formed between the Ub C-terminus and the intein which needed undergo a reversibly intramolecular trans-thioesterification (N-S acyl shift), the desired intermediate ((HAUb75-MESNa) could be isolated through incubation of fusion proteins immobilized onto the chitin column with excessive β-mercapto ethane sulfonic acid sodium salt (MESNa). Subsequently, HAUb–derived probes would be generated; when MESNa was replaced by a desired C-terminal thiol-reactive group (Fig. **3A**). According to the difference in C-terminal thiol-reactive group, seven HAUb-derived probes were synthesized, including four Michael acceptor-derived probes, vinyl methyl sulfone (HAUbVS), vinyl methyl ester (HAUb-VME), vinyl phenyl sulfone (HAUbVSPh) and vinyl cyanide (HAUbVCN); and three alkylhalide-containing inhibitors, chloroethyl (HAUbCl), bromoethyl (HAUbBr2) and bromopropyl (HAUbBr3) [27, 28] (Fig. **3B**). The HAUb–derived probes could be directly incubated with cell lysates, followed by labeling protein complexes containing modified enzymes are retrieved *via* anti-HA immunoprecipitation and separated by SDS-PAGE, then the retrieved proteins are identified by MS/MS [27-31] (Fig. **3C**).

Using this approach and an EL4 mouse thymoma cell line, Borodovsky *et al.* identified 23 active DUBs which belonged to the UCH and UBP DUBs subclasses and some polypeptides which could be modified by HAUb-derived probes with no sequence homology to any known UBP or UCH enzymes [27]. Among the 23 identified UCH and UBP DUBs: UCH37 and USP14 appear to interact with the

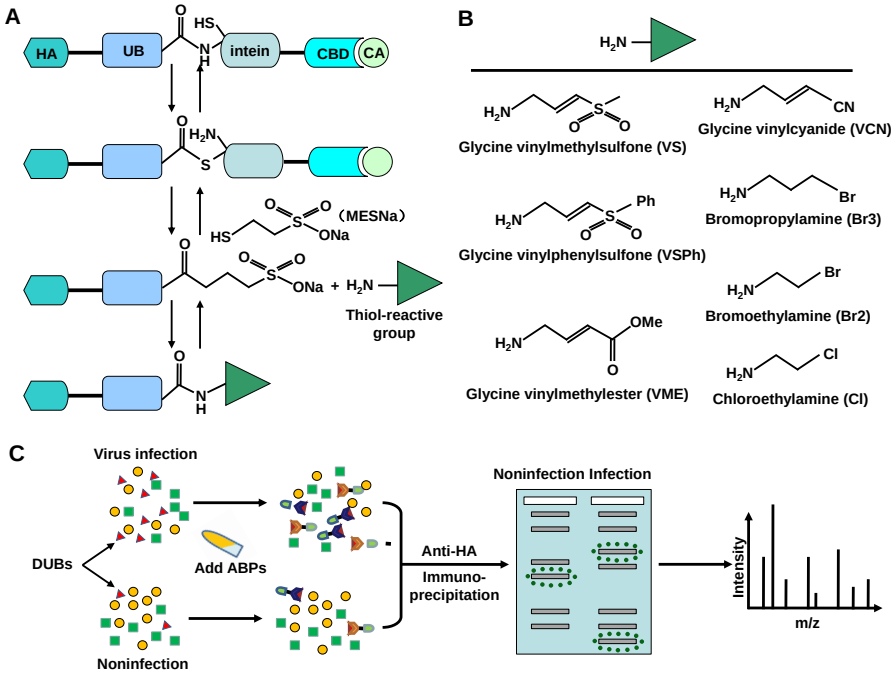

Fig. (3). (**A**) Synthesis of the HAUb–derived probes with the intein-based chemical ligation method. A fusion protein consisting of an N-terminal influenza hemagglutinin (HA)-tagged Ub (HAUb) lacking of Gly76 fused with an intein domain and a chitin-binding domain is firstly expressed in *E. coli*. Because a thioester could be formed between the Ub C-terminus and the intein which needed undergo a reversibly intramolecular trans-thioesterification (N-S acyl shift), the desired intermediate (HAUb75-MESNa) could be isolated through incubation of fusion proteins immobilized onto the chitin column with excessive β-mercapto ethane sulfonic acid sodium salt (MESNa). Subsequently, HAUb–derived probes are generated, based on that MESNa is replaced by a desired C-terminal thiol-reactive group. CBD, chitin-binding domain; CA, chitin agarose [13, 27, 28]. (**B**) The table of C-terminal thiol-reactive group [13, 27, 28]. (**C**) The HAUb–derived probes could be directly incubated with cell lysates, followed by labeling protein complexes containing modified enzymes are retrieved *via* anti-HA immunoprecipitation and separated by SDS-PAGE, subsequently the retrieved proteins are identified by MS/MS. Therefore, it can directly compare the overall level of DUBs pre- and post- virus infection.

proteasome 19S regulatory complex [66, 67]; CYLD, a tumor suppressor gene mutated in hereditary cylindromatosis, was the first demonstration of USP-like activity in this research. CYLD is recently verified to negatively regulate NF-κB pathway *via* deubiquitylating IκB kinase gamma, K63-polyubiquitinated TRAF2 and TRAF6 [14]. It is equally noteworthy that transcription of CYLD is induced by activation of the NF-κB and MKK3/6-p38 pathways [68]. Among the HAUb-derived probe modified polypeptides with no sequence homology to any known UBP or UCH enzymes, HSCP263 (otubain 1) which contained an OTU domain, was the first identified member of OTU DUBs subclass. Followed this work, a series of OTU DUBs have been identified such as A20, DUBA, otubain-2 and cezanne. Currently, OTU owns the second most members in mammalian DUB family [9-16].

An abundance of researches have demonstrated that virus can take advantage of ubiquitylation and deubiquitylation system for their own needs [17-25]. Virus can recruit the host cell's DUBs or encode DUBs itself to promote virus life cycle progression and interfere with host cell defense mechanisms. It makes us easier to understand the function and mechanism of DUBs through studying and researching the interactions between viruses and DUBs. Activity-based proteins profiling provides a more convenient method to dynamically monitor the state of cell's enzymes and HAUb-derived probes have been widely used as a smart tool to profile different kinds of USPs in virus-infected cells. In what follows we will review the recent advances in the applications of HAUb-derived probes in virus-infected cells in detail.

PROFILE THE ACTIVITIES OF DUBs OF VIRUS INFECTION CELLS *VIA* HAUB-DERIVED PROBES

HAUb-derived probes are firstly applied to study virus-related DUBs in Epstein–Barr virus (EBV)-immortalized lymphoblastoid cell lines [69]. Ovva *et al.* synthesized HAUbBr2 (bromoethylamine functionalized probe) and HAUbVME (vinylmethyl ester functionalized probe) to identify active USPs in normal, virus-infected and tumor-derived human cells. They identified 11 active USPs in Burkitt's lymphoma (BL) and EBV-transformed lymphoblastoid cell line, including USP5, 7, 9, 13 and 15, and UCH-L1 [69]. EBV, a cancer-causing virus, plays a primary role in the pathogenesis of human malignancies including BL, Hodgkin's lymphoma and nasopharyngeal carcinoma [70]. To investigate the effect of EBV on USP activity, HAUbVME probe was used to monitor the process of lymphoblastoid transformation of freshly isolated B cells by *in vitro* EBV infection [69]. The activity of six cellular DUBs, including UCH37, UCH-L3, UCH-L1, USP7, USP9X and USP15, was up-regulated in the process of lymphoblastoid transformation at different time points post infection [69]. Interestingly, USP7 has been confirmed to

interact with EBNA1 (Epstein-Barr Nuclear Antigen-1) of EBV [71]. EBNA1 is essential for the transcription and replication of the EBV genome and the segregation of EBV genomes with chromosomes during mitosis [23]. EBNA1 is responsible for viral persistence, the immortalization and transformation of host cells stimulated by EBV [23]. EBNA1, with a higher affinity to USP7 than P53, can inhibit the deubiquitylation of P53 and reduce the level of intracellular P53 for promoting the survival of host cells [72]. Additionally, USP7 can also prevent the auto-ubiquitylation induced proteasomal degradation to herpes simplex virus type 1 (HSV-1) regulatory protein ICP0, which is required for the efficient initiation of lytic infection by stimulating the reactivation of quiescent viral genomes [26]. Another two up-regulated DUBs, USP9X and UCH-L1, are able to bind to and stabilize beta-catenin, which is the central effector of the Wnt pathway and further known to be stabilized in latency type III EBV-transformed B cells [73-75].

Utilizing a similar strategy, Hemagglutinin-tagged Ub-vinyl methyl ester (HAUb-VME) probe was used to profile the activities of dividual USPs in biopsies of human papillomavirus (HPV) carrying cervical carcinoma and adjacent normal tissue [76]. Rolen *et al.* compared the activity of DUB in HPV positive and negative cervical carcinoma cell lines and HPV E6/E7 immortalized human keratinocytes to assess the contribution of HPV proteins in the regulation of DUBs [76]. A complex pattern of USP activity was observed in the tumor as well as normal tissues, including UCH-1, UCH-L3, UCH37, USP15, USP9X/Fam and USP7/HAUSP. The activity of both UCH-L3 and UCH37 was significantly increased in 76% of the tumors while UCH-L1 was decreased in 48% of the tumors examined compared to their normal counterparts [76]. Further more, the activities of UCH-L3, UCH37 and USP9X/Fam were consistently increased, while USP7/HAUSP activity was absent in the HPV18 positive biopsies [76]. In order to test whether the activity of specific USPs can be directly correlated to expression of the HPV, Rolen *et al.* immortalized the primary human keratinocytes through infection with murine retroviral expression vectors carrying the HPV 18 E6 and E7 genes under the control of the CMV immediate early promoter. The activity of all USPs was up-regulated following E6/E7 transduction, which suggested that these enzymes were important for virus-induced growth transformation [76].

IDENTIFICATION OF VIRAL DUBs BY HAUB-DERIVED PROBES

Kattenhorn *et al.* designed a HA-tagged Ub-vinylmethyl ester (HAUbVME) probe to profile DUB activity in lysates of primary fibroblasts infected with herpes simplex virus-1 (HSV-1), and identified a virus-encoded DUB, $UL36^{USP}$. $UL36^{USP}$ is the first identified DUBs expressed by virus *via* the strategy of activity-based proteins profiling [77]. $UL36^{USP}$ (about 500 amino acid residues) is the N-terminal fragment of UL36 (also called VP1/2 or ICP1/2), the large tegument protein (3164 amino acid residues) which is a constituent of the amorphous proteinaceous layer located between the nucleocapsid and the envelope of the herpesvirus particle [23, 78]. $UL36^{USP}$ can specifically disassemble lysine-48 but not lysine-63 polyubiquitin chains. The activity of $UL36^{USP}$ can be induced only when it is cleaved from full-length UL36, which often occurs late during viral replication [29, 77]. Although UL36 lacks obvious similarity to any known DUBs, the conservation of cysteine box and histidine box which exhibits in UL36 homolog from α-, β-, and γ- herpesvirus genomes, suggests that UL36 should belong to cysteine protease DUB families [23, 78].

Using activity-based proteins profiling or analogous labeling experiments, the DUB activity of $UL36^{USP}$ homologues is confirmed in other members of the Herpesviridae family, for example: pUL48 in human cytomegalovirus (HCMV), M48 in murine cytomegalovirus (MCMV), ORF64 in murine gammaherpesvirus 68 (MHV), MDV^{USP} in Marek's disease virus (MDV), pUL36 in pseudorabies virus (PrV) and BPLF1 in Epstein-Barr virus (EBV) [57, 59, 77-82]. Although the DUB activity of UL36 is implicated in virus pathogenesis, the function of UL36 remains poorly defined [23]. For example, a lower virus yields and delayed development of cytopathic effects were observed when mutation of either the putative catalytic cysteine or histidine residue in pUL48 of HCMV [57]. Besides, mutation of the MDV DUB active site cysteine can inhibit lymphoma formation and metastasis in chicken [81], while similar mutants in pUL36 can decrease the viral neuroinvasion [82]. Because the large tegument protein tightly bounded to the nucleocapsid after infection by fusion of the viral envelope with the cell membrane, some scientists speculated that UL36 may play a role in either or both of entry of the virus, as well as for viron assembly and release [5, 17, 23].

APPLICATION OF OTHER CHEMISTRY-BASED FUNCTIONAL PROTEOMICS IN THE IDENTIFICATION OF VIRUS-RELATED DUBs

Ub-aldehyde is generated after borohydrate reduction of an Ub-UCH thioester, wherefore structure of Ub-aldehyde is similar to the acyl-enzyme intermediate generated when the cysteine of the core domain of DUBs performs a nucleophilic attack on the scissile bond of ubiquitin [28]. Ub-aldehyde is a potent and specific inhibitor of DUBs, and is often used to solve the crystal structure of DUBs [36, 83, 84]. Based on these, Ub-aldehyde can act as a specific probe for DUBs. The probe has been applied to identify the DUBs in adenovirus-infected HeLa cells by Balakirev *et al.* They first found that the activity of DUBs was increased in infected HeLa cells compared with the noninfected by *in vitro* DUB assay. Subsequently, the biot-Ubal was synthesized to label and purify the deubiquitylating proteases. Using this method, Balakirev *et al.* firstly demonstrated the DUB activity of an adenain DUB—adenovirus L3 23K proteinase (Avp), which played an important role in the processing of viral precursor proteins during virion maturation [85]. Another DUB which was identified by affinity purification using biotin-conjugated ubiquitin aldehyde is otubain1 [86], which is the first DUB to be implicated in the regulation of T-cell anergy [87].

CONCLUSIONS

It is currently clear that virus can recruit the host's DUBs or encode DUBs itself to hijack the ubiquitylation system of

host cells to bypass host cell defense mechanisms and promote virus life cycle progression according to their own needs. Deregulation of DUBs also has been involved in other diseases such as cancer, neurological disorders and bacterial infection. Systematically investigating the interactions between viruses and DUBs results in our integrated understanding of the way of various viruses in manipulating DUBs to their advantage and a more clear-cut delineation of the underlying molecular mechanisms of deubiquitylation system of host cells. This information will make it easier to explore the role of DUBs in other disease's pathogenesis and design drugs for treatment. Activity-based protein profiling, which can directly monitor functional regulation in complex proteomes, is a potent tool in identification of novel DUBs and DUB-associated components. It can detect multiple DUBs in parallel, which can facilitate direct comparison of the overall level of DUBs pre- and post- virus infection. Although accumulated virus-related DUBs have been defined, the substrates and physiological roles of most of them are still elusive. More effectual and specific probes or inhibitors to single DUB are needed for exploring the precise mechanisms of virus-related DUBs.

ACKNOWLEDGEMENTS

This work was supported by grants from the National 973 Basic Research Program of China (2011CB910703, 2012CB518900), the National Science and Technology Major Project (2012ZX09501001-003) and Chinese NSFC (81072022, 81172173).

ABBREVIATIONS

Ub	=	Ubiquitin
DUBs	=	Deubiquitylating enzymes
MS/MS	=	Tandemmass spectrometry
UCH	=	The ubiquitin C-terminal hydrolase
USP	=	The ubiquitin- specific protease
IFN	=	The type 1 interferon
ABP	=	The activity-based probe
EBV	=	Epstein–Barr virus

REFERENCES

[1] Weissman, A. M. Themes and variations on ubiquitylation. *Nat. Rev. Mol. Cell Bio.*, **2001**, *2*(3), 169-178.

[2] Haglund, K.; Dikic, I. Ubiquitylation and cell signaling. *EMBO J.*, **2005**, *24*(19), 3353-3359.

[3] Huang, T. T.; D'Andrea, A. D. Regulation of DNA repair by ubiquitylation. *Nat. Rev. Mol. Cell Bio.*, **2006**, *7*(5), 323-334.

[4] Di Fiore, P. P.; Polo, S.; Hofmann, K. When ubiquitin meets ubiquitin receptors: a signalling connection. *Nat. Rev. Mol. Cell Bio.*, **2003**, *4*(6), 491-497.

[5] Isaacson, M. K.; Ploegh, H. L. Ubiquitination, ubiquitin-like modifiers, and deubiquitination in viral infection. *Cell Host Microbe*, **2009**, *5*(6), 559-570.

[6] Liu, Y. C.; Penninger, J.; Karin, M. Immunity by ubiquitylation: A reversible process of modification. *Nat. Rev. Immunol.*, **2005**, *5*(12), 941-952.

[7] Ravid, T.; Hochstrasser, M. Diversity of degradation signals in the ubiquitin-proteasome system. *Nat. Rev. Mol. Cell Bio.*, **2008**, 9(9), 679-U25.

[8] Nakayama, K. I.; Nakayama, K. Ubiquitin ligases: cell-cycle control and cancer. *Nat. Rev. Cancer*, **2006**, *6*(5), 369-381.

[9] Reyes-Turcu, F. E.; Ventii, K. H.; Wilkinson, K. D., Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu. Rev. Biochem.*, **2009**, *78*, 363-397.

[10] Amerik, A. Y.; Hochstrasser, M. Mechanism and function of deubiquitinating enzymes. *BBA-Mol. Cell Res.*, **2004**, *1695*(1-3), 189-207.

[11] Wing, S. S. Deubiquitinating enzymes - the importance of driving in reverse along the ubiquitin-proteasome pathway. *Int. J. Biochem. Cell Biol.*, **2003**, *35*(5), 590-605.

[12] Hussain, S.; Zhang, Y.; Galardy, P. J. DUBs and cancer The role of deubiquitinating enzymes as oncogenes, non-oncogenes and tumor suppressors. *Cell Cycle*, **2009**, *8*(11), 1688-1697.

[13] Love, K. R.; Catic, A.; Schlieker, C.; Ploegh, H. L. Mechanisms, biology and inhibitors of deubiquitinating enzymes. *Nat. Chem. Biol.*, **2007**, *3*(11), 697-705.

[14] Sun, S. C. Deubiquitylation and regulation of the immune response. *Nat. Rev. Immunol.*, **2008**, *8*(7), 501-511.

[15] Nijman, S. M. B.; Luna-Vargas, M. P. A.; Velds, A.; Brummelkamp, T. R.; Dirac, A. M. G.; Sixma, T. K.; Bernards, R. A genomic and functional inventory of deubiquitinating enzymes. *Cell*, **2005**, *123*(5), 773-786.

[16] Reyes-Turcu, F. E.; Wilkinson, K. D. Polyubiquitin binding and disassembly By deubiquitinating enzymes. *Chem. Rev.*, **2009**, *109*(4), 1495-1508.

[17] Edelmann, M. J.; Kessler, B. M. Ubiquitin and ubiquitin-like specific proteases targeted by infectious pathogens: Emerging patterns and molecular principles. *BBActa-Mol. Basis Dis.*, **2008**, *1782*(12), 809-816.

[18] Gao, G.; Luo, H. L. The ubiquitin-proteasome pathway in viral infections. *Can. J. Physiol. Pharmacol.*,**2006**, *84*(1), 5-14.

[19] Edelmann, M. J.; Kessler, B. M. Ubiquitin and ubiquitin-like specific proteases targeted by infectious pathogens: Emerging patterns and molecular principles. *BBActa-Mol Basis Dis.*, **2008**, *1782*(12), 809-816.

[20] Rytkonen, A.; Holden, D. W. Bacterial interference of ubiquitination and deubiquitination. *Cell Host Microbe*, **2007**, *1*(1), 13-22.

[21] Zhou, H. L.; Monack, D. M.; Kayagaki, N.; Wertz, I.; Yin, J. P.; Wolf, B.; Dixit, V. M. Yersinia virulence factor YopJ acts as a deubiquitinase to inhibit NF-kappa B activation. *J. Exp. Med.*, **2005**, *202*(10), 1327-1332.

[22] Artavanis-Tsakonas, K.; Misaghi, S.; Comeaux, C. A.; Catic, A.; Spooner, E.; Duraisingh, M. T.; Ploegh, H. L. Identification by functional proteomics of a deubiquitinating/deNeddylating enzyme in *Plasmodium falciparum*. *Mol. Microbiol.*, **2006**, *61*(5), 1187-1195.

[23] Lindner, H. A. Deubiquitination in virus infection. *Virology*, **2007**, *362*(2), 245-256.

[24] Shackelford, J.; Pagano, J. S. Tumor viruses and cell signaling pathways: Deubiquitination versus ubiquitination. *Mol. Cellular Biol.*, **2004**, *24*(12), 5089-5093.

[25] Shackelford, J.; Pagano, J. S. Role of the ubiquitin system and tumor viruses in AIDS-related cancer. *BMC Biochemistry*, **2007**, *8* (Suppl 1), S8.

[26] Boutell, C.; Canning, M.; Orr, A.; Everett, R. D. Reciprocal activities between herpes simplex virus type 1 regulatory protein ICP0, a ubiquitin E3 ligase, and ubiquitin-specific protease USP7. *J. Virol.*, **2005**, *79*(19), 12342-12354.

[27] Borodovsky, A.; Ovaa, H.; Kolli, N.; Gan-Erdene, T.; Wilkinson, K. D.; Ploegh, H. L.; Kessler, B. M. Chemistry-based functional proteomics reveals novel members of the deubiquitinating enzyme. *Chem. Biol.*, **2002**, *9*(10), 1149-1159.

[28] Hemelaar, J.; Galardy, P. J.; Borodovsky, A.; Kessler, B. A.; Ploegh, H. L.; Ovaa, H. Chemistry-based functional proteomics: Mechanism-based activity-profiling tools for ubiquitin and ubiquitin-like specific proteases. *J. Proteome Res.*, **2004**, *3*(2), 268-276.

[29] Liu, R.; Wang, K.; Yuan, K.; Wei, Y.; Huang, C. Integrative oncoproteomics strategies for anticancer drug discovery. *Expert Rev. Proteomics*, **2010**, *7*(3), 411-429.

[30] Kessler, B. M. Putting proteomics on target: activity-based profiling of ubiquitin and ubiquitin-like processing enzymes. *Expert Rev. of Proteomics*, **2006**, *3*(2), 213-221.

[31] Yuan, K. F.; Lei, Y. L.; Huang, C. H. Application of chemistry-based functional proteomics to screening for novel drug targets. *Combin. Chem. High Throughput Screen.*,**2010**, *13*(5), 414-421.

[32] Schlieker, C.; Korbel, G. A.; Kattenhorn, L. M.; Ploegh, H. L. A deubiquitinating activity is conserved in the large tegument protein of the Herpesviridae. *J. Virol.*, **2005**, *79*(24), 15582-15585.

[33] Roff, M.; Thompson, J.; Rodriguez, M. S.; Jacque, J. M.; Baleux, F.; ArenzanaSeisdedos, F.; Hay, R. T. Role of I kappa B alpha ubiquitination in signal-induced activation of NF-kappa B *in vivo*. *J. Biol. Chem.*, **1996**, *271*(13), 7844-7850.

[34] Kam, Y. A.; Xu, W.; DeMartino, G. N.; Cohen, R. E. Editing of ubiquitin conjugates by an isopeptidase in the 26S proteasome. *Nature*, **1997**, *385*(6618), 737-740.

[35] Butterworth, M. B.; Edinger, R. S.; Ovaa, H.; Burg, D.; Johnson, J. P.; Frizzell, R. A. The deubiquitinating enzyme UCH-L3 regulates the apical membrane recycling of the epithelial sodium channel. *J. Biol. Chem.*, **2007**, *282*(52), 37885-37893.

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

[37] Leggett, D. S.; Hanna, J.; Borodovsky, A.; Crosas, B.; Schmidt, M.; Baker, R. T.; Walz, T.; Ploegh, H.; Finley, D. Multiple associated proteins regulate proteasome structure and function. *Molecular Cell*, **2002**, *10*(3), 495-507.

[38] Nanao, M. H.; Tcherniuk, S. O.; Chroboczek, J.; Dideberg, O.; Dessen, A.; Balakirev, M. Y. Crystal structure of human otubain 2. *Embo Reports*, **2004**, *5*(8), 783-788.

[39] Hutti, J. E.; Turk, B. E.; Asara, J. M.; Ma, A.; Cantley, L. C.; Abbott, D. W. I kappa B kinase beta phosphorylates the K63 deubiquitinase A20 to cause feedback inhibition of the NF-kappa B pathway. *Mol. Cell. Biol.*, **2007**, *27*(21), 7451-7461.

[40] Enesa, K.; Zakkar, M.; Chaudhury, H.; Luong, L. A.; Rawlinson, L.; Mason, J. C.; Haskard, D. O.; Dean, J. L. E.; Evans, P. C. NF-kappa B suppression by the deubiquitinating enzyme Cezanne - A novel negative feedback loop in pro-inflammatory signaling. *J. Biol. Chem.*, **2008**, *283*(11), 7036-7045.

[41] Balakirev, M. Y.; Tcherniuk, S. O.; Jaquinod, M.; Chroboczek, J. Otubains: a new family of cysteine proteases in the ubiquitin pathway. *Embo Reports*, **2003**, *4*(5), 517-522.

[42] Burnett, B.; Li, F. S.; Pittman, R. N. The polyglutamine neurodegenerative protein ataxin-3 binds polyubiquitylated proteins and has ubiquitin protease activity. *Hum. Mol. Genet.*, **2003**, *12*(23), 3195-3205.

[43] Koulich, E.; Li, X. H.; DeMartino, G. N. Relative structural and functional roles of multiple deubiquitylating proteins associated with mammalian 26S proteasome. *Mol. Biol. Cell*, **2008**, *19*(3), 1072-1082.

[44] Cope, G. A.; Suh, G. S. B.; Aravind, L.; Schwarz, S. E.; Zipursky, S. L.; Koonin, E. V.; Deshaies, R. J. Role of predicted metalloprotease motif of Jab1/Csn5 in cleavage of Nedd8 from Cul1. *Science*, **2002**, *298*(5593), 608-611.

[45] McCullough, J.; Clague, M. J.; Urbe, S. AMSH is an endosome-associated ubiquitin isopeptidase. *J. Cell Biol.*, **2004**, *166*(4), 487-492.

[46] Oliveira, A. M.; Hsi, B. L.; Weremowicz, S.; Rosenberg, A. E.; Dal Cin, P.; Joseph, N.; Bridge, J. A.; Perez-Atayde, A. R.; Fletcher, J. A. USP6 (Tre2) fusion oncogenes in aneurysmal bone cyst. *Cancer Res.*, **2004**, *64*(6), 1920-1923.

[47] Durkop, H.; Hirsch, B.; Hahn, C.; Foss, H. D.; Stein, H. Differential expression and function of A20 and TRAF1 in Hodgkin lymphoma and anaplastic large cell lymphoma and their induction by CD30 stimulation. *J. Pathol.*, **2003**, 200(2), 229-239.

[48] Leroy, E.; Boyer, R.; Auburger, G.; Leube, B.; Ulm, G.; Mezey, E.; Harta, G.; Brownstein, M. J.; Jonnalagada, S.; Chernova, T.; Dehejia, A.; Lavedan, C.; Gasser, T.; Steinbach, P. J.; Wilkinson, K. D.; Polymeropoulos, M. H. The ubiquitin pathway in Parkinson's disease. *Nature*, **1998**, *395*(6701), 451-452.

[49] Migone, T. S.; Humbert, M.; Rascle, A.; Sanden, D.; D'Andrea, A.; Johnston, J. A. The deubiquitinating enzyme DUB-2 prolongs cytokine-induced signal transducers and activators of transcription activation and suppresses apoptosis following cytokine withdrawal. *Blood*, **2001**, *98*(6), 1935-1941.

[50] Ritchie, K. J.; Hahn, C. S.; Kim, K. I.; Yan, M.; Rosario, D.; Li, L.; de la Torre, J. C.; Zhang, D. E. Role of ISG15 protease UBP43 (USP18) in innate immunity to viral infection. *Nat. Med.*, **2004**, *10*(12), 1374-1378.

[51] Malakhova, O. A.; Kim, K. I.; Luo, J. K.; Zou, W. G.; Kumar, K. G. S.; Fuchs, S. Y.; Shuai, K.; Zhang, D. E. UBP43 is a novel regulator of interferon signaling independent of its ISG15 isopeptidase activity. *EMBO J.*, **2006**, *25*(11), 2358-2367.

[52] Zheng, D. H.; Chen, G.; Guo, B. C.; Cheng, G. H.; Tang, H. PLP2, a potent deubiquitinase from murine hepatitis virus, strongly inhibits cellular type I interferon production. *Cell Res.*, **2008**, *18*(11), 1105-1113.

[53] Devaraj, S. G.; Wang, N.; Chen, Z.; Tseng, M.; Barretto, N.; Lin, R.; Peters, C. J.; Tseng, C. T. K.; Baker, S. C.; Li, K. Regulation of IRF-3-dependent innate immunity by the papain-like protease domain of the severe acute respiratory syndrome coronavirus. *J. Biol. Chem.*, **2007**, *282*(44), 32208-32221.

[54] Hagglund, R.; Roizman, B. Role of ICP0 in the strategy of conquest of the host cell by herpes simplex virus 1. *J. Virol.*,**2004**, *78*(5), 2169-2178.

[55] Devaraj, S. G.; Wang, N.; Chen, Z.; Tseng, M.; Barretto, N.; Lin, R.; Peters, C. J.; Tseng, C. T. K.; Baker, S. C.; Li, K. Regulation of IRF-3-dependent innate immunity by the papain-like protease domain of the severe acute respiratory syndrome coronavirus. *J. Biol. Chem.*, **2007**, *282*(44), 32208-32221.

[56] Everett, R. D.; Meredith, M.; Orr, A. The ability of herpes simplex virus type 1 immediate-early protein Vmw110 to bind to a ubiquitin-specific protease contributes to its roles in the activation of gene expression and stimulation of virus replication. *J. Virol.*, **1999**, *73*(1), 417-426.

[57] Wang, J. L.; Loveland, A. N.; Kattenhorn, L. M.; Ploegh, H. L.; Gibson, W. High-molecular-weight protein (pUL48) of human cytomegalovirus is a competent deubiquitinating protease: Mutant viruses altered in its active-site cysteine or histidine are viable. *J. Virol.*,**2006**, *80*(12), 6003-6012.

[58] Chen, Z. B.; Wang, Y. H.; Ratia, K.; Mesecar, A. D.; Wilkinson, K. D.; Baker, S. C. Proteolytic processing and deubiquitinating activity of papain-like proteases of human coronavirus NL63. *J. Virol.*, **2007**, *81*(11), 6007-6018.

[59] Whitehurst, C. B.; Ning, S. B.; Bentz, G. L.; Dufour, F.; Gershburg, E.; Shackelford, J.; Langelier, Y.; Pagano, J. S. The Epstein-Barr Virus (EBV) deubiquitinating enzyme BPLF1 reduces EBV ribonucleotide reductase activity. *J. Virol.*, **2009**, *83*(9), 4345-4353.

[60] Brown, M. T.; Mangel, W. F. Interaction of actin and its 11-amino acid C-terminal peptide as cofactors with the adenovirus proteinase. *Febs Letters*, **2004**, *563*(1-3), 213-218.

[61] Schmidinger, H.; Hermetter, A.; Birner-Gruenberger, R. Activity-based proteomics: enzymatic activity profiling in complex proteomes. *Amino Acids*, **2006**, *30*(4), 333-350.

[62] Fonovic, M.; Bogyo, M. Activity based probes for proteases: Applications to biomarker discovery, molecular imaging and drug screening. *Curr. Pharm. Des.*, **2007**, 13(3), 253-261.

[63] Paulick, M. G.; Bogyo, M. Application of activity-based probes to the study of enzymes involved in cancer progression. *Curr. Opin. Genet. Dev.*, **2008**, *18*(1), 97-106.

[64] Uttamchandani, M.; Li, J. Q.; Sun, H.; Yao, S. Q. Activity-based protein profiling: New developments and directions in functional proteomics. *ChemBioChem*, **2008**, *9*(5), 667-675.

[65] Hu, M.; Li, P. W.; Li, M. Y.; Li, W. Y.; Yao, T. T.; Wu, J. W.; Gu, W.; Cohen, R. E.; Shi, Y. G. Crystal structure of a UBP-family deubiquitinating enzyme in isolation and in complex with ubiquitin aldehyde. *Cell*, **2002**, *111*(7), 1041-1054.

[66] Li, T. W.; Duan, W.; Yang, H. Y.; Lee, M. K.; Mustafa, F. B.; Lee, B. H.; Teo, T. S. Identification of two proteins, S14 and UIP1, that interact with UCH37. *Febs Letters*, **2001**, *488*(3), 201-205.

[67] Borodovsky, A.; Kessler, B. M.; Casagrande, R.; Overkleeft, H. S.; Wilkinson, K. D.; Ploegh, H. L. A novel active site-directed probe specific for deubiquitylating enzymes reveals proteasome association of USP14. *EMBO J.*, **2001**, *20*(18), 5187-5196.

[68] Yoshida, H.; Jono, H.; Kai, H.; Li, J. D. The tumor suppressor cylindromatosis (CYLD) acts as a negative regulator for toll-like receptor 2 signaling *via* negative cross-talk with TRAF6 and TRAF7. *J. Biol. Chem.*, **2005**, *280*(49), 41111-41121.

[69] Ovaa, H.; Kessler, B. M.; Rolen, U.; Galardy, P. J.; Ploegh, H. L.; Masucci, M. G. Activity-based ubiquitin-specific protease (USP) profiling of virus-infected and malignant human cells. *Proc. Natl. Acad. Sci USA*, **2004**, *101*(8), 2253-2258.

[70] Tsurumi, T.; Fujita, M.; Kudoh, A. Latent and lytic Epstein-Barr virus replication strategies. *Rev. Med. Virol.*, **2005**, *15*(1), 3-15.

[71] Holowaty, M. N.; Frappier, L. HAUSP/USP7 as an Epstein-Barr virus target. *Biochem. Soc. Trans.*, **2004**, *32*, 731-732.

[72] Saridakis, V.; Sheng, Y.; Sarkari, F.; Holowaty, M. N.; Shire, K.; Nguyen, T.; Zhang, R. G.; Liao, J.; Lee, W.; Edwards, A. M.; Arrowsmith, C. H.; Frappier, L. Structure of the p53 binding domain of HAUSP/USP7 bound to Epstein-Barr nuclear antigen 1: Implications for EBV-mediated immortalization. *Mol. Cell*, **2005**, *18*(1), 25-36.

[73] Taya, S.; Yamamoto, T.; Kanai-Azuma, M.; Wood, S. A.; Kaibuchi, K. The deubiquitinating enzyme Fam interacts with and stabilizes beta-catenin. *Genes to Cells*, **1999**, *4*(12), 757-767.

[74] Bheda, A.; Yue, W.; Gullapalli, A.; Whitehurst, C.; Liu, R. S.; Pagano, J. S.; Shackelford, J. Positive reciprocal regulation of ubiquitin C-terminal hydrolase L1 and beta-catenin/TCF signaling. *Plos One*, **2009**, 4(6), e5955.

[75] Everly, D. N.; Kusano, S.; Raab-Traub, N. Accumulation of cytoplasmic beta-catenin and nuclear glycogen synthase kinase 3 beta in Epstein-Barr virus-infected cells. *J. Virol.*,**2004**, *78*(21), 11648-11655.

[76] Rolen, U.; Kobzeva, V.; Gasparjan, N.; Ovaa, H.; Winberg, G.; Kisseljov, F.; Masucci, M. G. Activity profiling of deubiquitinating enzymes in cervical carcinoma biopsies and cell lines. *Mol. Carcinog.*, **2006**, *45*(4), 260-269.

[77] Kattenhorn, L. M.; Korbel, G. A.; Kessler, B. M.; Spooner, E.; Ploegh, H. L. A deubiquitinating enzyme encoded by HSV-1 belongs to a family of cysteine proteases that is conserved across the family herpesviridae. *Mol. Cell*, **2005**, *19*(4), 547-557.

[78] Schlieker, C.; Weihofen, W. A.; Frijns, E.; Kattenhorn, L. M.; Gaudet, R.; Ploegh, H. L. Structure of a herpesvirus-encoded cysteine protease reveals a unique class of deubiquitinating enzymes. *Mol. Cell*, **2007**, *25*(5), 677-687.

[79] Schlieker, C.; Korbel, G. A.; Kattenhorn, L. M.; Ploegh, H. L. A deubiquitinating activity is conserved in the large tegument protein of the Herpesviridae. *J. Virol.*, **2005**, *79*(24), 15582-15585.

[80] Gredmark-Russ, S.; Isaacson, M. K.; Kattenhorn, L.; Cheung, E. J.; Watson, N.; Ploegh, H. L. A gammaherpesvirus ubiquitin-specific protease is involved in the establishment of murine gammaherpesvirus 68 infection. *J. Virol.*, **2009**, *83*(20), 10644-10652.

[81] Jarosinski, K.; Kattenhorn, L.; Kaufer, B.; Ploegh, H.; Osterrieder, N. A herpesvirus ubiquitin-specific protease is critical for efficient T cell lymphoma formation. *Proce. Natl. Acad. Sci USA*, **2007**, *104*(50), 20025-20030.

[82] Boettcher, S.; Maresch, C.; Granzow, H.; Klupp, B. G.; Teifke, J. P.; Mettenleiter, T. C. Mutagenesis of the active-site cysteine in the ubiquitin-specific protease contained in large tegument protein pUL36 of Pseudorabies virus impairs viral replication *in vitro* and neuroinvasion *in vivo*. *J. Virol.*,**2008**, *82*(12), 6009-6016.

[83] Hershko, A.; Rose, I. A. Ubiquitin-aldehyde: a general inhibitor of ubiquitin-recycling processes. *Proc. Natl. Acad. Sci. USA*, **1987**, *84*(7), 1829-1833.

[84] Hu, M.; Li, P. W.; Li, M. Y.; Li, W. Y.; Yao, T. T.; Wu, J. W.; Gu, W.; Cohen, R. E.; Shi, Y. G. Crystal structure of a UBP-family deubiquitinating enzyme in isolation and in complex with ubiquitin aldehyde. *Cell*, **2002**, *111*(7), 1041-1054.

[85] Balakirev, M. Y.; Jaquinod, M.; Haas, A. L.; Chroboczek, J. Deubiquitinating function of adenovirus proteinase. *J. Virol.*, **2002**, *76*(12), 6323-6331.

[86] Balakirev, M. Y.; Tcherniuk, S. O.; Jaquinod, M.; Chroboczek, J. Otubains: a new family of cysteine proteases in the ubiquitin pathway. *Embo Reports*, **2003**, *4*(5), 517-522.

[87] Soares, L.; Seroogy, C.; Skrenta, H.; Anandasabapathy, N.; Lovelace, P.; Chung, C. D.; Engleman, E.; Fathman, C. G. Two isoforms of otubain 1 regulate T cell anergy *via* GRAIL. *Nat. Immunol.*, **2004**, *5*(1), 45-54.

Received: January 10, 2011 **Revised: March 30, 2011** **Accepted: April 6, 2011**