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Presenilins and calcium signaling – systems biology to the rescue

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Abstract

Mutations in presenilins result in familial Alzheimer's disease (FAD). Presenilins encode a catalytic subunit of γ -secretase complex, and FAD mutations in presenilins alter γ -secretase activity. Many FAD mutations in presenilins also affect intracellular calcium signaling. To explain these results it was proposed that presenilins encode endoplasmic reticulum (ER) calcium leak channels, and that this function is disrupted by FAD mutations. This hypothesis has been controversial. Two recent reports provide new evidence for the calcium leak channel hypothesis. One group reported the presence of putative ion-conduction pore in the high resolution crystal structure of bacterial presenilin homologue PSH1. Another group identified an essential role of presenilins in mediating ER calcium leak in unbiased cell-based screen for calcium homeostasis modulators. These results should enable the field to move forward and to focus on exploring connections between FAD mutations in presenilins, changes in γ -secretase and ER Ca^{2+} leak functions and development of the disease.

Presenilin 1 (PS1) and presenilin 2 (PS2) are 50-kDa proteins that contain nine transmembrane (TM) domains and reside in the endoplasmic reticulum (ER) membrane. The assembly of presenilins with nicastrin, APh-1, and Pen-2 forms the γ -secretase complex, which is transported to the cell surface and endosomes to cleave several substrates, including the amyloid precursor protein (APP). More than 180 missense mutations identified in the *PSEN1* gene and 20 mutations in the *PSEN2* gene result in familial Alzheimer's disease (FAD). γ -Secretases generate amyloid- β peptide, the main constituent of the amyloid plaques in the brains of both FAD and sporadic AD patients, and most attention of the AD field has been focused on studies of γ -secretase function of presenilins. There is however increasing evidence that presenilins also have γ -secretase-independent functions. One of these functions is related to calcium (Ca^{2+}) signaling. The connection between FAD mutations in presenilins and abnormal Ca^{2+} signaling was initially observed in studies with fibroblasts from FAD patients almost 20 years ago (1) and has been replicated many times in variety of experimental systems. However, a mechanistic explanation for these findings has been lacking.

Our laboratory proposed that in addition to acting as a γ -secretase, presenilins also function as passive low conductance endoplasmic reticulum (ER) Ca^{2+} leak channels (2). We further demonstrated that ER Ca^{2+} leak function of presenilins was disrupted by many FAD mutations, leading to elevated ER Ca^{2+} levels and excessive Ca^{2+} release (2, 3). Alternative hypotheses have also been proposed - one group suggested that presenilins affect ER Ca^{2+} signaling by directly gating inositol(1,4,5)-trisphosphate receptor (InsP_3R) (4) and another group suggested that presenilins modulate activity of ER Ca^{2+} pump (5). Because these three groups used similar experimental approaches and methods, but obtained contradictory results, it was difficult to understand the source of discrepancy, resulting in controversy (6). Considering that most of the AD field favored presenilin actions as a γ -secretase, this unresolved controversy continued to marginalize Ca^{2+} signaling function of presenilins since the Ca^{2+} signaling investigators could not agree.

New perspectives to this conundrum were recently provided by other scientists using different approaches. One major breakthrough came from determination of the crystal structure of archaeal presenilin homologue PSH1 (7). This paper is a real *tour de force* that offers the first atomic resolution information about the three-dimensional structure of presenilins. The resolution of the structure is sufficiently high to visualize a large, water-filled hole that traverses the entire protein across the lipid bilayer. The hole is surrounded by TMD2, TMD3, TMD5, and TMD7. The authors state that this hole is large enough to allow passage of small ions (7). Our previous mutagenesis data suggested that the ion-conducting pore of presenilins is lined up by residues of TMD7, but not TMD6 (8), consistent with the structure of PSH1. Although future work is needed, the water-filled cavity in the PSH1 structure is the most likely candidate for the ion conducting pore in the Ca^{2+} leak channel.

The second major breakthrough came from the application of systems biology approach (9). These authors set out to develop a quantitative model of cellular Ca^{2+} homeostasis. To achieve this goal they performed single-cell Ca^{2+} imaging studies and developed a set of differential equations that describes major Ca^{2+} pumps and leaks in HEK293 cells. Using an unbiased approach, they transfected 250 candidate shRNAi in the cells and used the developed mathematical model to quantify the effects of knockdown on Ca^{2+} pump and leak rates. Such an unbiased and highly sensitive approach enabled them to identify proteins involved in the elusive ER Ca^{2+} leak pathway. Knocking down presenilin-2 or Orai2 dramatically reduced ER Ca^{2+} leak rate and knocking down PSENEN (Pen-2) greatly increased ER Ca^{2+} leak rate. The knockdown of Pen-2 is known to inhibit proteolytic processing of presenilins and it is expected to increase the holoprotein form of the protein. Only the holoprotein form of presenilins supports ER Ca^{2+} leak (2), so that results with PSENEN (Pen-2) knockdown are most likely explained by accumulation of presenilin holoprotein in the ER.

These two new independent lines of evidence (7, 9) appear to support the “ER Ca^{2+} leak channel” hypothesis (2). This hypothesis states that presenilin proteins exist in 2 different states and play different roles in different subcellular compartments. Cleaved (or “mature”) presenilin forms a γ -secretase complex together with nicastrin, APH-1, and Pen-2, which is located in endosomal compartments and at the plasma membrane (Fig 1A). Holoprotein (or “immature”) presenilin forms the ER Ca^{2+} leak channel in the ER (Fig 1A). Which function

is affected by FAD mutations in presenilins? This is a key question for the proposed model (Fig 1A), which we attempted to address previously (3). Our conclusion was that some mutations in presenilins, such as the PS1- E9 mutation, primarily affect γ -secretase function and result in highly elevated A β 42/A β 40 ratio (Fig 1B). Some mutations, such as the PS1-M146V mutation, primarily affect ER Ca²⁺ leak function and result in ER Ca²⁺ overload (Fig 1B). Some mutations, such as the PS1-L166P mutation, affect both functions which result in ER Ca²⁺ dysregulation and increase A β 42/A β 40 ratio (Fig 1B). Clinical phenotypes of presenilin mutant families are quite heterogeneous (10), and the fact that various point mutations affect either one or both functions may help to explain this heterogeneity. We had noted previously that mutations that have strong effects on γ -secretase function of presenilins (such as PS1- E9 and PS1-L166P) appear to segregate with cotton wool plaques and spastic paraparesis phenotype (CWP/SP), whereas mutations that affect primarily ER Ca²⁺ leak function (such as PS1-M146V) are not (3) (Fig 1B).

Obviously, additional scientific and clinical studies are needed to test and develop these ideas, but the two recent publications (7, 9) should facilitate the exploration of how FAD mutations in presenilins change γ -secretase and ER Ca²⁺ leak functions, and development of Alzheimer's disease.

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