Deficiency or Mutation of Presenilin Genes Lead to the Dysregulation of Amino Acid Sensing by mTORC1

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Atenuated auto-lysosomal system has been associated with Alzheimer’s disease (AD), yet the underlying molecular mechanisms leading to this impairment are largely unknown. We show that the amino acid sensing of mechanistic target of Rapamycin complex 1 (mTORC1) is dysregulated in cells deficient in Presenilin, a protein associated with AD. In these cells, mTORC1 is constitutively tethered to lysosomal membranes, unresponsive to starvation, and inhibitory to TFEB-mediated clearance due to a reduction in Sestrin 2 expression. Normalization of Sestrin 2 levels through overexpression or elevation of nuclear calcium degenerate in long-term starvation assays. These results propose an altered mechanism for nutrient sensing in presenilin deficiency and underline the importance of clearance pathways in the onset of AD.

Figure 1: Model of Presenilin Function in mTORC1/TFEB Signaling through Regulation of Calcium/Sestrin Levels. Presenilins as or part of calcium ‘leaky’ channels on ER membranes impact cytotoxic and nuclear calcium levels which regulate Sestrin 2 gene expression. Sestrin 2 inhibits mTORC1 activity. High Sestrin 2 levels are required in all conditions to maintain mTORC1 activity in presenilin deficient cells, and inhibit TFEB-mediated clearance.

Figure 2: Defective amino acid sensing of mTORC1 in PSKO Cells, Independently of mTORC1 Localization. (A) mTORC1 activity is lower in PSKO cells compared to controls at baseline and after inhibition with rapamycin. Normalization of mTORC1 activity is seen in PSKO cells transfected with WT PS1 or with expression of exogenous Sestrin2-HA. (B) Intracellular calcium levels are elevated in PSKO cells compared to controls, and normalized to control levels after expression of exogenous Sestrin2-HA. (C) Biochemical viability assays show a significant reduction of ATP levels in PS1M146L or PS1A246E before the treatment with CI treatment (middle panel) or expression of exogenous Sestrin2-HA in neuronal cultures inhibited their degeneration.

Figure 3: Lysosomal Calcium Signaling in PS KO Cells. (A) Lysosomal calcium imaging in PS-KO (PS1M146L and A246E) cells show fragmented nuclei in leucine starved FAD (PS1M146L shown here) neurons (64%±7.6% of all cells, p=0.005). Arrows depict cells with nuclear cc3 and pyknotic nuclei, arrow head points to soma with highly accumulated cc3 in magnified region. (B) Autophagy flux assays show an attenuation of LC3II levels in baseline or after Chloroquine (CQ) treatment in PS1M146L and A246E, respectively at baseline: 2.67±0.3, 3.11±0.4, p=0.01 and after CQ: 2.21±0.25, 2.11±0.3, p=0.001. (C) LC3II levels of both cell lines to each other (CI+VPA: LC3,control: 5.1±0.8 vs LC3,si.PS1: 4.9±0.9, p=0.1; CI: LC3,control: 3.1±0.2 vs LC3,si.PS1: 2.9±0.2, p=0.01). (D) PS regulates Sestrin 2 transcript levels are low in PS knock-out cells an increase with expression of exogenous PS1WT-Flag constructs, while AD-specific controls are low at baseline and increase with expression of exogenous Sestrin2-HA constructs.

Figure 4: TFEB Driven Clearance Functions Are Impaired Due To Low Sestrin 2 Levels In Presenilin Deficient Cells. (A) Unlike in WT cells, moderate expression of exogenous TFEB-Flag proteins in PSDKO cells fail to sufficiently induce CLEAR activity and cellular clearance, antagonizing degeneration. (B) Isogenic, iPSC-derived human neurons expressing SCAP-HA, AGO2-HA, and Sestrin2 construct in derived human neurons show a reduction of LC3II and p62 levels in PSDKO cells in an autophagy flux assay. WT and PSDKO cells

Figure 5: Presenilin Deficiency, Attenuating the CLEAR Network Activity. (A) Normalization of Sestrin 2 levels through overexpression or elevation of nuclear calcium degenerate in long-term starvation assays. These results propose an altered mechanism for nutrient sensing in presenilin deficiency and underline the importance of clearance pathways in the onset of AD.