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Drosophila Model in the Study Role of UCH-L1

Dang Thi Phuong Thao

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Abstract

UCH-L1 (ubiquitin carboxyl-terminal hydrolase L1) is a protein, which plays an important role in ubiquitin-proteasome system. Many previous reports showed the relation between UCH-L1 and neurodegenerative diseases, diabetes, as well as cancer. However, the mechanism still remains unclear. In the aim to investigate the functions and regulatory mechanism of UCH-L1 in living organism, *Drosophila melanogaster* model was utilized to examine the role of UCH-L1. This chapter provides a summary on recent findings related to the roles of UCH-L1 based on the model. First, abnormal expression of *Drosophila* ubiquitin carboxyl-terminal hydrolase (dUCH) leads to the defects on fly tissue development and function. Gain function of dUCH in the eye imaginal discs induced a rough eye phenotype in the adult, partly resulting from induction of caspase-dependent apoptosis, upset of photoreceptor cell distribution and ommatidium apical mispatterning. Interestingly, the dUCH overexpression of induced rough eye phenotype was completely recused by co-expression either Sevenless or Draf of the mitogen-activated protein kinase pathway. Besides, knockdown dUCH in dopaminergic neurons resulted in some Parkinson's disease—like phenotypes in fly. Taken together, those findings in *Drosophila* model contributed a significant dUCH in tissue development and function.

Keywords: *Drosophila melanogaster*, UCH-L1, human diseases, eye development, anti-dUCH antibody

1. Introduction

Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), a protein of 223 amino acids (aa), weighs about 24,824 Da, a period lasting for more than 48 half-hour. UCH-L1 is an abundant protein in neurons, accounting for 1–2% of the total protein in the human brain [1]. In addition to the brain, UCH-L1 is also expressed strongly in the peripheral nervous system,

including sensory and nervous system activity. UCH-L1 belongs to remove the tagged enzyme (deubiquitinating enzyme (DUB)), an important protein in ubiquitin proteasome system (UPS). UCH-L1 hydrolases the peptide bond between ubiquitins and also plays a function as a ligase when it be in dimer form [2, 3]. UCH-L1 is an enzyme which binds to the polyubiquitin chains and released the single ubiquitin in the ubiquitin proteasome system. However, when UCH-L1 is in binary form, UCH-L1 leads to the formation of a polyubiquitin chain linked through lysine 63 (K63). Although the main activity of UCH-L1 is still unclear, UCH-L1 has been believed to play its role through maintaining a pool of free monomeric ubiquitin which is important for the function of ubiquitin proteasome system [4]. Abnormal function of UCH-L1 leads to the reduction of protein degradation, followed by the accumulation of ubiquitinated protein [5–7]. UCH-L1, therefore, may relate to many biological processes which dependent to ubiquitination including DNA repair, cell signalling, trafficking, endocytosis and degradation.

In 1998, a missense mutation of UCH-L1 (I93M) was first identified in a German family with Parkinson's disease (PD) [8]. By contrast, another variant of UCH-L1 (S18Y) was discovered as a factor in the risk reduction of PD [9]. Other studies also found that UCH-L1 was related to abnormal accumulation and aggregation of α-synuclein which leads to formation of Lewy bodies [3]. Furthermore, gracile axonal dystrophy (GAD) mouse which carries a deletion within UCH-L1 gene manifested motor ataxia, axonal degeneration and a reduction in the monoubiquitin level in neurons [10–12].

On the other hand, many studies indicated that UCH-L1 involved too many types of human cancer [4]. High expression of UCH-L1 was found in many types of cancers such as breast cancer, non-small cell lung cancer [13, 14]. UCH-L1 expression can be self-upregulated via oncogenic β-catenin/TCF activation. The UCH-L1 upregulates oncogenic β-catenin by which feedback regulates the expression of *uch-l1* gene [15]. UCH-L1 may also promote cancer metastasis via β-catenin-induced epithelial-to-mesenchymal transition [16, 17]. High levels of UCH-L1 may promote oncogenic transformation, invasion and metastasis, and the function of UCH-L1 might due to the enhancement of Akt signalling in vitro and in vivo [16, 18, 19].

By contrast, UCH-L1 had been also reported as a tumor suppressor in many other studies. The downregulation of UCH-L1 was observed in various types of cancer such as esophageal cancer, breast cancer, prostate cancer and pancreatic cancer [20–24]. Reduction in UCH-L1 expression leads to cell proliferation arrest and p53-mediated apoptosis [22, 25].

In humans, the gene coding for UCH-L1 is located in the short arm of chromosome 4 at position 14, from base pair 40,953,685 to 40,965,202, 11,518 base pairs long [26]. In *Drosophila melanogaster*, ubiquitin carboxyl-terminal hydrolase (dUCH) encoded by CG4265 gene is a homolog of human UCH-L1 (hUCH-L1). The identity and similarity between dUCH and hUCH-L1 are 44.5 and 75.7%, respectively. In this chapter, we provide a summary on recent findings related to the roles of UCH-L1 in living organisms by *Drosophila* models. Those findings indicated that dUCH (ortholog of human UCH-L1 in *Drosophila*) plays an important role tissue development and involves in Parkinson's disease.

2. *Drosophila* model in the study role of UCH-L1

2.1. Homolog of human ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in *Drosophila melanogaster*

The survey of the *Drosophila* genome database allowed an identification of the CG4265 as a homolog of the human UCH-L1. The CG4265 gene, named as dUCH (*Drosophila* ubiquitin carboxyl-terminal hydrolase), encodes a 224-amino-acid protein that shows 44.5% identity and 75.7% similarity with human UCH-L1. The Cys residue at amino acid (aa) position 90 and the His residue at aa 161, both of which are essential for hydrolase activity of human UCH-L1 [27–29], are conserved in *Drosophila melanogaster* along with several other species including *Mus musculus* and *Caenorhabditis elegans* (**Figure 1**).

2.2. Generation of anti-dUCH antibody

Since *Drosophila melanogaster* has been shown to be a compatible model for studying human diseases, the UCH-L1 homologous protein in *Drosophila melanogaster* (dUCH) is utilized for analyzing the role of UCH-L1 in living system. Thereby, anti-dUCH antibody is essential for research and needs to be generated. The produced anti-dUCH antibody was shown to have high specificity and sensitivity to the dUCH protein. The affinity of the antibody is 1:320,000 at 7.81 ng/µl antigen concentration. The 1:40,000 dilution-produced antibodies can detect antigen at a low concentration 0.98 ng/µl [30]. Besides, the antibody showed a high specificity for

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hUCH-L1    1 --MQLKPMEINPEMLNKVLSRLGVAGQWRFVDVLGLEEESLGSVPAPACALLLLFPLTAQ 58
mUCH-L1    1 --MQLKPMEINPEMLNKVLAKLGVAGQWRFADVLGLEEETLGSVPSPACALLLLFPLTAQ 58
dUCH       1 -MLTWTPLESNPEVLTKYIHKLGVSPAWSVTDVIGLEDDTLEWIPRPVKAFILLFPCSET 59
cUBH-1     1 MAAPWTPLESNPSVINPMIEKMGVSGVK-TVDVLFFDDESIGK---PQHAVILCFPEYKK 56

hUCH-L1   59 HENFRKKQIEELKGQEVSPKVYFMKQTIGNSCGTIGLIHAVANNQDKLGFEDGSVLKQFL 118
mUCH-L1   59 HENFRKKQIEELKGQEVSPKVYFMKQTIGNSCGTIGLIHAVANNQDKLEFEDGSVLKQFL 118
dUCH      60 HRAEEHDRIKEVEEQ-HPEDLFYMRQFTHNACGTVALIHSVANNKEV--DIDRGVLKDFL 116
cUBH-1    57 VDEIMKPIYEQ--AKAADDSVFFMKQKISNACGTFALFHSLANLEDRINLGDGSFA-KWL 113

hUCH-L1  119 SETEKMSPEDRAKCFEKNEAIQAAHDAVAQEGQCRV--DDKVNFHFILFNNVDGHLYELD 176
mUCH-L1  119 SETEKLSPEDRAKCFEKNEAIQAAHDSVAQEGQCRV--DDKVNFHFILFNNVDGHLYELD 176
dUCH     117 EKTASLSPEERGRALEKDEKFTADHEALAQEGQTNAANHEKVIHHFIALVNKEGTLYELD 176
cUBH-1   114 AEAKKVGIEERSDFLANNAELAGIHAAAATDGQTAP--SGDVEHHFICFVGKNGILYEID 171

hUCH-L1  177 GRMPFPVNHGASSEDTLLKDAAKVCREFTEREQGEVRFSAVALCKAA- 223
mUCH-L1  177 GRMPFPVNHGASSEDSLLQDAAKVCREFTEREQGEVRFSAVALCKAA- 223
dUCH     177 GRKSFPIKHGPTSEETFVKDAAKVCKEFMARDPNEVRFTVLALTAAQQ 224
cUBH-1   172 SRRPFAREIGPTSDATLVKDAGAACQHLIEKLD-NVSFSAIAVVNQ-- 216
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Figure 1. Amino acid sequences of UCH-L1 protein between human (hUCH-L1), mouse (mUCH-L1), *Drosophila* (dUCH-L1) and *C. elegans* (cUBH-L1). The identity and similarity between human and *Drosophila* were 44.5 and 75.7%, respectively. Identical amino acids are shaded in dark grey, and similar amino acids are shaded in light grey. The red letters indicate the identical amino acids at active sites. Clustal Omega (1.2.4) multiple sequence alignment was applied.

Drosophila either in Western blot or in immunostaining. When the dUCH was overexpressed in fly eye imaginal discs using the GAL4/UAS system, the dUCH protein level was specifically recognized by the anti-dUCH antibody, and the antibody sensitivity showed different levels of the dUCH target protein in *Drosophila* tissues either in Western blot or in immunostaining (**Figure 2**). Success in producing dUCH antibody provides a good material for further experiments in the study role of UCH-L1 by *Drosophila* model.

2.3. *Drosophila* model for studying the UCH-L1 role in tissue development

Being a member of ubiquitin proteasome system (UPS), UCH-L1 is thought to be involved in many different processes in living organisms, such as cell proliferation and differentiation. In *Drosophila* model, tissue-specific knockdown of dUCH resulted in abnormal phenotype in adult flies. When dUCH was knocked down in posterior area of eye imaginal discs by the combination of GMR-Gal4 driver and UAS-duchIR cassette (GMR-Gal4 > UAS-duchIR), the *duch* knocked-down adult compound eye exhibited a rough eye phenotype, and ommatidium was bulged and sticked together, while the control fly showed a normal phenotype. Knockdown dUCH in the thorax by Pnr-Gal4 driver gave hair-deformed defection. The wing of the knocked-down dUCH flies also showed some extraordinary phenotype as the vein in the wing disappeared or deformed. When TH-Gal4 drives the synthesis of *duch* dsRNA
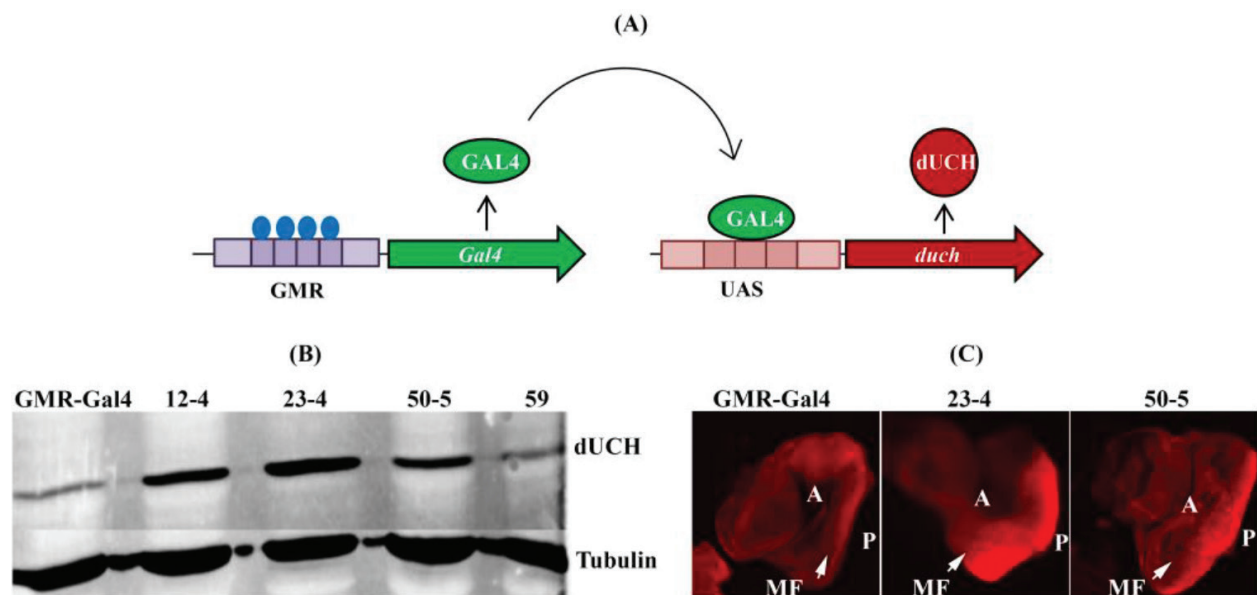

Figure 2. Generation of polyclonal anti-dUCH antibody for studying UCH-L1 function in *Drosophila melanogaster* model. (A) GAL4/UAS system is used for overexpressing dUCH protein in transgenic flies. Gal4 protein was expressed under GMR driving promoter in *Drosophila* posterior eye imaginal discs. Then, the expressed Gal4 bound to UAS element on the upstream of duch gene in transgenic *Drosophila* and caused the *duch* gene expression. (B) Western blot analysis of total protein from eye imaginal discs with polyclonal anti-dUCH antibody (above) and monoclonal anti-alpha tubulin antibody (below). GMR-Gal4: total protein from eye imaginal discs of transgenic fly, which showed dUCH endogenous protein. 12-4, 23-4, 50-5, 59: total protein from four different transgenic fly lines, which overexpresses dUCH protein under GMR-Gal4 driver. (C) Immunohistochemistry analysis on eye imaginal discs from the third instar *Drosophila melanogaster* larvae with polyclonal anti-dUCH antibody. GMR-Gal4: eye imaginal discs of transgenic fly, which showed dUCH endogenous protein. 23-4, 50-5: eye imaginal discs from two different transgenic fly lines, which overexpresses dUCH protein under GMR-Gal4 driver.

in *Drosophila* brain tissue, the third larval crawling ability was strongly defected (**Figure 3**). Emphatically, knockdown dUCH in whole bodies of the flies by Act5C-Gal4 resulted in pupal lethal effects. These observations strongly suggested that the dUCH plays an important role in maintaining normal *Drosophila* tissue development.

On the other hand, overexpression of dUCH in *Drosophila melanogaster* showed an apoptosis induction in eye imaginal discs and resulted in rough eye phenotype in adult flies. The apoptosis induction was vanished by co-expression of P35, a vacuolar viral protein that inhibits downstream effecter caspases. The apoptosis induction is followed by compensatory proliferation (**Figure 4**) [31].

Furthermore, dUCH overexpression also caused the upset in distribution of photoreceptor clusters in fly pupal retina (**Figure 5**).

In *Drosophila* pupal retinae, the ommatidia were arranged precisely. Different cell types appeared in typical shape and position. However, overexpression of dUCH in pupal retinae increased apical mispatterning. In many regions of dUCH-overexpressing retinae, ommatidia showed defects in alignment and orientation. Cone cell clusters are in different sizes and distorted. In addition, the morphology of pigment cells was aberrant. Defects in the shape and the number of primary pigment cells were detected. The shape of secondary and tertiary pigment cells (interommatidial pigment cells) was altered. In addition to the morphological
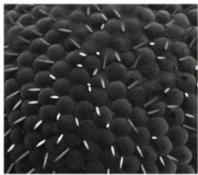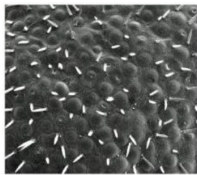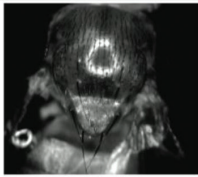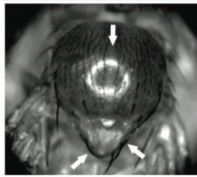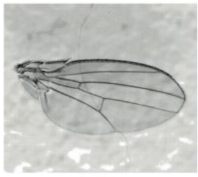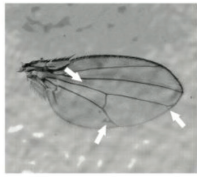

GAL4 line	Expression of GAL4	Phenotype		
		Control	Knockdown of dUCH	
Act5C	All tissues	Normal	Pupal lethal	
GMR	Cells behind the morpho-genetic furrow of eye dics			Rough eye
Pnr	Dorsal wing dics			Disappeared/ deformed some hairs in thorax
MS1096	Dorsal wing dics			Disappeared/ deformed some veins in wings
TH	Dopaminergic cells			Locomotor dysfunction in the third larvae

Figure 3. Tissue-specific knockdown of dUCH resulted in defects in adult flies.

Figure 4. Overexpression of dUCH induces caspase-dependent apoptosis in eye imaginal discs. (a–e) Scanning electron micrographs of adult compound eyes. (a'-e') Immunostaining of the eye imaginal discs with anti-active caspase-3 antibody. (a,a') GMR-GAL4; (b,b') GMR-GAL4;UAS-dUCH/+; (c,c') GMR-GAL4;UAS-dUCH/+;UAS-P35/+; (d,d')GMR-GAL4;UAS-dUCH/+;UAS-LacZ/+; (e,e') GMR-GAL4; UAS-P35/+. Note the increased number of caspase-3 positive cells (brackets) behind the morphogenetic furrow of eye discs overexpressing dUCH (b') and the lack of signals detected in eye discs co-expressing both dUCH and P35 (c'). The arrow indicates the morphogenetic furrow (MF). The bars are for 50 μm.

changes, the alignment of these cells was confused. In many regions, adjacent ommatidia were separated by more than one layer of interommatidial pigment cells. As a consequence, ommatidia in abnormal region did not maintain hexagonal shape. Bristles were misplaced, possibly due to the aberrance of pigment cells (**Figure 6**).

Interestingly, co-expressing dUCH with Sevenless or Draf in eye imaginal discs could suppress the rough eye phenotype induced by overexpressing dUCH. It is therefore likely that overexpression of dUCH downregulates the MAPK pathway, resulting in impairment of eye development (**Figure 7**) [31].

2.4. *Drosophila* model for studying the UCH-L1 role in Parkinson's disease

UCH-L1 was first linked to PD when mutation UCH-L1I93M was found in two siblings from a family with autosomal dominant PD [8]. Transgenic mice that overexpression of UCH-L1I93M showed an accumulation of α-synuclein with ubiquitin in the brain [3]. UCH-L1-deficient
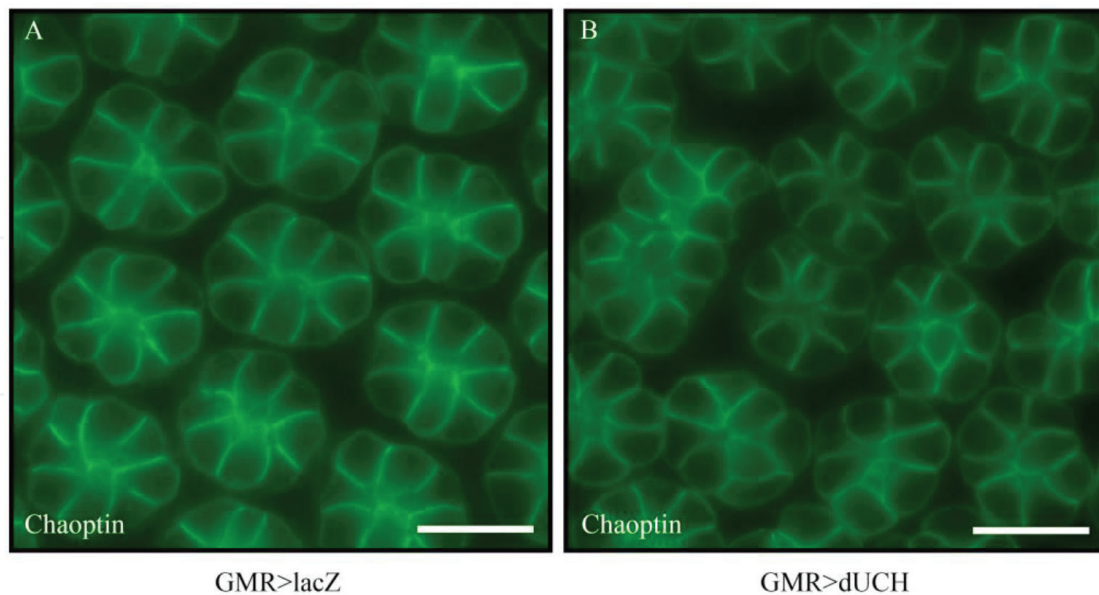
Figure 5. Immunostaining of retinae at 42 h after puparium formation (APF) with anti-chaoptin antibody. (A) Control retina and (B) dUCH-overexpressing retina. The bars indicate 10 μm.

mice showed neuronal loss in the spinal gracile tract and exhibit early development sensory and progressive motor ataxia [7]. However, another mutation UCH-L1S18Y is dedicated that decreased rick in PD by antioxidant and neuron-protective function [32]. Therefore, the mechanism of UCH-L1 still remains unclear. In *Drosophila* model, specific knockdown dUCH in dopaminergic neuron caused a degeneration of DA neurons and resulted in locomotor dysfunctions (**Figures 8** and **9**).

2.5. Materials and methods

2.5.1. Fly stocks

Fly stocks were maintained at 25°C on standard food containing 0.7% agar, 5% glucose and 7% dry yeast. Wild-type strain Canton-S was obtained from the Bloomington *Drosophila* Stock Center (BDSC). RNAi lines carrying UAS-dUCH-IR fusion (GD#26468) for knockdown *Drosophila* ubiquitin carboxyl-terminal hydrolase (dUCH, CG4265) were received from the Vienna *Drosophila* Resource Center (VDRC). GAL4 drivers were used to perform the targeted knockdown of dUCH in various tissues of *D. melanogaster*: Act5C-GAL4 (BDSC#3954), GMR-GAL4 (line #16), MS1096-GAL4 (BDSC#8860), pnr-GAL4 (BDSC#3039) and TH-GAL4 (BDSC#8848).

2.5.2. Western immunoblot analysis

Wild-type and transgenic adult flies carrying GMR-GAL4 > UAS-dUCH were frozen in liquid nitrogen and homogenized in a solution containing 50 mM Tris-HCl (pH 7.5); 5 mM $MgCl_2$; 150 mM NaCl; 10% glycerol; 0.1% Triton X-100; 0.1% NP-40; 1 mM phenylmethylsulfonyl fluoride; 5 mM β-mercaptoethanol; 10 g/ml each of aprotinin, leupeptin and pepstatin A; and 1 g/ml each of antipain, chymostatin and phosphoramidon. Homogenates were centrifuged, and extracts (200 g of protein) were electrophoretically separated on SDS-polyacrylamide gels containing 10%
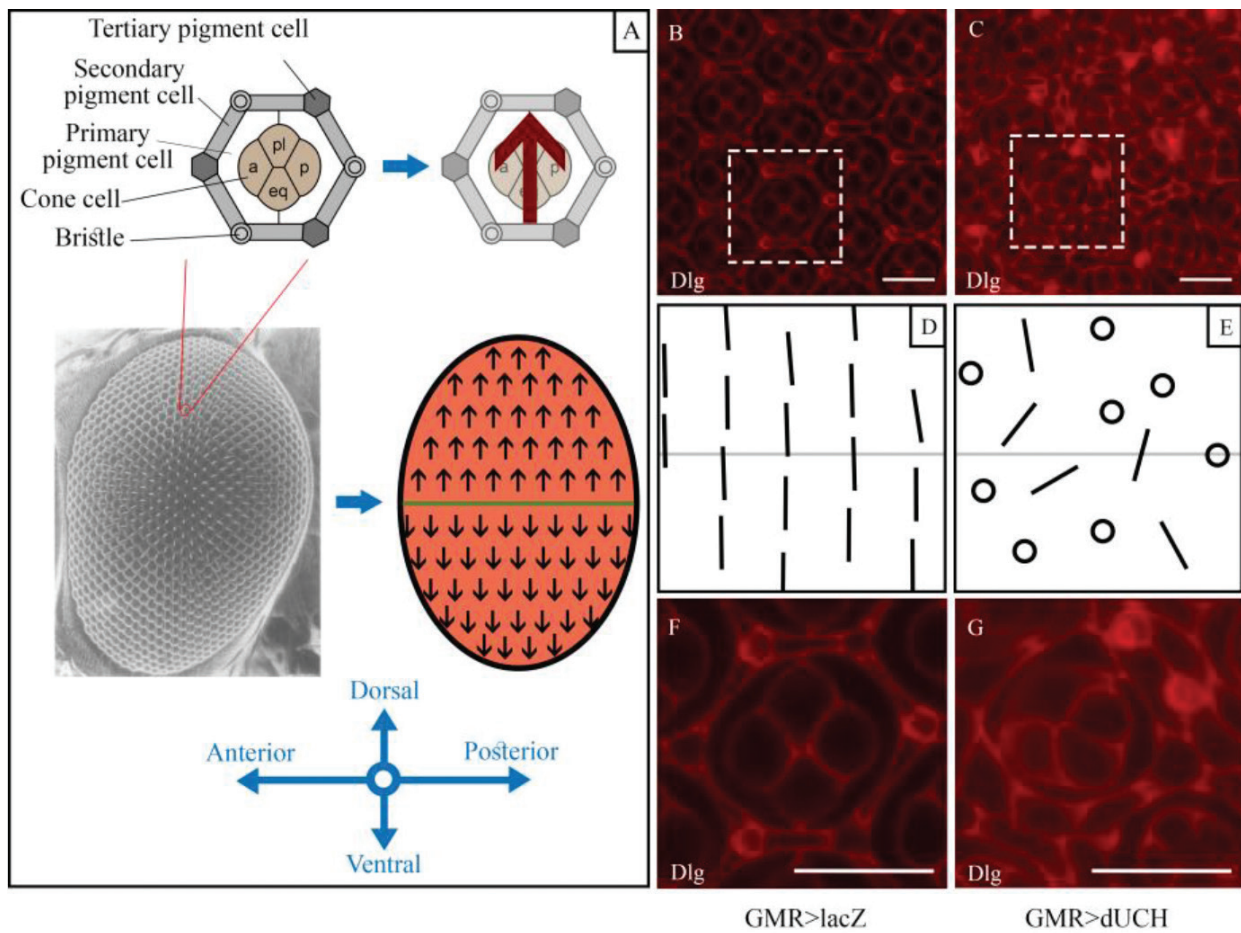
Figure 6. Overexpression of dUCH-induced apical mispatterning of 42 h APF retinae. (A) Normal adult *Drosophila* eye schematically representing orientation of the ommatidia with the green line representing the equator and schematically representing cross-sectional structure of a pupal ommatidium at the apical level with a, anterior cone cell; p, posterior cone cell; pl., polar cone cell; eq, equatorial cone cell. Red arrow marks equatorial-polar axis. (B-C) Immunostaining of retinae at 42 h APF with anti-Dlg antibody, (B) control retina and (C) dUCH-overexpressing retina. (D-E) Diagrams show orientation of the ommatidia in control fly (D) and dUCH-overexpressing fly (E). Black segments represent apical orientation of the ommatidia, black circles represent unclear cases and grey lines represent the anterior-posterior axis of the retinae. (F-G) The magnification of the ommatidia in control fly (F) and dUCH-overexpressing fly (G). Bars in all figures indicate 10 μm.

acrylamide and then transferred to polyvinylidene difluoride membranes (Bio-Rad). The blotted membranes were blocked with TBS/0.05% Tween-20 containing 5% skim milk for 1 h at 25°C, followed by incubation with rabbit polyclonal anti-dUCH at 1:1000 dilution or mouse monoclonal anti-α tubulin (Developmental Studies Hybridoma Bank (DSHB)) at 1:5000 dilution for 16 h at 4°C. After washing, the membranes were incubated with HRP-conjugated secondary antibodies (GE Healthcare Bioscience) at 1:10,000 dilution for 1 h at 25°C. Detection was performed with ECL Western blotting detection reagents (GE Healthcare Bioscience), and images were analyzed with a Lumivision Pro HSII image analyzer (Aisin Seiki).

2.5.3. Immunostaining

Larval and adult brains were dissected in cold phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde at 25°C for 15 min. After washing with 0.3% PBS-T (PBS containing 0.3% Triton-X100) twice, the samples were blocked in blocking solution (0.15% PBS-T containing
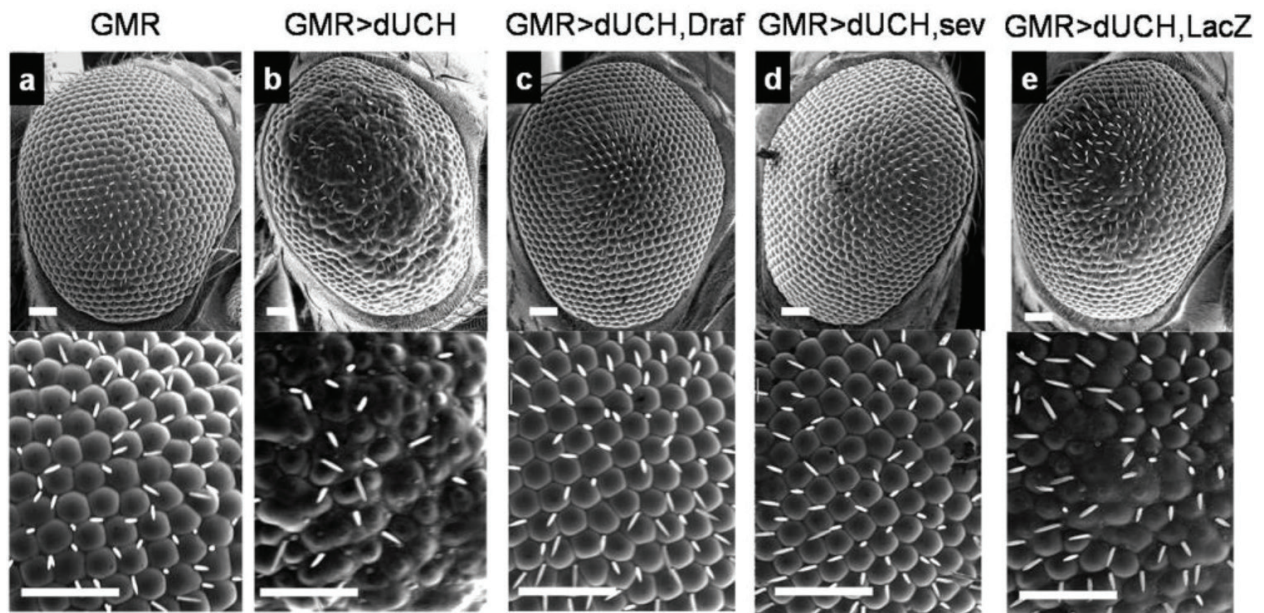
Figure 7. Suppression of the dUCH-induced rough eye phenotype by co-expression of sev or Draf. (a) GMR-GAL4;+; (b) GMR-GAL4;UAS-d;CH/+; (c) GMR-GAL4;UAS-dUCH/+;hsp-Draf/+; (d) GMR-GAL4/hsp-sev;UAS-dUCH/+; (e) GMR-GAL4;+;UAS-LacZ/+. Magnifications are 200× for the upper and 700× for the lower panels. Flies were reared at 28°C. The bars indicate 50 μm.

10% normal goat serum) at 25°C for 20 min. Samples were then incubated with the following primary antibodies diluted in blocking solution: rabbit anti-*Drosophila* ubiquitin carboxyl-terminal hydrolase (anti-dUCH; 1:500) at 4°C for 16 h or rabbit anti-tyrosine hydroxylase (anti-TH; 1:250; Millipore, AB152) at 4°C for 20 h. After washing with 0.3% PBS-T, samples were incubated with secondary antibodies conjugated with Alexa 488 or FITC (1500, Invitrogen) at 25°C for 2 h and then washed and mounted in VECTASHILED Antifade Mounting Medium (Vector Laboratories, Japan). Finally, the samples were inspected by a confocal laser scanning microscope (Olympus FluoView FV10i or Olympus BX41 Microscope).

2.5.4. Crawling assay

Male larvae in the early third instar stage were collected randomly and washed with PBS to discard food traces. After that, larvae were transferred to agar plates containing 2% agar with a density of 2–4 larvae per plate. The movement of larvae was recorded by a digital camera for 60 s. The recorded videos were then converted into AVI type by MOV to AVI converter (Pazera Jacek, Poland) and then analyzed by ImageJ (NIH, USA) with wrMTrck plugin (developed by Dr. Jesper Søndergaard Pedersen) to track larval movement and draw motion paths.

2.5.5. Climbing assay

Newly eclosed adult male flies were collected and transferred to conical tubes which have heights of 15 cm and diameters of 2 cm. After that, the tubes were tapped to collect the flies to the bottom, and the length of time to record the movement of flies was 30 s. The procedures were repeated five times and recorded by a digital camera. For all of the climbing experiments, the height which each fly climbed to was scored as follows: 0 (less than 2 cm),
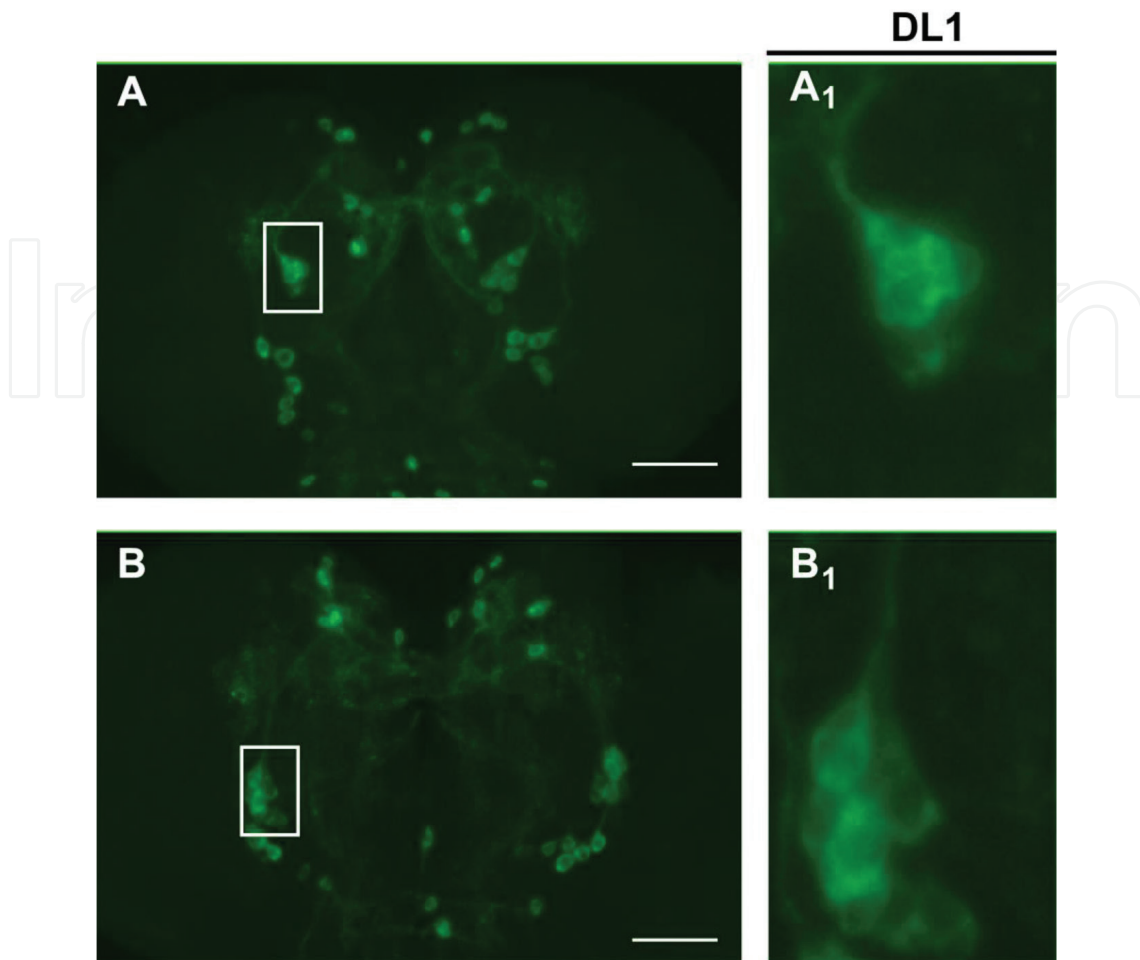
Figure 8. Loss of DL1 dopaminergic (DA) neurons in dUCH knockdown brain lobe. DA neuron clusters in the third instar larval were stained by anti-tyrosine hydroxylase antibody (anti-TH (green)). (A) Whole brain lobe with DA clusters in dUCH knockdown fly: TH-GAL4/UAS-dUCH-IR (TH > dUCH-IR). (A1) The magnification of DL1 DA cluster in knockdown fly brain lobe. (B) Whole brain lobe with DA clusters in control fly: TH-GAL4/+. (B1) The magnification of DL1 DA cluster in control fly brain lobe.

1 (between 2 and 4 cm), 2 (between 4 and 6 cm), 3 (between 6 and 8 cm), 4 (between 8 and 10 cm) and 5 (more than 10 cm). The climbing assay was performed every 5 days until all flies lose their locomotor abilities.

2.5.6. Conclusion and perspective

UCH-L1 was known as a complex and unclear function protein. It has several irrelevant activities as hydrolase and ligase, which are also related to ubiquitin. Previous reports showed that abnormal UCH-L1 functioning, caused by mutations or change in levels of protein expression. Those reports also implied that UCH-L1 could have many negative effects, with impacts on cell proliferation, cell cycling and cell death through activation of many genes [33, 34]. In this chapter, some data compatibly demonstrated that overexpression of dUCH, a homolog of human UCH-L1 in *Drosophila melanogaster*-induced apoptosis, interfered eye development by upset distribution of photoreceptor cell distribution and caused
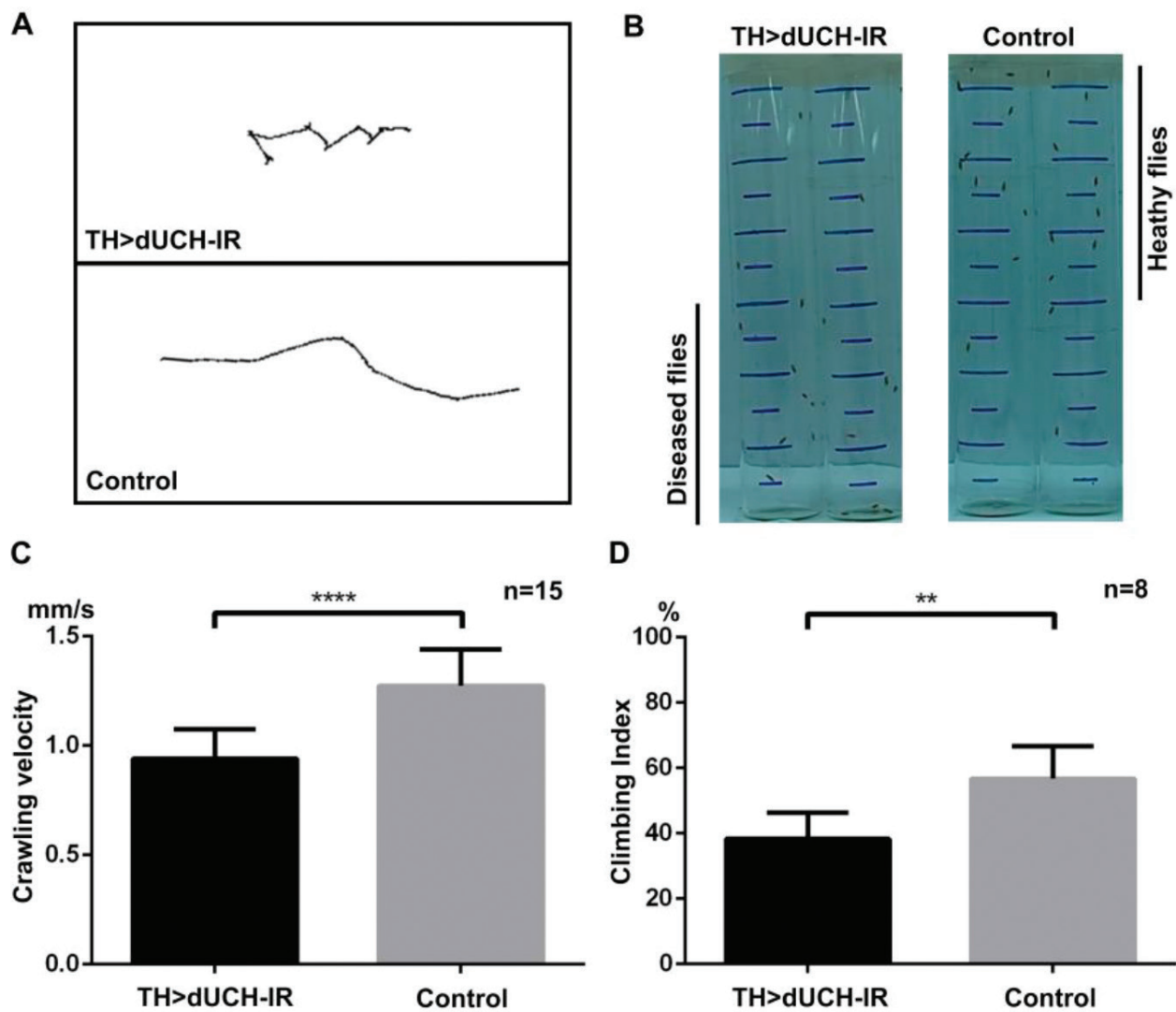
Figure 9. The dysfunction in locomotor in dopaminergic neuron-specific dUCH knockdown flies. (A) Motion paths of larvae: control and dUCH knockdown larvae (TH > dUCH-IR). Knockdown larvae exhibit shorter and disorder crawling paths (upper panel) compared to control (below panel). (B) Climbing assay for measurement of adult fly locomotor ability. (C) Crawling velocity of control (TH) and knockdown larvae (TH > dUCH-IR). Knockdown larvae showed the reduction in crawling pace and parametric unpaired t test with Welch's correction, ****p < 0.0001; error bars present SD. (D) Climbing ability of control (TH) and dUCH knockdown adult flies (TH > dUCH-IR). Knockdown flies start to exhibit the decline in climbing ability at 5 days after eclosion, repeatedly measuring two way ANOVA with Bonferroni's post hoc test, **p < 0.01; error bars present SEM.

apical mispatterning in ommatidium. The effects of dUCH overexpression may involve in mitogen-activated protein kinase pathway. On the other hand, knockdown dUCH resulted in defect of tissue development and function. Particularly, knockdown dUCH in dopaminergic neuron impaired fly locomotion and degenerated dopaminergic neurons. Besides the *Drosophila* model's benefits, as well as the correlation between *Drosophila* UCH (dUCH) and human UCH (UCH-L1), these data strongly demonstrated that *Drosophila melanogaster* is an advantage model to investigate the functions and regulatory mechanism of UCH-L1 in living organism.

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References

[1] Wilkinson KD, Lee KM, Deshpande S, Duerksen-Hughes P, Boss JM, et al. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. Science. 1989;**246**:670-673

[2] Larsen CN, Krantz BA, Wilkinson KD. Substrate specificity of deubiquitinating enzymes: ubiquitin C-terminal hydrolases. Biochemistry. 1998;**37**(10):3358-3368

[3] Liu Y, Fallon L, Lashuel HA, Liu Z, Lansbury PT Jr. The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. Cell. 2002;**111**(2):209-218

[4] Hurst-Kennedy J, Chin LS, Li L. Ubiquitin C-terminal hydrolase L1 in tumorigenesis. Biochemistry Reaserch International. 2012;**2012**:123706

[5] Tan YY, Zhou HY, Wang ZQ, Chen SD. Endoplasmic reticulum stress contributes to the cell death induced by UCH-L1 inhibitor. Molecular and Cellular Biochemistry. 2008;**318**(1-2):109-115

[6] Bifsha P, Landry K, Ashmarina L, Durand S, Seyrantepe V, Trudel S, Quiniou C, Chemtob S, Xu Y, Gravel RA, Sladek R, Pshezhetsky AV. Altered gene expression in cells from patients with lysosomal storage disorders suggests impairment of the ubiquitin pathway. Cell Death and Differentiation. 2007;**14**(3):511-523

[7] Saigoh K, Wang YL, Suh JG, Yamanishi T, Sakai Y, Kiyosawa H, Harada T, Ichihara N, Wakana S, Kikuchi T, Wada K. Intragenic deletion in the gene encoding ubiquitin carboxy-terminal hydrolase in gad mice. Nature Genetics. 1999;**23**(1):47-51

[8] Leroy E et al. The ubiquitin pathway in Parkinson's disease. Nature. 1998;**395**:451-452. DOI: 10.1038/26652

[9] Lincoln S et al. Low frequency of pathogenic mutations in the ubiquitin carboxy-terminal hydrolase gene in familial Parkinson's disease. Neuroreport. 1999;**10**:427-429

[10] Yamazaki K, Wakasugi N, Tomita T, Kikuchi T, Mukoyama M, et al. Gracile axonal dystrophy (GAD), a new neurological mutant in the mouse. Proceedings of the Society for Experimental Biology and Medicine. 1988;**187**:209-215

[11] Ichihara N, Wu J, Chui DH, Yamazaki K, Wakabayashi T, et al. Axonal degeneration promotes abnormal accumulation of amyloid beta-protein in ascending gracile tract of gracile axonal dystrophy (GAD) mouse. Brain Research. 1995;**695**:173-178

[12] Osaka H, Wang YL, Takada K, Takizawa S, Setsuie R, et al. Ubiquitin carboxy-terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron. Human Molecular Genetics. 2003;**12**:1945-1958

[13] Miyoshi Y, Nakayama S, Torikoshi Y, Tanaka S, Ishihara H, Taguchi T, Tamaki Y, Noguchi S. High expression of ubiquitin carboxy-terminal hydrolase-L1 and -L3 mRNA predicts early recurrence in patients with invasive breast cancer. Cancer Science. 2006;**97**(6):523-529

[14] Hibi K, Westra WH, Borges M, Goodman S, Sidransky D, Jen J. PGP9.5 as a candidate tumor marker for non-small-cell lung cancer. The American Journal of Pathology. 1999;**155**(3):711-715

[15] Bheda A, Shackelford J, Pagano JS. Expression and functional studies of ubiquitin C-terminal hydrolase L1 regulated genes. PLoS One. 2009;**4**(8):e6764

[16] Jang MJ, Baek SH, Kim JH. UCH-L1 promotes cancer metastasis in prostate cancer cells through EMT induction. Cancer Letters. 2011;**302**(2):128-135

[17] Kim K, Lu Z, Hay ED. Direct evidence for a role of beta-catenin/LEF-1 signaling pathway in induction of EMT. Cell Biology International. 2002;**26**(5):463-476

[18] Ma Y, Zhao M, Zhong J, Shi L, Luo Q, Liu J, Wang J, Yuan X, Huang C. Proteomic profiling of proteins associated with lymph node metastasis in colorectal cancer. Journal of Cellular Biochemistry. 2010;**110**(6):1512-1519

[19] Hussain S, Foreman O, Perkins SL, Witzig TE, Miles RR, van Deursen J, Galardy PJ. The de-ubiquitinase UCH-L1 is an oncogene that drives the development of lymphoma in vivo by deregulating PHLPP1 and Akt signaling. Leukemia. Official Journal of the Leukemia Society of America, Leukemia Research Fund, U.K. 2010;**24**(9):1641-1655

[20] Tokumaru Y, Yamashita K, Kim MS, Park HL, Osada M, Mori M, Sidransky D. The role of PGP9.5 as a tumor suppressor gene in human cancer. International Journal of Cancer. Journal International du Cancer. 2008;**123**(4):753-759

[21] Takase T, Hibi K, Yamazaki T, Nakayama H, Taguchi M, Kasai Y, Ito K, Akiyama S, Nagasaka T, Nakao A. PGP9.5 overexpression in esophageal squamous cell carcinoma. Hepato-Gastroenterology. 2003;**50**(53):1278-1280

[22] Xiang T, Li L, Yin X, Yuan C, Tan C, Su X, Xiong L, Putti TC, Oberst M, Kelly K, Ren G, Tao Q. The ubiquitin peptidase UCHL1 induces G0/G1 cell cycle arrest and apoptosis through stabilizing p53 and is frequently silenced in breast cancer. PLoS One. 2012; **7**(1):e29783

[23] Ummanni R, Jost E, Braig M, Lohmann F, Mundt F, Barett C, Schlomm T, Sauter G, Senff T, Bokemeyer C, Sultmann H, Meyer-Schwesinger C, Brummendorf TH, Balabanov S. Ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) is a potential tumour suppressor in prostate cancer and is frequently silenced by promoter methylation. Molecular Cancer. 2011;**10**:129

[24] Sato N, Fukushima N, Maitra A, Matsubayashi H, Yeo CJ, Cameron JL, Hruban RH, Goggins M. Discovery of novel targets for aberrant methylation in pancreatic carcinoma using high-throughput microarrays. Cancer Research. 2003;**63**(13):3735-3742

[25] Caballero OL, Resto V, Patturajan M, Meerzaman D, Guo MZ, Engles J, Yochem R, Ratovitski E, Sidransky D, Jen J. Interaction and colocalization of PGP9.5 with JAB1 and p27(Kip1). Oncogene. 2002;**21**(19):3003-3010

[26] Meray RK, Lansbury PT Jr. Reversible monoubiquitination regulates the Parkinson disease-associated ubiquitin hydrolase UCH-L1. The Journal of Biological Chemistry. 2007;**282**(14):10567-10575

[27] Larsen CN, Price JS, Wilkinson KD. Substrate binding and catalysis by ubiquitin C-terminal hydrolases: Identification of two active site residues. Biochemistry. 1996; **35**(21):6735-6744

[28] Case A, Stein RL. Mechanistic studies of ubiquitin C-terminal hydrolase L1. Biochemistry. 2006;**45**(7):2443-2452

[29] Boudreaux DA, Maiti TK, Davies CW, Das C. Ubiquitin vinyl methyl ester binding orients the misaligned active site of the ubiquitin hydrolase UCHL1 into productive conformation. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:9117-9122. DOI: 10.1073/pnas.0910870107

[30] Tram NTQ, Trang NTT, Thao DTP, Thuoc TL. Production of polyclonal anti-dUCH (drosophila ubiquitin carboxyl-terminal hydrolase) antibodies. Monoclonal Antibodies in Immunodiagnosis and Immunotherapy. 2013;**32**(2):105-112

[31] Thao DT, An PN, Yamaguchi M, LinhThuoc T. Overexpression of ubiquitin carboxyl terminal hydrolase impairs multiple pathways during eye development in Drosophila melanogaster. Cell and Tissue Research. 2012;**348**(3):453-463

[32] Ragland M, Hutter C, Zabetian C, Edwards K. Association between the ubiquitin carboxyl-terminal esterase L1 gene (UCHL1) S18Y variant and Parkinson's Disease: A HuGE review and meta-analysis. American Journal of Epidemiology. 2009;**170**:1344-1357. DOI: 10.1093/aje/kwp288

[33] Chu KY, Li H, Wada K, Johnson JD. Ubiquitin C-terminal hydrolase L1 is required for pancreatic beta cell survival and function in lipotoxic conditions. Diabetologia. 2012; **55**:128-140

[34] Kabuta T, Mitsui T, Takahashi M, Fujiwara Y, Kabuta C, et al. Ubiquitin C-terminal hydrolase L1 (UCH-L1) acts as a novel potentiator of cyclin-dependent kinases to enhance cell proliferation independently of its hydrolase activity. The Journal of Biological Chemistry. 2013;**288**:12615-12626