Presenilins: A novel link between intracellular calcium signaling and lysosomal function?

Ilya Bezprozvanny[1,2]

[1]Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75390
[2]Laboratory of Molecular Neurodegeneration, St. Petersburg State Technical University, 195251 St. Petersburg, Russia

Mutations in presenilins (PS), transmembrane proteins encoding the catalytic subunit of γ-secretase, result in familial Alzheimer's disease (FAD). Several studies have identified lysosomal defects in cells lacking PS or expressing FAD-associated PS mutations, which have been previously attributed to a function for PS in lysosomal acidification. Now, in this issue, Coen et al. (2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201201076) provide a series of results that challenge this idea and propose instead that presenilins play a role in calcium-mediated lysosomal fusion.

Lysosomes are the primary degradative organelles in all cell types. The degradative function of lysosomes is particularly important in nondividing cells, such as neurons. Several diseases associated with lysosome dysfunction, termed lysosomal storage diseases (LSDs), have been identified, most of them affecting brain function (Schultz et al., 2011). Conversely, many neurodegenerative disorders also exhibit hallmarks of LSDs. Alzheimer's disease (AD) is an important example where lysosomal and autophagic dysfunction have been observed in neurons by multiple investigators (Schultz et al., 2011). However, a clear mechanistic link between AD and lysosomal and autophagic processes has been missing. Previously, Lee et al. (2010) had proposed that such a link could be provided by a novel function of the presenilin-1 protein (PS1), which is encoded by the gene most frequently mutated in familial AD (FAD). Using PS1 knockout (KO) blastocysts, they provided evidence in support of the hypothesis that PS1 functions as a specific chaperone for the V0a1 subunit of v-ATPase by facilitating its folding, stability, and N-glycosylation by the oligosaccharyl–transferase (OST) complex in the rough ER (Fig. 1 A). They further suggested that FAD-causing mutations in PS1 have similar effects, thereby decreasing the amount of V0a1 subunit delivered to the lysosomes in PS1-FAD cells. As a result, lysosomes fail to become sufficiently acidified, impairing their degradative capacity and causing an LSD-like phenotype (Fig. 1 A).

The PS1-FAD model proposed by Lee et al. (2010) is conceptually similar to the situation in action myoclonus-renal failure syndrome (AMRF). The AMRF syndrome results from point mutations in lysosomal integral membrane protein LIMP-2 (Berkovic et al., 2008; Blanz et al., 2010). Normal function of LIMP-2 is to act as a sorting receptor for β-glucocerebrosidase (β-GC; Fig. 1 B). AMRF-causing mutations impair LIMP-2 association with β-GC, resulting in inefficient transport of β-GC to lysosomes (Fig. 1 B). Similar to AMRF, Lee et al. (2010) proposed that when PS1 is mutated, v-ATPase V0a1 N-glycosylation and trafficking to lysosomes is impaired, resulting in LSD, autophagic dysfunction, and neurodegeneration (Fig. 1 A). Interestingly, mutations in the V0a1 orthologue in flies cause adult-onset neurodegeneration, which indicates that neuron-specific v-ATPase function is required for neuronal maintenance and that defects in lysosomal acidification are indeed incompatible with neuronal survival (Williamson et al., 2010).

The role of presenilins in lysosomal function has been recently reevaluated by three different groups. Although some results in these papers contradict each other, these papers raise doubt about the validity of the model proposed by Lee et al. (2010). The first paper (Neely et al., 2011) confirmed autophagic deficits in cells lacking both isoforms of PS (double KO; DKO) but did not observe a deficit in lysosomal acidification in these cells using LysoTracker red. The second paper (Zhang et al., 2012) did not measure lysosomal pH specifically, but found no difference in an mean pH of acidic vacuolar organelles in PS DKO cells. The third paper (see Coen et al. in this issue), demonstrated that lysosomes in PS DKO cells acidified normally using several reagents (LysoTracker red, LysoSensor DND160, and pH-sensitive fluorescein derivatives). The use of several independent measurements by Coen et al. (2012) in these experiments makes a strong case against acidification defects in PS DKO cells. Furthermore, Zhang et al. (2012) and Coen et al. (2012) dispute some other key findings of Lee et al. (2010). Both groups demonstrate that lysosomal processing of endogenous Cathepsin D is not affected in PS1 KO, PS DKO, or PS1-FAD cells (Coen et al., 2012; Zhang et al., 2012). Our own group obtained similar results in experiments with PS DKO mouse embryonic fibroblasts (MEFs) and PS cDKO (conditional DKO) hippocampal neuronal cultures (unpublished data). Both groups demonstrated that N-glycosylation of the V0a1 subunit is

Correspondence to Ilya Bezprozvanny: Ilya.Bezprozvanny@utsouthwestern.edu

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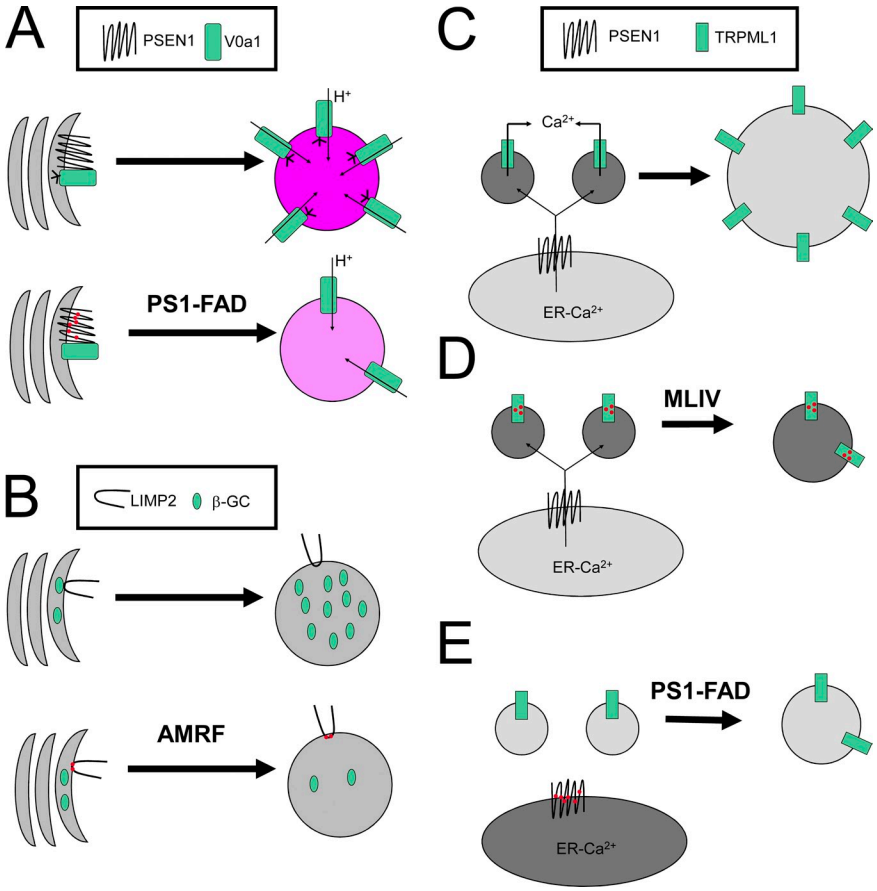

Figure 1. **Potential causes of lysosomal defects in neurological disorders.** (A) Presenilin 1 (PS1) KO or FAD mutations impair N-glycosylation of V0a1 (V0a1 subunit of v-ATPase). In the absence of N-glycosylation, V0a1 does not traffic to lysosomes, causing defects in lysosomal acidification. Based on Lee et al. (2010). Pink and dark pink indicate differences in intralysosomal H^+ concentrations. (B) LIMP-2 mutations impair trafficking of β-glucocerebrosidase (β-GC) to lysosomes in action myoclonus-renal failure syndrome (AMRF). Based on Berkovic et al. (2008) and Blanz et al. (2010). (C) Lysosomal Ca^{2+} release via TRPML1 channels triggers lysosomal fusion. (D) Mutations in TRPML1 result in impaired lysosomal Ca^{2+} release and impaired lysosomal fusion in mucolipidosis IV (MLIV). Based on LaPlante et al. (2004). (E) PS1 KO or FAD mutations impair ER Ca^{2+} leak and result in reduced Ca^{2+} loading into lysosomes. Reduced lysosomal Ca^{2+} levels result in impaired lysosomal fusion. Based on Tu et al. (2006) and Coen et al. (2012). (C–E) Gray and dark gray correspond to different Ca^{2+} concentrations in the ER and lysosomes.

unaffected in PS1 KO and PS DKO cells (Coen et al., 2012; Zhang et al., 2012).

There are several potential technical issues that may be responsible for discrepancies between these recent studies and experiments by Lee et al. (2010). Such issues include the choice of cell line under investigation; Lee et al. (2010) performed most of the experiments with PS1 KO blastocyst-derived (BD) cell line BD15, which had been developed previously (Lai et al., 2003). Neely et al. (2011) and Coen et al. (2012) performed most of the studies with PS DKO MEF cells and PS1 KO primary neurons, whereas Zhang et al. (2012) used PS1 KO and PS DKO embryonic stem (ES) cells and PS cDKO neurons in their experiments. These groups also used different methods and different reagents to quantify lysosomal pH levels and to perform biochemical analysis of the V0a1 N-glycosylation state and subcellular localization. One can expect that most of these technical issues will be sorted out and the sources of discrepancy identified. A strong argument against the concept of a key role for N-glycosylation in the trafficking of vacuolar ATPase (V-ATPase) comes from experiments in which Coen et al. (2012) demonstrate that a version of V0a1 ATPase with its unique N-glycosylation site mutated is still properly targeted and functional in cell cultures or in a transgenic fly model in vivo (Coen et al., 2012). Because loss of the *Drosophila melanogaster* V0a1 function causes degeneration (Williamson et al., 2010), this later result raises serious doubts that the N-glycosylation event is as important for V0a1 targeting and function, as had been postulated by Lee et al. (2010). One potential caveat with these later studies is that these experiments have been performed with overexpressed mutant V0a1 ATPase, which may have overridden the mechanism by which this protein, at endogenous levels, is targeted to the lysosomes.

In light of this serious challenge to the hypothesis proposed by Lee et al. (2010; Fig. 1 A), what then can be an explanation for the lysosomal defects consistently observed in PS DKO and PS1-FAD cells? Neely et al. (2011) conclude that presenilins act at the level of autophagosome–lysosomal interaction or lysosomal function (Neely et al., 2011). They confirmed the finding by Lee et al. (2010) that the function of presenilins in autophagy is not related to their γ-secretase activity, but do not describe a specific mechanism that connects presenilins and the autophagic/lysosomal pathway (Neely et al., 2011). Using a transcription profiling approach, Zhang et al. (2012) discovered highly significant elevation of coordinated lysosomal expression and regulation (CLEAR) network genes (Sardiello et al., 2009) in the excitatory cortical and hippocampal neurons from PS cDKO mice (Zhang et al., 2012). These findings confirm that neuronal lysosomes are affected in the absence of presenilins, but again provide no mechanistic information about potential causes of such alterations. Zhang et al. (2012) speculate that in the absence of γ-secretase function, lysosomal degradation machinery is "jammed" by unprocessed membrane protein stubs from the late endosomes and Golgi compartments, resulting in compensatory up-regulation of CLEAR network genes. This is a plausible hypothesis that may indeed explain lysosomal abnormalities in PS DKO cells that completely lack

γ-secretase activity. This hypothesis is however not likely to explain the cause of lysosomal abnormalities in PS1-FAD cells, where γ-secretase activity is usually only mildly altered.

The study by Coen et al. (2012) does offer a novel mechanistic hypothesis that may account for lysosomal abnormalities in PS DKO and PS1-FAD cells. These authors argue that although lysosomal acidification is normal in these cells, lysosomal fusion is impaired. Defects in lysosomal fusion may lead to impaired delivery of lysosomal fusion enzymes from endosomal compartments and abnormal cargo distribution between lysosomes and autophagosomes, resulting in an LSD-like phenotype. Previous results by the same group already pointed to a potential connection between presenilins and lysosomal fusion (Esselens et al., 2004), although defective fusion has not been observed in studies of PS1 KO cells by other groups (Wilson et al., 2004; Lee et al., 2010). The authors now argue that lysosomal fusion events may require Ca^{2+} release from lysosomes and that impaired Ca^{2+} signaling in PS DKO and PS1-FAD cells may potentially be responsible for lysosomal fusion defects in these cells. To test this hypothesis, they performed experiments with Gly–Phe–β-naphtylamide (GPN) tripeptide, which causes osmotic lysis of late endosomes and lysosomes. Using GPN peptide, they demonstrated that the content of lysosomal Ca^{2+} stores was significantly reduced in PS DKO MEF cells and in PS1 KO neurons (Coen et al., 2012). Importantly, lysosomal Ca^{2+} content could be rescued by stable retroviral transduction of PS DKO MEF cells with PS1 or γ-secretase activity–defective PS1 mutant, indicating that the observed lysosomal Ca^{2+} phenotypes do not depend on γ-secretase activity of presenilins. Coen et al. (2012) did not directly demonstrate that the rescue of lysosomal Ca^{2+} loading also rescues the lysosomal fusion defect, but these results are consistent with their previous findings, which indicated that presenilins have a role in lysosomal fusion that is not dependent on their γ-secretase activity (Esselens et al., 2004).

The model proposed by these authors is analogous to pathogenesis of mucolipidosis IV (MLIV), a neurological disorder that usually presents during the first year of life with blindness, cognitive impairment, and psychomotor delays. The MLIV results from the loss of function mutations in the TRPML1 channel, which mediates Ca^{2+} release from the lysosomes (LaPlante et al., 2004). TRPML1-mediated Ca^{2+} release is a major fusogenic signal for late endosomes and lysosomes (Fig. 1 C), and in the absence of this signal lysosomal fusion does not occur, leading to an LSD phenotype (Fig. 1 D). The model suggested by Coen et al. (2012) is analogous to MLIV, but instead of deficiency in TRPML1 function the deficiency appears to be at the step of Ca^{2+} loading into lysosomes (Fig. 1 E).

What is the connection between presenilins and Ca^{2+} loading to lysosomes? One obvious possibility is that the lysosomal acidification defect (Lee et al., 2010) causes reduced Ca^{2+} loading into lysosomes, as lysosomal H^+ and Ca^{2+} homeostasis are closely related. However, Coen et al. (2012) did not observe a lysosomal acidification defect in their experiments with PS DKO cells, so a different model needs to be proposed to explain their result. I would like to speculate that reduced Ca^{2+} loading into lysosomes may potentially result from impaired ER Ca^{2+} leak function (Fig. 1 E). In previous studies, our laboratory demonstrated that presenilins act as ER Ca^{2+} leak channels, and that the ER Ca^{2+} leak function of presenilins is disrupted by many FAD mutations (Tu et al., 2006). An idea that presenilins function as ER Ca^{2+} leak channels is also not without controversy (Bezprozvanny et al., 2012; Shilling et al., 2012), but experimental evidence for this idea has been provided so far in multiple lines of investigation performed in a variety of experimental systems (Nelson et al., 2007, 2010, 2011; Zhang et al., 2010). I would like to propose that reduced Ca^{2+} leak from ER in PS DKO and PS1-FAD cells makes less Ca^{2+} available for loading into lysosomes, leading to impaired lysosomal fusion and LSD (Fig. 1 E). The ER Ca^{2+} pool is much greater than the lysosomal Ca^{2+} pool, and one can expect that even small fluctuations in ER Ca^{2+} handling can have large effects on the lysosomal Ca^{2+} load. We previously demonstrated that the ER Ca^{2+} leak function of presenilins is γ-secretase independent (Tu et al., 2006). Thus, the ability of the γ-secretase activity–defective PS1 mutant to rescue Ca^{2+} loading into lysosomal compartments in PS DKO MEF cells (Coen et al., 2012) is consistent with this hypothesis. Obviously, this hypothesis is at present highly speculative and needs to be tested experimentally, but it may finally help to reconcile the two most established γ-secretase–independent functions of presenilins: their role in ER Ca^{2+} signaling and their role in lysosomal function.

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