Autophagy 7:7, 788-789; July 2011; © 2011 Landes Bioscience

Therapeutic effects of remediating autophagy failure in a mouse model of Alzheimer disease by enhancing lysosomal proteolysis

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The extensive autophagic-lysosomal pathology in Alzheimer disease (AD) brain has revealed a major defect in the proteolytic clearance of autophagy substrates. Autophagy failure contributes on several levels to AD pathogenesis and has become an important therapeutic target for AD and other neurodegenerative diseases. We recently observed broad therapeutic effects of stimulating autophagic-lysosomal proteolysis in the TgCRND8 mouse model of AD that exhibits defective proteolytic clearance of autophagic substrates, robust intralysosomal amyloid-β peptide (Aβ) accumulation, extracellular β-amyloid deposition and cognitive deficits. By genetically deleting the lysosomal cysteine protease inhibitor, cystatin B (CstB), to selectively restore depressed cathepsin activities, we substantially cleared Aβ, ubiquitinated proteins and other autophagic substrates from autolysosomes/lysosomes and rescued autophagic-lysosomal pathology, as well as reduced total Aβ40/42 levels and extracellular amyloid deposition, highlighting the underappreciated importance of the lysosomal system for Aβ clearance. Most importantly, lysosomal remediation prevented the marked learning and memory deficits in TgCRND8 mice. Our findings underscore the pathogenic significance of autophagic-lysosomal dysfunction in AD and demonstrate the value of reversing this dysfunction as an innovative therapeutic strategy for AD.

Key words: autophagy, lysosome, cathepsin, cystatin B, proteolysis, Alzheimer disease, transgenic

Abbreviations: Aβ, amyloid-β peptide; AD, Alzheimer disease; APP, amyloid precursor protein; AV, autophagic vacuole; Cat, cathepsin; CBKO, cystatin B knockout; CstB, cystatin B; WT, wild type

Submitted: 03/06/11

Revised: 03/15/11

Accepted: 03/24/11

DOI: 10.4161/auto.7.7.15596

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Punctum to: Yang DS, Stavrides P, Mohan PS, Kaushik S, Kumar A, Ohno M, et al. Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits. Brain 134:258–77; PMID: 21186265; DOI: 10.1093/brain/awq341.

The autophagy and endocytic pathways converging on the lysosome are major routes for the processing of amyloid precursor protein (APP) and the generation and degradation of Aβ and β-cleaved C-terminal fragments. A continuum of endosomal-autophagic-lysosomal abnormalities in neurons of the AD brain is well established, including very early appearing swelling of neuronal endosomes reflecting pathologically accelerated endocytosis, increased lysosome biogenesis and striking autophagic-lysosomal pathway pathology characterized by robust accumulation of autophagic vacuoles (AVs) and lysosomes in dystrophic neurites throughout the AD brain. These AVs, most of which contain cathepsins, are filled with incompletely digested "waste" proteins (including Aβ), implying that lysosomal proteolysis is defective. Growing genetic and biochemical evidence has identified lysosomal proteolysis failure as the principal basis for autophagy dysfunction in AD.

Although boosting autophagy induction has been used, with some promise, to delay disease onset in AD mouse models, we have considered that remediation at the lysosomal level may be necessary in AD once proteolysis becomes impaired.

To provide proof of principle that selectively targeting lysosomal dysfunction may be a therapeutic approach, we investigated TgCRND8 mice overexpressing mutant human APP695. Our evaluations of this mouse model documented marked deficits of autophagic-lysosomal function. Neurons in affected brain regions of TgCRND8 mice, but not wild-type mice (WT), exhibit grossly enlarged cathepsin (Cat) D-positive lysosomal compartments and relatively fewer normal-sized lysosomes. These giant neuronal lysosomal compartments appear ultrastructurally as electron-dense, single-membrane-limited vesicles of 1.5 to 5.0 μm diameter containing amorphous granular and membranous material and a minor lipopigment component and are distinguishable from lipofuscin. By double-immunofluorescence labeling, these compartments contain markers of both autophagosomes/autolysosomes and late endosomes. Collectively, these studies identified enlarged compartments as autolysosomes.

Further evidence indicated that enlarged autolysosomes are filled with incompletely digested autophagic substrates, reflecting inefficient degradation by lysosomal hydrolases. AV and lysosome fractions isolated from the brains of TgCRND8 mice contain abnormally high levels of LC3-II, ubiquitinated proteins and Aβ detected by immunoblotting. These antigens are also abundant in giant autolysosomes of CA1 neurons of TgCRND8 brain labeled immunocytochemically. Cathepsin activities measured in extracts of TgCRND8 brain are also significantly lowered relative to those in WT brains.

Given this evidence for impaired lysosomal proteolysis, we sought to restore more normal cathepsin activities in lysosomes of TgCRND8 mice by deleting the gene for CstB, an endogenous inhibitor of cysteine proteases. Using a highly specific affinity-purified polyclonal antibody, we showed that CstB mainly localizes to lysosomal compartments in mouse neurons. CstB deletion, as expected, partially relieves the suppression of multiple cathepsins, including, surprisingly, CatD: enzymatic activities of CatB, CatL and CatD are higher in CstB knockout mouse (CBKO) brains than in WT brains. Moreover, the rate of degradation of long-lived proteins is raised over WT levels in mouse primary fibroblasts and neurons from CBKO mice.

CstB Deletion in TgCRND8 Promotes the Clearance of Proteins Accumulating Abnormally in Lysosomal Compartments

In TgCRND8 mice bred with CBKO mice (CBKO/TgCRND8), enzymatic activities of CatB, CatL and CatD in brain homogenates are elevated over those in TgCRND8 mice. Both CBKO and CBKO/TgCRND8 mice display higher CatD specific enzymatic activity (i.e., CatD enzymatic activity per unit CatD protein) than TgCRND8 mice. In addition, we observed higher levels of CatD in isolated brain AVs and lysosomes from CBKO mice (compared to WT) and from CBKO/TgCRND8 mice (compared to TgCRND8). By contrast, levels of Aβ, ubiquitinated proteins and LC3-II are reduced markedly in the same AV and lysosome fractions from CBKO/TgCRND8 mice compared to those in TgCRND8, indicating that lysosomal protein degradation/clearance in TgCRND8 is improved by CstB deletion.

CstB Deletion in TgCRND8 Reverses Lysosomal System Pathology and Amyloid Pathology

The enhanced clearance of autophagic substrates from abnormal autolysosomes has substantial ameliorative effects on the neuropathology in TgCRND8 mice. Giant autolysosomes in hippocampal neurons disappear while normal-sized lysosomes become more numerous, yielding an essentially normal intracellular lysosomal pattern in CBKO/TgCRND8 mice. CstB deletion also eliminates intralysosomal Aβ-immunostaining in CBKO/TgCRND8 hippocampus. Extracellular amyloid load, detected immunocytochemically with antibodies to Aβ40 or Aβ42, also diminishes in the brains of CBKO/TgCRND8 compared to TgCRND8 and is associated with 40% reduced levels of Aβ40 and Aβ42, measured by ELISA. CstB deletion does not interfere with APP processing; levels of APP holoprotein, sAPPα or sAPPβ are unchanged, supporting the conclusion that the CstB deletion effects are likely exerted at the level of Aβ clearance.

CstB Deletion in TgCRND8 Restores Learning and Memory Functions

In a hippocampus-dependent contextual fear conditioning paradigm, TgCRND8 exhibits a reduced duration of freezing in comparison to WT, while CBKO and CBKO/TgCRND8 show durations of freezing similar to WT, indicating improved contextual memory in CBKO/TgCRND8. Similarly, in an odor habituation test of memory function, TgCRND8 mice display an increased latency to habituate to novel odors, while CBKO/TgCRND8, similar to WT and CBKO, habituate more rapidly to novel odors.

Conclusions

Our findings highlight the pathogenic significance of lysosomal system dysfunction in AD and establish proof of concept that enhancing lysosomal function ameliorates neuropathology and cognitive deficits in AD models while increasing the degradation of Aβ and other potentially toxic autophagic substrates, and therefore shows considerable promise as a therapeutic approach for AD.

Acknowledgements

This work was supported by the National Institute on Aging [P01 AG017617 to R.A.N.] and the Alzheimer's Association [IIRG-08-90771 to D.S.Y.].