Biochemical and Biophysical Research Communications 415 (2011) 24–29

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The serine protease HtrA2 cleaves UCH-L1 and inhibits its hydrolase activity: Implication in the UCH-L1-mediated cell death

Dae-Wook Park [1], Min-Kyung Nam [1], Hyangshuk Rhim *

Department of Biomedical Sciences, College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea
Department of Medical Life Sciences, College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea

ARTICLE INFO

Article history:
Received 24 September 2011
Available online 6 October 2011

Keywords:
HtrA2
Serine protease
UCH-L1 cleavage
UCH-L1 hydrolase inhibition
PD-associated genes
Tumor cell death

ABSTRACT

Ubiquitin (Ub) carboxyl-terminal hydrolase L1 (UCH-L1) has dual functions, such as hydrolase activity on the chemical bonds formed by the C-terminal Gly of Ub and dimerization-dependent ubiquitin ligase activity. Accumulating evidence suggests that dual activities of UCH-L1 were intimately associated with Parkinson's diseases (PD) and cancer. However, the molecular mechanism that regulates UCH-L1 enzymatic activity has not yet been fully elucidated. The serine protease high temperature requirement A2 (HtrA2), a PD-associated gene, is important in regulating cell survival as well as apoptosis. Using in vitro and in vivo cleavage assays, we have demonstrated that UCH-L1 is a natural substrate for the serine protease HtrA2 in the apoptotic pathway. Notably, we show that released, cytosolic HtrA2 decreases UCH-L1 protein level and its hydrolase activity through HtrA2-mediated cleavage of UCH-L1 under apoptotic conditions. These findings suggest that the HtrA2-mediated cleavage of UCH-L1 may play important roles in regulating the fine balance between cell growth and cell death.

1. Introduction

Ubiquitin (Ub) carboxyl-terminal hydrolase L1 (UCH-L1) is a cysteine protease and belongs to the family of UCHs that catalyze the hydrolysis of amides, ester, and thioester bonds formed by the C-terminal Gly of Ub [1]. In terms of its hydrolase activity, several previous studies have shown that UCH-L1 plays important roles in generating and stabilizing the free Ub monomers, allowing the Ub monomers to be recycled [2–4]. On the other hand, UCH-L1 has an opposing enzymatic activity of its hydrolase activity, which is a dimerization-dependent Ub ligase activity that can catalyze the joining of Ub monomers by forming a new chemical bond between the C-terminus of Ub with K63 of the other Ub [5].

Several recent studies suggest that UCH-L1 plays important roles in cancer [6–8]. A previous genome-wide expression profiling and biochemical study has described that the *UCH-L1* gene was frequently silenced in all nasopharyngeal carcinoma and suggested that UCH-L1 may serve as a tumor suppressor by interacting with and stabilizing a canonical tumor suppressor p53 by deubiquitinating p53 [9]. As an additional evidence for the tumor suppressor function of UCH-L1, its overexpression induces apoptosis in breast cancer cells [10]. On the contrary, a recent transgenic mouse study provides the first in vivo evidence that UCH-L1 has oncogenic properties in the development of lymphoma through activating the Akt pathway [11]. Identification of the role of UCH-L1 in tumorigenesis has been complicated by its opposite functions that it is capable of acting both as a tumor suppressor as well as an oncogene, which can act differentially depending upon types and growth stages of tumors [12,13]. Nevertheless, these studies suggest that UCH-L1 may play a critical role in regulating the optimal balance between cell death and cell survival; however, the molecular mechanism that regulates UCH-L1 protein level and its enzymatic activity has not yet been fully elucidated.

The high temperature requirement A2 (HtrA2) has been known as a Parkinson's disease (PD)-associated serine protease, which is localized in the mitochondria under normal conditions; however, the processed, mature HtrA2 is released into the cytosol in response to various cellular stresses [14]. This released cytosolic HtrA2 promotes cell death via two different mechanisms: a caspase-independent manner through its ability to function as a serine protease as well as a caspase-dependent manner by antagonizing XIAP-mediated caspase-3 cascade [15]. To date, several studies have delineated the substrates of HtrA2, such as XIAP, HAX-1, APP, WTS and WT1, and characterized their functions in regulating key cellular processes, such as cell proliferation, survival, and cell death, through the HtrA2 proteolytic system [16–20]. In our previous study, we have shown that the serine protease HtrA2 directly cleaves one of PD-associated proteins, E3 ubiquitin ligase Parkin, and thus inhibits its ligase activity [21]. In this context, the enzyme–substrate relationship between HtrA2

* Corresponding author at: Department of Biomedical Sciences, College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea. Fax: +82 2 594 2385.
E-mail address: hrhim@catholic.ac.kr (H. Rhim).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.09.148

and UCH-L1, also known as a PD-associated protein [22], was investigated. Here, we have demonstrated that UCH-L1 is a natural substrate for the serine protease HtrA2 in the apoptotic pathway and that HtrA2-mediated cleavage of UCH-L1 impaired its hydrolase activity. Our findings suggest that the HtrA2 and UCH-L1 enzyme–substrate pair may be critical regulatory factors for maintaining a balance between cell death and cell survival.

2. Materials and methods

Details regarding reagents, plasmid construction, cell culture, lactate dehydrogenase (LDH) release assay, immunoblot analyses, immunofluorescence assay, GST fusion protein purification are provided in Supplementary data.

2.1. In vitro cleavage assay

In vitro HtrA2 cleavage assay for UCH-L1 was performed as follows; GST–UCH-L1 (1 μM) was incubated with GST–HtrA2 (10 μM) in a final 100 μl of cleavage buffer [50 mM Tris–HCl (pH 7.5), 1 mM DTT] for 16 h at 37 °C. After reaction, the supernatant was separated by centrifugation and resolved by 13% SDS–PAGE, and the cleaved products were visualized by staining with Coomassie Brilliant Blue dye and by IB assay with anti-UCH-L1.

The cleaved UCH-L1 fragments were transferred electrophoretically onto a polyvinylidene difluoride (PVDF) membrane, and the transferred fragments were stained with Ponceau S solution. A 14-kDa fragment was excised from the membrane, and its N-terminal amino acid residues were identified by using the Procise 491 protein sequencer (Applied Biosystems, Korea Basic Science Institute).

2.2. Measurement of UCH-L1 hydrolase activity

UCH-L1 hydrolase assay was performed as described in a previous study [5] with a slight modification. In vitro reaction mixtures or cell lysates were added to the UCH-L1 substrate solution containing 125 nM of the fluorogenic substrate ubiquitin C-terminal 7-amido-4-methylcoumarin (Ub-AMC) in 100 μl of hydrolysis buffer [50 mM Tris–HCl (pH 7.5), 0.5 mM EDTA, 5 mM DTT], and the cleavage reaction of AMC from Ub-AMC was carried out using a 96-well black assay plate (Greiner Bio-One) at RT. The hydrolase activity of UCH-L1 was determined by measuring the rate of conversion of Ub-AMC to ubiquitin and free AMC for 5 min by fluorescence emission at 460 nm (λ_{ex} = 380 nm) using Synergy Mx monochromator-based multi-mode microplate reader (BioTek). Values are means ± SEM of three independent experiments and are expressed in arbitrary fluorescence unit (AFU).

3. Results and discussion

3.1. Loss of UCH-L1 function induces cell death

A previous study has shown that inhibition of UCH-L1 hydrolase activity induces the aggregation of ubiquitin-conjugated proteins and thus enhances cell death in primary neurons [23]. Moreover, a recent study reported that LDN-57444 (LDN), an inhibitor of UCH-L1 hydrolase activity, induces cell death through ER stress-mediated accumulation of ubiquitinated proteins in neuroblastoma cells [24]. These findings raise the possibility that UCH-L1 may be involved in regulating cell survival and cell death. To examine the effects of the UCH-L1 hydrolase activity on cell death, we selected two distinct human ovarian carcinoma cell lines, UCH-L1-negative OVCAR-5 and UCH-L1-positive OVCAR-8, as UCH-L1 is expressed exclusively in the ovary as well as in the brain [2] (Fig. 1A). We measured the amounts of LDH released from cells treated with LDN, as its release reflects a loss of the integrity of the cell membrane. In both cell types with or without UCH-L1, there were no significant increases of LDH release in cells treated with genipin used as a negative control, comparable to those treated with DMSO as a vehicle control. Notably, UCH-L1-positive OVCAR-8 cells showed a 7-fold increase in LDH release in response to LDN, but only a 2-fold increase in UCH-L1-negative OVCAR-5 cells, compared with control. In addition, the LDN-treated OVCAR-8 cells exhibited dramatic morphological changes. These results demonstrate that the UCH-L1 hydrolase activity in UCH-L1-positive cancer cells plays a key role in induction of cell death and provide evidence that its activity is required for the regulation of cell survival.

3.2. HtrA2 directly cleaves UCH-L1 and inhibits its hydrolase activity in vitro

Loss of the UCH-L1 function, specially its hydrolase activity, is known to impair ubiquitin proteasome system (UPS) [24]. Notably, we presented that this UCH-L1 inactivation caused toxicity in UCH-L1-positive cells (Fig. 1A, Supplementary Fig. 1). However, the molecular mechanism by which the UCH-L1 hydrolase activity is regulated in the apoptotic pathway still remains largely unknown. We have previously shown that two PD-associated proteins, serine protease HtrA2 and E3 ubiquitin ligase Parkin, serve as the physiologically relevant enzyme–substrate pair in the cytosol through the release of HtrA2 from the mitochondria upon apoptotic stimulus, such as staurosporine (STS), a potent protein kinase inhibitor [21]. Consistent with our previous study, we also found that endogenous level of the UCH-L1, which has been originally known as a PD-associated protein, was markedly decreased in HEK293 cells under STS-induced apoptosis (Fig. 1B). We therefore postulate that UCH-L1 would be intimately associated with the HtrA2-dependent proteolytic system. To test this notion, we investigated whether HtrA2 can cleave UCH-L1 by incubating both HtrA2 and UCH-L1 in vitro (Fig. 2). The in vitro cleavage reaction was initiated with both GST-fusion proteins bound to glutathione Sepharose 4B beads to more easily discriminate the cleaved UCH-L1 fragments from the reaction sample. After completion of the reaction, the supernatant (sup) was separated from the protein-bound glutathione beads (ppt) by centrifugation and analyzed by staining with Coomassie Brilliant Blue dye (Fig. 2A). The level of UCH-L1 protein was decreased in the presence of proteolytically active HtrA2 serine protease (lane 4), but not in the protease-inactive mutant S306A (lane 5) and UCH-L1 alone (lane 1). Notably, we detected a fragment with apparent molecular mass of about 14 kDa (lane 4). To identify the specificity of the 14-kDa fragment, the reaction samples shown in Fig. 2A were analyzed by IB assay with the Ab that specifically recognizes UCH-L1 (Fig. 2B). The 14-kDa fragment was specifically recognized by anti-UCH-L1 Ab, and additional two faint bands with molecular masses of 11 and 10 kDa were also detected. However, it was hard to detect any UCH-L1 fragments from the ppt fraction by using IB assay with UCH-L1 Ab (data not shown). Because the sup fraction was separated from GST-fusion proteins bound to glutathione Sepharose 4B beads, these immuno-specific fragments is likely to be derived through endoproteolytic cleavage at the C-terminal region of UCH-L1.

Subsequently, to identify the cleavage site in UCH-L1, we analyzed N-terminal amino acid sequence of the 14 kDa UCH-L1 fragment (Supplementary Fig. 2). Although the N-terminal amino acid sequencing result was slightly ambiguous, showing XANQX, we can infer that the valine (V99) at position 99 appears to be a putative amino acid residue on the N-terminal side of the cleavage site in UCH-L1 based on both the sequencing result and molecular mass of the cleaved fragment. Taken together, the results indicate

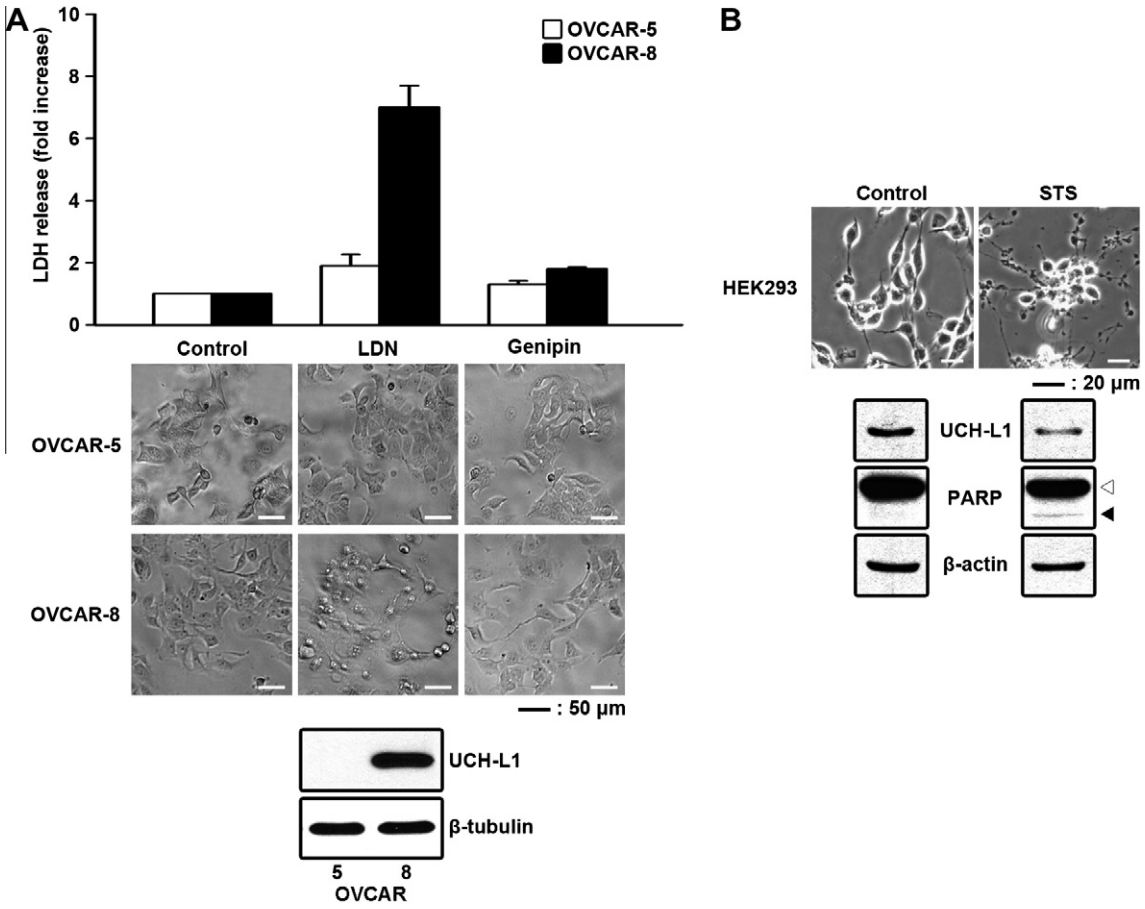

Fig. 1. Inhibition of UCH-L1 hydrolase activity induces cell death. (A) High sensitivity of UCH-L1-positive OVCAR-8 to LDN. Human ovarian cancer cells, OVCAR-5 and -8, were incubated for 2 h with 20 μM LDN-57444 (LDN), an inhibitor of UCH-L1 hydrolase activity, or 50 μM genipin used as a negative control, which are dissolved in DMSO as a vehicle control (refer to control). The amount of LDH released into the culture medium was measured using CytoTox-One Homogenous Membrane Integrity assay kit and expressed as fold increase relative to control (control refers to 1). Data are mean ± SEM of three independent experiments. (B) A decrease of UCH-L1 levels in STS-induced cell death. HEK293 cells were treated with 1 μM STS for 8 h, and the level of UCH-L1 was analyzed by IB and normalized with β-actin.

that HtrA2 can directly cleave UCH-L1 in a serine protease activity-dependent manner and that the 14-kDa HtrA2-mediated UCH-L1 cleavage product corresponds to the C-terminal region encompassing amino acid 99-223 of UCH-L1. Because the catalytic triad of UCH-L1 comprises cysteine 90 (C90), histidine 161 (H161), and aspartate 176 (D176) (Supplementary Fig. 2) [25], HtrA2-mediated UCH-L1 cleavage may accompany irreversible destruction of UCH-L1 catalytic activity, leading to the loss of its enzymatic activities for executing physiological functions. To test this notion, we performed a general deubiquitinating assay using Ub-AMC as a fluorogenic substrate (Fig. 2C). As we proposed, a 60% decrease in the hydrolase activity of UCH-L1 was detected in the presence of HtrA2 (wt), whereas no significant decrease was found in the presence of HtrA2 (S306A). These results indicate that the inhibition of the hydrolase activity of UCH-L1 results from the HtrA2-mediated UCH-L1 cleavage in vitro.

3.3. HtrA2-mediated UCH-L1 cleavage elicits the decrease of UCH-L1 protein level and the inhibition of its activity in vivo

UCH-L1 and HtrA2 must encounter in the same subcellular region as the first step to assess the potential biological relevance of the UCH-L1 cleavage by HtrA2 observed in vitro. First, we co-transfected HEK293T cells with the plasmids encoding UCH-L1 and HtrA2 and examined subcellular localizations of both proteins by IFA (Fig. 3A). UCH-L1 and HtrA2 were primarily localized to two different subcellular regions, the cytosol and the mitochondria, respectively, under normal conditions (Fig. 3A, control), as described in previous studies that UCH-L1 is predominantly localized to the cytoplasm of neurons, specially dopaminergic neurons in the substantia nigra [26]; however, HtrA2 is primarily localized to the mitochondria [21]. Because HtrA2 is released from the mitochondria into the cytosol in response to apoptotic stimuli, such as STS, we treated HEK293T cells with 0.5 μM STS and analyzed the localizations of both HtrA2 and UCH-L1 by IFA (Fig. 3A, STS). STS induced the release of HtrA2 into the cytosol, where it was colocalized with UCH-L1, allowing the first step of the HtrA2-mediated cleavage of UCH-L1 reaction in cells.

Next, to determine whether HtrA2 serine protease has any proteolytic activity on UCH-L1 in the cytosol, the HtrA2-deficient ($HtrA2^{-/-}$) and wild-type ($HtrA2^{+/+}$) MEFs were selected, and endogenous levels of UCH-L1 were assessed under the same condition shown in Fig. 3A (Fig. 3B, 0.5 μM STS). In many experimental studies on cell lines, STS, which is widely used as an intracellular stress inducer of apoptosis [27,28], has been found to induce the mitochondrial release of pro-apoptotic molecules, such as cytochrome *c*, the second mitochondria-derived activator of caspases (SMAC/DIABLO) and HtrA2, the third activator of caspases and to cause mitochondrial depolarization [29]. The UCH-L1 level was not altered when HtrA2 still resided in the mitochondria (0, 0.1 μM STS). In contrast, the UCH-L1 level was dramatically decreased in proportion to the increase in the concentration of STS, showing a decrease of 60% at 0.5 μM STS (Supplementary Fig. 3). Interestingly, this UCH-L1 proteolytic processing appears to occur

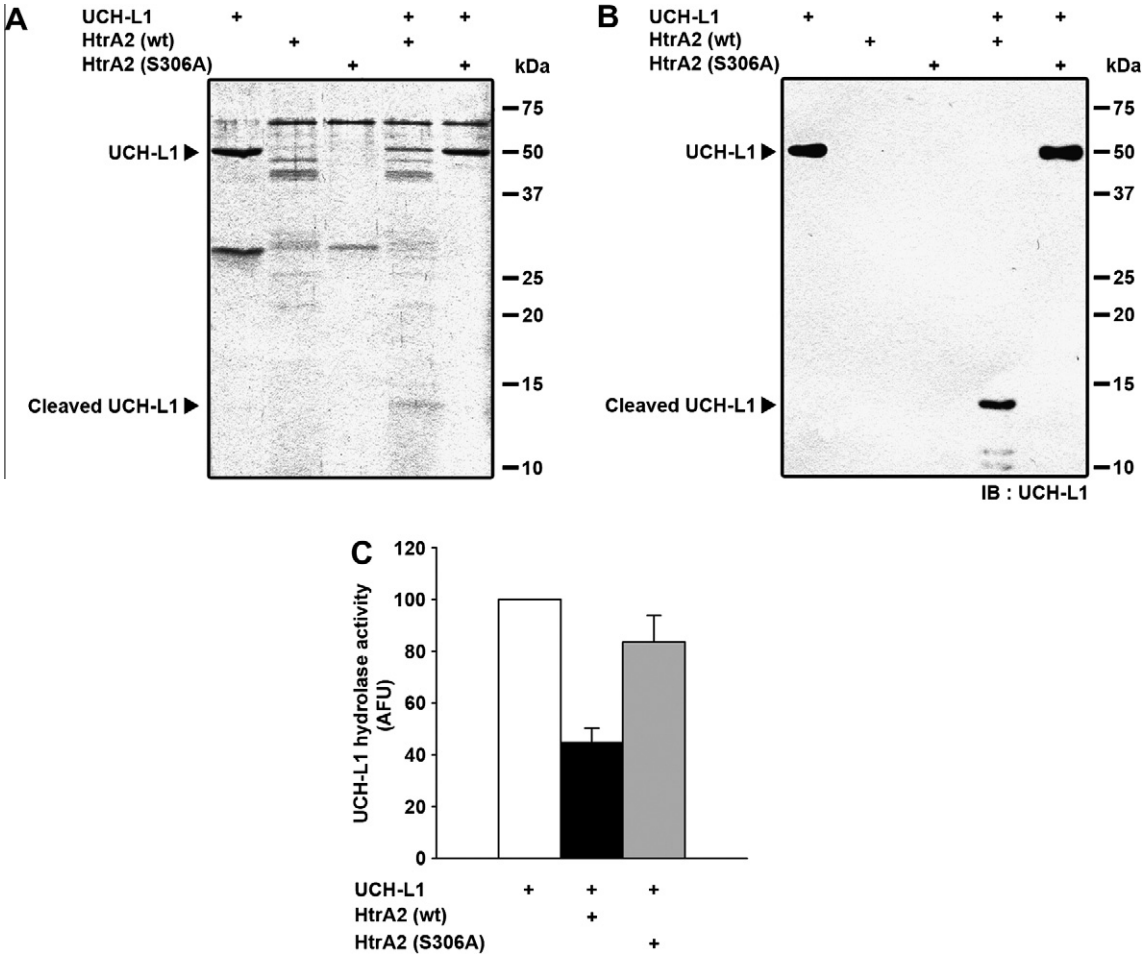

Fig. 2. The UCH-L1 hydrolase activity is decreased by HtrA2-mediated UCH-L1 cleavage in vitro. UCH-L1 and HtrA2 were expressed as GST-fusion proteins in *Escherichia coli* and purified by selective binding to glutathione 4B beads. The purified UCH-L1 protein (1 μM) was incubated with either wt or mutant from (S306A) of the HtrA2 protein (10 μM) for 16 h at 37 °C. The reaction samples were resolved by 15% SDS–PAGE, visualized by staining with Coomassie Brilliant Blue (A) and analyzed by IB with the anti-UCH-L1 Ab (B). (C) UCH-L1 hydrolase activity in vitro. UCH-L1 hydrolase activity was measured by fluorometric assay using Ub-AMC, a general deubiquitinating enzyme substrate. The reaction mixtures similar to those shown in (A) (40 nM UCH-L1 and 400 nM HtrA2) were incubated with 125 nM Ub-AMC. Values are mean ± SEM of three independent experiments and are expressed in arbitrary fluorescence unit (AFU).

by the HtrA2 serine protease that is released from the mitochondria into the cytosol, but not by a caspase-dependent manner in MEFs, because change in the UCH-L1 level was not observed in the *HtrA2*$^{-/-}$ MEFs under both normal and apoptotic conditions (Fig. 3B, right panel). However, we could hardly detect the 14-kDa UCH-L1 fragment in MEFs found in the in vitro cleavage reaction. The 14-kDa C-terminal fragment may be more susceptible to degradation in apoptotic conditions and thus rapidly degrade in the cytosol via UPS [30]. In parallel, to exclude any discrepancy between *HtrA2*$^{+/+}$ and *HtrA2*$^{-/-}$ MEFs and further investigate the specificity of HtrA2 on the effect of the decrease in the UCH-L1 level, production of the active HtrA2 serine protease was prevented in the *HtrA2*$^{+/+}$ MEFs by transfection with the small interfering RNA (siRNA) specific for *HtrA2* mRNA (si*HtrA2*) (Fig. 3C). The decrease of UCH-L1 (lane 2) was almost completely abolished when the endogenous level of HtrA2 protein was markedly reduced by about 90% (lane 4).

To validate that HtrA2-mediated UCH-L1 cleavage has effects on the hydrolase activity of UCH-L1 in living cells, Ub-AMC (125 nM) was incubated with cell lysates (2 μg) extracted from control and STS-treated MEFs (Fig. 3D). Consistent with the results of in vitro deubiquitinating assay shown in Fig. 2C, we observed a significant level of decrease, about 33% of UCH-L1 hydrolase activity under apoptotic condition in which HtrA2 is released into the cytosol. These results indicate that the phenomena observed in Fig. 1, which reflect cell death induced by inhibition of UCH-L1 hydrolase activity and decrease of UCH-L1 level under apoptotic condition, are mediated by HtrA2 through reaction with UCH-L1 in the cytosol.

3.4. HtrA2 cleaves UCH-L1 in a serine protease-dependent manner

In order to verify that the serine protease activity of HtrA2 is responsible for the cleavage of UCH-L1 in vivo, we co-transfected HEK293 cells with the plasmid encoding UCH-L1 and a wild-type (wt) or S306A, proteolytically inactive HtrA2 mature form (M-HtrA2) and analyzed the expression pattern of UCH-L1 by IB with anti-HA (Fig. 4A). In particular, these mature forms of HtrA2 have no mitochondrial targeting sequence (MTS), such that they can be localized in the cytosol where the HA-tagged UCH-L1 resides. Consistent with the above result (Fig. 3B), IB analysis revealed that overexpression of the M-HtrA2 (wt) resulted in over 90% decrease in the amount of HA-tagged UCH-L1 (lane 2), whereas this phenomenon was not observed in the presence of the M-HtrA2 (S306A) mutant (lane 3). Finally, to substantiate whether the cleavage of UCH-L1 following STS treatment occurs in an HtrA2 serine protease-dependent manner, we used Ucf-101, a cell-permeable furfurylidine–thiobarbituric acid, which acts as a potent, specific

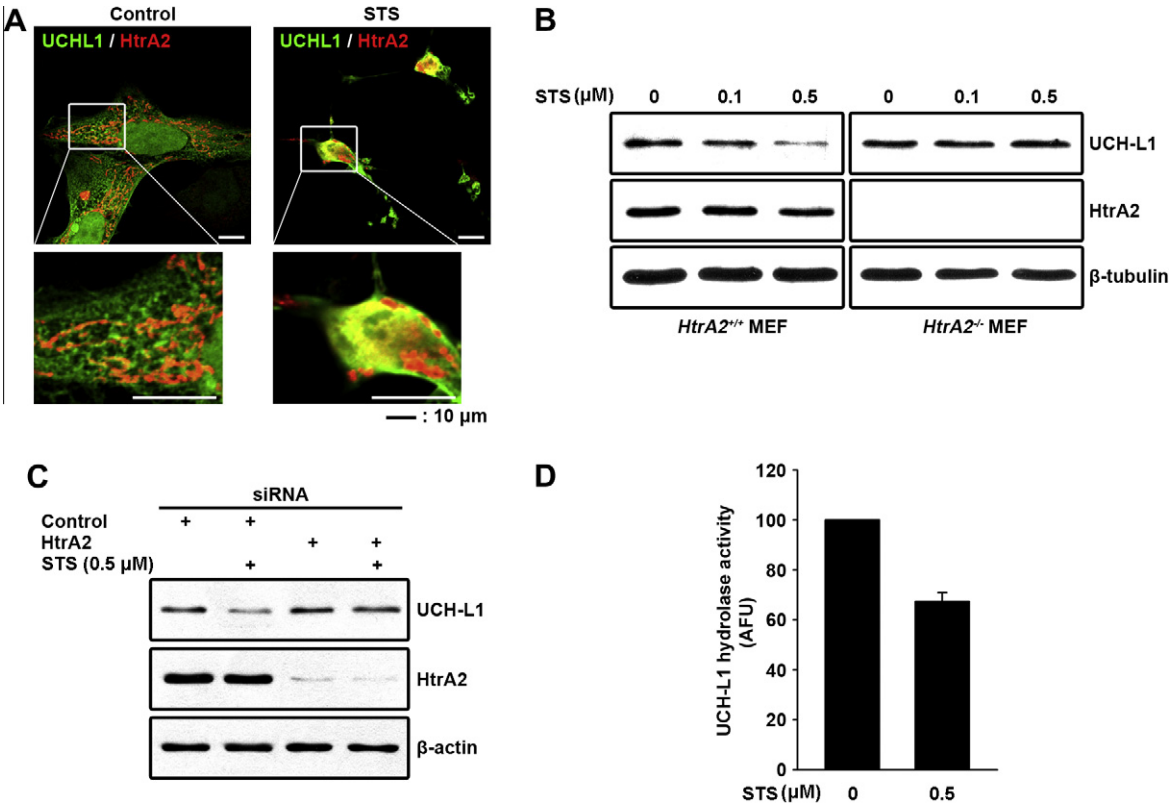

Fig. 3. HtrA2-mediated cleavage of UCH-L1 in the STS-induced cell death condition. (A) Co-localization of UCH-L1 and HtrA2 in the cytosol. The pF-HtrA2 (S306A) and pUCH-L1-EGFP plasmids were co-transfected into HEK293T cells. At 18 h post-transfection, cells were treated with 0.5 μM STS dissolved in DMSO (as a vehicle control) for 8 h, followed by immunostaining with anti-UCH-L1 and anti-HtrA2 Abs. Subcellular localization of UCH-L1 (green) and HtrA2 (red) was detected by confocal microscopy. (B) Cleavage of UCH-L1 in wild-type (*HtrA2*$^{+/+}$) MEFs. *HtrA2*$^{+/+}$ and *HtrA2*$^{-/-}$ MEFs were treated with the indicated concentrations of STS for 8 h. Cell lysates were analyzed by IB with the specific Abs against the indicated proteins. (C) The specificity of HtrA2-mediated cleavage of UCH-L1. MEFs were transfected with 20 nM of the indicated siRNAs. At 72 h post-transfection, cells were treated with 0.5 μM STS for 8 h and cell lysates were analyzed by IB. (D) UCH-L1 hydrolase activity in MEFs. Wild-type (*HtrA2*$^{+/+}$) MEFs were treated with DMSO as a vehicle control or 0.5 μM STS for 8 h, and total cellular proteins (2 μg) were incubated with 125 nM Ub-AMC. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

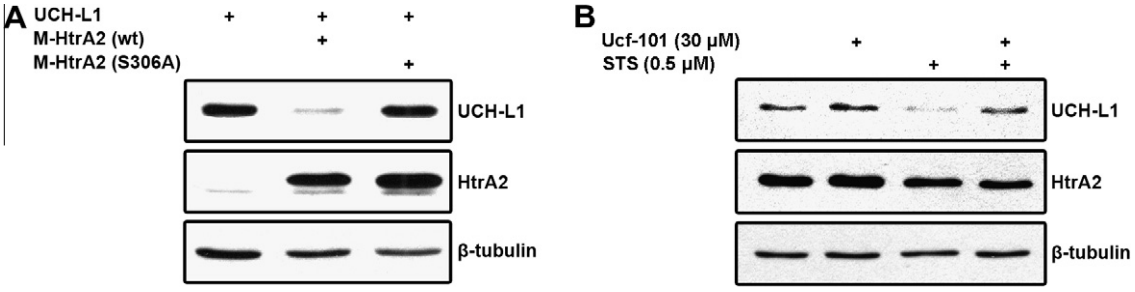

Fig. 4. The HtrA2 proteolytic activity is essential for cleavage of UCH-L1 in cells. (A) Cleavage of UCH-L1 by proteolytically active HtrA2. HEK293 cells were co-transfected with plasmid encoding the indicated proteins. At 18 h post-transfection, cell lysates were analyzed by IB. (B) Abrogation of HtrA2-mediated cleavage of UCH-L1 by inhibition of HtrA2 protease activity. Wild-type (*HtrA2*$^{+/+}$) MEFs were pretreated with Ucf-101, an HtrA2-specific protease inhibitor, for 30 min, followed by treatment with STS for 8 h. Cell lysates were prepared and analyzed by IB.

inhibitor of the serine protease HtrA2 [31]. *HtrA2*$^{+/+}$ MEFs were treated with Ucf-101 for 30 min to inhibit the serine protease activity of endogenous HtrA2, followed by treatment with STS to release HtrA2 into its action site (Fig. 4B). In the absence of HtrA2, the protein level of UCH-L1 was not affected by the inhibition of HtrA2 activity and/or mitochondrial release of pro-apoptotic molecules (Supplementary Fig. 4). On the contrary, in the present of HtrA2, HtrA2 cleaved endogenous UCH-L1 when released from the mitochondria into the cytosol in response to STS (Fig. 4B, lane 3). Notably, the STS-induced decrease of UCH-L1 level was abolished by blocking the HtrA2 serine protease activity (lane 4). Collectively, the serine protease HtrA2 serves as a specific and key player for controlling the level and hydrolase activity of UCH-L1 during the apoptotic pathway.

In conclusion, we have demonstrated that UCH-L1 serves as a natural substrate for the serine protease HtrA2 in apoptotic pathway and that its hydrolase activity is regulated through direct cleavage of UCH-L1 by HtrA2 released from the mitochondria under apoptotic condition. Our study provides evidence that the HtrA2 and UCH-L1 enzyme–substrate reaction is one of key factors in regulating cell death induced by loss-of-UCH-L1 function and suggest that the UCH-L1 and HtrA2 pair may play important roles in regulating the balance between cell death and cell survival.

Acknowledgments

This study was supported by a Grant of the Korea Health Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (A101099).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.09.148.

References

[1] C.N. Larsen, J.S. Price, K.D. Wilkinson, Substrate binding and catalysis by ubiquitin C-terminal hydrolases: identification of two active site residues, Biochemistry 35 (1996) 6735–6744.
[2] H. Osaka, Y.L. Wang, K. Takada, S. Takizawa, R. Setsuie, H. Li, Y. Sato, K. Nishikawa, Y.J. Sun, M. Sakurai, T. Harada, Y. Hara, I. Kimura, S. Chiba, K. Namikawa, H. Kiyama, M. Noda, S. Aoki, K. Wada, Ubiquitin carboxy-terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron, Hum. Mol. Genet. 12 (2003) 1945–1958.
[3] I.N. Day, R.J. Thompson, UCHL1 (PGP 9.5): neuronal biomarker and ubiquitin system protein, Prog. Neurobiol. 90 (2010) 327–362.
[4] S.V. Todi, H.L. Paulson, Balancing act: deubiquitinating enzymes in the nervous system, Trends Neurosci. 34 (2011) 370–382.
[5] Y. Liu, L. Fallon, H.A. Lashuel, Z. Liu, P.T. Lansbury Jr., The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility, Cell 111 (2002) 209–218.
[6] K. Hibi, W.H. Westra, M. Borges, S. Goodman, D. Sidransky, J. Jen, PGP9.5 as a candidate tumor marker for non-small-cell lung cancer, Am. J. Pathol. 155 (1999) 711–715.
[7] H.J. Kim, Y.M. Kim, S. Lim, Y.K. Nam, J. Jeong, K.J. Lee, Ubiquitin C-terminal hydrolase-L1 is a key regulator of tumor cell invasion and metastasis, Oncogene 28 (2009) 117–127.
[8] M.J. Jang, S.H. Baek, J.H. Kim, UCH-L1 promotes cancer metastasis in prostate cancer cells through EMT induction, Cancer Lett. 302 (2011) 128–135.
[9] L. Li, Q. Tao, H. Jin, A. van Hasselt, F.F. Poon, X. Wang, M.S. Zeng, W.H. Jia, Y.X. Zeng, A.T. Chan, Y. Cao, The tumor suppressor UCHL1 forms a complex with p53/MDM2/ARF to promote p53 signaling and is frequently silenced in nasopharyngeal carcinoma, Clin. Cancer Res. 16 (2010) 2949–2958.
[10] W.J. Wang, Q.Q. Li, J.D. Xu, X.X. Cao, H.X. Li, F. Tang, Q. Chen, J.M. Yang, Z.D. Xu, X.P. Liu, Over-expression of ubiquitin carboxy terminal hydrolase-L1 induces apoptosis in breast cancer cells, Int. J. Oncol. 33 (2008) 1037–1045.
[11] S. Hussain, O. Foreman, S.L. Perkins, T.E. Witzig, R.R. Miles, J. van Deursen, P.J. Galardy, The de-ubiquitinase UCH-L1 is an oncogene that drives the development of lymphoma in vivo by deregulating PHLPP1 and Akt signaling, Leukemia 24 (2010) 1641–1655.
[12] J. Yu, Q. Tao, K.F. Cheung, H. Jin, F.F. Poon, X. Wang, H. Li, Y.Y. Cheng, C. Rocken, M.P. Ebert, A.T. Chan, J.J. Sung, Epigenetic identification of ubiquitin carboxyl-terminal hydrolase L1 as a functional tumor suppressor and biomarker for hepatocellular carcinoma and other digestive tumors, Hepatology 48 (2008) 508–518.
[13] A. Bheda, J. Shackelford, J.S. Pagano, Expression and functional studies of ubiquitin C-terminal hydrolase L1 regulated genes, PLoS ONE 4 (2009) e6764.
[14] L. Vande Walle, M. Lamkanfi, P. Vandenabeele, The mitochondrial serine protease HtrA2/Omi: an overview, Cell Death Differ. 15 (2008) 453–460.
[15] L.A. Pradelli, M. Beneteau, J.E. Ricci, Mitochondrial control of caspase-dependent and -independent cell death, Cell. Mol. Life Sci. 67 (2010) 1589–1597.
[16] S.M. Srinivasula, S. Gupta, P. Datta, Z. Zhang, R. Hegde, N. Cheong, T. Fernandes-Alnemri, E.S. Alnemri, Inhibitor of apoptosis proteins are substrates for the mitochondrial serine protease Omi/HtrA2, J. Biol. Chem. 278 (2003) 31469–31472.
[17] L. Cilenti, M.M. Soundarapandian, G.A. Kyriazis, V. Stratico, S. Singh, S. Gupta, J.V. Bonventre, E.S. Alnemri, A.S. Zervos, Regulation of HAX-1 anti-apoptotic protein by Omi/HtrA2 protease during cell death, J. Biol. Chem. 279 (2004) 50295–50301.
[18] H.J. Park, S.S. Kim, Y.M. Seong, K.H. Kim, H.G. Goo, E.J. Yoon, S. Min do, S. Kang, H. Rhim, Beta-amyloid precursor protein is a direct cleavage target of HtrA2 serine protease. Implications for the physiological function of HtrA2 in the mitochondria, J. Biol. Chem. 281 (2006) 34277–34287.
[19] S. Kuninaka, S.I. Iida, T. Hara, M. Nomura, H. Naoe, T. Morisaki, M. Nitta, Y. Arima, T. Mimori, S. Yonehara, H. Saya, Serine protease Omi/HtrA2 targets WARTS kinase to control cell proliferation, Oncogene 26 (2007) 2395–2406.
[20] J. Hartkamp, B. Carpenter, S.G. Roberts, The Wilms' tumor suppressor protein WT1 is processed by the serine protease HtrA2/Omi, Mol. Cell 37 (2010) 159–171.
[21] H.M. Park, G.Y. Kim, M.K. Nam, G.H. Seong, C. Han, K.C. Chung, S. Kang, H. Rhim, The serine protease HtrA2/Omi cleaves Parkin and irreversibly inactivates its E3 ubiquitin ligase activity, Biochem. Biophys. Res. Commun. 387 (2009) 537–542.
[22] E. Leroy, R. Boyer, G. Auburger, B. Leube, G. Ulm, E. Mezey, G. Harta, M.J. Brownstein, S. Jonnalagada, T. Chernova, A. Dehejia, C. Lavedan, T. Gasser, P.J. Steinbach, K.D. Wilkinson, M.H. Polymeropoulos, The ubiquitin pathway in Parkinson's disease, Nature 395 (1998) 451–452.
[23] Z. Li, F. Melandri, I. Berdo, M. Jansen, L. Hunter, S. Wright, D. Valbrun, M.E. Figueiredo-Pereira, Delta12-prostaglandin J2 inhibits the ubiquitin hydrolase UCH-L1 and elicits ubiquitin-protein aggregation without proteasome inhibition, Biochem. Biophys. Res. Commun. 319 (2004) 1171–1180.
[24] Y.Y. Tan, H.Y. Zhou, Z.Q. Wang, S.D. Chen, Endoplasmic reticulum stress contributes to the cell death induced by UCH-L1 inhibitor, Mol. Cell. Biochem. 318 (2008) 109–115.
[25] C. Das, Q.Q. Hoang, C.A. Kreinbring, S.J. Luchansky, R.K. Meray, S.S. Ray, P.T. Lansbury, D. Ringe, G.A. Petsko, Structural basis for conformational plasticity of the Parkinson's disease-associated ubiquitin hydrolase UCH-L1, Proc. Natl Acad. Sci. USA 103 (2006) 4675–4680.
[26] M. Basso, S. Giraudo, D. Corpillo, B. Bergamasco, L. Lopiano, M. Fasano, Proteome analysis of human substantia nigra in Parkinson's disease, Proteomics 4 (2004) 3943–3952.
[27] H.J. Chae, J.S. Kang, J.O. Byun, K.S. Han, D.U. Kim, S.M. Oh, H.M. Kim, S.W. Chae, H.R. Kim, Molecular mechanism of staurosporine-induced apoptosis in osteoblasts, Pharmacol. Res. 42 (2000) 373–381.
[28] C.A. Belmokhtar, J. Hillion, E. Segal-Bendirdjian, Staurosporine induces apoptosis through both caspase-dependent and caspase-independent mechanisms, Oncogene 20 (2001) 3354–3362.
[29] S.W. Tait, D.R. Green, Mitochondria and cell death: outer membrane permeabilization and beyond, Nat. Rev. Mol. Cell Biol. 11 (2010) 621–632.
[30] E.J. Dudek, K.J. Lampi, J.A. Lampi, F. Shang, J. King, Y. Wang, A. Taylor, Ubiquitin proteasome pathway-mediated degradation of proteins: effects due to site-specific substrate deamidation, Invest. Ophthalmol. Vis. Sci. 51 (2010) 4164–4173.
[31] L. Cilenti, Y. Lee, S. Hess, S. Srinivasula, K.M. Park, D. Junqueira, H. Davis, J.V. Bonventre, E.S. Alnemri, A.S. Zervos, Characterization of a novel and specific inhibitor for the pro-apoptotic protease Omi/HtrA2, J. Biol. Chem. 278 (2003) 11489–11494.