MINI-REVIEW

Power and Promise of Ubiquitin Carboxyl-terminal Hydrolase 37 as a Target of Cancer Therapy

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Abstract

Ubiquitin carboxyl-terminal hydrolase 37 (UCH37, also called UCHL5), a member of the deubiquitinating enzymes, can suppress protein degradation through disassembling polyubiquitin from the distal subunit of the chain. It has been proved that UCH37 can be activated by proteasome ubiqutin chain receptor Rpn13 and incorporation into the 19S complex. UCH37, which has been reported to assist in the mental development of mice, may play an important role in oncogenesis, tumor invasion and migration. Further studies will allow a better understanding of roles in cell physiology and pathology, embryonic development and tumor formation, hopefully providing support for the idea that UCH37 may constitute a new interesting target for the development of anticancer drugs.

Keywords: UCH37 - deubiquitination - proteasome - protein interaction - tumor therapy target

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Introduction

The ubiquitin-26S proteasome system is the main non-lysosomal route for intracellular protein degradation in eukaryotes. The 26S proteasome is a proteolytic complex which consists of two subcomplexes: the barrel-shaped core complex (the 20S proteasome) and the 19S regulatory complex (also known as PA700). The proteasome recognizes substrate via its multiubiquitin chain, followed by ATP-dependent unfolding and translocation of the substrate from the regulatory particle into the core particle to be degraded (Hershko and Ciechanover, 1998; Pickart, 2001; Glickman and Ciechanover, 2002; Wilkinson, 2002; Goldberg, 2003). But ubiquitin groups which bound with the substrate are mostly not delivered into the core particle and broken down with substrate, which is regulated by the deubiquitinating enzymes (DUBs). DUBs, which are capable of removing Ub from the protein substrates, are also involved in lots of biological processes such as transcriptional regulation, growth and differentiation, and oncogenesis (Wilkinson, 1997; Chung and Baek, 1999). Ubiquitin carboxyl-terminal hydrolase 37 (UCH37, also called UCHL5), a member of the deubiquitinating enzymes, can suppress protein degradation through disassembling polyubiquitin from the distal subunit of the chain. It has been proved that UCH37 can be activated by proteasome ubiqutin chain receptor Rpn13 and incorporation into the 19S complex. UCH37, which has been reported to assist in the mental development of mice, may play an important role in oncogenesis, tumor invasion and migration (Guterman and Glickman, 2004; Fang et al., 2010; Lee et al., 2011; Fang et al., 2013).

Discovery of UCH37

In 1997, Cohen and coworkers reported a 19S-associated isopeptidase that could selectively disassemble polyubiquitin chain from the distal end of poorly ubiquitinated protein conjugates. This isopeptidase, a protein with molecular mass of 37K named UCH37 (also called UCH-L5), functions to disassemble Lys48-linked poly-ubiquitin from the distal end of the chain, and is thought to be involved in editing ubiquitinated substrates according to the length of polyubiquitin chains rather than the structure of the target proteins themselves. The protein can also disassemble Lys6- and Lys11-linked polyubiquitin, but not α-linked di-ubiquitin or poly-ubiquitin with introduced mutations (Leu8Ala and Ile44Ala) (Lam et al., 1997a; Lam et al., 1997b).

Molecular structure

UCH37 gene is located in 1q32 of the chromosome, the full-length of the human UCH37 cDNA consisting of 1818 nucleotides, including a 236-nucleotide 5'-untranslated region (UTR), a 990-nucleotide open reading frame, and a 592-nucleotide 3'-UTR. The possible transcription promoter was calculated by NNPP version 2.2 (Reese, 2001) as "cctggggc**cg**cacaaaaggctcccagc**cg**gcctcc**cg**caatgtcctcacc" in the -329 to -280 forward the 5'-UTR. The underline nucleotides were predicted as the methylated sites. Then the human UCH37 amino acid sequence was compared

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Table 1. Predicted phosphorylation sites on UCH37 protein

Substrate	Position	Score	Kinase prediction	Context
Ser	72	0.994	AKT1, PKA, PKB, ATM, GSK-3, PKC, IKK, PKG, RSK, STK4, CHK1, PDK	VVQD**S**RLDT
	131	0.865	AKT1, PKA, ATM, PKC, PKG, PLK1, RSK, STK4, CHK1, CK1	GLAL**S**NSDV
	156	0.997	AKT1, PKA, ATM, Aurora, PKG, RSK, STK4, CHK1, CK1, PDK, CK2	DTKT**S**AKEE
	212	0.991	AKT1, PKA, PKB, ATM, PKC, Aurora, PKG, CaM, MAPK, RSK, CDK, STK4, CHK1, PDK, CK2	IQKY**S**EGEI
	258	0.909	AKT1, ATM, PKG, RSK, STK4, CHK1	NSML**S**AIQS
Thr	76	0.638	GRK, PKB, PKC, CDK	SRLD**T**IFFA
	110	0.576	GRK, PKB, PKC, CDK, CK2, PDK	HLGE**T**LSEF
Tyr	168	0.515	Fgr, ALK, PDGFR, BTK, Ret, IGF1R, EPH, IR, JAK2, TYK2, Fes, ZAP70, FGFR1	HFVS**Y**VPVN

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        1       20 24 28 38 42 45 49 68 75   94 101      112119134142153     190198211
Mus     MS--SN···K···R···R···L···S···K···V···V···ET···V···THQDVH···S···Q···D···S···TKTP···A···T···YSEGEIRFNL
Rattus  MS--SN···E···G···R···L···N···K···V···V···ET···V···THQDVH···S···Q···D···S···TKTS···A···T···YSEGEIRFNL
Homo    MT--GN···E···G···R···L···N···K···V···V···DT···V···THQDVH···S···Q···D···S···TKTS···A···S···YSEGEIRFNL
Bos     MT--GN···E···G···R···L···N···K···V···V···DT···V···THQDVH···S···Q···D···S···AKTA···A···S···YSEGEIRFNL
Gallus  MAGGSS···E···G···R···L···N···K···I···V···DT···V···AHQDIR···S···Q···E···S···AKSS···S···S···----------
Danio   MA--GS···E···G···K···M···N···N···V···I···DQ···I···THPDML···T···N···E···G···AKST···V···S···YSEGEIRFNL

                                    249253     259264      275283306 314
Mus     MAIVSDRKMIYEQKIAELQRQLAE···D···GSTV···A···VARNQM···V···I···HQ···EKAKEKQNAKKAQETK 329  96%
Rattus  MAIVSDRKMIYEQKIAELQRQLAE···D···GSTV···A···VARNQM···V···I···HQ···EKIFSCRGRNL----- 324  94%
Homo    MAIVSDRKMIYEQKIAELQRQLAE···D···GNSM···A···VAKNQM···V···I···HQ···EKAKEKQNAKKAQETK 329  100%
Bos     MAIVSDRKMIYEQKIAELQRQLA-···D···GSNM···A···VAKNQM···V···I···HK···EKAKEKQNAKKAQETK 328  98%
Gallus  ------------------------···E···SSNM···S···VAKYQM···N···I···HQ···EKAKEKQNAKKVQEAK 297  92%
Danio   MAIVSDRKMIYEKKIVELQAQLTE···D···SGNH···S···IAKYQL···N···V···YQ···EKAKEKQSAKKIQEAK 329  88%
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Figure 1. Comparison of the UCHL5(UCH37) in Different Species. The underlined sequences indicate the KEKE-like motif. GeneBank accession numbers of each sequence are as follows: Homo sapiens, NP_057068.1; Bos Taurus, NP_776906.2; Mus musculus, NP_062508.1; Rattus norvegicus, NP_001012149.1; Gallus gallus, NP_001006530.1; Danio rerio, NP_998249.1

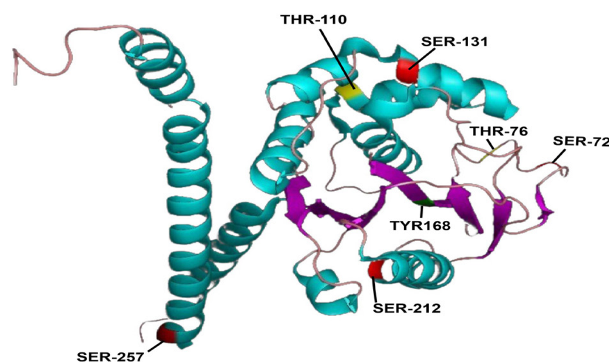

Figure 2. The Crystal Structure of the UCH37 and the Phosphorylation Locations on the UCH37. The N-terminal catalytic domain is composed of a central six-stranded anti-parallel β-sheet and seven α-helices on either side of the sheet, causing it to form a bilobal structure, and the C-terminal domain comprises α-helices 8-10. This model shows that predicted Ser and Thr sites have high surface accessibility for phosphorylation. The Ser, Thr and Tyr residues are denoted by red, yellow and green colors

with those from other species deposited in GenBank database using ClustalW2 software (Larkin et al., 2007), we noticed that human UCH37 shared 98% (vs. Bos), 96% (vs. Mus), 94% (vs. Rattus), 92% (vs. Gallus), and 88% (vs. Danio) of identity (Figure 1). UCH37 in all of these species have conserved catalytic amino acid residues, which indicates that the UCH37 may perform ubiquitin C-terminal hydrolase activity. UCH37 consists of two functional domains, a catalytic domain (UCH-domain, residues 1-226) and a C-terminal domain (tail-domain, 227-329). The crystal structure of N-terminal catalytic domain shows that it is composed of a central six-stranded anti-parallel β-sheet, with seven α-helices on either side of the sheet causing it to form a bilobal structure (Figure 2).

The S-shaped loop, which is the active-site crossover loop of UCH37, consisting of residues 142-163, is disordered. It would be reordered if ubiquitin was bound and uncovering the S-shaped loop with the C-terminus of ubiquitin just like UCH-L3, indicating that large substrates can not pass through the loop on UCH37N. Helix-3, which comprises a wall on the edge of the putative substrate-binding site (P'-site), is collapsed in UCH37N, resulting in a broader V-shaped trough when compared with other UCHs. This suggests that UCH37 can distinguish substrates with different features, such as larger substrates (Nishio et al., 2009). UCH37 has a long crossover loop (>14 residues). It is the loop length and potentially loop-chain flexibility which play a important role in the catalytic activity and substrate specificity of a UCH for isopeptide Ub chain (Zhou et al., 2011). The C-terminal domain comprises α-helices 8-10, and a helical segment comprising residues 306-311 (Burgie et al., 2011).

In biological systems, protein localization, activity, their interaction with other proteins and overall turnover is determined by post translational modifications (PTMs). Phosphorylation is one important PTM. Phosphorylation potential for human UCH37 and kinase specific phosphorylation sites were predicted using NetPhos 2.0 and KinasePhos 2.0 (Blom et al., 1999; Wong et al., 2007). The minimum threshold value used to predict phosphorylation is 0.5 for NetPhos 2.0. A total of 8 sites showed high potential for phosphorylations. Amongst these 5 were Ser, 2 Thr and 1 was Tyr (Table 1). In spite of Ser 156, 7 predicted sites were highly conserved in mammals (Figure 2). We drew the 3D structure of UCH37 and assessed the possible surface accessibility of UCH37 for the phosphorylation by PyMOL. We found that Ser 72, 212 and 257, and Thr 76 and 110 as "exposed" surfaces. This information depicts that Ser and Thr residues have high access to phosphorylation and may play an important role in protein localization, activity and degradation.

Protein-protein interactions between UCH37 and other proteins

The proteins that interact with UCH37 were first reported in 2001. Li T et al. (2001) identified two proteins that interacted with UCH37 using the yeast two-hybrid screen: S14, which is a subunit of 19S, and UIP1 (UCH37

Table 2. Predicted Consequential Pairing of Target Region and miRNA

Classification	Predicted miRNA	Position	Predicted consequential pairing of target region (top) and miRNA (bottom)
Conserved	hsa-miR-586	Position 134-140 of UCHL5 3' UTR	5' ...GCCUGUCUUGUCUUU**AAUGCAUG**... | | | | | | | 3' CCUGGAUUUUUAUG**UUACGUAU**
	hsa-miR-657	Position 162-168 of UCHL5 3' UTR	5' ...AUAAACUUCUUCCCU**ACCUGC**AU... | | | | | | | 3'GGAUCUCUCCCACUCU**UGGACGG**
	hsa-miR-1322	Position 166-172 of UCHL5 3' UTR	5' ...ACUUCUUCCCUACCU**GCAUCAUG**... | | | | | | | 3' GUCGUAGUCGU**CGUAGUAG**
Poorly conserved	hsa-miR-429	Position 405-411 of UCHL5 3' UTR	5' ...UAACGGAAAAAAAAU**CAGUAUU**U... | | | | | | | 3' UGCCAAAAUGGUCU**GUCAUAA**U
	hsa-miR-200c	Position 405-411 of UCHL5 3' UTR	5' ...UAACGGAAAAAAAAU**CAGUAUU**U... | | | | | | | 3' AGGUAGUAAUGGGCC**GUCAUAA**U
	hsa-miR-200b	Position 405-411 of UCHL5 3' UTR	5' ...UAACGGAAAAAAAAU**CAGUAUU**U... | | | | | | | 3' AGUAGUAAUGGUCC**GUCAUAA**U

TargetScan predicted 3 conserved sites and 39 poorly conserved sites, while microRNA.org and miRDB both predicted 8 potential poorly conserved sites. 3 of the poorly conserved sites appeared in all of the three results

Figure 3. Proteasome-assocaited UCH37 in the Cytoplasm is Active, While Only a Fraction of Nuclear UCH37 is Active. In the cytoplasm, hRpn13's C terminal domain binds UCH37's C terminal tail via KEKE motifs, relieving UCH37 autoinhibition, and hRpn13's N terminal domain binding the polyubiquitin chain and hRpn2. UCH37 in this model recruited to the proteasome and disassembling polyubiquitin from the distal subunit of the chain while hRpn13 binds the proximal subunit. In the nucleus, UCH37 is associated with hINO80 via its C-terminal tail and the N-terminal domain of NFRKB. In hINO80, UCH37 is held in an inactive state; however, it can be activated by transient interaction of the INO80 complex with the proteasome or hRpn13

interacting protein 1). The C-terminal extension of UCH37 is vital for the interaction and furthermore, the interaction between UIP1 and UCH37 was much stronger than that between S14 and UCH37.

Recent studies have showed that hRpn13/Adrm1/GP110, a subunit of the 19S particle, binds to UCH37 enhancing its isopeptidase activity (Figure 3). The C-terminal regions of hRpn13 and UCH37 containing KEKE motifs are involved in their physical interaction (Hamazaki et al., 2006; Qiu et al., 2006; Yao et al., 2006). The N-terminal domain of hRpn13, binds the polyubiquitin chains and hRpn2. It has been revealed that hRpn13 has a canonical pleckstrin-homology fold in the amino-terminal domain which consists of a seven-stranded β-sandwich structure. The structure is capped by the carboxy-terminal α-helix. So the authors called this domain "pleckstrin-like receptor for ubiquitin" (Pru) (Saeki and Tanaka, 2008; Schreiner et al., 2008). Interestingly, UCH37 can also bind to the proteasome through Rpn10/S5a, another ubiquitin receptor of the proteasome beyond hRpn13 (Deveraux et al., 1994; Stone et al., 2004). This receptor is located at the hinge between the lid and base of the 19S complex, and close to where UCH37 lies.

It is also found that in the nucleus UCH37 is combined with the human Ino80 chromatin-remodeling complex (hINO80), which is an ATP-dependent chromatin remodeling complex that alters nucleosome positioning on DNA during both transcription and DNA repair (Figure 3) (Cai et al., 2007). UCH37 is associated with hINO80 through its C-terminal tail and N-terminal domain of NFRKB, the N-terminal third (residues 1-476) of NFRKB which is a DNA-binding transcriptional activator. The interaction between NFRKB and full-length UCH37 has the same feature with the interaction between UCH37 and hRpn13 (Yao et al., 2008; Zediak and Berger, 2008). Wicks et al. (2005; 2006) found that UCH37 could bind weakly to Smad2 and Smad3, and strongly to Smad7.

Regulation of UCH37

Much literature has reported the structure and interactant of UCH37, but little focuses on the upperstream regulations of UCH37. DNA methylation is an epigenetic mechanism regulates gene expression. The changes in the methylation pattern including the methylation of CpG islands and primers, which are normally unmethylated, may lead to the silencing of endogenic genes (Sulewska et al., 2007). It has been predicted that there are three possible methylation sites in the UCH37 promoter. When the promoter region has a high methylation status, the gene transcription is usually suppressed. MicroRNAs are small endogenous RNAs that derive from distinctive hairpin precursors in various organisms. After incorporation into a silencing complex, which contains at its core an Argonaute

protein, a miRNA can pair to an mRNA and thereby specify the post-transcriptional repression of that protein-coding message, either by transcript destabilization, translational repression, or both. Many sites that match the miRNA seed (nucleotides 2-7), particularly those in 3' untranslated regions (3'UTRs), are preferentially conserved (Baek et al., 2008). For the prediction of possible sites for miRNA binding, TargetScan, microRNA.org and miRDB were used (Betel et al., 2008; Wang, 2008; Wang and El Naqa, 2008; Friedman et al., 2009). A total of 3 conserved sites and 3 poorly conserved sites showed high potential possibility as the target region (Table 2).

Previous studies have showed that neither UCH37 itself nor UCH37-Adrm1 or UCH37-hRpn13-hRpn2 complexes can hydrolyse Lys48-linked di-ubiquitin efficiently; rather, conjunction with the 19S complex is necessary to enable hydrolysis of polyubiquitin chains (Yao et al., 2006). hRpn13 can bind the C-terminal tail of UCH37 and relieves its autoinhibition. It hasn't been known how hRpn13 activates UCH37, but its strong ubiquitin-binding affinity may contribute by increasing UCH37's affinity for the substrates when in the hRpn13 complex and by orienting neighboring ubiquitin moieties in a configuration that is optimal for hydrolysis. hRpn13's ability to modulate the relative orientation of its Pru- and UCH37-binding domain may contribute a driving force in UCH37 catalysis (Chen et al., 2010). Although the Rpn10/S5a subunit also appears to interact with UCH37 in the 19S complex, this interaction fails to activate UCH37 (Holzl et al., 2000; Stone et al., 2004; Liu et al., 2007). In hINO80, UCH37 is held in an inactive state; however, it can be activated by transient interaction of the INO80 complex with the proteasome or hRpn13 in a "hit-and-run" manner without disrupting its association with INO80 (Figure 3) (Yao et al., 2008; Zediak and Berger, 2008).

A new study on the structure of C-terminal extension of UCH37 by Burgie SE et al found that the crystallographic tetramer of UCH37 predicted its autoinhibition, as Helix 9 would occlude the ubiquitin binding-site. Activation appeared to be regulated in part by the C-terminal domain of UCH37 as removal of the residues 238-329 provided enhanced hydrolase activity. In the absence of BSA, UCH37 specific activity was relatively low, and was highly dependent upon the UCH37 concentration (Burgie et al., 2011). These results suggest that additives that could stabilize UCH37 solubility through a direct binding event, for example, Rpn13 or chemical modifications of UCH37 that may yield a more soluble form; for example, removal of the C-terminal tail could enhance UCH37's activity. However, these hypotheses need further verification.

The degradation way of UCH37 has not been reported yet. Whether it is degraded by lysosomal route or ubiquitin-26S proteasome system also needs further investigation.

Regulation effects

Several studies, performed both *in vivo* and *in vitro*, have suggested that UCH37 can suppress protein degradation through disassembles polyubiquitin from the distal subunit of the chain, shortening it such that poorly ubiquitinated substrates can be rescued from being degraded (Lam et al., 1997b; Husnjak et al., 2008; Koulich et al., 2008; Schreiner et al., 2008; Jacobson et al., 2009). In contrast, a recent study has suggested that UCH37 may promote the degradation of specific proteasome substrates instead, such as nitric oxide synthase and IκB-α (Mazumdar et al., 2010). Little is known in addressing these seeming contradictions. It is likely that UCH37 may inhibit the degradation of some substrates while promote the degradation of others. Recent study showed that only ubiquitinated loosely-folded proteins, after becoming bound to the 26S, interacted with Ubp6/Usp14 or Uch37 to activate ATP hydrolysis and enhance their own destruction (Peth et al., 2013).

In view of hINO80 as an ATP-dependent nucleosome remodeling complex which is involved in transcriptional regulation, possible substrates for UCH37 are the histones H_2A and H_2B and transcriptional factors. It is also possible that UCH37 activity affects nucleosome remodeling by hINO80 (Cai et al., 2007; Zediak and Berger, 2008). TGF-β (transforming growth factor-β) signals through serine/threonine kinase receptors and intracellular Smad transcription factors. An important regulatory step involves specific ubiquitination by Smurfs (Smad-ubiquitin regulatory factors), members of the HECT (homologous to E6-associated protein C-terminus) ubiquitin ligase family, which mediate the proteasomal degradation of Smads and/or receptors. The interaction between Smads and UCH37 could potentially counteract Smurf-mediated ubiquitination. Importantly, Smad7 can act as an adaptor able to recruit UCH37 to the type I TGF-βreceptor. Consequently, UCH37 dramatically up-regulates TGF-β-dependent gene expression by deubiquitinating and stabilizing the type I TGF-β receptor (Figure 2) (Wicks et al., 2005; Wicks et al., 2006). UCH37 knockdown significantly inhibits the activity of a TGF-β-dependent gene reporter and selectively decreases levels of some TGF-β-dependent target genes, notably p21, a protein which plays a key role in cell cycle arrest by preventing G1/S cell cycle progression and inhibits proliferation, and PAI-1, a scaffold protein that has been shown to act as a tumor suppressor and induce apoptosis via caspase-3, during the early phase of TGF-β receptor activation. Yet UCH37 knockdown significantly impairs cell migration through abolishes the TGF-β-induced expression of MMP-2 and PAI-1, which are thought to play a key role in TGF-β-dependent cell migration and tumor invasion, at both early and late stages of TGF-β receptor activation (Cutts et al., 2011). Thus, up-regulation of UCH37 and related DUBs observed in several cancers may play an important role in late stage of tumor development. Chen Z et al has showed that the ratio of Bax/Bcl-2 is higher in silencing of UCH37 than in that of control group after that of UCH37 in A549 cells. Meanwhile, experiments with the A549 cell line disclose that silencing of UCH37 could induce efficiently A549 cell apoptosis through activation of caspase-9 and caspase-3. On the other hand, over-expression of UCH37 leads to the opposite effect (Chen et al., 2011).

Table 3. Comparison of Three DUBs Associated with the Regulatory Particle

	RPN11	UCH37	USP14
Family belonging	JAMM family	UCH family	USP family
Cleaving site	The base of the ubiquitin chain	The distal tip of the ubiquitin chain	The distal tip of the ubiquitin chain
Deubiquitinating moment	Somewhat "late" in the reaction pathway	Commencing upon docking of the substrate to the proteasome	Commencing upon docking of the substrate to the proteasome
Result	Promoting substrate degradation	Suppressing protein degradation Promoting the degradation of specific proteasome substrates	Suppressing substrate degradation

Clinical significance

In addition to the impact of TGF-β-dependent gene expression and an important role UCH37 plays in apoptotic by altering Bax/Bcl-2 ratio and enzymatic activities of caspase-9 and caspase-3, some studies have shown its potential role in oncogenesis. Rolén et al. (2006) have found that the activity of the C-terminal hydrolases UCH37 is up-regulated in the majority of tumor tissues compared with the adjacent normal tissues. We have showed that the quantity of UCH37 rises in hepatocellular carcinoma (HCC) using a functional proteomic analysis to screen UCH37-interacting proteins in HCC, thus identifying glucose-regulated protein 78, essential for cell viability (Hirohashi et al., 2006), as one interacting with UCH37. It was also found that UCH37 was a predictor for time to recurrence of HCC. And *in vitro*, UCH37 could promote cell migration and invasion through deubiquitinating PRP19, an essential RNA splicing factor, in HCC cell lines (Fang et al., 2013). Kapuria V et al have found that WP1130, a partly selective DUB inhibitor which directly inhibits DUB activity of USP5, USP9x, USP14 and UCH37, could mediate inhibition of tumor-activated DUBs resulting in down-regulation of anti-apoptotic and up-regulation of proapoptotic proteins (Kapuria et al., 2010). The relationship between the UCH37 expression level and the outcomes of esophageal squamous cell carcinoma (ESCC) patients was found by our group recently (Chen et al., 2012). The protein expression level of UCH37 was higher in the cancer tissue than in para-tumorous tissue and was overexpressed in the tumor tissues of recurrent patients. The result of multivariate analysis also showed us that UCH37 can be a predictor for overall survival (OS) and disease-free survival (DFS) and has the potential power to predict the ESCC recurrence (Chen et al., 2012). All of the evidences suggest that the up-regulation of UCH37 maybe play an important role in oncogenesis through promoting some Proto-oncogenes' expression and stem cell-like characteristics in the cell.

It has been reported that UCH37 and Rpn13, the regulators of the proteasome pathway, also play a vital role in the mental development of mice; deleted UCH37 can result a severe defect in embryonic brain development in prenatal lethality in mice, which suggests the physiological role of UCH37 in murine development (Al-Shami et al., 2010). It will be interesting to find out whether lacking UCH37 would have significant impact on the development of other organs.

D'Arcy and his group (D'Arcy et al., 2011) discovered that b-AP15, a proteasome inhibitor that abrogates the deubiquitinating activity of the 19S regulatory particle, inhibited the activity of two 19S regulatory-particle-associated deubiquitinases, UCH37 and USP14, resulting in accumulation of polyubiquitin. The tumor progression and organ infiltration can be inhibited by the treatment of b-AP15 in four different solid tumor models in vivo and an acute myeloid leukemia model, indicating that the deubiquitinating activity of UCH37 maybe a new anticancer drug target.

Comparison with other DUBs

Substrate deubiquitination on the proteasome is mediated by three distinct deubiquitinating enzymes (DUBs) associated with the regulatory particle: RPN11, UCH37, and USP14. Here we compare the characteristics of the three DUBs (Table 3) (Lam et al., 1997b; Verma et al., 2002; Yao and Cohen, 2002; Hanna et al., 2006; Koulich et al., 2008; Jacobson et al., 2009; Lee et al., 2010; Lee et al., 2011).

An obvious question that arises is why there're two DUBs, UCH37 and USP14, which share similar enzymatic properties on the proteasome. To answer this question, we shall first know the distinctions between UCH37 and USP14. RPN11 and UCH37 have been found to discriminate strongly among different types of chain linkages, and this may be true of USP14 as well. Chain length may be equally critical. A third substrate feature that may determine USP14 susceptibility is the amenability of the substrate to the proteasome-directed unfolding (Hanna et al., 2006; Lee et al., 2010). The persistence of the proteasome-substrate interaction can be controlled by USP14 in a noncatalytic function of the protein. And USP14 can also regulate the opening of the substrate translocation channel in the CP (Peth et al., 2009). So there're three possible reasons to have both UCH37 and USP14 on the proteasome. Perhaps two DUBs on the proteasome can enhance the effect of protein degradation suppressing. And if one was inhibited, the other one can ensure the effects in the normal level. On the other hand, their substrates are mutually complementary which covers all kinds of features. Last but not least, the two DUBs may work in coordination. USP14 slowing substrate degradation allows a longer exposure of the substrate to the trimming activity by UCH37, which leads a more stable way to suppress substrate from degradation.

Future directions

Although the previous literature indicates a growing focus on deubiquitinating enzymes, the studies in this area are at a preliminary stage. UCH37, as a member of the DUBs, has received a lot of investigation on its structure, substrate, function and clinical significance. But questions

still remain to be answered, such as how the quality of UCH37 is controlled; how UCH37 is activated; what role the phosphorylation plays; what the degradation way of UCH37 is; what role UCH37 plays in INO80 complex; what the target genes are; what role UCH37 plays in oncogenesis and what the pathway of UCH37 is to induce cancer.

Conclusions

In conclusion, UCH37, a member of the deubiquitinating enzymes, can suppress protein degradation by disassembling polyubiquitin from the distal subunit of the chain. It has been proved that UCH37 can be activated with proteasome ubiqutin chain receptor Rpn13 and via their incorporation into the 19S complex. UCH37, which has been reported to assist in the mental development of mice, may play an important role in oncogenesis, tumor invasion and migration. Further studies on UCH37 will allow us to get a deep insight into cell physiology and pathology, embryonic development and tumor formation, and further support the idea that UCH37 may constitute a new interesting target for the development of anticancer drugs.

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References

Al-Shami A, Jhaver KG, Vogel P, et al (2010). Regulators of the proteasome pathway, Uch37 and Rpn13, play distinct roles in mouse development. *PLoS One*, **5**, e13654.

Baek D, Villen J, Shin C, et al (2008). The impact of microRNAs on protein output. *Nature*, **455**, 64-71.

Betel D, Wilson M, Gabow A, Marks DS, Sander C (2008). The microRNA.org resource: targets and expression. *Nucleic Acids Res*, **36**, D149-53.

Blom N, Gammeltoft S, Brunak S (1999). Sequence and structure-based prediction of eukaryotic protein phosphorylation sites. *J Mol Biol*, **294**, 1351-62.

Burgie SE, Bingman CA, Soni AB, Phillips GN, Jr. (2011). Structural characterization of human Uch37. Proteins.

Cai Y, Jin J, Yao T, et al (2007). YY1 functions with INO80 to activate transcription. *Nat Struct Mol Biol*, **14**, 872-4.

Chen X, Lee BH, Finley D, Walters KJ (2010). Structure of proteasome ubiquitin receptor hRpn13 and its activation by the scaffolding protein hRpn2. *Mol Cell*, **38**, 404-15.

Chen Y, Fu D, Xi J, et al (2012). Expression and Clinical Significance of UCH37 in Human Esophageal Squamous Cell Carcinoma. *Dig Dis Sci*, **57**, 2310-7.

Chen Z, Niu X, Li Z, et al (2011). Effect of ubiquitin carboxy-terminal hydrolase 37 on apoptotic in A549 cells. *Cell Biochem Funct*, **29**, 142-8.

Chung CH, Baek SH (1999). Deubiquitinating enzymes: their diversity and emerging roles. *Biochem Biophys Res Commun*, **266**, 633-40.

Cutts AJ, Soond SM, Powell S, Chantry A (2011). Early phase TGFbeta receptor signalling dynamics stabilised by the deubiquitinase UCH37 promotes cell migratory responses. *Int J Biochem Cell Biol*, **43**, 604-12.

D'Arcy P, Brnjic S, Olofsson MH, et al (2011). Inhibition of proteasome deubiquitinating activity as a new cancer therapy. *Nat Med*, **17**, 1636-40.

Deveraux Q, Ustrell V, Pickart C, Rechsteiner M (1994). A 26 S protease subunit that binds ubiquitin conjugates. *J Biol Chem*, **269**, 7059-61.

Fang Y, Fu D, Shen XZ (2010). The potential role of ubiquitin c-terminal hydrolases in oncogenesis. *Biochim Biophys Acta*, **1806**, 1-6.

Fang Y, Fu D, Tang W, et al (2013). Ubiquitin C-terminal Hydrolase 37, a novel predictor for hepatocellular carcinoma recurrence, promotes cell migration and invasion via interacting and deubiquitinating PRP19. *Biochim Biophys Acta*, **1833**, 559-72.

Fang Y, Mu J, Ma Y, et al (2012). The interaction between ubiquitin C-terminal hydrolase 37 and glucose-regulated protein 78 in hepatocellular carcinoma. *Mol Cell Biochem*, **359**, 59-66.

Friedman RC, Farh KK, Burge CB, Bartel DP (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*, **19**, 92-105.

Glickman MH, Ciechanover A (2002). The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev*, **82**, 373-428.

Goldberg AL (2003). Protein degradation and protection against misfolded or damaged proteins. *Nature*, **426**, 895-9.

Guterman A, Glickman MH (2004). Deubiquitinating enzymes are IN/(trinsic to proteasome function). *Curr Protein Pept Sci*, **5**, 201-11.

Hamazaki J, Iemura S, Natsume T, et al (2006). A novel proteasome interacting protein recruits the deubiquitinating enzyme UCH37 to 26S proteasomes. *EMBO J*, **25**, 4524-36.

Hanna J, Hathaway NA, Tone Y, et al (2006). Deubiquitinating enzyme Ubp6 functions noncatalytically to delay proteasomal degradation. *Cell*, **127**, 99-111.

Hershko A and Ciechanover A (1998). The ubiquitin system. *Annu Rev Biochem*, **67**, 425-79.

Hirohashi Y, Wang Q, Liu Q, et al (2006). p78/MCRS1 forms a complex with centrosomal protein Nde1 and is essential for cell viability. *Oncogene*, **25**, 4937-46.

Holzl H, Kapelari B, Kellermann J, et al (2000). The regulatory complex of Drosophila melanogaster 26S proteasomes. Subunit composition and localization of a deubiquitylating enzyme. *J Cell Biol*, **150**, 119-30.

Husnjak K, Elsasser S, Zhang N, et al (2008). Proteasome subunit Rpn13 is a novel ubiquitin receptor. *Nature*, **453**, 481-8.

Jacobson AD, Zhang NY, Xu P, et al (2009). The lysine 48 and lysine 63 ubiquitin conjugates are processed differently by the 26 s proteasome. *J Biol Chem*, **284**, 35485-94.

Kapuria V, Peterson LF, Fang D, et al (2010). Deubiquitinase inhibition by small-molecule WP1130 triggers aggresome formation and tumor cell apoptosis. *Cancer Res*, **70**, 9265-76.

Koulich E, Li X, DeMartino GN (2008). Relative structural and functional roles of multiple deubiquitylating proteins associated with mammalian 26S proteasome. *Mol Biol Cell*, **19**, 1072-82.

Lam YA, DeMartino GN, Pickart CM, Cohen RE (1997a).

Specificity of the ubiquitin isopeptidase in the PA700 regulatory complex of 26 S proteasomes. *J Biol Chem*, **272**, 28438-46.

Lam YA, Xu W, DeMartino GN, Cohen RE (1997b). Editing of ubiquitin conjugates by an isopeptidase in the 26S proteasome. *Nature*, **385**, 737-40.

Larkin MA, Blackshields G, Brown NP, et al (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, **23**, 2947-8.

Lee BH, Lee MJ, Park S, et al (2010). Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature*, **467**, 179-84.

Lee MJ, Lee BH, Hanna J, King RW, Finley D (2011). Trimming of ubiquitin chains by proteasome-associated deubiquitinating enzymes. *Mol Cell Proteomics*, **10**, R110 003871.

Li T, Duan W, Yang H, et al (2001). Identification of two proteins, S14 and UIP1, that interact with UCH37. *FEBS Lett*, **488**, 201-5.

Liu CH, Goldberg AL, Qiu XB (2007). New insights into the role of the ubiquitin-proteasome pathway in the regulation of apoptosis. *Chang Gung Med J*, **30**, 469-79.

Mazumdar T, Gorgun FM, Sha Y, et al (2010). Regulation of NF-kappaB activity and inducible nitric oxide synthase by regulatory particle non-ATPase subunit 13 (Rpn13). *Proc Natl Acad Sci U S A*, **107**, 13854-9.

Nishio K, Kim SW, Kawai K, et al (2009). Crystal structure of the de-ubiquitinating enzyme UCH37 (human UCH-L5) catalytic domain. *Biochem Biophys Res Commun*, **390**, 855-60.

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

Peth A, Kukushkin N, Bosse M, Goldberg AL (2013). Ubiquitinated proteins activate the proteasomal ATPases by binding to Usp14 or Uch37. *J Biol Chem*, **288**, 7781-90.

Pickart CM (2001). Mechanisms underlying ubiquitination. *Annu Rev Biochem*, **70**, 503-33.

Qiu XB, Ouyang SY, Li CJ, et al (2006). hRpn13/ADRM1/GP110 is a novel proteasome subunit that binds the deubiquitinating enzyme, UCH37. *EMBO J*, **25**, 5742-53.

Reese MG (2001). Application of a time-delay neural network to promoter annotation in the Drosophila melanogaster genome. *Comput Chem*, **26**, 51-6.

Rolen U, Kobzeva V, Gasparjan N, et al (2006). Activity profiling of deubiquitinating enzymes in cervical carcinoma biopsies and cell lines. *Mol Carcinog*, **45**, 260-9.

Saeki Y, Tanaka K (2008). Cell biology: two hands for degradation. *Nature*, **453**, 460-1.

Schreiner P, Chen X, Husnjak K, et al (2008). Ubiquitin docking at the proteasome through a novel pleckstrin-homology domain interaction. *Nature*, **453**, 548-52.

Stone M, Hartmann-Petersen R, Seeger M, et al (2004). Uch2/Uch37 is the major deubiquitinating enzyme associated with the 26S proteasome in fission yeast. *J Mol Biol*, **344**, 697-706.

Sulewska A, Niklinska W, Kozlowski M, et al (2007). DNA methylation in states of cell physiology and pathology. *Folia Histochem Cytobiol*, **45**, 149-58.

The PyMOL Molecular Graphics System: [http://www.pymol.org/citing], Version 1.3, http://www.pymol.org/export.

Verma R, Aravind L, Oania R, et al (2002). Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome. *Science*, **298**, 611-5.

Wang X (2008). miRDB: a microRNA target prediction and functional annotation database with a wiki interface. *RNA*, **14**, 1012-7.

Wang X, El Naqa IM (2008). Prediction of both conserved and nonconserved microRNA targets in animals. *Bioinformatics*, **24**, 325-32.

Wicks SJ, Grocott T, Haros K, et al (2006). Reversible ubiquitination regulates the Smad/TGF-beta signalling pathway. *Biochem Soc Trans*, **34**, 761-3.

Wicks SJ, Haros K, Maillard M, et al (2005). The deubiquitinating enzyme UCH37 interacts with Smads and regulates TGF-beta signalling. *Oncogene*, **24**, 8080-4.

Wilkinson KD (1997). Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. *FASEB J*, **11**, 1245-56.

Wilkinson KD (2002). Cell biology: unchaining the condemned. *Nature*, **419**, 351-3.

Wong YH, Lee TY, Liang HK, et al (2007). KinasePhos 2.0: a web server for identifying protein kinase-specific phosphorylation sites based on sequences and coupling patterns. *Nucleic Acids Res*, **35**, W588-94.

Yao T, Cohen RE (2002). A cryptic protease couples deubiquitination and degradation by the proteasome. *Nature*, **419**, 403-7.

Yao T, Song L, Jin J, et al (2008). Distinct modes of regulation of the Uch37 deubiquitinating enzyme in the proteasome and in the Ino80 chromatin-remodeling complex. *Mol Cell*, **31**, 909-17.

Yao T, Song L, Xu W, et al (2006). Proteasome recruitment and activation of the Uch37 deubiquitinating enzyme by Adrm1. *Nat Cell Biol*, **8**, 994-1002.

Zediak VP, Berger SL (2008). Hit and run: transient deubiquitylase activity in a chromatin-remodeling complex. *Mol Cell*, **31**, 773-4.

Zhou ZR, Zhang YH, Liu S, Song AX, Hu HY (2011). Length of the active-site crossover loop defines the substrate specificity of ubiquitin C-terminal hydrolases for ubiquitin chains. *Biochem J*, **441**, 143-9.