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Invited review

Emerging roles of deubiquitinating enzymes in human cancer[1]

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Key words

Abstract

Protein modifications by the covalent linkage of ubiquitin have significant involvement in many cellular processes, including stress response, oncogenesis, viral infection, transcription, protein turnover, organelle biogenesis, DNA repair, cellular differentiation, and cell cycle control. Protein ubiquitination and subsequent degradation by the proteasome require the participation of both ubiquitinating enzymes and deubiquitinating enzymes. Although deubiquitinating enzymes constitute a large family in the ubiquitin system, the study of this class of proteins is still in its infant stage. Recent studies have revealed a variety of molecular and biological functions of deubiquitinating enzymes and their association with human diseases. In this review we will discuss the possible roles that deubiquitinating enzymes may play in cancers.

Introduction

The post-translational modification of proteins by the covalent linkage of ubiquitin targets those proteins for degradation by the proteasome, and this process involves the participation of both ubiquitinating enzymes and deubiquitinating enzymes. Although deubiquitinating enzymes constitute a large family in the ubiquitin–proteasome system, their biological functions and pathophysiological roles remain largely unknown and have only just begun to be understood. In recent years, more and more studies have revealed the involvement of deubiquitinating enzymes in cancers as well as in other diseases. In this review, we will mainly focus on the emerging roles of this class of enzymes in the development and progression of cancers.

Molecular functions of deubiquitinating enzymes

Ubiquitin-mediated protein degradation and lysosomal protein degradation are the 2 mechanisms for intracellular protein turnover. The ubiquitin-mediated mechanism is further classified into the ubiquitin/proteasome pathway and ubiquitin/aggresome pathway. The ubiquitin/proteasome system is the major non-lysosomal proteolytic mechanism in the cytosol and nucleus of eukaryotic cells, lysosomal, and the ubiquitin/aggresome pathway. An important function of this mechanism is the degradation of abnormal proteins generated under normal and stress conditions. The substrates of this system include many cystolic, nucleic, and integral membrane proteins, such as transcription factors, cell cycle regulators, and signal transducers[1]. The degradation of a protein via the ubiquitin–proteasome pathway involves 2 successive steps: (1) the conjugation of multiple ubiquitin moieties to the substrate; and (2) the degradation of the tagged protein by the 26S proteasome complex.

The enzymatic reactions of the ubiquitin pathway have mostly been elucidated. In these reactions, proteins are targeted for degradation by covalent ligation to ubiquitin, a 76 amino acid polypeptide that is universally distributed among eukaryotes and highly conserved. The ubiquitin–protein conjugation requires the sequential execution of 3 types of enzymes (Figure 1). The C-terminal glycine residue of ubiquitin is activated in an ATP-dependent process by an ubiquitin-activating enzyme, E1. Activated ubiquitin is then transmitted to a cysteine residue of an ubiquitin-conjugating enzyme (ubiquitin carrier protein), E2. In the last step catalyzed by an ubiquitin-protein ligase (E3), ubiquitin is linked by its C-terminus in an amide isopeptide linkage to a e-amino group of the lysine residues of the substrate protein. This conjugation cascade is highly substrate specific.
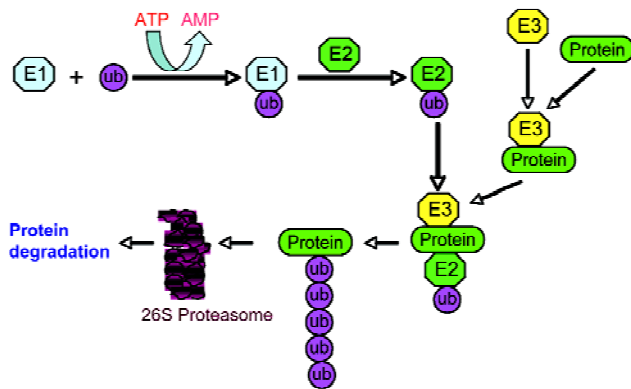
Figure 1. Enzymatic reactions of the ubiquitin pathway.

The modification of proteins by ubiquitin is reversible. Ubiquitinated proteins are not only subject to degradation, but also to deubiquitination. When tagged proteins are subjected to degradation by the 26S proteasome complex, it requires the release of free and reusable ubiquitin. The deubiquitination reactions, which require accurate proteolytic processing at the C-terminal glycine of ubiquitin, are catalyzed by deubiquitinating enzymes (Figure 2). The substrates for these enzymes include precursor forms of ubiquitin, conjugates resulting from non-specific side reactions of E1-S-ubiquitin/E2-S-ubiquitin with cellular nucleophiles, covalently-linked ubiquitin substrates, and poly-ubiquitin chains. Deubiquitinating enzymes are isopeptidases and consist of 2 classes: ubiquitin C-terminal hydrolases and ubiquitin-specific processing proteases. Ubiquitin C-terminal hydrolases are papain-like thiol proteases with a 230 amino acid core catalytic domain and share significant sequence similarity to the neuron-specific human protein PGP9.5 (UCH-L1). These enzymes cleave ubiquitin derivatives with small or disor-

Figure 2. Deubiquitinating enzymes catalyze the release of ubiquitin from ubiquitinated proteins.

dered C-terminal domains[2,3]. Most of this class of enzymes is relatively small with a molecular weight less than 40 kDa. Ubiquitin C-terminal hydrolases are known to have important roles in development and neural function. For example, during the establishment of long-term synaptic facilitation in Aplysia, the induction of a protein with UCH enzyme activity was observed[4]. Ubiquitin-specific processing proteases are a much larger group of thiol proteases and are very divergent with a molecular weight ranging from 50 to 250 kDa. This class of enzymes is known to be involved in the regulation of signal transduction, growth, and development[3]. Both deubiquitinating enzyme classes have a specific, tight binding site for ubiquitin. Although the exact and specific physiological roles of deubiquitinating enzymes have not been fully established, the following functions are generally accepted: (1) processing of ubiquitin precursors; (2) recycling of adventitiously trapped catalytic intermediates; (3) proofreading of protein ubiquitination; (4) recycling of ubiquitin from polyubiquitinated proteins following commitment to degradation; (5) keeping proteasomes free of ubiquitin chains; and (6) maintaining free ubiquitin concentrations[1]. The functions and molecular basis of deubiquitination and deubiquitinating enzymes are increasingly being understood over the past several years. For example, it is now known that deubiquitination of protein substrates is tightly coupled with degradation in the proteasome[5,6], and the inactivation of the deubiquitinating enzyme can completely prevent protein degradation[5]. However, due to the large number of members in the deubiquitinating enzyme family, it is not surprising to see that different members execute totally different functions. It was found that UBP6, a proteasome-associated cysteine protease, delays protein degradation[7]. More interestingly, a recent study demonstrated that ubiquitin C-terminal hydrolase-L1 possesses ligase activity as well as hydrolase activity, and these 2 opposing enzymatic activities affect the degradation of a-synuclein and susceptibility to Parkinson's disease[8].
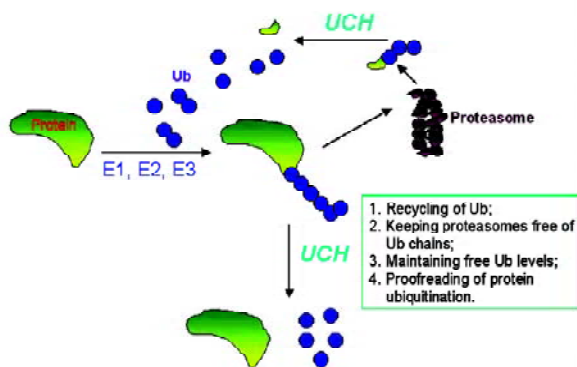
Aberrant expression of deubiquitinating enzymes in human cancers

Several types of deubiquitinating enzymes were found to be upregulated in cancer cells. Ubiquitin C-terminal hydrolase-L1, identical to the neuron-specific protein gene product PGP9.5 and widely expressed in neuronal tissues (1%–2% of brain proteins) at all stages of neuronal differentiation was originally suggested as a neuroendocrine marker[9]. Two decades ago there were reports that tumors of neuroendocrine origin, such as small cell carcinoma, showed positive staining for PGP9.5[10,11]. Recently, Jen and colleagues exam-

ined the expression of PGP9.5 in normal lung epithelium, lung tumor cell lines, and 98 resected primary non-small-cell lung carcinomas and observed that the increased expression of PGP9.5 was specifically associated with cancer development [12]. In primary non-small-cell lung carcinomas, 54% (53/98) of the cases had positive PGP9.5 staining, and this protein was present in 44% (29/66) of stage I and 75% (24/32) of stages II and IIIA non-small-cell lung carcinomas[12]. The high expression of PGP9.5 also occurs in other types of cancer. The evaluation of the expression of PGP9.5 in 69 resected ductal carcinomas of the pancreas and in normal pancreatic tissues revealed a significant negative correlation between the overexpression of PGP9.5 and postoperative survival[13]. PGP9.5-negative pancreatic cancer patients had significantly better survival rates compared with those who were PGP9.5 positive[13]. In colorectal cancer, the same group also examined the expression of PGP9.5 in primary colorectal cancers and correlated the results with the pathological features[14]. In colorectal cancer, 46% (33/74) of the specimens showed positive staining with PGP9.5 in most tumor cells, whereas no PGP9.5 expression was detected in adjacent normal epithelium. A correlation analysis revealed 2 significant differences in maximal tumor size and the extent of the tumor[14]. More recently, the overexpression of ubiquitin C-terminal hydrolase-L1 was reported in human myeloma and medullary thyroid carcinoma cells[15]. These studies indicate that the expression of PGP9.5 is closely associated with cancer progression, and suggest that this protein could be a useful a tumor marker for diagnosis and prognosis. In our studies on the multidrug resistance phenotype, we found that ubiquitin C-terminal hydrolase-L1 is overexpressed in several drug-resistant cancer cell lines. However, the exact role of ubiquitin C-terminal hydrolase-L1 (PGP9.5) and its regulation in cancer remains unknown. Al-Katib *et al* demonstrated that ubiquitin C-terminal hydrolase-L1 expression could be induced by the phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate, and by bryostatin, in acute lymphoblastic leukemia and suggested a role of this enzyme in B-cell differentiation[16,17]. PGP9.5 was shown to interact and colocalize with JAB1, a Jun activation domain-binding protein that can bind to p27^{kip1}[18]. This interaction and colocalization was suggested to contribute to the degradation of p27^{kip1}[18], a cyclin-dependent kinase inhibitor whose loss in epithelial cancers was significantly correlated with the pathological tumor grade and with high-grade, poorly-differentiated tumors showing a significantly lower p27 protein than their well-differentiated counterparts[19]. The decreased level of p27^{kip1} is frequently observed in human cancers, including breast, lung, prostate, colon, skin, and ovarian cancers[20–22], and has been

found to correlate with cancer development and poor survival[19]. These observations also provide a clue to the implication of UCH-L1 in cancer development and progression. In addition, deubiquitinating enzymes are upregulated in Burkitt's lymphoma, a highly malignant B cell neoplasma, and some other types of malignancies[23,24].

Deubiquitinating enzymes: oncogenes or tumor suppressors?

Alterations in the affluence of important regulatory proteins are the frequently observed defects in malignant cells. The gain in the function of oncogenes or loss of tumor suppressor proteins is the umbilical mechanism by which cells escape normal control on proliferation. Because of the broad involvement of the ubiquitin–proteasome proteolysis in the regulation of protein turnover, this pathway may play a vital role in cancer development and progression. In fact, considerable insight into the linkage of the ubiquitin–proteasome pathway with cancer has been gained in recent years. For example, the tumor suppressor p53 is tightly regulated by the ubiquitin–proteasome pathway[25,26]. p53 is an unstable nuclear protein with a half-life of 30 min in normal cells. After cellular stress or DNA damage, p53 is stabilized through the downregulation of its degradation, leading to growth arrest or apoptosis. The ubiquitination-proteasome pathway has also been reported to play a critical role in the pathogenesis of breast cancer by affecting the downregulation of growth factor receptors, such as EGFR/ErbB-1, Neu/ErbB-2, and ErbB-3/HER3[27,28]. Nuclear factor-kappa B (NF-κB) plays a pivotal role in many aspects of tumor development, progression, and therapy, and its activation relies primarily on the ubiquitination-mediated degradation of its inhibitor IκB[29]. The role the deubiquitinating enzymes play in the regulation of those oncogenes or tumor suppressor genes represents an emerging and interesting research area, and novel functions of a variety of deubiquitinating enzymes have begun to be uncovered. Gu *et al* discovered that herpesvirus-associated ubiquitin-specific protease [HAUSP, or ubiquitin-specific protease (USP)7] possesses ubiquitin hydrolase activity that can stabilize p53 by deubiquitinating the tumor suppressor, suggesting a tumor suppressor function of this deubiquitinating enzyme[30]. In contrast, using the genetic disruption approach, Cummins and Vogelstein demonstrated that MDM2, rather than p53, is the HAUSP substrate, and the deubiquitination of MDM2 by HAUSP leads to the destabilization of p53[31]. Gu *et al* later also found that HAUSP can deubiquitinate and stabilize MDM2 in a p53-independet manner, suggesting that HAUSP plays a dynamic role in the

p53–MDM2 pathway[32]. Therefore, HAUSP may have both tumor suppressive and oncogenic effects, depending on different circumstances.

USP2a is a deubiquitinating enzyme that is overexpressed in prostate cancer and plays an important role in regulating tumor cell survival. Recently, it was found that this isopeptidase can deubiquitinate and stabilize fatty acid synthase, an anti-apoptotic protein whose overexpression is often observed in progressive human tumors[33]. Therefore, USP2a may act as an oncogenic protein in tumor formation or cancer progression. USP11 physically associates with and deubiquitinates BRCA2, but its pro-survival function to DNA damage is independent of its effect on the deubiquitination of BRCA2[34]. USP28 has also been found to be involved in DNA damage response; however, its role is to promote DNA damage-induced apoptosis through regulating the pro-apoptotic Chk2–p53–PUMA pathway[35]. On the other hand, BAP1, a ubiquitin C-terminal hydrolase, shows growth inhibitory effects on cancer cells, and this tumor suppressor function depends on its interaction with breast cancer susceptibility gene product BRCA1[36]. Although the precise mechanism of this interaction is not clear, the deubiquitinating activity of BAP1 is believed to play a key role.

In a high-throughput RNA interference screen to identify deubiquitinating enzymes involved in cancer, Brummelkamp et al found that the cylindromatosis tumor suppressor (CYLD), which is mutated in familial cylindromatosis, is a USP[37]. CYLD can bind to NEMO (IKKg), deubiquitinate, and inactivate TNF-receptor-associated factors, leading to the inhibition of NF-κB activation. The suppression of CYLD results in the activation of NF-κB and resistance to apoptosis. The deubiquitinating activity of CYLD, its interaction with NEMO, and the inhibitory effect on NF-kB activation, were also discovered in another 2 independent studies[38,39]. Therefore, deubiquitinating enzymes can be either tumor suppressor genes depending on their molecular functions and their roles in diverse signaling pathways. In addition, deubiquitinating enzymes have been reported to be involved in the regulation of cell differentiation, apoptosis, and other physiological or pathological processes[40–42] (Table 1). With further investigations on deubiquitinating enzymes, it can be expected that more insight of the roles and functions of this class of enzymes will be uncovered.

Deubiquitinating enzymes and other cancer-related problems

Pain occurs in approximately 60%–80% of cancer patients with terminal disease, particularly in patients with metastatic cancer. Although pain in cancer patients can be attributed to many factors, direct tumor involvement is the most common cause. There is evidence that ubiquitin hydrolases might contribute to the occurrence of cancer pain. Angioleiomyoma is a solitary subcutaneous tumor characterized by pain in about half of patients with this tumor. In angioleiomyoma patients suffering from pain, small nerve fiber immunoreactivity for ubiquitin C-terminal hydrolase-L1 was observed within the capsule (77%) and tumor stroma (69%)[43]. Similar observations were reported in other tumors, such as gastrointestinal stromal tumors, fibrosarcoma, and melanoma[44]. These observations suggest that ubiquitin C-terminal hydrolase-L1 as a part of peripheral components plays a role, although as yet unclear yet in cancer-associated pain.

Tumor antigens, which are mostly proteins or peptides expressed or presented by tumor cells, act as signals for immune effectors (T cells and antibodies) to differentiate them from normal tissues. Several deubiquitinating enzymes have been reported to participate in the immune response to cancer. For instance, the expression of ubiquitin C-terminal hydrolase-L1 was found to correlate with T-status in non-small-cell lung cancer[45]. In a proteomic analysis of sera from lung cancer patients, ubiquitin C-terminal hydrolase-L1 was identified as a tumor antigen that induces a humoral immune response[46]. More recently, another ubiquitin hydrolase, ubiquitin C-terminal hydrolase-L3, was found to be involved in the humoral immune response in human colon cancer. Yet the exact roles that these ubiqutin hydrolases play in immune response remain to be elucidated. Because of the structural and functional similarity, one might expect that the involvement of more deubiquitinating enzymes in the immune response to cancer will be uncovered.

Future perspective

Although deubiquitinating enzymes comprise the largest known family of enzymes in the ubiquitin system, at present, little is known about the physiological or pathological roles of those proteins. While deubiquitinating enzymes share a common fundamental biochemical function, they nevertheless serve as controllers of highly diverse cellular processes. In particular, a large number of ubiquitin ligases have been identified and their association with diseases, such as cancer, has begun to be uncovered. For example, the oncogene MDM2 is known to serve as a ubiquitin ligase for p53 and promotes its degradation. The drugs targeting MDM2 have already been developed[47]. With increasing understanding of the functions and roles of the enzymes involved in protein ubiquitination and deubiquitination, we

Table 1. Deubiquitinating enzymes and their possible involvement in cancers.

Deubiquitinates	Involvement in cancer	References
UCH-L1	Overexpressed in lung cancers, pancreatic cancer, colorectal cancer, myeloma, medullary thyroid carcinoma; possibly associated with tumor progression, pain, and immune response	12, 13, 14, 15, 40, 41, 42, 43
HAUSP or USP7	Tumor suppressor or oncogene (unclear)	30, 31, 32
USP2a	Stabilizes fatty acid synthase; possibly an oncogene	33
BAP1	Possesses tumor suppressor functions	36
USP11	Survival-promoting to DNA damage	34
USP28	Promotes DNA damage-induced apoptosis	35
CYLD	Functions as a tumor suppressor	37, 38, 39
DUB-2	Anti-apoptotic activity	41
UBP43	Involved in myeloid cell differentiation	40
HUMORF8	Oncogenic activity	42

can expect to know more about what these ubiquitin-specific processing proteases and ubiquitin C-terminal hydrolases exactly do during cancer development and progression. This knowledge will certainly provide insight into whether some of those enzymes can serve as prognostic tumor markers or therapeutic targets for cancer. Drugs targeting the ubiquitin–proteasome pathway (such as velcade) have already been proven to be very effective against some types of cancers[48–52]. Compounds that inhibit the activity of some ubiquitin C-terminal hydrolases have also been reported[53], but their implications in the treatment of cancer or other diseases remain to be investigated. It is conceivable that drugs targeting deubiquitinating enzymes will sooner or later be used in treating cancers and other human diseases.

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