Published in final edited form as:

Brain Res. 2012 December 13; 1489: 8–16. doi:10.1016/j.brainres.2012.10.036.

PRESENILIN-NULL CELLS HAVE ALTERED TWO-PORE CALCIUM CHANNEL EXPRESSION AND LYSOSOMAL CALCIUM; IMPLICATIONS FOR LYSOSOMAL FUNCTION

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Abstract

Presenilins are necessary for calcium homeostasis and also for efficient proteolysis through the autophagy/lysosome system. Presenilin regulates both endoplasmic reticulum calcium stores and autophagic proteolysis in a γ -secretase independent fashion. The endo-lysosome system can also act as a calcium store, with calcium efflux channels being recently identified as two-pore channels 1 and 2. Here we investigated lysosomal calcium content and the channels that mediate calcium release from these acidic stores in presenilin knockout cells. We report that presenilin loss leads to a lower total lysosomal calcium store despite the buildup of lysosomes found in these cells. Additionally, we find alterations in two-pore calcium channel protein expression, with loss of presenilin preventing the formation of a high molecular weight species of TPC1 and TPC2. Finally, we find that treatments that disturb lysosomal calcium release lead to a reduction in autophagy function yet lysosomal inhibitors do not alter two-pore calcium channel expression. These data indicate that alterations in lysosomal calcium in the absence of presenilins might be leading to disruptions in autophagy.

Keywords

Presenilin; Lysosome; Calcium; Two-pore channel; Autophagy

1. INTRODUCTION

Presenilins are multi-spanning transmembrane proteins best known for their function in the γ -secretase complex. Through its enzymatic function, presenilins cleave many type-1 transmembrane proteins such as amyloid precursor protein, Notch, and cadherins (Selkoe and Wolfe, 2007). Additionally, presenilins have functions outside of γ -secretase, mostly mediated by physical interaction of presenilins with the proteins they affect. Two of the most notable γ -secretase independent functions of presenilins are the regulation calcium homeostasis and autophagy (Green et al., 2008; LaFerla, 2002; Lee et al., 2010; Leissring et al., 1999a; Leissring et al., 1999b; Neely et al., 2011; Tu et al., 2006).

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Presenilin interacts with a number of calcium channels and pumps including sarco-ER Ca^{2+} ATPase (SERCA) (Green et al., 2008; Wakabayashi et al., 2009), the inositol 1,4,5-trisphosphate (IP_3) receptor (Cheung et al., 2008), and the ryanodine receptor (Chan et al., 2000; Rybalchenko et al., 2008). Through these interactions presenilins are vital to calcium homeostasis in cells by affecting ER calcium stores, as well as capacitive calcium entry (Leissring, 2000; Yoo et al., 2000), and also mitochondrial stores (Zampese et al., 2011).

Presenilins are also crucial for efficient proteolysis via autophagy (Lee et al., 2010; Neely et al., 2011). During autophagy, protein aggregates and damaged organelles are brought to the lysosome for degradation. Proper autophagy is vital to cell survival, neuronal health, and clearance of long-lived proteins and protein aggregates (Berger et al., 2006; Deretic, 2011; Hara et al., 2006; Komatsu et al., 2006; Mortimore and Pösö, 1987; Onodera and Ohsumi, 2005; Ravikumar et al., 2008; Sarkar et al., 2009). Our previous data indicated that presenilins function in autophagy at the step of lysosome fusion to autophagosomes or lysosome function (Neely et al., 2011). Of note, calcium fluxes have been shown to regulate autophagy induction, especially via the IP_3 receptor (Decuypere et al., 2011; Sarkar and Rubinsztein, 2006).

In addition to calcium fluxes regulating the induction of autophagy, lysosomes act as calcium stores, and possess energy dependent calcium efflux channels, known as two-pore channels (TPC). TPCs are sensitive to the second messenger nicotinic acid adenine dinucleotide phosphate (NAADP) (Brailoiu et al., 2009; Brailoiu et al., 2010; Calcraft et al., 2009; Ogunbayo et al., 2011; Ruas et al., 2010; Zong et al., 2009). The two forms of TPCs in mammals are TPC1, localized both endosomes and lysosomes, and TPC2, localized to lysosomes only (Brailoiu et al., 2009; Brailoiu et al., 2010; Calcraft et al., 2009). These channels are predicted to have two repeated domains of six transmembrane regions each, a structure that corresponds to half a voltage sensitive $\text{Ca}^{2+}/\text{Na}^+$ channel (Furuichi et al., 2001; Ishibashi et al., 2000). Due to this, it is speculated that TPCs function as dimers (Ishibashi et al., 2000). Recently, TPCs have been confirmed to form both homo- and heterodimers (Rietdorf et al., 2011). Little is known about the role of these channels in lysosome function, or on the autophagic processes that deliver autophagosomes to the lysosome. Fusion of organelles within the endo-lysosomal system is calcium-dependent and therefore lysosomal calcium stores may regulate autophagosome-lysosome fusion (Holroyd et al., 1999; Pryor et al., 2000). Recently, NAADP was shown to regulate autophagy in astrocytes (Pereira et al., 2011). TPCs have also been implicated in trafficking vesicles within the endo-lysosomal system and so may be involved in lysosome biogenesis (Ruas et al., 2010). It is interesting to note that the mechanism by which calcium is stored in the endo-lysosomal system is not clear but it seems to be dependent on a proton gradient (Christensen et al., 2002).

Given the role of presenilins in autophagy, we hypothesized that calcium dyshomeostasis due to presenilin loss leads to autophagic deficits. Although much is known about how presenilin loss affects ER and extracellular calcium, the effect of presenilin loss on lysosomal calcium stores and the channels that mediate lysosomal calcium release has not been thoroughly explored. Therefore, we investigated both of these characteristics in presenilin knockout cells. Recently, Coen et al. found that presenilin-deficient cells have a decrease in lysosomal calcium content (Coen et al., 2012). In our studies, we similarly observed a decrease in the total lysosomal calcium store despite the buildup of lysosomes in presenilin double knockout cells. We then expanded on these findings to discover that, in the absence of presenilins, TPC expression is altered, specifically with a decrease in molecular weights corresponded to the dimeric channel. These changes in TPCs indicate dysfunction in the absence of presenilins. Finally, we find that TPC inhibition causes a reduction in autophagy function but lysosomal inhibitors do not alter TPC expression. These data

indicate that alterations in lysosomal calcium and TPCs in the absence of presenilins is a possible mechanism for their autophagy dysfunction.

2. RESULTS

2.1 Presenilin loss reduces lysosomal calcium levels

Our previous data indicated that presenilin knockout cells exhibit alterations in calcium homeostasis and reduction of autophagic function (Green et al., 2008; Neely et al., 2011). Since lysosomal calcium alterations can affect autophagy function (Pereira et al., 2011), we sought to examine whether presenilin knockout cells have abnormal amounts of lysosomal calcium. Hence, we measured the levels of cytosolic calcium in response to glycyl-L-phenylalanine 2-naphthylamide (GPN). GPN is a dipeptide substrate for cathepsin C and causes osmotic lysis of lysosomes to demonstrate lysosomal calcium content (Haller et al., 1996; Jadot et al., 1984; Pandey et al., 2009). We found that presenilin-double knockout (PSDKO) mouse embryonic fibroblasts have lower magnitude of response (ΔR) compared with control cells (Fig. 1 A–B). This result confirms recent published data demonstrating a decrease in lysosomal calcium in presenilin-deficient cells (Coen et al., 2012).

2.2 Presenilin loss alters of TPC protein expression

Since presenilin knockout cells have a decreased lysosomal calcium store, we next investigated protein expression of two lysosomal calcium channels, TPC1 and TPC2. We found that protein expression of both TPC1 and TPC2 was altered in PSDKO cells (Fig. 1, C–D). PSDKO cells had an increase in TPC1 at the molecular weight corresponding to the glycosylated monomer (low MW) while they have a marked decrease in expression at the molecular weight corresponding to a dimer (high MW) (Fig. 1, C–D). Correspondingly, similar results were obtained when investigating the expression of TPC2. PSDKO cells had no change in expression of the glycosylated monomer while they had a similarly robust decrease in expression of the size corresponding to the dimer. These results are intriguing, as it seems that PSDKO cells have a reduction in both TPC1 and TPC2 in the hypothesized functional form of these proteins. Importantly, TPC dimer formation was recently confirmed by co-immunoprecipitation, mass spectrometry, and FRET imaging (Rietdorf et al., 2011). These data strongly indicate that presenilin loss not only leads to changes in overall lysosomal calcium store but also the levels of both TPC1 and TPC2 channels, which mediate calcium efflux from lysosomes.

We next investigated whether presenilin single knockout would have the same effect as presenilin double knockout. We find that presenilin-1 or -2 knockout cells have the same expression phenotype for TPC1 (Fig. 1, E–F). Both cell types have a reduction in the high molecular weight species of TPC1 but show no consistent change in the low molecular weight species. These data indicate that both presenilin-1 and 2 seem to be important for proper formation of the putative dimer of TPC1. Interestingly, we found that presenilin-1 knockout exclusively affected TPC2 expression while presenilin-2 knockout had no effect. There is a reduction in expression in monomeric TPC2 in presenilin-1 knockout cells, yet no change in the high molecular weight species (Fig. 1, E–F). It is possible that in presenilin-mediated dimerization of TPC2, either presenilin-1 or 2 can function alone. Therefore, with the loss of just one presenilin, the other will compensate and allow for proper dimerization.

2.3 Lysosome numbers and volume are increased in presenilin knockout cells, but TPC1-GFP and TPC2-GFP puncta are decreased

To assess TPC1 localization and distribution, we transiently transfected control and PSDKO cells with TPC1-GFP (a generous gift from Dr. Sandip Patel, University College London), and co-stained with the dye LysoTrackerRed. Z-stacks were performed via confocal

microscopy and then 3D modeling performed with Bitplane Imaris software, to calculate the volumes and numbers of LysotrackerRed and TPC1-GFP. Typical captures are shown in Fig. 2A–F, for both control and PSDKO cells. A three dimensional composite of the Z-stack is shown for control cells and PSDKO cells (Fig. 2A, D, respectively). The respective composites with LysotrackerRed puncta reveal stark increases in lysosomal numbers in PSDKO cells (Fig. 2B, E). Volume and puncta counts revealed stark increases in lysosomal number and total lysosomal volume per cell in PSDKO cells (Fig. 2G), as well as increases in the average volume per puncta (Fig. 2H). We stratified these results further, and separated LysotrackerRed puncta based on size. These analyses revealed that PSDKO cells had increased lysosome numbers across all volume categories (Fig. 2I). Expressed as percentages of total lysosomes per cell we found that PSDKO cells have comparatively more large and small lysosomes than control cells, but less average sized puncta (Fig. 2J). These results confirm our previous studies showing increased LysotrackerRed in PSDKO cells, and provide additional information about the absolute numbers and volumes of these puncta. Taken together with the calcium release evoked by GPN in these cells, PSDKO cells have nearly three times the lysosomal volume per cell than a control fibroblast, yet still have reduced GPN sensitive calcium stores.

TPC1-GFP fluorescence produced puncta, which were, for the most part, distinct from LysotrackerRed, in both control and PSDKO cells (Fig. 2A–F, and 2O–T). TPC1-GFP puncta were far more numerous than LysotrackerRed puncta in both cell lines, as were the average volumes (Fig. 2K). Volume analysis shows no differences in the number of TPC1-GFP puncta per cell between control and PSDKO cells, but a small and significant reduction in average TPC1-GFP volume (Fig. 2K). This appears to be due to a slight, yet significant, reduction in the average volume per puncta (Fig. 2L). These results show that the loss of presenilin has subtle effects on the distribution of TPC1-GFP, when transiently expressed, in addition to the effects characterized on endogenous TPC1 in PSDKO cells.

We performed a similar experiment using TPC2-GFP. Typical Z-stack composites and 3D modeling of TPC2-GFP and LysotrackerRed puncta can be seen in Fig. 3A–F. Analysis shows significant reductions in TPC2-GFP puncta per cell (Fig. 3G), but no differences in the average volume per TPC2-GFP puncta (Fig. 3H). These results show that TPC2-GFP distribution is altered in PSDKO cells.

2.4 Reducing TPC2 function leads to disrupted autophagy

Our data show that presenilin knockout leads to altered lysosomal calcium and changes in two-pore channel expression and dimerization. We previously demonstrated that presenilin knockout cells also have deficits in autophagy (Neely et al., 2011). Hence, we next investigated if the alterations in TPCs could conceivably lead to autophagy dysfunction. To test this, we treated control cells with the TPC2 channel antagonist, Ned-19. Eight-hour Ned-19 treatment led to a reduction in LC3-II expression, a marker for autophagosomes (Fig. 4A–B), and a reduction in the rate of long-lived protein proteolysis, as measured through radioactivity (Fig. 4C). These results confirm that reducing TPC2 function can negatively impact autophagy and provide a connection between alterations in TPCs and autophagy found in presenilin knockout cells.

2.5 Abolishing lysosome function does not change TPC expression

Although our data thus far indicate that changes in TPCs in presenilin knockout cells might lead to autophagy dysfunction, we also wanted to determine if lysosome inhibition could change TPC expression. Control fibroblasts were treated with leupeptin/ammonium chloride, a potent lysosomal inhibitor cocktail, for 7 hours. Although we were able to confirm lysosomal inhibition through a buildup LC3-II, we did not find any alterations in

TPC expression (Fig. 4D–E). These data suggest that the changes in TPC expression present in presenilin knockout are not caused by deficits in lysosomal function

3. DISCUSSION

The presenilins regulate many important cellular processes, through γ -secretase dependent and independent mechanisms. Two γ -secretase independent processes are the regulation of calcium homeostasis, and efficient proteolysis through the autophagy-lysosome system. We previously showed that presenilins interact with the SERCA, to enable efficient pumping of cytosolic calcium into the endoplasmic reticulum stores (Green et al., 2008), an interaction that has been replicated in multiple studies (Wakabayashi et al., 2009). In addition, we showed that the presenilins are necessary for proper lysosomal-dependent proteolysis, with their absence leading to autophagosome accumulation (Neely et al., 2011). Here, we demonstrate that the absence of presenilins has implications not only for ER calcium stores and signaling, but also on the amount of calcium stored in lysosomal GPN-sensitive stores (Jadot et al., 1984), a result that has also been observed by others (Coen et al., 2012). Furthermore, presenilin-null cells have three times more lysosomal volume, suggesting that each lysosome has substantially reduced calcium content than a lysosome in control cells. Although little is known about the role that lysosomal calcium plays in protein degradation, including facilitation and initiation of autophagy, it is known that lysosomal calcium is necessary for fusion and trafficking within the endolysosomal system (Holroyd et al., 1999; Pryor et al., 2000; Ruas et al., 2010) and also that NAADP can regulate autophagy (Pereira et al., 2011).

Although we are unable to explore if the lack of presenilins could affect the filling of lysosomes, we found that loss of presenilins had profound implications for both TPC1 and TPC2. These channels are proposed to function as dimers, based on their similarity to $\text{Ca}^{2+}/\text{Na}^{+}$ channels (Furuichi et al., 2001; Ishibashi et al., 2000), and recently biochemical proof of this has been shown (Rietdorf et al., 2011). In our control fibroblasts, we clearly detect TPC1 and TPC2 proteins at weights consistent with dimers. When both presenilins were deleted, these dimers were reduced, suggesting that presenilins play a role in the dimerization of both TPCs. While our data suggest that these high molecular weight bands detected with both TPC1 and TPC2 antibodies are dimers, there may be alternative explanations as well.

What are the implications of presenilin-mediated TPC dimerization on lysosomal calcium and autophagic/lysosomal proteolysis? We demonstrated that TPC2 inhibition with Ned-19 reduced the rate long-lived protein degradation, suggesting that impaired TPC function would impact autophagy. It is important to note that presenilin loss results in impaired proteolysis via the lysosome corresponding with a build up of LC3-II – the opposite effect to Ned-19. Hence, alterations in TPCs do not account for the full effect of presenilins on autophagy. Additionally, impairing autophagy does not alter TPC expression and therefore, altered TPC expression in presenilin knockout cells is unlikely due to impaired autophagy. Hence, presenilins appear to be involved in setting lysosomal calcium levels possibly through regulation of TPC expression and dimerization and this could be part of a mechanism by which presenilins function in autophagy. Since we are unable to explore lysosomal filling, as these mechanisms are undefined, we cannot discount a role for presenilins in establishing a lysosomal calcium gradient. Our data expands the understanding of the basic cell biology role of wild-type presenilins in autophagy and calcium homeostasis. In the scope of this study, we did not investigate the effect of presenilin familial Alzheimer's disease mutations on the dimerization of TPCs. Given that autophagy function is detrimentally affected with presenilin mutations (Lee et al., 2010), it is possible that TPC dimerization is also affected. Additionally, the intimate connection

between calcium and amyloid-beta production and the location of TPCs within the lysosome, a site of amyloid-beta production, suggests a possible further link between these pathways and Alzheimer's disease pathogenesis. Clearly, the role of TPCs in both familial and sporadic Alzheimer's disease should be the focus of further investigation. Although the exact mechanism by which presenilins affect lysosomal calcium is still unclear, our data establish a never before described connection between presenilins and TPCs.

4. EXPERIMENTAL PROCEDURE

Cell culture

Mouse embryonic fibroblasts (Bart de Strooper, Katholieke Universiteit Leuven, Leuven, Netherlands) were maintained with DMEM (Gibco), 1% Penicillin/Streptomycin (Gibco), and 10% FBS (Gibco). When appropriate, cells were treated with leupeptin/NH₄Cl for 6hrs at 37°C for 6 hours or 4 μ M trans-Ned-19 (Enzo Life Sciences) at 37°C for 24 hours.

Western Blotting

Western blotting was performed as previously described (Neely et al., 2011). Antibodies used in this study include LC3 (MBL Int), TPC1 (Novus), TPC2 (Novus) PS1 (Novus), PS2 (Novus), and Actin (Sigma).

Calcium imaging

Cells were loaded with 2.5 μ M fura2-AM (Sigma) with 0.005% pluronic acid in Ca²⁺ imaging solution for one hour at room temperature in the dark. Cells were then washed and imaged using the InCyt Im2 Ration imaging system (Intracellular Imaging, Inc.) using excitation at 340 and 380nm. Cells were recorded until baseline was established and then GPN was added to a final bath concentration of 100 μ M.

TPC-GFP expression

Cells were transiently transfected with TPC1-GFP or TPC2-GFP (a generous gift from Dr. Sandip Patel, University College London) using lipofectamine2000 (Invitrogen). Cells were allowed to express for 24–48 hrs. When appropriate, cells were loaded with 100nM LysoTrackerRed (Invitrogen) to label acidic organelles for 30 minutes at 37°C. Cells were fixed with 4% paraformaldehyde for 20 minutes prior to coverslipping with fluoromount-g (Southern Biotech).

Long-lived proteolysis assay

This protocol was adapted from (Bauvy et al., 2009) and performed as previously described (Neely et al., 2011). When media was replaced during chase period, cells were washed extensively and media was replaced with 10mM valine and 4 μ M trans-Ned-19 (Enzo Life Sciences) when applicable.

Confocal microscopy

Images were acquired on the Leica DM2500 confocal microscope. Acquisition software used was LAS_AF (Leica Microsystems). Slides were prepared using fluoromount mounting media (Southern Biotech) and images were acquired at room temperature. Fluorochromes used are listed under the specific experiment. Z step size was 0.5 μ m. At least ten different fields of view were collected for each condition with 5–25 cells per field.

Bitplane Imaris software analysis

Analysis of confocal images was performed using the surfaces module of Bitplane Imaris software ver. 7.3.0. Parameters for each analysis were as follows: LysoTrackerRed-threshold

values between 28.9–207.4, estimated diameter 0.7 μm , and diameter between 0.2–2.5 μm , TPC1-GFP-threshold values between 8.4–73.08, estimated diameter 0.4 μm , and diameter between 0.2–2.5 μm , and TPC2-GFP-threshold values between 4.5–73.1, estimated diameter 0.7 μm , and diameter between 0.2–2.5 μm . Only GFP positive cells were used for analysis of TPC1-GFP and TPC2-GFP data.

Statistics

Data are presented as mean + 1 SEM, with n = number of samples examined. An unpaired Student's T-test or ANOVA was used to determine statistical significance ($p < 0.05$). The specific statistical analyses used are indicated within the figure legends. Graphpad Prism was used for statistical analysis.

Acknowledgments

We appreciate the gift of the TPC-GFP constructs from Dr. Sandip Patel (University College London). We are grateful to Dr. Masashi Kitazawa for his helpful guidance and discussions. This work was supported in part by NIH grants: 021928 and AG027544 awarded to FML.

Abbreviations

TPC	two-pore calcium channel
NAADP	nicotinic acid adenine dinucleotide phosphate
GPN	glycyl-L-phenylalanine 2-naphthylamide
PSDKO	presenilin double knockout

References

- Bauvy, C.; Meijer, A.J.; Codogno, P. Autophagy in Mammalian Systems, Part B. Vol. 452. Elsevier; 2009.
- Berger Z, Ravikumar B, Menzies FM, Oroz LG, Underwood BR, Pangalos MN, Schmitt I, Wullner U, Evert BO, O'Kane CJ, Rubinstein DC. Rapamycin alleviates toxicity of different aggregate-prone proteins. Human molecular genetics. 2006; 15:433–42. [PubMed: 16368705]
- Brailoiu E, Churamani D, Cai X, Schrlau MG, Brailoiu GC, Gao X, Hooper R, Boulware MJ, Dun NJ, Marchant JS, Patel S. Essential requirement for two-pore channel 1 in NAADP-mediated calcium signaling. J Cell Biol. 2009; 186:201–9. [PubMed: 19620632]
- Brailoiu E, Rahman T, Churamani D, Prole DL, Brailoiu GC, Hooper R, Taylor CW, Patel S. An NAADP-gated Two-pore Channel Targeted to the Plasma Membrane Uncouples Triggering from Amplifying Ca^{2+} Signals. J Biol Chem. 2010; 285:38511–6. [PubMed: 20880839]
- Calcraft PJ, Ruas M, Pan Z, Cheng X, Arredouani A, Hao X, Tang J, Rietdorf K, Teboul L, Chuang KT, Lin P, Xiao R, Wang C, Zhu Y, Lin Y, Wyatt CN, Parrington J, Ma J, Evans AM, Galione A, Zhu MX. NAADP mobilizes calcium from acidic organelles through two-pore channels. Nature. 2009; 459:596–600. [PubMed: 19387438]
- Chan SL, Mayne M, Holden CP, Geiger JD, Mattson MP. Presenilin-1 mutations increase levels of ryanodine receptors and calcium release in PC12 cells and cortical neurons. J Biol Chem. 2000; 275:18195–200. [PubMed: 10764737]
- Cheung KH, Shineman D, Muller M, Cardenas C, Mei L, Yang J, Tomita T, Iwatsubo T, Lee VM, Foscett JK. Mechanism of Ca^{2+} disruption in Alzheimer's disease by presenilin regulation of InsP_3 receptor channel gating. Neuron. 2008; 58:871–83. [PubMed: 18579078]
- Christensen KA, Myers JT, Swanson JA. pH-dependent regulation of lysosomal calcium in macrophages. J Cell Sci. 2002; 115:599–607. [PubMed: 11861766]
- Coen K, Flannagan RS, Baron S, Carraro-Lacroix LR, Wang D, Vermeire W, Michiels C, Munck S, Baert V, Sugita S, Wuytack F, Hiesinger PR, Grinstein S, Annaert W. Lysosomal calcium

- homeostasis defects, not proton pump defects, cause endo-lysosomal dysfunction in PSEN-deficient cells. *J Cell Biol.* 2012; 198:23–35. [PubMed: 22753898]
- Decuypere JP, Bultynck G, Parys JB. A dual role for Ca(2+) in autophagy regulation. *Cell Calcium.* 2011
- Deretic V. Autophagy in immunity and cell-autonomous defense against intracellular microbes. *Immunol Rev.* 2011; 240:92–104. [PubMed: 21349088]
- Furuichi T, Cunningham KW, Muto S. A putative two-pore channel AtTPC1 mediates Ca(2+) flux in Arabidopsis leaf cells. *Plant Cell Physiol.* 2001; 42:900–5. [PubMed: 11577183]
- Green KN, Demuro A, Akbari Y, Hitt BD, Smith IF, Parker I, LaFerla FM. SERCA pump activity is physiologically regulated by presenilin and regulates amyloid beta production. *The Journal of cell biology.* 2008; 181:1107–1116. [PubMed: 18591429]
- Haller T, Dietl P, Deetjen P, Volkl H. The lysosomal compartment as intracellular calcium store in MDCK cells: a possible involvement in InsP3-mediated Ca2+ release. *Cell Calcium.* 1996; 19:157–65. [PubMed: 8689673]
- Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-migishima R, Yokoyama M, Mishima K, Saito I, Okano H, Mizushima N. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature.* 2006; 441:885–889. [PubMed: 16625204]
- Holroyd C, Kistner U, Annaert W, Jahn R. Fusion of endosomes involved in synaptic vesicle recycling. *Mol Biol Cell.* 1999; 10:3035–44. [PubMed: 10473644]
- Ishibashi K, Suzuki M, Imai M. Molecular cloning of a novel form (two-repeat) protein related to voltage-gated sodium and calcium channels. *Biochem Biophys Res Commun.* 2000; 270:370–6. [PubMed: 10753632]
- Jadot M, Colmant C, Wattiaux De Coninck S, Wattiaux R. Intralysosomal hydrolysis of glycyl-L-phenylalanine 2-naphthylamide. *Biochem J.* 1984; 219:965–70. [PubMed: 6743255]
- Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, Tanaka K. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature.* 2006; 441:880–884. [PubMed: 16625205]
- LaFerla FM. Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. *Nature reviews. Neuroscience.* 2002; 3:862–72.
- Lee JH, Yu WH, Kumar A, Lee S, Mohan PS, Peterhoff CM, Wolfe DM, Martinez-Vicente M, Massey AC, Sovak G, Uchiyama Y, Westaway D, Cuervo AM, Nixon RA. Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. *Cell.* 2010; 141:1146–58. [PubMed: 20541250]
- Leissring MA, Parker I, LaFerla FM. Presenilin-2 mutations modulate amplitude and kinetics of inositol 1, 4,5-trisphosphate-mediated calcium signals. *J Biol Chem.* 1999a; 274:32535–8. [PubMed: 10551803]
- Leissring MA, Paul BA, Parker I, Cotman CW, LaFerla FM. Alzheimer's presenilin-1 mutation potentiates inositol 1,4,5-trisphosphate-mediated calcium signaling in *Xenopus* oocytes. *Journal of neurochemistry.* 1999b; 72:1061–8. [PubMed: 10037477]
- Leissring MA. Capacitative calcium entry deficits and elevated luminal calcium content in mutant presenilin-1 knockin mice. *J Cell Biol.* 2000; 149:793–798. [PubMed: 10811821]
- Mortimore GE, Pösö AR. Intracellular protein catabolism and its control during nutrient deprivation and supply. *Annual review of nutrition.* 1987; 7:539–64.
- Neely KM, Green KN, LaFerla FM. Presenilin is necessary for efficient proteolysis through the autophagy-lysosome system in a gamma-secretase-independent manner. *J Neurosci.* 2011; 31:2781–91. [PubMed: 21414900]
- Ogunbayo OA, Zhu Y, Rossi D, Sorrentino V, Ma J, Zhu MX, Evans AM. Cyclic adenosine diphosphate ribose activates ryanodine receptors, whereas NAADP activates two-pore domain channels. *J Biol Chem.* 2011; 286:9136–40. [PubMed: 21216967]
- Onodera J, Ohsumi Y. Autophagy is required for maintenance of amino acid levels and protein synthesis under nitrogen starvation. *The Journal of biological chemistry.* 2005; 280:31582–6. [PubMed: 16027116]

- Pandey V, Chuang CC, Lewis AM, Aley PK, Brailoiu E, Dun NJ, Churchill GC, Patel S. Recruitment of NAADP-sensitive acidic Ca^{2+} stores by glutamate. *Biochem J*. 2009; 422:503–12. [PubMed: 19548879]
- Pereira GJ, Hirata H, Fimia GM, do Carmo LG, Bincoletto C, Han SW, Stilhano RS, Ureshino RP, Bloor-Young D, Churchill G, Piacentini M, Patel S, Smaili SS. Nicotinic acid adenine dinucleotide phosphate (NAADP) regulates autophagy in cultured astrocytes. *J Biol Chem*. 2011
- Pryor PR, Mullock BM, Bright NA, Gray SR, Luzio JP. The role of intraorganellar Ca^{2+} in late endosome-lysosome heterotypic fusion and in the reformation of lysosomes from hybrid organelles. *J Cell Biol*. 2000; 149:1053–62. [PubMed: 10831609]
- Ravikumar B, Duden R, Rubinsztein DC. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Human Molecular Genetics*. 2008;1107–1117.
- Rietdorf K, Funnell TM, Ruas M, Heinemann J, Parrington J, Galione A. Two-pore channels form homo- and heterodimers. *J Biol Chem*. 2011
- Ruas M, Rietdorf K, Arredouani A, Davis LC, Lloyd-Evans E, Koegel H, Funnell TM, Morgan AJ, Ward JA, Watanabe K, Cheng X, Churchill GC, Zhu MX, Platt FM, Wessel GM, Parrington J, Galione A. Purified TPC isoforms form NAADP receptors with distinct roles for Ca^{2+} signaling and endolysosomal trafficking. *Curr Biol*. 2010; 20:703–9. [PubMed: 20346675]
- Rybalchenko V, Hwang SY, Rybalchenko N, Koulen P. The cytosolic N-terminus of presenilin-1 potentiates mouse ryanodine receptor single channel activity. *Int J Biochem Cell Biol*. 2008; 40:84–97. [PubMed: 17709274]
- Sarkar S, Rubinsztein DC. Inositol and IP_3 levels regulate autophagy: biology and therapeutic speculations. *Autophagy*. 2006; 2:132–4. [PubMed: 16874097]
- Sarkar S, Ravikumar B, Floto RA, Rubinsztein DC. Rapamycin and mTOR-independent autophagy inducers ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies. *Cell Death and Differentiation*. 2009;46–56. [PubMed: 18636076]
- Selkoe DJ, Wolfe MS. Presenilin: running with scissors in the membrane. *Cell*. 2007; 131:215–21. [PubMed: 17956719]
- Tu H, Nelson O, Bezprozvanny A, Wang Z, Lee SF, Hao YH, Serneels L, De Strooper B, Yu G, Bezprozvanny I. Presenilins form ER Ca^{2+} leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell*. 2006; 126:981–93. [PubMed: 16959576]
- Wakabayashi T, Craessaerts K, Bammens L, Bentahir M, Borgions F, Herdewijn P, Staes A, Timmerman E, Vandekerckhove J, Rubinstein E, Boucheix C, Gevaert K, De Strooper B. Analysis of the gamma-secretase interactome and validation of its association with tetraspanin-enriched microdomains. *Nat Cell Biol*. 2009; 11:1340–6. [PubMed: 19838174]
- Yoo AS, Cheng I, Chung S, Grenfell TZ, Lee H, Pack-Chung E, Handler M, Shen J, Xia W, Tesco G, Saunders AJ, Ding K, Frosch MP, Tanzi RE, Kim TW. Presenilin-mediated modulation of capacitative calcium entry. *Neuron*. 2000; 27:561–72. [PubMed: 11055438]
- Zampese E, Fasolato C, Kipanyula MJ, Bortolozzi M, Pozzan T, Pizzo P. Presenilin 2 modulates endoplasmic reticulum (ER)-mitochondria interactions and Ca^{2+} cross-talk. *Proc Natl Acad Sci U S A*. 2011; 108:2777–82. [PubMed: 21285369]
- Zong X, Schieder M, Cuny H, Fenske S, Gruner C, Rotzer K, Griesbeck O, Harz H, Biel M, Wahl-Schott C. The two-pore channel TPCN2 mediates NAADP-dependent Ca^{2+} -release from lysosomal stores. *Pflugers Arch*. 2009; 458:891–9. [PubMed: 19557428]

- Presenilin loss leads a reduction in overall lysosomal calcium content.
- Presenilins regulate expression of two-pore lysosomal calcium channels.
- Inhibition of lysosomal calcium channels decreases autophagic function.
- Thus, presenilin regulation of lysosomal calcium modulates autophagy.

Figure 1. Altered lysosomal calcium and TPC expression with presenilin knockout

A, Calcium response in control and presenilin 1 and 2 knockout (PSDKO) mouse embryonic fibroblasts shown as a ratio of 340/380nm excitation vs. time. Time of 100 μ M GPN addition is denoted by gray bar. B, PSDKO fibroblasts show a reduced maximal response (ΔR) calculated from the average of 60 seconds of baseline subtracted from the highest response of each cell. Error bars represent $\pm$ SEM, $n=5-6$ plates, total number of cells shown on graph, Unpaired Student's T-Test, *, $p<0.05$. C-D, Presenilin double knockout (DKO) fibroblasts have alterations in two-pore channels by immunoblot. Quantification of western blots in A was performed by densitometric analysis, and is presented as percent of control, normalized to actin. Error bars represent $\pm$ SEM, $n=4$, Unpaired Student's T-Test, *, $p<0.05$. E-F, Presenilin single knockout fibroblasts (1KO and 2KO) have alterations in two-pore channels by immunoblot. Quantification of western blots in C was performed by densitometric analysis, and is presented as percent of control, normalized to actin. Error bars represent $\pm$ SEM, $n=3-4$, One-way ANOVA analysis, post hoc comparison Tukey's HSD, *, $p<0.05$.

Figure 2. Increased lysosome number and volume with presenilin knockout but decreased TPC1-GFP

A, Original confocal image of control murine fibroblasts transfected with TPC1-GFP and loaded with LysotrackerRed. B, Image in A with volume modeling of LysotrackerRed. C, Image in A with volume modeling of both LysotrackerRed and TPC1-GFP. D, Original confocal image of PSDKO murine fibroblasts transfected with TPC1-GFP and loaded with LysotrackerRed. E, Image in D with volume modeling of LysotrackerRed. F, Image in D with volume modeling of both LysotrackerRed and TPC1-GFP. G, PSDKO cells have increased LysotrackerRed puncta/cell and average LysotrackerRed volume/cell as analyzed using Imaris software. Error bars represent +SEM, n=7–9 fields of view, 5–25 cells/field, Unpaired Student's T-Test, *, p<0.05. H, PSDKO cells have increased average lysosome volume compared to control cells. Error bars represent +SEM, n=7–9 fields of view, 5–25 cells/field, Unpaired Student's T-Test, *, p<0.05. I, Presenilin knockout cells have increased lysosomes of all sizes. J, Puncta size as represented as percent of the total puncta show that presenilin knockout cells have increases in large and small puncta while control cells have a larger percentage clustered around the average size. K, PSDKO cells have decreased TPC1-GFP volume/cell as analyzed using Imaris software. Error bars represent +SEM, n=7–9 fields of view, 5–25 cells/field, Unpaired Student's T-Test, *, p<0.05. L, PSDKO cells have decreased average TPC1-GFP puncta volume compared to control cells. Error bars represent +SEM, n=7–9 fields of view, 5–25 cells/field, Unpaired Student's T-Test, *, p<0.05. M–N,

TPC1-GFP distribution in control and PSDKO cells. O-T, TPC1-GFP distribution in conjunction with LysotrackerRed in control and PSDKO cells.

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Figure 3. Presenilin knockout cells have decreased TPC2-GFP puncta

A, Original confocal image of control murine fibroblasts transfected with TPC2-GFP and loaded with LysotrackerRed. B, Image in A with volume modeling of LysotrackerRed. C, Image in A with volume modeling of both LysotrackerRed and TPC2-GFP. D, Original confocal image of PSDKO murine fibroblasts transfected with TPC2-GFP and loaded with LysotrackerRed. E, Image in D with volume modeling of LysotrackerRed. F, Image in D with volume modeling of both LysotrackerRed and TPC2-GFP. G, PSDKO cells have decreased TPC2-GFP puncta/cell and volume/cell as analyzed using Imaris software. Error bars represent +SEM, n=7–9 fields of view, 5–25 cells/field, Unpaired Student's T-Test, *, p<0.05. H, PSDKO does not have an effect on average TPC2-GFP puncta volume compared to control cells. I–J, TPC2-GFP distribution in control and PSDKO cells. K–P, TPC2-GFP distribution in conjunction with LysotrackerRed in control and PSDKO cells.

Figure 4. TPC2 antagonist causes impairment in autophagy

A–B, 4 μ M Ned-19 treatment for 24 hours causes a reduction in LC3-II. Quantification of western blots in A was performed by densitometric analysis, and is presented as percent of control, normalized to actin. Error bars represent +SEM, n=4, Unpaired Student's T-Test, *, p<.05. C, Ned-19 treatment results in reduced long lived protein proteolysis. Error bars represent +SEM, n=4, Unpaired Student's T-Test, *, p<.05. D–E, Leupeptin/NH₄Cl treatment leads to a buildup of LC3-II but does not affect TPC expression.