Presenilin, Notch, and the genesis and treatment of Alzheimer's disease

Dennis J. Selkoe*

Center for Neurologic Diseases, Harvard Medical School, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, HIM 730, Boston, MA 02115

Elucidation of the proteolytic processing of the amyloid β-protein precursor (APP) has revealed that one of the two proteases (γ-secretase) that cleave APP to release amyloid β-protein (Aβ) is likely to be presenilin. Presenilin also mediates the γ-secretase-like cleavage of Notch receptors to enable signaling by their cytoplasmic domains. Therefore, APP and Notch may be the first identified substrates of a unique intramembranous aspartyl protease that has presenilin as its active-site component. In view of the evidence for a central role of cerebral build-up of Aβ in the pathogenesis of Alzheimer's disease, this disorder appears to have arisen in the human population as a late-life consequence of the conservation of a critical developmental pathway.

The modern era of scientific research on the pathogenesis of Alzheimer's disease (AD) began in the mid 1960s with the first electron microscopic descriptions of the ultrastructure of the classical brain lesions Alzheimer noted in 1906: extracellular amyloid plaques and intraneuronal neurofibrillary tangles. In the mid 1970s, deficits of specific neurotransmitter systems in the brain tissue of AD victims started to be described, beginning with the cholinergic system. In the mid 1980s, the biochemical compositions of the proteinaceous filaments making up the amyloid plaques and the neurofibrillary tangles became known, followed by the cloning of the β-amyloid precursor protein (APP). And in the 1990s, mutations or polymorphisms in certain genes were shown to predispose humans to AD, and their effects on the proteolytic processing of APP to the amyloid β-protein (Aβ) and the fate of Aβ were elucidated. These and related advances, including the development of cell culture and mouse models to study both the production and the cytotoxicity of Aβ, have brought us to the verge of human trials of antiamyloid therapies.

Although research on AD was initially characterized by phenomenological observations, the situation has changed dramatically in the last decade, and a specific and rigorous molecular explanation has come increasingly into focus. A particularly exciting development has been the recent realization that the fundamental basis of AD appears to relate directly to certain signaling mechanisms that are crucial for normal development in all multicellular organisms. Indeed, the degree of progress is such that one can now begin to understand why and how AD arose in the human population. Here, I will review work from my collaborators and me that we believe helps support this provocative view of the origin of AD and how one might ultimately prevent the disorder.

Materials and Methods

All of the methods used in the studies summarized in this presentation have been published in recent reports (1–8).

Presenilins as Mediators of Intramembranous Proteolysis of APP and Notch

Elevated cerebral levels of Aβ peptides, particularly those ending at amino acid 42 ($A\beta_{42}$), are an early and invariant feature of all forms of AD (reviewed in ref. 9). As a result, understanding the detailed mechanism by which two proteolytic activities designated β-secretase and γ-secretase cleave APP to liberate Aβ peptides has been a central goal of our work since the original discovery of the normal cellular production of Aβ (10–12). Because most of the missense mutations in APP linked to a rare form of early-onset AD, as well as all of the known mutations in presenilin (PS) 1 and 2, have been found to selectively alter the γ-secretase cleavage of APP to heighten $A\beta_{42}$ production, my colleagues and I have focused in particular on the identity and nature of γ-secretase. A key observation from our perspective was the finding that small amounts of full-length APP could be coimmunoprecipitated with presenilin in whole lysates and isolated microsomal vesicles from cells expressing transfected or endogenous PS1 (7, 13). Although this finding generated controversy (14), it served as a major impetus for our hypothesizing that presenilin participates intimately as part of the catalytic complex by which γ-secretase mediates the putative intramembranous proteolysis of APP (7, 15). An alternative hypothesis for the role of presenilin in the γ-secretase mechanism is that it does not form complexes with APP, but rather acts as a mediator of membrane trafficking that brings the components of the γ-secretase reaction together (14, 16). However, when we examined the maturation of holoAPP through the secretory pathway (i.e., the timing of the acquisition of N- and O-linked sugars), we were unable to detect any difference in this secretory processing between cells that express PS1 and those from PS1 knockout embryos that entirely lack it (17). Likewise, subcellular fractionation on discontinuous iodixanol gradients of cells that express or lack PS1 showed no definable difference in the vesicular distribution of the two major APP C-terminal fragments that are created by the actions of β- and α-secretase (referred to as C99 and C83, respectively) and are the immediate substrates of γ-secretase (6). We extended the original observation of DeStrooper *et al.* (18) that the absence of PS1 in knockout cells sharply elevates the amount of C83 and C99 in fractionated microsomes, but we observed no change in their subcellular localization. Taken together, these results suggested to us that direct participation of presenilin in the γ-secretase catalytic complex was a more tenable mechanism than an indirect role in the trafficking of the components of the reaction (7).

Another biochemical finding in our work that strongly favored the former hypothesis was the observation that FAD-causing missense mutations in either APP or PS1 were associated with decreased efficacy (i.e., increased IC_{50}s) of peptidomimetic inhibitors of γ-secretase designed by my collaborator, Michael Wolfe, as transition state analogs for an aspartyl protease

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Abbreviations: PS, presenilin; AD, Alzheimer's disease; APP, β-amyloid precursor protein; Aβ, amyloid β-protein; TM, transmembrane domain.

*E-mail: selkoe@cnd.bwh.harvard.edu.

Table 1. Evidence supporting presenilin as the active site component of γ-secretase

Predicted characteristics of γ-secretase	Corresponding features of PS
Aspartyl protease (requires two aspartates)	There are two completely conserved aspartates in presenilins—required for γ-secretase function
Intramembranous proteolysis	The two aspartates are within the membrane
Cleavage occurs near the middle of the membrane	The two aspartates are near the middle of TM6 and TM7
Aspartyl proteases have an acidic pH optimum	De novo Aß generation in PS-containing microsomes occurs optimally at mildly acidic pH (≈6.4)
γ-Secretase binds to its substrates	PS forms complexes with APP and, in particular, with C99 and C83
An intramembranous protease needs a structure for membrane entry of water	PS has an 8-TM structure that could form a pore and admit water
Deletion of γ-secretase must obviate proteolysis	Deletion of PS1 and PS2 obviates all intramembranous proteolysis of Notch and C99
Transition state mimic inhibitors should bind directly to the active site of the protease	APP transition state mimics bind directly and specifically to PS heterodimers
Such inhibitors should bind intimately to the target protease	Photactivatable groups located <12 Å from the active-site binding moiety at the N and C termini of an inhibitor bind to the NTF and CTF of PS, respectively (22)

mechanism (5, 17). Although the increases in IC_{50} were modest, they were highly reproducible and statistically significant. When we attempted to devise a model to explain this observation, we found it difficult to understand how a function of presenilin in protein trafficking (e.g., of γ-secretase or APP) could account for the negative effects of single missense mutations in presenilin on γ-secretase inhibitor potency. It seemed more probable that these shifts in inhibitor potency when presenilin was mutant denoted a conformational alteration of a site within the γ-secretase complex in which presenilin somehow directly participated.

A further finding that turned out to be critical in supporting the first over the second hypothesis of PS function (above) was the nature of the designed peptidomimetic compounds that inhibited γ-secretase (5, 19). These were ≈4–6 residue peptidomimetics based on the $A\beta_{42-43}$ cleavage region within the APP transmembrane domain. Difluoro alcohol or difluoro ketone moieties were installed at the P1 position as noncleavable, transition state mimicking functionalities, and the effects on Aβ generation in intact whole cells were examined. Dose-dependent inhibitions of $A\beta_{40}$ and $A\beta_{42}$ were seen (19), and a rough structure–activity relationship could be derived by substituting certain of the residues in these peptidomimetics (5). Importantly, the inhibitory activity of the difluoro alcohol moiety signified that these transition state mimics were acting on an aspartyl protease rather than one of the other major classes of known proteases. The results of these designed inhibitor studies, along with molecular modeling, led to a hypothesis that γ-secretase cleavage involves a helical substrate (the APP transmembrane domain) and an unusual aspartyl protease that cleaves the substrate within the phospholipid bilayer (5).

Taken together, the various observations summarized above supported the concept that presenilin was intimately involved in the γ-secretase cleavage of APP and led to close inspection of the presenilin sequence for a possible proteolytic motif (4). The observation of two (and only two) intramembranous aspartyl residues that were predicted to be approximately in the center of transmembrane domain (TM)-6 and TM7 in all members of the presenilin family, flanking the site in the proximal TM6–TM7 loop that undergoes endoproteolysis (20), led to the hypotheses that presenilin was itself γ-secretase and that it might also effect the endoproteolytic cleavage (a proposed autoproteolysis; ref. 4). Mutagenesis of either of the TM aspartates in PS1 to alanine or glutamate resulted in a marked decrease in endogenous PS1 endoproteolytic fragments as a result of their "replacement" (21) by the apparently noncleavable asp-mutant exogenous PS1 (4). Likewise, C83 and C99 levels were markedly elevated, and Aβ and p3 levels were reduced sharply (4). These results suggested that mutation of a single TM aspartate residue produced essentially the same APP biochemical phenotype in a cell as deleting the entire PS1 gene (18). Furthermore, the incubation of isolated microsomes from either wild-type or asp-mutant-expressing cells with a C99 cDNA in an *in vitro* transcription/translation reaction showed that new Aβ peptide could be generated by the microsomes expressing wild-type PS1 at pH 6.4 but much less so at neutral pH (7.4), whereas no Aβ generation was detected from the microsomes expressing asp-mutant PS1 (4).

These results suggested that PS1 was either an unusual "diaspartyl" cofactor for γ-secretase or was itself γ-secretase, an unprecedented intramembranous aspartyl protease that was activated by autoproteolysis. Because reconstitution of γ-secretase activity—i.e., Aβ generation from purified components (PS1 and the C99 substrate) in phospholipid vesicles—could not be achieved without knowledge of other protein cofactors required for the γ-secretase reaction, our laboratories took an alternative approach to confirm presenilin as the γ-secretase. Using the aforementioned peptidomimetic transition state analogs, we were able to show that these inhibitors, when tagged with biotin and modified with a covalent crosslinking moiety, could bind directly to presenilin heterodimers in cell lysates, isolated microsomes, and intact cells (2). Simultaneous binding studies by Li *et al.* (22), using potent γ-secretase inhibitors that emerged from screening of compound libraries on Aβ-secreting cells, support the conclusion that presenilin heterodimers are the direct molecular target of active-site directed inhibitors of γ-secretase.

When all of the results summarized above are considered together, there is now very strong evidence that presenilin contains the active site of γ-secretase (Table 1). Although there is no precedent before this work for an intramembranous aspartyl protease, the cleavage of sterol regulatory element binding protein (SREBP) is effected by an unusual polytopic metalloprotease called site 2 protease that appears to have its active site within one of its TM domains (23). The parallels in the proteolytic processing of SREBP, APP, and the Notch family of cell-surface receptors (24–26) have led to the concept of regulated intramembrane proteolysis (RIP), by which integral membrane proteins undergo unusual scissions within the phospholipid bilayer (27). To ultimately achieve Aβ generation from purified components, we are currently attempting to purify further the presenilin/γ-secretase complex and identifying protein partners that may be necessary for proteolytic activity. One such candidate PS partner is the recently described nicastrin polypeptide (28).

Discussion

The results emerging from these studies of the relationship of the presenilins to γ-secretase suggest not only a novel proteolytic mechanism that may process numerous other transmembrane proteins (29) but also specific molecular targets for treating and preventing AD. Indeed, γ-secretase inhibitors have recently advanced from preclinical development into early human trials.[†] Our findings predict that many γ-secretase inhibitors, including all that are directed against the active site, will bind to presenilin heterodimers. Given the clear evidence that presenilin mediates not only the intramembranous proteolysis of APP but also that of Notch to release the cytoplasmic signaling domain of Notch to the nucleus (24–26), it would be particularly advantageous to find members of the presenilin/γ-secretase complex that principally modulate either the APP or the Notch cleavage. This would offer the prospect of more specificity toward Aβ inhibition, with less likelihood of side effects arising from down-regulating Notch signaling. However, there may well be other, still unknown single transmembrane proteins that are substrates of presenilin, so not all of the potential toxicity associated with γ-secretase inhibition may be attributable to interference with Notch signaling. We are currently searching for such additional presenilin substrates. Inhibition of β-secretase, recently identified by several groups (30–34), is an alternative approach for chronically lowering cerebral Aβ levels.

In conclusion, chronic partial (e.g., 30–40%) inhibition of γ-secretase or β-secretase remains a rational and compelling strategy to slow and ultimately prevent AD. A separate approach that is also highly attractive is the use of Aβ immunization to enhance clearance of Aβ from the brain (35–37).

[†]Molinoff, P. B., Felsenstein, K. M., Smith, D. W. & Barten, D. M. (2000) *Aβ modulation: The next generation of AD therapeutics* (World Alzheimer's Congress 2000, Washington, DC, July 9–13, 2000), abstr. no. 615.

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