Communicative & Integrative Biology 6:5, e25179; September/October 2013; © 2013 Landes Bioscience

GSK-3 and lysosomes meet in Alzheimer's disease

Limor Avrahami and Hagit Eldar-Finkelman*

Department of Human Molecular Genetics and Biochemistry; Sackler School of Medicine; Tel Aviv University; Tel Aviv, Israel

Keywords: GSK-3, lysosome, Alzheimer's disease, Aβ pathology, presenilin-1

Abbreviations: GSK-3, glycogen synthase kinase-3; AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, beta amyloid; PS1, presenilin-1

Aberrant regulation of glycogen synthase kinase-3 (GSK-3) is implicated in Alzheimer's disease (AD), but the mechanisms involved remain elusive. Our recent study shows that GSK-3 impairs lysosomal acidification and that inhibition of GSK-3 re-acidified lysosomes in brains of AD mice. This effect was accompanied by reductions in β-amyloid pathology and amelioration of cognitive deficits. Presenilin-1 (PS1) is an essential factor in lysosomal acidification. To determine whether the inhibition of GSK-3 restores lysosomal malfunction caused by dysfunctional PS1, we treated MEF cells deficient in presenilin proteins (MEF-PS1/2$^{-/-}$) with a selective substrate competitive GSK-3 inhibitor, L803-mts. L803-mts enhanced the acidic lysosomal pool in MEF-PS1/2$^{-/-}$ cells and increased levels of activated cathepsin D in the lysosomes. We conclude that GSK-3 and PS1 operate via similar mechanisms to disrupt lysosomal acidification. Importantly, these data indicate that GSK-3 inhibitors have potential in treatment of conditions associated with defective PS1.

Lysosomes are the primary degradative components responsible for clearing intracellular waste products and damaged proteins, their proper activity is vital for the well-being of the cell.[1] The enzymatic degradation that occurs in lysosomes is highly dependent on the lysosomal acidic pH, which is maintained by the vacuolar ATPase (v-ATPase) proton pumps. Impaired lysosomal activity was initially observed in heredity lysosome storage diseases, and recent studies have demonstrated a tight link between lysosomes and neurodegenerative diseases. Of particular interest is the role of lysosomes in Alzheimer's disease (AD): A decline in lysosomal activity is observed in aging brains, and defects in lysosomal acidification are associated with typical AD pathology of fibrillogenic β amyloid (Aβ) deposits.[2-5]

Accumulation of Aβ plaques is a key hallmark in AD pathogenesis. The 40- or 42-residue Aβ peptides that make up the plaques are generated by sequential proteolysis of the amyloid precursor protein (APP) by β-secretase, β-site APP Cleaving Enzyme 1 (BACE1), and presenilin-dependent γ-secretase.[6,7] Reducing the accumulation of Aβ deposition is thus believed to be a useful therapeutic strategy. Disruption in lysosomal acidification resulted in enhanced Aβ pathology and reduced cognitive ability in AD mouse models,[2-5] giving rise to the hypothesis that restoring lysosomal acidity reverse AD symptoms.

Glycogen synthase kinase-3 (GSK-3) is an evolutionary conserved serine/threonine kinase expressed as two isozymes, GSK-3α and GSK-3β. GSK-3 is emerging an important drug target in AD therapy. Excessive phosphorylation of GSK-3 targets such as the microtubule-associated protein tau, collapsin response mediator proteins (CRMPs) and β-catenin is implicated in mechanisms contributing to AD pathogenesis.[8-10] Indeed, treatment with GSK-3 inhibitors reverses AD symptoms in various animal models.[11] An initial study connected GSK-3α isozyme with Aβ production via enhanced γ-secretase-mediated APP proteolysis.[12] To gain further insights into the role of GSK-3 in Aβ pathology, we used the "5XFAD" mouse model. These mice co-express a total of five familial AD mutations in APP and presenilin-1 (PS1) and develop massive cerebral Aβ loads.[13] We treated these mice nasally with L803-mts, a selective, substrate-competitive GSK-3 inhibitor developed in our laboratory. We found that treatment with L803-mts reduces Aβ pathology and ameliorates cognitive deficits.[14] We also showed that L803-mts restores the lysosomal acidification that was severely impaired in the brains of the 5XFAD mice.[14] This effect was independent of autophagy indicating that lysosomes play a major role in the catabolic disposal of Aβ loads under these conditions.

Recent studies implicated PS1 in controlling lysosomal acidification.[15] We asked whether inhibition of GSK-3 can "repair" lysosomal malfunction caused by dysfunctional PS1. We treated MEF cells deficient in presenilin proteins (MEF-PS1/2$^{-/-}$)[16] with L803-mts. After the treatment cells were stained with LysoTracker Red, a dye that accumulates in acidified organelles, and imaged by confocal microscopy. L803-mts increased the number of acidified lysosomes and intensity of staining as compared with control untreated cells (**Fig. 1**). We next examined the levels of Cathepsin D (CatD), a principle lysosomal protease that is activated in the acidified lysosomal environment.

*Correspondence to: Hagit Eldar-Finkelman; Email: heldar@post.tau.ac.il
Submitted: 04/18/13; Accepted: 05/25/13
Citation: Avarhami L, Eldar-Finkelman H. GSK-3 and lysosomes meet in Alzheimer's disease. Commun Integr Biol 2013; 6: e25179; http://dx.doi.org/10.4161/cib.25179

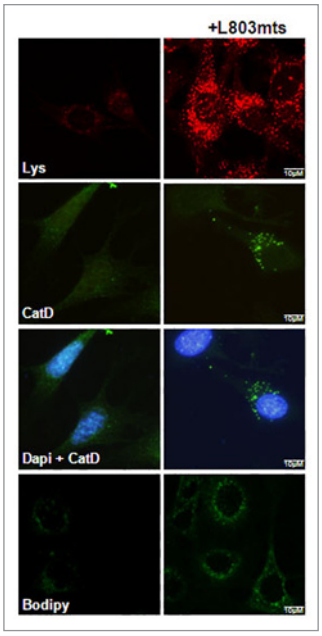

Figure 1. Inhibition of GSK-3 restores impaired lysosomal acidification caused by disrupted PS proteins. MEF-PS1/2$^{-/-}$ cells were treated with L803-mts (40 μM, 6 h) and screened by the following lysosomal markers: live-cell imaging of cells stained with Lysotracker-Red (Lys, top panel); fixed cells immunostained with CatD antibody (middle panels); and live-cell imaging of cells stained with pepstatin A BODIPY (bottom panel).

Immunofluorescence analysis with anti-CatD antibody showed a low level, diffuse signal in the untreated cells. In contrast, L803-mts increased CatD signal (**Fig. 1**). To examine whether CatD was more active in L803-mts treated cells, cells were stained with pepstatin A BODIPY, which binds specifically to the active form of CatD. The BODIPY signal was enhanced by L803-mts, confirming that L803-mts restored lysosomal acidification in these cells. We conclude that GSK-3 and PS1 likely operate via similar mechanisms that impair lysosomal acidification, perhaps through disrupted glycosylation of v-ATPase V0a1 subunit; this glycosylation is critically important for v-ATPase assembly in the lysosome membrane.[15] Another important conclusion from this study is that GSK-3 inhibition should provide benefit in treating conditions associated with defective PS1.

Additional work that was published in parallel to our publication demonstrated the role of GSK-3 in regulating Aβ pathology, but suggested different mechanisms that involved either reduction in β-site APP cleaving enzyme-1, BACE1, expression[17] or enhancement in APP processing via lysosome biogenesis.[18] Altogether, GSK-3 is clearly a prominent factor that contributes to accumulation of Aβ loads in the AD brain. The mechanisms involved are likely dependent on the cellular context including the levels and/or activities of additional factors that contribute to Aβ pathology such as APP, PS1 and lysosomes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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