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Review

Presenilin: RIP and beyond

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ABSTRACT

Over the years the presenilins (PSENs), a family of multi-transmembrane domain proteins, have been ascribed a number of diverse potential functions. Recent *in vivo* evidence has supported the existence of PSEN functions beyond its well-established role in regulated intramembrane proteolysis. In this review, we will briefly discuss the ability of PSEN to modulate cellular signaling pathways through γ-secretase cleavage of transmembrane proteins. Additionally, we will critically examine the proposed roles of PSEN in the regulation of β-catenin function, protein trafficking, calcium regulation, and apoptosis.

Contents

Ever since the discovery of *presenilin* (PSEN) mutations in families with autosomal dominant Alzheimer's disease (AD), the function of these enigmatic proteins has been the subject of intense investigation. This global effort has led to the confluence of the seemingly disparate fields of embryonic development and adult neurodegeneration as investigators discovered that PSENs formed the catalytic component of γ-secretase, a novel intramembrane aspartyl protease (along with Nicastrin (Nct), Aph-1, and Pen-2) [1]. Not only is γ-secretase involved in the proteolysis of amyloid precursor protein (APP) thereby generating the C-terminus of the pathogenic Aβ42 peptide, it is also responsible for the S3 cleavage that liberates the soluble Notch intracellular domain (NICD) to translocate to the nucleus to affect transcription of its target genes.

To date, more than 50 γ-secretase substrates have been identified [2] and it has been suggested that γ-secretase-mediated cleavage does not depend on the sequence of a type-I membrane protein, but rather on the size of its extracellular domain [3]. Additionally, it has been claimed that a few type-II transmembrane proteins and even one multipass transmembrane protein, GluR3, can become substrates of γ-secretase although these results need to be independently verified [2]. Based on this apparent promiscuity we proposed that γ-secretase may function as "the proteasome of the membrane" [4], with the important biological

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function of clearing protein domains from cellular membranes. For a minority of substrates, γ-secretase may play a central role in a signaling paradigm that has been termed regulated intramembrane proteolysis (RIP) [5]. RIP activates signaling pathways, such as Notch, by allowing intracellular domains (ICDs) to translocate to the nucleus. Alternatively, RIP may turn off signaling events in which the transmembrane anchored protein is responsible for signaling and cleavage terminates the signal. For example, DCC-induced neurite outgrowth is enhanced by γ-secretase inhibitors and not observed with over-expression of the DCC-ICD [6]. A third possibility is that cleavage may serve as a switch between signaling modes with the transmembrane form activating one pathway at the membrane and the soluble ICD carrying out a different function in another cellular compartment. An example of this may be ErbB4.

An extremely important point in regards to RIP, largely ignored by most investigators, is that the fate of any ICD is dependent on its N-terminus that dictates the stability of the cleaved products. According to the N-end rule, only ICDs whose N-terminus evades ubiquitination (methionine or valine) escape degradation, whereas fragments beginning in other residues undergo rapid proteasomal degradation [7–9]. Since most investigators over-express ICD fragments initiating with a methionine, "signaling" capability of γ-secretase substrates is grossly overestimated as they have artificially stabilized ICDs that would otherwise be unstable due to N-end rule degradation [10,11]. This explains the abundance of valines in the transmembrane domain (TMD) of known γ-secretase substrates and hampers efforts to identify novel substrates; ICDs with unstable N-terminal residues are difficult to detect without complete inhibition of the proteasome. This may result in a number of substrates being erroneously considered resistant to γ-secretase because their ICD fragments could not be detected due to their rapid turnover by the proteasome.

Beyond their role in γ-secretase, PSENs have been suggested to carry out a wide range of functions. However, *in vivo* evidence for γ-secretase-independent functions has been scant due to the dominant Notch loss-of-function phenotypes caused by PSEN deficiency in many organ systems. The moss *Physcomitrella patens* (*P. patens*), an organism that lacks Notch but contains homologues for all γ-secretase components, was used to ask if γ-secretase-independent functions of PSENs exist in plants [12]. *P. patens* lacking the *P. patens* Presenilin (PpPS) gene displayed a number of intriguing phenotypes, which were not only rescued by expression of human PSEN but interestingly, were also rescued with catalytically dead aspartyl mutant PSEN. These phenotypes indicated the existence of a conserved, γ-secretase-independent function of human PSEN. This conclusion is buttressed by genetic evidence in mammals for an *in vivo* function of PSEN beyond its role in γ-secretase. Mice deficient for both PSEN 1 and 2 display a more severe somite phenotype than that seen with Aph-1, Nct, or Pen-2 knock-out mice or in animals deficient for Notch signaling (Notch receptor double mutants or RBP-Jκ mutants that lack all canonical Notch signaling [13]). Specifically, mice in which Notch signaling or other members of γ-secretase have been removed were able to generate anterior somites, whereas PSEN1/PSEN2 double mutants did not, indicating that PSENs contribute to generation of anterior somites beyond their role in γ-secretase.

This review will discuss the current evidence for the diversity in PSEN function and its role in RIP. Bearing in mind the stability issue raised above, we will not attempt to address the potential functions of PSENs in regulating all of the currently known substrates as this list is continuously expanding and has been the focus of other reviews [2,14]. Instead, we will briefly discuss the role of PSENs in RIP through focusing on three well-studied substrates that represent the modes of RIP-mediated signaling mentioned above. Lastly, we will provide an overview of PSEN functions beyond its role in γ-secretase, namely in the regulation of Wnt signaling and adhesion, protein trafficking, calcium regulation, and apoptosis. A guide to the topics that we cover in this review is displayed in Fig. 1.

1. Role of PSENs in RIP: three select substrates

1.1. Notch

The best understood biological function of PSENs is their critical role in activating Notch receptors via RIP. Notch is a receptor in an evolutionarily conserved pathway mediating short-range communication used by all metazoans at various stages of development and in the adult. Briefly, the Notch receptor binds to its ligand, presented by neighboring cells. The force involved in resolving this complex leads to shedding of the extracellular domain, allowing cleavage by γ-secretase at the cell surface or in an early endosome [15,16]. Upon cleavage, which can occur at several scissile bonds [11], NICD is released into the cytosol. Those that can escape N-end rule degradation (NICD-V starting at valine 1744) are translocated to the nucleus where they bind RBP-Jκ and Mastermind to activate various target genes. Although four Notch homologues (Notch 1–4) exist in mammals, and redundancy between the homologues is often seen, each Notch protein does appear to have unique functions. For instance, Notch2 but not Notch1 is required for kidney development in mammals [17], and only mutations in Notch3 are known to cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) in aging humans [18]. Many additional aspects of Notch biology, regulation and the diverse physiological functions were topics of other excellent reviews and will not be discussed here [19–22].

Importantly, several human diseases and syndromes have been shown to be associated with the Notch pathway such as Alagille syndrome, aortic valve disease, and spondylocostal dysostosis as well as cancer such as T-cell acute lymphoblastic leukemia (T-ALL) and the degenerative disease CADASIL [18,23–27]. All are caused by mutations in Notch pathway components such as Notch1, 2, and 3, Jagged-1, Delta-like 3, and Lunatic fringe. Mutations of PSENs are known to not only cause familial forms of Alzheimer's disease (FAD) and affect APP cleavage, but also decrease Notch signaling to varying degrees [28,29]. No human PSEN mutations have been shown to contribute to any known Notch-related syndromes, arguing that γ-secretase activity is in excess relative to the RIP needs of Notch for most of the adult life. Whether Notch is involved in the pathogenesis of AD is unknown, however, Notch contributes to synaptic plasticity [30–32] and reduced Notch activity could be involved in late-onset cognitive decline [33,34]. This ambiguity has fueled a debate in the field on whether pathogenic mutations in PSEN should be considered loss- or gain-of-function [35].

It is intriguing that PSENs also cleave Notch ligands, Jagged and Delta, following their ectodomain shedding [36–38]. Although no requirement for γ-secretase has been detected in signal-sending cells [39], methionine-stabilized Jagged-ICD and Delta-like1-ICD were observed to translocate to the nucleus and play a role in AP1 and Smad-mediated signaling, respectively [37,40], raising the possibilities that these may reflect bi-directional signaling [37]. However, since no physiological roles have been demonstrated, they are more likely to fall under the general "proteasome"-like activity of γ-secretase.

1.2. APP

APP is another well-studied substrate of γ-secretase although its biological function is still under considerable debate [41]. Shedding of the APP extracellular domain is constitutive, ligand independent and can be mediated either by the inducible α-secretase or β-secretase. Much of APP proteolysis occurs intracellularly. Although