PERSPECTIVE

Modification of ubiquitin C-terminal hydrolase L1 by reactive lipid species: role in neural regeneration and diseases of aging

Role of ubiquitin C-terminal hydrolase L1 (UCHL1) in brain function: Ubiquitin is used by a variety of cellular systems to tag proteins for transport to various organelles. There are a number of enzymes in the ubiquitin-proteasome pathway (UPP) that tag abnormally folded proteins with ubiquitin for transport to the proteasome for degradation. UCHL1 is a neuron-specific enzyme constituting over 1% of brain protein that can both ligate ubiquitin to proteins and hydrolyze ubiquitin (Ub) from proteins allowing for ubiquitin recycling (Setsuie and Wada, 2007). UCHL1 has several functions in neurons, including some that are specific for neuronal function (**Figure 1**). UCHL1 may tag abnormal unfolded proteins for transport to the proteasome as part of the neuronal UPP. Although it may play a role in the neuronal UPP, there are a variety of other deubiquitinases that are expressed in many other cell types such as UCHL3 that may serve this function in neurons. UCHL1 interacts with a number of cytoskeletal, axonal and synaptic proteins suggesting that it may have other neuron-specific functions. Mutations or deletion of UCHL1 produce prominent axonal pathology and white matter abnormalities in rodents, suggesting that UCHL1 is important in axonal and synaptic function in addition to the UPP (Kabuta et al., 2008). Furthermore, UCHL1 interacts with synaptic proteins suggesting that it may have a role in transporting synaptic vesicles to the plasma membrane central to neurotransmitter release. Inhibition of UCHL1 activity blocks long term potentiation in hippocampus (Gong et al., 2006). These and other observations suggest that UCHL1 is required for these neuron-specific functions rather than degradation of unfolded proteins. UCHL1 has been associated with the pathogenesis of a number of neurodegenerative diseases. A mutation in UCHL1 (Parkin 5) has been associated with familial Parkinson's disease (PD). Furthermore, UCHL1 may be involved in the pathogenesis of Alzheimer's disease (AD) (Setsuie and Wada, 2007). Oxidative modification and down-regulation of UCHL1 has been detected in idiopathic PD and AD brains (Choi et al., 2004). Genetic disruption of UCHL1 produces degeneration of motor neurons similar to those found in amyotrophic lateral sclerosis (ALS) (Bilguvar et al., 2013). These results suggest that UCHL1 activity may be important preserving axonal and synaptic function in a variety of disorders.

Modification of the UCHL1 protein structure by reactive lipids and neuronal cell death: Reactive lipid species such as prostaglandins and isoprostanes have been implicated in the pathogenesis of stroke and many other brain diseases (Liu et al., 2013). Reactive lipid species such as 15-deoxy- $\Delta^{12,14}$ -prostaglandin J2 (15dPGJ2) are produced after cerebral ischemia and are capable of covalently modifying cysteine residues on certain proteins. 15dPGJ2 produces dramatic changes in the structure and function UCHL1 (Koharudin et al., 2010). Covalent modification of the cysteine 152 (C152) of UCHL1, but not other UCHL1 cysteines, unfolds the protein resulting in aggregation of UCHL1 and loss of its hydrolase activity.

To test whether binding of reactive lipids and other substrates to the C152 in UCHL1 is important in neural injury, a knock-in mouse bearing a cysteine 152 to alanine mutation (UCHL1-C152A) was constructed. These mice and colony wild type controls were used to obtain cortical neuron enriched cultures. Primary neurons were used for cell viability assays, western blotting for ubiquitinated (Ub-) protein detection, and immunocytochemical neurite detection using an anti-neurofilament L antibody. UCHL1-C152A neurons were protected from cell death induced by 5 μM 15dPGJ2, and had less accumulation and aggregation of Ub-proteins than wild type controls. 15dPGJ2-induced neurite damage was also significantly decreased in UCHL1-C152A neurons compared to wild type after 24 hours of incubation with 1.25 μM 15dPGJ2 (Liu et al., 2015). These results suggest that binding of 15dPGJ2 and other reactive lipids to the C152 of UCHL1 exacerbates injury, particularly to neurites. Furthermore, binding of reactive lipids to C152 disrupts the UPP resulting in accumulation of Ub-proteins and exacerbates cell death. The above study also suggests an important protective role of UCHL1-C152A in neuronal survival.

Implications for neural injury and repair in stroke, traumatic brain injury (TBI) and neurodegenerative diseases: In pathological conditions such as cerebral ischemic trauma, many reactive lipids are produced which may inactivate UCHL1 and exacerbate injury to neuritis (Liu et al., 2013). Preventing the binding of these substrates to UCHL1 could prevent its inactivation and could be an effective novel therapeutic approach in stroke and TBI where there is extensive axonal injury and disruption of synaptic function. The C152 site of UCHL1 is a specific site for covalent modification and inactivation. Compounds that compete for substrate binding at this site could ameliorate axonal and synaptic dysfunction after stroke and TBI. Another approach is to replace inactivated UCHL1 in neurons with functional UCHL1 protein. The wild type UCHL1 protein has been fused with the prothrombin domain of the HIV-transactivator protein (TAT) which allows the UCHL1-TAT fusion protein (TAT-UCHL1) to readily transduce neurons *in vitro* and *in vivo*. Treatment of neurons with TAT-UCHL1 decreased neuronal death after hypoxia *in vitro* (Liu et al., 2011). Thus, future studies addressing whether treatment with wild type TAT-UCHL1 or TAT-UCHL1 bearing the C152A mutation is useful in reducing axonal injury and improving synaptic function in TBI and stroke models may be warranted.

These approaches may also be useful in treatment of neurodegenerative diseases such as AD and PD. Formation of neuritic plaques is one of the hallmarks of AD and absence of UCHL1 expression has been linked with amyloid β protein accumulation in the UCHL1-null gad mice. Gong et al. (2006) found that treatment with TAT-UCHL1 reversed the decrement in hippocampal long term potentiation in a mouse model of AD. Furthermore, systemic treatment with TAT-UCHL1 improved memory function. Overexpression of UCHL1 or N-terminal truncated UCHL1 has been reported to delay AD and PD progression *in vivo* (Kim et al., 2014). Therefore, treatment with TAT-UCHL1 or inhibitors that prevent binding of reactive lipids to the C152 site of UCHL1 may be useful in a variety of neurodegenerative diseases. Stroke and neurodegenerative diseases are a major burden in the aging population, and these mechanisms may also be important in age-related changes in normal brain aging.

This work was supported in part by NIH R01 NS37459-15. The author thanks Marie Rose for preparation of the figure, and Hao Liu and Ms. Rose for helpful comments.

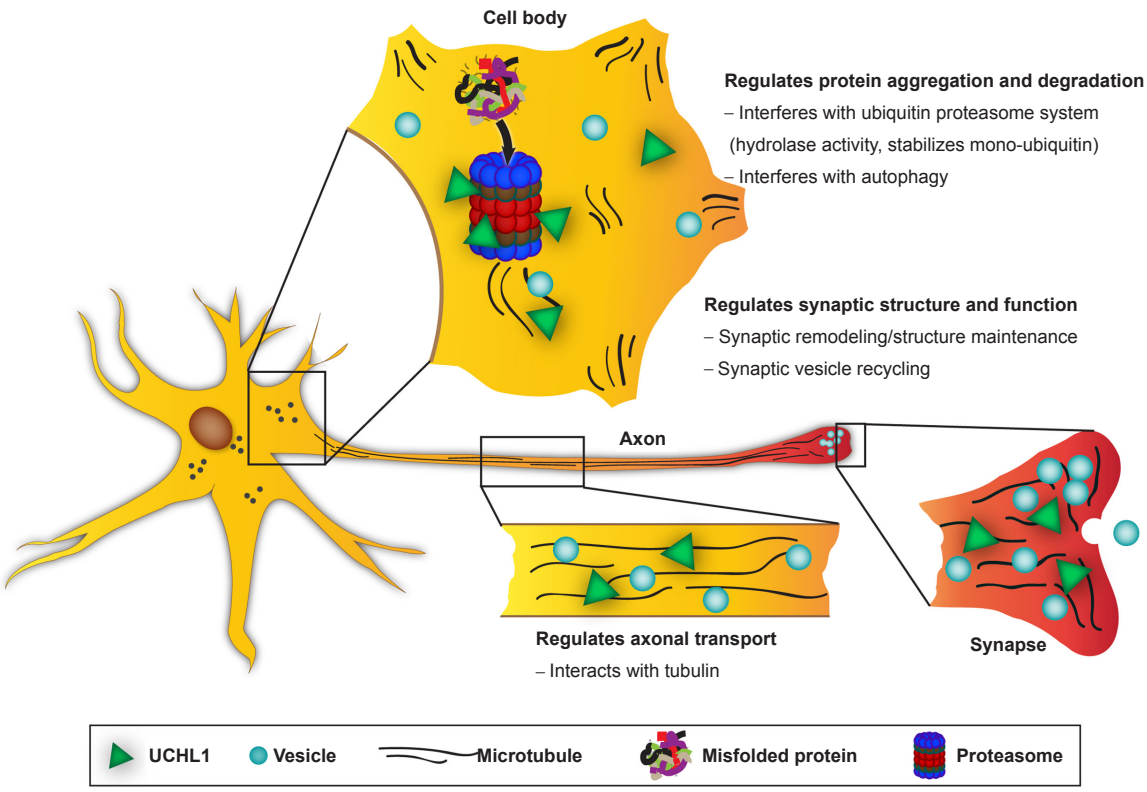

Figure 1 Schematic diagram illustrating role of ubiquitin C-terminal hydrolase L1 (UCHL1) in cell body, axon and synapse.

Steven H. Graham*

Geriatric Research Educational and Clinical Center, V.A. Pittsburgh Healthcare System, PA, USA

***Correspondence to:** *Steven H. Graham, M.D., Ph.D., Steven.Graham@va.gov.*
***Accepted:** 2016-06-07*
***orcid:** 0000-0002-1718-2547 (Steven H. Graham)*
***doi:** 10.4103/1673-5374.184482*
***How to cite this article:** Graham SH (2016) Modification of ubiquitin C-terminal hydrolase L1 by reactive lipid species: role in neural regeneration and diseases of aging. Neural Regen Res 11(6):908-909.*

References

Bilguvar K, Tyagi NK, Ozkara C, Tuysuz B, Bakircioglu M, Choi M, Delil S, Caglayan AO, Baranoski JF, Erturk O, Yalcinkaya C, Karacorlu M, Dincer A, Johnson MH, Mane S, Chandra SS, Louvi A, Boggon TJ, Lifton RP, Horwich AL, et al. (2013) Recessive loss of function of the neuronal ubiquitin hydrolase UCHL1 leads to early-onset progressive neurodegeneration. Proc Natl Acad Sci U S A 110:3489-3494.

Choi J, Levey AI, Weintraub ST, Rees HD, Gearing M, Chin LS, Li L (2004) Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. J Biol Chem 279:13256-13264.

Gong B, Cao Z, Zheng P, Vitolo OV, Liu S, Staniszewski A, Moolman D, Zhang H, Shelanski M, Arancio O (2006) Ubiquitin hydrolase Uch-L1 rescues beta-amyloid-induced decreases in synaptic function and contextual memory. Cell 126:775-788.

Kabuta T, Furuta A, Aoki S, Furuta K, Wada K (2008) Aberrant interaction between Parkinson disease-associated mutant UCH-L1 and the lysosomal receptor for chaperone-mediated autophagy. J Biol Chem 283:23731-23738.

Kim HJ, Jeong JE, Baek JY, Jeong J, Kim S, Kim YM, Kim Y, Nam JH, Huh SH, Seo J, Jin BK, Lee KJ (2014) N-terminal truncated UCH-L1 prevents Parkinson's disease associated damage. PLoS One 9:e99654.

Koharudin LM, Liu H, Di Maio R, Kodali RB, Graham SH, Gronenborn AM (2010) Cyclopentenone prostaglandin-induced unfolding and aggregation of the Parkinson disease-associated UCH-L1. Proc Natl Acad Sci U S A 107:6835-6840.

Liu H, Li W, Ahmad M, Miller TM, Rose ME, Poloyac SM, Uechi G, Balasubramani M, Hickey RW, Graham SH (2011) Modification of ubiquitin-C-terminal hydrolase-L1 by cyclopentenone prostaglandins exacerbates hypoxic injury. Neurobiol Dis 41:318-328.

Liu H, Li W, Rose ME, Hickey RW, Chen J, Uechi GT, Balasubramani M, Day BW, Patel KV, Graham SH (2015) The point mutation UCH-L1 C152A protects primary neurons against cyclopentenone prostaglandin-induced cytotoxicity: implications for post-ischemic neuronal injury. Cell Death Dis 6:e1966.

Liu H, Li W, Ahmad M, Rose ME, Miller TM, Yu M, Chen J, Pascoe JL, Poloyac SM, Hickey RW, Graham SH (2013) Increased generation of cyclopentenone prostaglandins after brain ischemia and their role in aggregation of ubiquitinated proteins in neurons. Neurotox Res 24:191-204.

Setsuie R, Wada K (2007) The functions of UCH-L1 and its relation to neurodegenerative diseases. Neurochem Int 51:105-111.