BACE1 and Presenilin: Two Unusual Aspartyl Proteases Involved in Alzheimer's Disease

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Key Words

Alzheimer's disease · Knockout mice · β -Secretase · γ -Secretase

Abstract

Two enzymatic activities are required to generate the pathogenic β -amyloid (A β) peptide that accumulates in the brain of Alzheimer's disease patients. Both activities are carried out by two unusual aspartyl proteases known as β - and γ -secretase. Their therapeutic inhibition appears, therefore, a promising strategy to treat the disease. Transgenic mouse models in which the genes encoding the secretases have been ablated offer an invaluable tool, on the one hand, to gain more insights into the biological function of these proteases and, on the other hand, to predict the consequences that might be associated with enzyme inhibition *in vivo*.

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Two unusual aspartyl proteases are implicated in Alzheimer's disease (AD). These are BACE1 [1] and the presenilins (PS) 1 and 2 [2] and they perform the two consecutive cleavages of the amyloid precursor protein (APP) required to generate the toxic β -amyloid (A β) peptide. First, BACE1 cuts APP, a type I transmembrane protein, at the β -site in the ectodomain 28 amino acids from the

transmembrane region. BACE1 is an aspartyl protease that is unusual in that it contains a carboxy-terminal extension that harbors a transmembrane domain [3–6]. The enzyme is by itself necessary and sufficient to perform the cleavage. The BACE1-generated APP C-terminal fragment that remains membrane-bound is subsequently cleaved by a PS-containing γ -secretase complex at the γ -site within the transmembrane domain [7]. Although several membrane proteins have been shown to be processed within their transmembrane regions, it is not yet clear how hydrolysis can take place within the hydrophobic milieu of the membrane [8–11]. In addition to this unusual enzymatic property, PS-dependent cleavage, contrary to β -cleavage, requires the function of at least three additional proteins, Nicastrin [12], Pen-2 [13] and Aph-1 [14], that together with PS form the active γ -complex [15–17]. Despite the fact that direct proof that purified PS functions as a protease *in vitro* is still missing, many lines of evidence point strongly to PS as genuine aspartyl proteases [discussed extensively in 16, 18, 19]. The role played by the other components of the γ -complex in the cleaving reaction remains to be determined.

Alternatively to the BACE1/PS or β/γ -processing of APP that is known as 'amyloidogenic' since it results in A β formation, APP can be cleaved by α/γ -secretases in a nonamyloidogenic pathway. α -Secretases are membrane-bound metalloproteases that belong to the ADAM (a disintegrin and metalloprotease) family [20–22]. They cleave

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APP between the amino acids lysine 16 and leucine 17 of the A β domain (numbers are given considering the first amino acid of A β as position 1), therefore limiting A β formation. The membrane-bound carboxy-terminal fragment of APP is subsequently processed within the transmembrane domain by γ -secretase resulting in the secretion of p3, a small peptide that lacks the amino-terminal 16 amino acids of A β .

γ -Secretase as Therapeutic Target

Because the activity of β - and γ -secretases is required to generate the pathogenic A β peptide, inhibitors of these enzymes are potential therapeutic drugs to treat AD. Therefore, understanding the biological functions of these two unusual proteases and defining their natural substrates is enormously interesting not only from a purely basic scientific point of view, but in addition it is also crucial for the development of AD therapies aimed at lowering A β .

The physiological role of PSs has been extensively investigated in *Caenorhabditis elegans*, *Drosophila melanogaster* and *Mus musculus*. Deficiencies in PS genes cause severe lethal phenotypes that closely resemble those observed upon inactivation of the Notch signaling pathway [23]. Even partially deficient PS1 $\pm$ PS2 $^{-/-}$ mice that display an overall reduction in γ -secretase activity develop in adulthood a strong autoimmune phenotype [24]. The similarity in phenotypes of PS- and Notch-deficient animals can be explained by the fact that Notch receptors are substrates of γ -secretase and γ -cleavage is required to release the Notch intracellular domain from the membrane, which subsequently translocates into the nucleus and regulates gene transcription. The Notch pathway is responsible for complex cell fate decisions that occur during embryogenesis but also in adult life during T cell differentiation [25, 26] and neurite remodeling in the central nervous system [27]. Moreover, Notch and APP are not the only substrates for γ -secretase; in fact there is a growing list of type I transmembrane proteins that are processed by PS within their transmembrane regions. All the above data suggest that therapeutic inhibition of γ -secretase may lead to undesirable toxic side effects and, indeed, administration of a potent γ -secretase inhibitor to mice, beyond the expected decrease in plasma and brain A β levels, also resulted in marked defects in lymphocyte development and in the intestine villi and mucosa, most probably due to an inhibition of Notch processing [28]. The possibility, however, still exists of developing alterna-

tive drugs that rather than targeting the catalytic site of γ -secretase and thus affecting the cleavage of all substrates would specifically act on APP cleavage [81].

β -Secretase as Therapeutic Target

Unlike PS, genetic ablation of the BACE1 gene in mice is not associated with any gross phenotype [29–31], even in aged animals [32]. The only phenotype described thus far is some indicator of anxiety detected recently in more specific behavioral tests [33]. Moreover, BACE1 deficiency could prevent the learning and memory impairments and the cholinergic dysfunction observed in a transgenic mouse model for AD [34]. Whereas all these data highlight the therapeutic potential of BACE1 inhibition, important questions still need to be addressed to better predict the functional consequences (if any) of this action.

Several lines of evidence suggest that APP is not the only and probably not the main substrate for BACE1. First, the two proteins localize to different subcellular compartments in polarized cells limiting the access of BACE1 to its substrate. Whereas most BACE1 goes apically, the bulk of APP is sorted basolaterally [35]. Artificially targeting APP to the apical surface was sufficient to increase β -processing and A β generation. Similarly, the efficiency of BACE1 cleavage could be improved in non-polarized cells by increasing the relative exposure of the substrate APP to BACE1 via its targeting to the endosomal compartment [36]. Second, the cleavage site in APP is not optimal and artificial sequences have been found that are processed far more efficiently not only than wild-type APP but also than APP carrying the Swedish familial Alzheimer (FAD) mutation already known to be a better substrate for BACE1 [37]. It is, therefore, reasonable to assume that additional BACE1 substrates exist that are processed more efficiently than APP. Indeed two new substrates have been described for BACE1: P-selectin glycoprotein ligand-1 (PSGL-1) [38] and the sialyl transferase ST6Gal [39]. Both proteins are membrane-bound, as expected for a BACE1 substrate, and both have been ascribed a role in immune responses [40–42]. The evidence that they are substrates for BACE1 comes mainly from experiments in cultured cells, in conditions where both enzyme and substrate were overexpressed. Yet, whether these cleavages occur in vivo and their physiological significance, if any, remain to be determined. If PSGL-1 and ST6Gal are relevant physiological substrates of BACE1 and if their function is linked to the immune system, then the lack of any specific defect in BACE1-

deficient animals may simply reflect the specific pathogen-free conditions in which mice are housed. Alternatively, genetic redundancy and the activation of compensatory mechanisms could also account for the absence of phenotype in BACE1 null mice. No significant compensatory mechanism seems to be activated in brain for APP cleavage [29–31]; however, this result does not exclude the possibility that compensatory mechanisms operate to cleave other substrate(s). Resolving this issue is important because putative compensatory mechanisms activated during embryogenesis might not operate in the elderly when chronic BACE1 inhibition is considered as a treatment for AD.

Shortly after the discovery of BACE1, a homologous gene has been identified by database search and named BACE2 [6, 43–45]. BACE2 encodes a membrane-bound aspartyl protease that is 68% similar to BACE1 at the amino acid level and that can process APP at the β -site. BACE2 is then the nearest BACE1 homologue, but besides their amino acid homology and their common structural organization, the two enzymes differ in their tissue distribution [45–47], subcellular localization [48] and substrate specificity [45, 48, 49]. BACE2 is ubiquitously expressed in fetal and adult tissues, although the enzyme levels in brain are low [44, 45, 47]. Whether BACE2 affects, positively or negatively, the A β pool is a subject of debate. A positive contribution of BACE2 to brain A β levels has been suggested in two pathological conditions. First, because the BACE2 gene is located in the Down syndrome critical region of chromosome 21, it has been speculated that upregulation of BACE2 expression could be at least partially responsible for the higher levels of A β and the development of the AD-like neuropathology associated with this syndrome [43, 47, 50]. Second, based on the observation that BACE2 cleavage of APP at the β -site is more efficient when APP carries the Flemish FAD mutation, it has been proposed that BACE2-mediated APP cleavage might play a role in the development of AD in individuals carrying this mutation [45]. A number of observations suggest, in contrast, that BACE2 would function in vivo as an anti-amyloid protein that would limit the amount of A β generated by BACE1. Such a property would be explained by the capacity of BACE2 to cleave APP within the A β region. In fact both BACE1 and BACE2 cleave APP at internal sites within the A β domain. BACE1 cleaves between tyrosine 10 and glutamic acid 11 (β 11 position) and as a consequence of this cleavage an N-terminally truncated A β species is generated that is more prone to aggregation than full-length A β [51]. The internal BACE2 cleavage site is between residues

phenylalanine 19 and 20 and BACE2 cleavage at this position is more efficient than at the β 1-site [45, 48, 49]. If BACE2 cleaves preferably at this position in vivo, then BACE2 coexpression with BACE1 would result in decreased A β secretion. Indeed it has been shown that BACE2 overexpression reduces A β levels [44–46, 48] whereas BACE2 downregulation by RNAi elevates A β secretion [52]. These observations lead to the suggestion that BACE2 does not function in vivo as a β -secretase but rather as an α -like secretase that precludes A β formation [48, 49, 52, 53].

Despite some differences in substrate specificity, BACE1 and BACE2 cleave similar sequences and respond similarly to mutations introduced at the β -site in APP [45]. It is, therefore, likely that substrate-based inhibitors for BACE1 will also affect BACE2 function. This has important implications from a therapeutic point of view. On the one hand, if BACE2 negatively regulates A β levels, its nonselective inhibition could counteract the effect of BACE1 inhibition. On the other hand, although the natural substrate(s) for BACE2 are not known, the fact that the mRNA is widely expressed in fetal and adult tissues suggests it might fulfill essential functions. Moreover, whereas long-term BACE1 disruption does not seem to be associated with any gross phenotypic alteration, the physiological consequences of blocking both BACEs are thus far not known. BACE1 and BACE2 might process common substrates in vivo that have not yet been identified and this overlapping function(s) might be unique to these enzymes. The generation of BACE2- and BACE1/BACE2-deficient mice will help to address these questions.

Alternative Approaches for BACE1 Modulation

Alternatively to the nonselective inhibition of BACE1 activity, strategies can be envisioned that may target other aspects of BACE1 metabolism. Considerable progress has been made in our understanding of BACE1 biology since its initial discovery at the end of 1999 [3–6, 54]. In particular, some modes of modulating BACE1 protein levels as well as enzymatic activity have been proposed that might operate in vivo.

BACE1 protein levels and activity are increased in brains of patients with AD [55–59]; however, the levels of BACE1 transcript seem to be comparable in AD and nondemented controls [56, 60–62]. This suggests that BACE1 expression is regulated posttranscriptionally through a mechanism that is altered in AD. In this respect, the BACE1 transcript contains a 5'-untranslated region (5'-UTR) quite unusual for a cellular mRNA. It is more than 400 nucleotides long, contains three short open reading

frames and has a high GC content and hence the potential to fold into a stable secondary structure [63]. A ribosome-shunting mechanism has been proposed to explain how the translational machinery can access the BACE1 start codon. Ribosome shunting involves the recruitment of ribosomes in a cap-dependent manner and their subsequent nonlinear migration in which part of the 5'-UTR is skipped and ribosomes are directly translocated to a site at or close to the start codon of the major open reading frame [64]. The efficiency of shunting seems to be cell type-dependent, suggesting that cell-specific factors are involved in the modulation of BACE1 mRNA translation and raising the possibility that the process is altered in AD [63]. Identifying the mechanism of BACE1 mRNA translation and the factor(s) involved might reveal novel therapeutic targets to control BACE1 expression.

The increase in BACE1 protein observed in brains of AD patients can alternatively be explained by a decrease in protein turnover. There is thus far no solid indication that BACE1 protein is stabilized in AD brains, but a hypothesis can be drawn based on some data from cell culture studies. BACE1 is a rather stable protein with a half-life of about 16 h [65, 66]. Higher levels of the lipid second messenger ceramide in cultured cells led to stabilization of BACE1, which in turn resulted in an increase in BACE1-dependent APP products [66]. Because ceramide controls several biochemical events and its levels are elevated in AD brains, the authors propose that ceramide regulates A β generation in vivo by affecting the steady-state levels of BACE1 protein. Although more data are required to support this conclusion, the possibility that a signal transduction pathway having ceramide as second messenger can control BACE1 stability is quite appealing. Clearly further research is needed to identify the components of such a pathway and their modes of regulation.

In addition to the modulation of the levels of BACE1 protein, factors that directly affect enzyme activity have also been identified. Several lines of evidence suggest that β -secretase cleavage of APP takes place in rafts, specialized membrane microdomains rich in cholesterol and sphingolipids. First, cholesterol depletion that results in raft disruption caused a drastic decrease of β -cleavage in hippocampal neurons [67] and a strong increase of α -cleavage in various peripheral and neural cell lines [68]. Conversely, exposure of neurons and glial cells to cholesterol decreased α -secretase-mediated cleavage of APP [69]. Second, BACE1 protein has been shown to localize partially into rafts [70]. Finally, increasing the association of BACE1 with lipid rafts by either artificially adding a GPI anchor at the C-terminus of BACE1 ectodomain [71]

or by antibody cross-linking [72] was sufficient to stimulate β -processing. Therefore, affecting the association of BACE1 with rafts appears as an alternative to modulate the efficiency of β -cleavage. This could be theoretically achieved in an indirect way by modulating cholesterol levels in the brain, and indeed cholesterol-lowering drugs are already being tested in clinical trials. The rationale for using such drugs came originally from several epidemiological studies that showed a correlation between high blood cholesterol levels and a higher risk of developing AD [73–75]. Moreover, patients that have been treated with cholesterol-lowering statins were somehow protected against the disease [76, 77]. The protective role of statins in AD, although not definitively demonstrated, could then be the consequence of their indirect effect on BACE1 activity. An alternative and more direct way of modulating BACE1 compartmentalization into lipid rafts could theoretically be achieved via modulation of its interaction with proteins responsible for the localization of BACE1 into these microdomains. Although very speculative at this stage, at least two proteins have been proposed to play such a role: phospholipid scramblase 1 [78] and a GPI-anchored protein [79].

A novel and promising approach to control BACE1 activity has recently been described. BACE1 has been shown to bind to heparan sulfates (HS) both in vivo and in vitro and this binding resulted in inhibition of enzyme activity [80]. The inhibitory properties of the HS depended on the saccharide size and specific structural characteristics. HS inhibition in cultured cells was specific for β -cleavage of APP since no effect on α -cleavage was detected. The authors could show that HS function by blocking the access of the substrate to the enzyme's active site. Interestingly, inhibition of cellular HS synthesis resulted in a concomitant increase in BACE1 activity. These data are preliminary and at present far from practical therapeutic applications, but the possibility of using a natural, direct inhibitor to control BACE1 function definitively deserves further investigation.

Perspectives

The activity of BACE1 and PS is required to generate the pathogenic A β peptide that accumulates in the brain of AD patients. Clearly, blocking their activities appears to be a promising therapy to treat the disease. Transgenic mouse models in which the genes encoding the secretases have been ablated offer an invaluable tool, on the one hand, to gain more insights into the biological function of

these proteases and, on the other hand, to predict the consequences that might be associated with enzyme inhibition in vivo.

Genetic ablation of the PS genes in mice helped to identify the function of these enzymes in the Notch signalling pathway as well as their absolute requirement for the cleavage of Notch and APP within their transmembrane regions. The biological roles of Nicastrin, Pen-2 and Aph-1 as components of γ -secretase or their possible extra γ -secretase function remain to be determined. Several paralogs and alternatively spliced variants of at least PS and Aph-1 have been identified suggesting that γ -secretase is not an homogenous activity and the availability of mice deficient in γ -secretase components will help address these issues. Nicastrin- and Pen-2-deficient mice have been generated and they exhibit a phenotype consistent with inhibition of the Notch pathway. We are now awaiting the data for Aph-1-deficient mice.

The absence of any gross phenotype in BACE1 null mice converts this enzyme into a promising drug target. Pharmacologic inhibition of BACE1 does not appear, however, as straightforward as predicted. Moreover, ac-

tive site inhibitors are likely to affect in addition BACE2 function. The generation of BACE2 and BACE1/BACE2 double knockout mice is, therefore, crucial to help us predict the consequences of blocking BACE function in vivo and to gain more insights into the biological functions of these two proteases.

Other alternatives to the active site inhibitors for PS and BACE1 can be envisioned that target other aspects of the enzyme metabolism, including for example their interaction with the substrate, their localization in specific subcellular sites where cleavage takes place or the modulation of the expression of the enzymes. Preliminary data that such approaches are feasible are available and energy should be dedicated to their further investigation.

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