Overexpression of ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) delays Alzheimer’s progression in vivo

Mingming Zhang, Fang Cai, Shuting Zhang, Si Zhang & Weihong Song

Deposition of amyloid β protein (Aβ) to form neuritic plaques in the brain is the pathological hallmark of Alzheimer’s disease (AD). Aβ is produced by β- and γ-cleavages of amyloid β precursor protein (APP). Ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) is a de-ubiquitinating enzyme that cleaves ubiquitin at its carboxyl terminal. Dysfunction of UCHL1 has been reported in neurodegenerative diseases. However, whether UCHL1 affects Aβ production and AD progression remains unknown. Here we report that UCHL1 interacts with APP and regulates Aβ production. UCHL1 increases free ubiquitin level and accelerates the lysosomal degradation of APP by promoting its ubiquitination. Furthermore, we demonstrate that overexpression of UCHL1 by intracranial injection of UCHL1-expressing rAAV reduces Aβ production, inhibits neuritic plaque formation and improves memory deficits in AD transgenic model mice. Our study suggests that UCHL1 may delay Alzheimer’s progression by regulating APP degradation in a long-term fashion, and that overexpression of UCHL1 may be a safe and effective disease-modifying strategy to treat AD.

Alzheimer’s disease (AD) is the most common neurodegenerative disorder. Aβ, the central component of neuritic plaques, is produced from the amyloid β precursor protein (APP). Under physiological conditions, the majority of APP is processed by α-secretase within the Aβ domain in a non-amyloidogenic pathway. β-cleavage of APP by BACE1 at Asp1 site produces CTFβC99, which is subsequently processed by the presenilin-dependent γ-secretase complex to generate Aβ in the amyloidogenic pathway. APP is a type I transmembrane protein and matured in the ER and the Golgi apparatus before reaching the plasma membrane. It is then rapidly internalized to the endosome and sent to lysosome for degradation. Several AD-associated proteins, including BACE1, TMP21, PS1, aph-1 and nicastrin, are degraded by the ubiquitin proteasome system (UPS). Autophagy-lysosomal degradation may also require ubiquitin signaling, especially when the proteasome is dysfunctional. While it mainly undergoes lysosomal degradation, APP is also ubiquitinated and degraded by UPS. These findings suggest that ubiquitin proteasome signaling is important for APP processing and Aβ production.

Deubiquitinating enzymes (DUBs) cleave ubiquitin at its terminal carbonyl Gly-76, and the ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) belongs to the UCH family of DUBs. UCHL1 is expressed predominantly in the brain and neuroendocrine systems, and accounts for 1–2% of total brain soluble proteins. UCHL1 effectively hydrolyzes amino acids from ubiquitin and cleave di-ubiquitins. It also serves as an ubiquitin ligase at higher concentrations, adding ubiquitin to already mono-ubiquitinated proteins. In addition, it acts as a free ubiquitin stabilizer, providing ready-to-use ubiquitin for various cellular events. UCHL1 expression is tightly regulated and NFκB signaling modulates its expression. Dysfunction of UCHL1 has been reported in many neurodegenerative diseases. The J93M missense mutation in UCHL1 was identified in early-onset familial PD cases. The E7A recessive mutation resulted in childhood-onset progressive neurodegeneration. Genetic deletions of UCHL1 in mouse strain, the gad mice, led to phenotypes of gracile axonal dystrophy and premature death. Reduced UCHL1 protein level was found in sporadic AD brains, and overexpression of UCHL1 rescued learning and memory deficits in AD model mice by restoring LTP in the hippocampus. We have shown that UCHL1 regulates BACE1 degradation. However, whether UCHL1 affects Aβ production and AD progression in a long-term fashion remains unknown.

To determine whether UCHL1 regulates APP processing, double transgenic AD model mice APP23/PS45 were injected with AAV1-UCHL1-GFP or control virus at 7 weeks of age. Overexpression of UCHL1 significantly...
Facilitation of APP degradation by UCHL1 could be through the lysosomal or proteasomal pathway. We found that lysosomal inhibitor chloroquine significantly accumulated mature APP in the Haw cell (p<0.01) (Figure 3A, B). Surprisingly, proteasomal inhibitor MG132 decreased mature APP level (p<0.05) (Figure 3A, B). It may be due to the blockage of APP maturation by MG132. Supporting evidence included its little effect on newly synthesized immature APP (lower lane in Fig 3A) but substantial effect on mature APP (upper lane in Fig 3A). The experiments were replicated in 20E2 cells. Chloroquine treatment led to marked APP accumulation (p<0.05) (Figure 3C, D), while MG132 treatment reduced mature APP level (p<0.05) (Figure 3C, D). To investigate whether UCHL1 reduced APP protein level by the lysosomal degradation, Haw cells were transfected with UCHL1 and then treated with 100 μM chloroquine (Figure 3E). Lysosomal inhibition attenuated the effect of UCHL1 on APP protein level, resulting in abolishment of the UCHL1’s inhibitory effect on APP (p<0.05) (Figure 3F). We replicated the experiments and obtained same results in 20E2 cells (p<0.05) (Figure 3G and H). Our results indicated that UCHL1 promoted the degradation of APP via the lysosomal pathway.

To investigate whether UCHL1 physically interacts with APP, Haw cells were transfected with UCHL1 and then treated with chloroquine. Co-immunoprecipitation assay showed the interaction between UCHL1 and APP, and the interaction was potentiated by chloroquine (Figure 3I). To investigate whether UCHL1 promotes the ubiquitination and degradation of APP by regulating the free ubiquitin pool, Haw cells were co-transfected with pZ-UCHL1-st and ubiquitin-expression plasmid pCW7. Overexpression of UCHL1 significantly elevated free ubiquitin levels (p<0.05) (Figure 3J, L), and overexpression of ubiquitin further increased free ubiquitin levels (p<0.01) (Figure 3J, L). Mature APP level was reduced by UCHL1 as expected (p<0.05) (Figure 4I), and was further reduced by ubiquitin expression (p<0.01) (Figure 3J, K). The results indicate that the increased free ubiquitin level by UCHL1 may

Figure 1 | UCHL1 regulates APP processing and Aβ production in vivo. (A, B) APP CTFs in hippocampal tissues were lower in AAV1-UCHL1-GFP-infected than in AAV1-GFP-infected APP23/gad mice controls. N=8 for each group. **p<0.01 by Student’s t-test. (C) APP40 of AAV1-UCHL1-GFP-infected mice was significantly lower than control mice. N=5 for each group. *p<0.05 by Student’s t-test. (D, E) APP CTFs from hippocampal tissues of APP23/gad were higher than APP23 mice. N=8 for each group. *p<0.05 by Student’s t-test. The values are expressed as mean ± SEM.
be responsible for increasing ubiquitinated APP and decreasing mature APP.

Next we examined the effect of UCHL1 on Alzheimer’s development in vivo. APP23/PS45 mice were intracranially injected with AAV1-UCHL1-GFP or its control AAV1-GFP at 7 weeks of age. GFP and UCHL1 protein level were markedly increased in UCHL1-GFP virus-infected hippocampal area 10 weeks after injection (Supplemental Figure 1B and C). In mice that were unilaterally injected with AAV1-UCHL1-GFP, GFP was robustly expressed in dentate gyrus and CA3 of the hippocampus ipsilaterally (Supplementary Figure 1D). In addition, UCHL1 was co-expressed with GFP in AAV1-UCHL1-GFP-infected mice, but not in those infected with AAV1-GFP (Supplemental Figure 1E). UCHL1 overexpression significantly reduced the number of neuritic plaques in the hippocampal region (p<0.01) (Figure 4Aa and b, B) and in the neocortex area of APP23/PS45 mice (p<0.01) (Figure 4Ac and b, D). Thioflavin-S staining confirmed the reduction of plaque formation in UCHL1-overexpressed mice (Figure 4Ad and Cc, d). We also knocked down UCHL1 expression in APP23 transgenic mice by breeding APP23 mice with heterozygous gad mice. At 6 months old APP23 mice merely started to develop Aβ plaques, with at most 1 to 2 plaques per slice. The plaque numbers were increased in APP23/gad mice compared to APP23 (Figure 4E and F). The results indicate that knockdown of UCHL1 expression facilitates neuritic plaque formation in APP23 mice.

To investigate whether UCHL1 overexpression improves learning and memory deficit in AD model mice, Morris water maze was carried out in AAV-UCHL1-GFP-injected APP23/PS45 mice and the control group. There were no difference of the escape latency (p>0.05) (Figure 4G) or swimming speed (p>0.05) (Figure 4H) on day 1 of the visible platform test between the UCHL1-overexpressed and control groups, indicating that viral infection and UCHL1 overexpression did not affect the swimming ability or vision. In the hidden platform test UCHL1-overexpression significantly shortened escape latency of the AD mice (p<0.05) (Figure 4I). In the probe trial test, UCHL1-overexpressing mice spent markedly more time in the hidden platform quadrant (p<0.05) (Figure 4J). To further confirm UCHL1’s effect on learning and memory deficits, we crossed the gad mice with APP23. There were no difference of the escape latency (p>0.05) (Figure 4K) or swimming speed (p>0.05) in the visible platform test (Figure 4L) between APP23 and APP23/gad mice. In the hidden platform tests, APP23/gad mice showed significantly prolonged escape latency compared to APP23 mice (p<0.05) (Figure 4M). APP23/gad mice passed the virtual platform less frequently than APP23 mice in the hidden platform test (p<0.05) (Figure 4N). Taken together, these data demonstrated that UCHL1 expression rescued memory deficits in AD model mice, whereas reducing UCHL1 expression exacerbated the memory deficits in AD model mice.

Dysfunction of UCHL1 has been implicated in neurodegenerative diseases. Here we demonstrated that UCHL1 interacts with APP and that APP undergoes UCHL1-assisted ubiquitination followed by trafficking to lysosome for degradation. Our study showed that reduction of UCHL1 exacerbates AD-like pathology and behavioral...
performance in APP23/gad mice. The data suggests that the lower expression of UCHL1 may be partially responsible for Alzheimer pathophysiology and cognitive impairment. We delivered UCHL1 into the hippocampal region of the mice by intracranial injection of UCHL1-expressing AAV. Overexpression of UCHL1 reduced Aβ production, inhibited neuritic plaque formation and improved memory deficits in AD model mice. Long-term expression by AAV delivery has been verified with no significant adverse events from the viral delivery method. Our work is the first to suggest that UCHL1 delays AD progression in a long-term fashion, and that rAAV-mediated UCHL1 gene therapy to overexpress UCHL1 in the brain could be a promising disease-modifying strategy for AD therapeutics.

**Methods**

Experimental procedures were carried out in accordance with the guidelines and approved protocols by The University of British Columbia Animal Care and Use Committee and Biosafety Committee. To generate the adenovirus-associated virus (AAV) expressing UCHL1, the human UCHL1 cDNA sequence was cloned into the pAAV-26S-GFP-cDNA vector. APP23 transgenic mice carry human APP751 cDNA with the Swedish mutation and P385 transgenic mice carry human PS1 cDNA with the G34A mutation. The gracile axonal dystrophy (gad) mouse carries a spontaneous mutant with an in-frame deletion in exons 7 and 8 of UCHL1 and is equivalent to a UCHL1 knockout mouse model. APP and Aβ proteins were assayed in AAV-infected AD model mice.
mice or APP23/gad mice. Neuritic plaque formation was assessed by immunostaining with 4G8 antibody and Thioflavin-S staining. Morris water maze test was used to determine the learning and memory deficits. Full Methods and any associated references are described in Online Supplemental Data.

1. Deng, Y. et al. Amyloid-beta protein (Abeta) Glu11 is the major beta-secretase site of beta-site amyloid-beta precursor protein-cleaving enzyme 1 (BACE1), and shifting the cleavage site to Abeta Asp1 contributes to Alzheimer pathogenesis. *Eur J Neurosci* **37**, 1962–1969 (2013).

2. Koo, E. H., Squazzo, S. L., Selkoe, D. J. & Koo, C. H. Trafficking of cell-surface amyloid beta-protein precursor. 1. Secretion, endocytosis and recycling as detected by labeled monoclonal antibody. *J Cell Sci* **109** (Pt 5), 991–998 (1996).

3. Small, S. A. & Gandy, S. Sorting through the cell biology of Alzheimer's disease: intracellular pathways to pathogenesis. *Neuron* **52**, 15–31 (2006).

---

**Figure 4** | Overexpression of UCHL1 decreases plaque formation and improves learning and memory deficits. (A) Viruses were bilaterally injected into the hippocampal region of APP23/PS45 mice at the age of seven weeks old. The mice were then sacrificed at 17 weeks old. Neuritic plaques were detected by 4G8 antibody. The arrows indicate plaques in AAV1-GFP control virus (Aa) or AAV1-UCHL1-GFP injection (Ab). The plaques were confirmed by thioflavin-S staining (Ac and d). (B) Quantification of neuritic plaques in (A, a-b) by Image J (NIH). The value represents mean ± SEM. n = 8 for each group. **p < 0.01 by Student’s t-test. (C) Detection of neuritic plaques in the cortical region in AAV1-UCHL1-GFP -injected mice (Cb) and controls (Ca). The plaques were confirmed by thioflavin-S staining (Cc and d). Bar: 500 μm. (D) Quantification of neuritic plaques in (C, a-b), n = 8 for each group. **p < 0.01 by Student’s t-test. (E) Neuritic plaques in six-month-old APP23 and APP23/gad mice were examined by thioflavin-S staining. (F) Quantification of (E). n = 8 for each group. *p < 0.05 by Student’s t-test. (G-J) Eight weeks after AAV1-UCHL1-GFP or AAV1-GFP injection, APP23/PS45 mice were subjected to Morris water maze test at the age of 15 weeks old. n = 16 for AAV1-GFP group, n = 13 for AAV1-UCHL1-GFP group. On the first day of visible platform test, AAV1-UCHL1-GFP-injected mice displayed similar escape latency (G) and swimming speed (H) as control mice. *p = 0.05 by Student’s t-test. (I) During hidden platform test, AAV1-UCHL1-GFP mice exhibited shorter escape latency. *p < 0.05 by two-way ANOVA group comparison. (J) On day 6 of the probe trial, AAV1-UCHL1-GFP-injected mice spent more time in the target quadrant than controls. *p < 0.05 by Student’s t-test. The values are expressed as mean ± SEM. (K-N) Six-month-old APP23 or APP23/gad mice were subjected to Morris water maze test. n = 12 for APP23 group, n = 11 for APP23/gad group. (K) Escape latency and (L) swimming speed. P > 0.05 by Student’s t-test. (M) Hidden platform tests. *p < 0.05 by two-way ANOVA post-hoc Bonferroni test. (N) The probe trial on day 6. *p < 0.05 by Student’s t-test.
23. Yamazaki, K. et al. Degradation of BACE by the ubiquitin-proteasome pathway. *Faseb J* 18, 1571–1573 (2004).

22. Bilguvar, K. et al. Ubiquitin-proteasome pathway mediates degradation of APH-1. *J Neurochem* 99, 1403–1412 (2006).

21. Leroy, E. et al. Degradation of nicastrin involves both proteasome and lysosome. *J Neurochem* 101, 982–992 (2007).

13. Kaneko, M. et al. Protein synthesis is impaired in a transgenic mouse model for Alzheimer’s disease. *Neurobiol Aging* 19, S19–21 (1998).

12. Liu, Y. et al. TMP21 degradation is mediated by the ubiquitin-proteasome pathway. *Eur J Neurosci* 28, 1980–1988 (2008).

11. Haass, C. et al. Targeting of cellsurface beta-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments. *Nature* 357, 500–503 (1992).

10. Caporaso, G. L. et al. Chloroquine degradation and Parkinson’s disease susceptibility. *Nature* 459, 3924–3932 (2009).

9. Ohta, H. et al. The demonstration of new human neurone-specific protein detected by high-resolution two-dimensional polyacrylamide gel electrophoresis. *J Neurochem* 40, 1542–1547 (1983).

8. Wilson, P. O. et al. The immunolocalization of protein gene product 9.5 using rabbit polyclonal and mouse monoclonal antibodies. *Br J Exp Pathol* 69, 91–104 (1988).

7. Fraser, P. E. et al. Prosminil 1 is actively degraded by the 26S proteasome. *Neurobiol Aging* 19, S19–21 (1998).

6. He, G. et al. Hypoxia facilitates Alzheimer’s disease pathogenesis by up-regulating BACE1 gene expression. *Proc Natl Acad Sci U S A* 103, 18727–18732 (2006).

5. He, G. et al. NF-kappaB signaling inhibits ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson’s and Alzheimer’s diseases. *J Biol Chem* 278, 2781–2789 (2003).

4. Qing, H. et al. 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 (2004).

3. Song, W. et al. Hypoxia facilitates Alzheimer’s disease pathogenesis by up-regulating BACE1 gene expression. *Proc Natl Acad Sci U S A* 103, 18727–18732 (2006).

2. Zhang, M. et al. Control of BACE1 degradation and APP processing by ubiquitin carboxyl-terminal hydrolase L1. *J Neurochem* 120, 1129–1138 (2012).

1. Gracile axonal dystrophy (GAD), a new neurological mutant in order to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/