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REVIEW

The β-secretase enzyme BACE1 as a therapeutic target for Alzheimer's disease

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Abstract

Amyloid plaques are defining histopathologic lesions in the brains of Alzheimer's disease (AD) patients and are composed of the amyloid-beta peptide, which is widely considered to play a critical role in the pathogenesis of AD. The β-secretase, or β-site amyloid precursor protein cleaving enzyme 1 (BACE1; also called Asp2, memapsin 2), is the enzyme that initiates the generation of amyloid beta. Consequently, BACE1 is an attractive drug target for lowering cerebral levels of amyloid beta for the treatment or prevention of AD. Much has been learned about BACE1 since its discovery over 10 years ago. In the present article, we review BACE1 properties and characteristics, cell biology, *in vivo* validation, substrates, therapeutic potential, and inhibitor drug development. Studies relating to the physiological functions of BACE1 and the promise of BACE1 inhibition for AD will also be discussed. We conclude that therapeutic inhibition of BACE1 should be efficacious for AD, although careful titration of the drug dose may be necessary to limit mechanism-based side effects.

Discovery of BACE1, the Alzheimer's β-secretase

Autosomal dominant mutations in the genes for amyloid precursor protein (APP) and the presenilins (presenilin-1 and presenilin-2) cause familial Alzheimer's disease (AD) (reviewed in [1]), and these findings together with others suggest that the amyloid-beta (Aβ) peptide plays a central role in AD pathogenesis. Consequently, therapeutic approaches to lower brain Aβ levels should be efficacious for the treatment or prevention of AD. Aβ is generated through the sequential endoproteolysis of APP by the β-secretase and γ-secretase enzymes (reviewed in [2]). β-secretase cuts first at the N-terminus of Aβ; γ-secretase cleaves only thereafter to make the C-terminus of Aβ. Then Aβ is secreted from neurons to form amyloid plaques in the AD brain. Inhibition of β-secretase should thus decrease production of Aβ, the pathogenic form of the peptide.

Since the discovery of Aβ, the molecular identity of the β-secretase has been intensely sought because of its prime status as a drug target for AD. Prior to the enzyme's discovery, the properties of β-secretase activity in cells and tissues had been extensively characterized. In 1999 five groups reported the molecular cloning of the β-secretase [3], variously naming the enzyme BACE [4], Asp2 [5,6], or memapsin 2 [7] (herein, β-secretase will be referred to as β-site amyloid precursor protein cleaving enzyme 1 (BACE1)). The groups used different isolation methods (expression cloning, protein purification, genomics), yet all identified the same enzyme and agreed it possessed all the characteristics of β-secretase.

BACE1 cell biology

BACE1 is a type 1 transmembrane aspartic protease related to the pepsins and retroviral aspartic proteases [3-7]. BACE1 activity has a low optimum pH [4], and the enzyme is predominantly localized in acidic intracellular compartments (for example, endosomes, trans-Golgi) with its active site in the lumen of the vesicle [3-8]. The highest expression levels of BACE1 are found in neurons [3,4]. Importantly, BACE1 overexpression or BACE1 knockdown increases or decreases production of Aβ and β-secretase-cleaved APP fragments, respectively [4]. In addition, the activity of BACE1 on wild-type and mutant APP substrates is consistent with the sequence specificity of β-secretase. For example, BACE1 cleaves APP with the Swedish familial AD-causing mutation (APPswe) ~10-fold to 100-fold more efficiently than wild-type APP [3,4,9].

BACE1 is synthesized as a 501-amino-acid zymogen (containing a short prodomain) in the endoplasmic reticulum [3-7,10]. Within the lumen of the endoplasmic reticulum, BACE1 is subjected to simple glycosylation on four Asn residues [11] and transient acetylation on seven Arg residues [12]. Further addition of complex carbohydrates and removal of the BACE1 prodomain by furin convertases occur in the Golgi compartment [13-16].

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BACE1 is phosphorylated on Ser 498, and this phosphorylation together with a C-terminal acidic cluster dileucine motif (DXXLL) regulates BACE1 recycling between the cell surface and endosomal compartments [17-19].

BACE1 is S-palmitoylated on four Cys residues located at the junction of the transmembrane and cytosolic domains [15,20], and this modification facilitates BACE1 partitioning into lipid rafts. Increased targeting of BACE1 to the lipid raft was suggested to enhance β-secretase processing of APP [21,22]. A recent study, however, reported that nonraft-localized palmitoylation-deficient BACE1 is equally active in APP processing and Aβ secretion as is raft-associated palmitoylated BACE1 [20]. Although BACE1 can process APP in both raft and nonraft environments, a membrane-anchored version of a BACE1 transition-state inhibitor produced by linkage to a sterol moiety appeared more potent as a result of targeting to lipid rafts [23].

Golgi-localized γ-ear-containing ARF-binding (GGA) proteins interact with the BACE1 C-terminal DXXLL motif via a VHL domain and regulate trafficking of BACE1 between the late Golgi and early endosomes [24-26]. Depletion of GGA proteins by RNAi or disruption of phosphorylation of BACE1 on Ser498 increases accumulation of BACE1 in early endosomes, an acidic environment that favors BACE1 cleavage of APP and subsequent Aβ production [27-29]. Interestingly, GGA3 is a caspase-3 substrate and is degraded during neuronal apoptosis. In the brains of AD patients, in which neuronal apoptosis may occur, the levels of GGA3 are significantly decreased [28]. Reduced GGA3 levels not only increase localization of BACE1 to early endosomes, but also stabilize BACE1 by preventing its trafficking to lysosomes where it is degraded.

Intracellular localization of BACE1 can be altered by other regulatory factors in addition to the GGA proteins. Reticulon/Nogo family members have been identified as negative regulators of BACE1 [30,31]. Overexpression of reticulon proteins results in prolonged BACE1 retention in the endoplasmic reticulum with concomitant decrease in BACE1-mediated APP cleavage [32]. Sorting nexin 6 is another BACE1-associated protein that influences BACE1 subcellular localization and acts as a negative regulator of BACE1 activity [33]. Inhibition of sorting nexin 6 increases Aβ as well as retrograde transport of BACE1 to the trans-Golgi network. Sortilin is the most recently identified modulator of BACE1 trafficking [34]. When overexpressed, sortilin increases BACE1-mediated APP cleavage, while RNAi-mediated knockdown decreases Aβ. Future studies aimed at identifying the BACE1 interactome will yield fruitful information regarding novel proteins that control BACE1 trafficking, and hence Aβ production, which in turn could represent potential AD therapeutic targets.

In vivo validation of BACE1

Soon after BACE1 was discovered, its homolog BACE2 was identified [5,35]. BACE1 and BACE2 shared 64% amino acid sequence similarity, which suggested BACE2 was also a β-secretase. BACE2 was expressed at low levels in neurons and did not have the same cleavage activity as β-secretase, however, indicating it was a poor β-secretase candidate. To demonstrate that BACE1 was the β-secretase *in vivo*, *BACE*$^{-/-}$ mice were generated [36-39]. Initial reports indicated that *BACE1*$^{-/-}$ mice were viable and fertile, suggesting that therapeutic inhibition of BACE1 might be free of mechanism-based side effects. Recent studies, however, have shown that *BACE1*$^{-/-}$ mice are not completely normal (discussed below).

Importantly, Aβ production, amyloid pathology, electrophysiological dysfunction, and cognitive deficits were prevented when *BACE1*$^{-/-}$ mice were bred to APP transgenic mice [36,40-43]. Moreover, lentiviral delivery of BACE1 RNAi attenuated Aβ amyloidosis and cognitive deficits in APP transgenics [43,44]. These results demonstrated that BACE1 is the major, if not only, β-secretase enzyme in the brain. Taken together, BACE1 characterization and validation studies have unequivocally demonstrated that BACE1 is the authentic β-secretase in the brain and that it is a promising therapeutic target for lowering cerebral Aβ levels.

BACE1 substrates

In addition to APP, BACE1 has other substrates (Table 1), and identification of these substrates is useful not only for evaluation of potential mechanism-based toxicity arising from inhibition of BACE1 but also for designing potent and selective BACE1 inhibitors. Moreover, the diverse list of BACE1 substrates suggests a variety of BACE1 physiological functions.

All known BACE1 substrates are transmembrane proteins, such as Golgi-localized membrane-bound α2,6-sialyltransferase [45,46], IL-1 type II receptor [47], P-selectin glycoprotein ligand-1 [48], APP homologs Aβ precursor-like protein-1 and Aβ precursor-like protein-2 [49-51], low-density lipoprotein receptor-related protein [51,52], the voltage-gated sodium channel β1 to β4 subunits [53-55], neuregulin-1 [56,57] and neuregulin-3 [58]. Recently, an unbiased screen for novel BACE1 substrates identified 64 type I transmembrane proteins, three glycophosphatidylinositol-linked proteins and one type II transmembrane protein [51]. Although the majority of these putative BACE1 substrates have not been validated, several were found to be cleaved by BACE1 in cell culture, including ephrin type A receptor 5, Golgi phosphoprotein-4, leucine-rich repeats and immunoglobulin-like domain protein-2 (and -3), insulin-like growth factor 2 receptor and semaphorin-4C. Further studies will be required to determine whether these proteins are BACE1

Table 1. Validated BACE1 substrates and potential mechanism-based toxicity arising from BACE1 inhibition

Substrate	Toxicity
APP, APLP1 and APLP2	Impaired cell–cell signaling, injury recovery, synaptic function, memory
IL1R2	Abnormal inflammatory response
LRP	Impairments in lipid metabolism, degradation of proteases and activation of lysosomal enzymes
$Na_v\beta_{1-4}$	Altered neuronal excitability [76]
Neuregulin-1 and neuregulin-3	Impaired remyelination following nerve injury [58]
PSGL-1	Altered leukocyte recruitment during innate and adaptive immune response [77]
ST6Gal I	Hepatopathology, B-cell dysfunction

APLP, amyloid beta precursor-like protein; APP, amyloid precursor protein; BACE1, β-site amyloid precursor protein cleaving enzyme 1; IL1R2, IL-1 type II receptor; LRP, low-density lipoprotein receptor-related protein; $Na_v\beta_{1-4}$, voltage-gated sodium channel β1 to β4 subunits; PSGL-1, P-selectin glycoprotein ligand-1; ST6Gal I, Golgi-localized membrane-bound β-galactoside α2,6–sialyltransferase.

substrates at endogenous levels *in vivo*. Additional BACE1 substrates are likely to be discovered in the future.

BACE1 as a therapeutic target for Alzheimer's disease

Although *BACE1*$^{-/-}$ mice appear viable and fertile, the growing list of BACE1 substrates has suggested that less obvious phenotypes related to deficient BACE1 processing of substrates may exist. Indeed, eliminating BACE1 cleavage of neuregulin-1 in *BACE1*$^{-/-}$ mice causes reduced myelin sheath thickness of axons of both peripheral sciatic nerves [56,57] and central optic nerves [56]. *BACE1*$^{-/-}$ mice also display retarded remyelination of injured sciatic nerves [58]. In addition, recent studies have demonstrated that *BACE1*$^{-/-}$ mice exhibit increased frequency of spontaneous and kainite-induced seizures [59-61]. Hypomyelination and increased seizures observed in *BACE1*$^{-/-}$ mice have raised concerns that therapeutic BACE1 inhibition may be associated with similar untoward effects in humans. However, whether the hypomyelination and seizure phenotypes in *BACE1*$^{-/-}$ mice are caused by the lack of BACE1 activity in the adult or during embryonic or postnatal development is currently unknown.

Because of potential adverse side effects associated with strong inhibition or reduction of BACE1, investigators have tested whether a moderate decrease in BACE1 activity would provide benefits in the central nervous system while limiting mechanism-based toxicities. Laird and coworkers showed a significant reduction of Aβ deposition in brains of 12-month-old *APPswe;PS1DE9;BACE1*$^{+/-}$ mice as compared with that of *APPswe;PS1DE9;BACE1*$^{+/+}$ mice; however, no significant differences were observed in brains of 20-month-old *APPswe;PS1DE9;BACE1*$^{+/-}$ animals [43]. It is unclear why the older mice in this study did not show reduced amyloidosis. In a similar study, McConlogue and colleagues reported a significantly reduced Aβ burden in the brains of 13-month-old and 18-month-old *PDAPP;BACE1*$^{+/-}$ mice [62]. Although the two studies had differences, neither indicated a negative phenotype associated with the *BACE1*$^{+/-}$ mice. Taken together, these data suggest the exciting possibility that partial inhibition of BACE1 may effectively reduce Aβ deposition without mechanism-based toxicity.

BACE1 inhibitor development

Since the identification of BACE1, intense efforts have been underway to develop small-molecule BACE1 inhibitors as drugs for AD. First-generation BACE1 inhibitors were peptide-based mimetics (peptidomimetics) of the APP β-site that replaced the scissile amide bond with a nonhydrolizable transition state analog such as statine [3]. The X-ray structure of BACE1 co-crystallized with peptidomimetic inhibitors [63] greatly facilitated the rational design of BACE1 inhibitors. More recently, later-generation nonpeptidic compounds with low nanomolar half-maximal inhibitory concentration potencies have been generated (reviewed in [64-66]).

Although initial drug development efforts with peptidomimetic BACE1 inhibitors were encouraging, BACE1 has since proven to be a challenging medicinal chemistry target. There appear to be several reasons for this challenge. First, BACE1 has a large hydrophobic substrate-binding site designed to fit polypeptides, thus making it difficult to inhibit the enzyme with small nonpeptidic compounds that have desirable drug-like characteristics. Ideally, BACE1 inhibitor drugs should be of a molecular weight <500, orally bioavailable, metabolically stable, intrinsically potent, and highly selective for BACE1 over BACE2 and other aspartic proteases. This latter point is relevant because BACE1$^{-/-}$;BACE2$^{-/-}$ double knockout mice have enhanced postnatal lethality compared with BACE1$^{-/-}$ single knockout mice [39]. Compounds must also be hydrophobic enough to penetrate both plasma and intracellular membranes to gain access to the lumen of the compartment where the BACE1 active site is localized. Finally, efficacious BACE1 drugs would need to efficiently cross the blood–brain

barrier and achieve a high concentration in the cerebral parenchyma.

Despite these challenges, potent nonpeptidic small-molecule BACE1 inhibitors have shown success in lowering cerebral Aβ levels in mouse [67-69], hamster [70] and primate [71] models. Moreover, the biopharmaceutical company CoMentis (South San Francisco, CA, USA) recently announced the completion of the first human phase 1 clinical trial of a BACE1 inhibitor drug [72]. Other BACE1 inhibitor drug candidates will probably soon be entering into human clinical trials. An interesting alternative to small-molecule inhibitors entails the use of monoclonal antibodies to inhibit BACE1 enzymatic activity. Recent reports hint at the potential of antibodies that inhibit BACE1 cleavage of APP by either directly binding to BACE1 [73] or by binding to the β-secretase cleavage site of APP [74]. The latter has shown *in vivo* efficacy for decreasing Aβ in a murine model [75]. These encouraging results suggest that therapeutic approaches involving BACE1 inhibition for the treatment or prevention of AD may be a reality in the future. Given recent data hinting at important physiological roles for BACE1, however, careful titration of the BACE1 drug dosage may be necessary to minimize mechanism-based side effects.

Conclusions

Since the discovery of BACE1 over a decade ago, our knowledge about this enzyme has increased significantly. Progress has been made toward the development of small-molecule inhibitors capable of penetrating the blood–brain barrier and having sufficient potency to inhibit Aβ generation *in vivo*. There is also very early evidence for the prospect of anti-BACE1 monoclonal antibodies for AD treatment. Despite promising news of BACE1 inhibitor development, however, BACE1 therapeutics for routine AD treatment are not yet available. Although BACE1 small-molecule inhibitor medicinal chemistry and pharmacokinetics have proven challenging, the recent entry of at least one BACE1 inhibitor into clinical trial is an encouraging advance toward BACE1 therapeutic inhibition for the treatment of AD. Drug-makers and physicians should keep in mind that BACE1 appears to have important physiological functions, perhaps requiring careful titration of the BACE1 inhibitor drug dose to minimize potential mechanism-based toxicity. Finally, advancing our understanding of the roles of BACE1 in health and disease will facilitate the development of novel therapies for AD and may shed light on the etiology of this devastating disease and other disorders of the nervous system.

Abbreviations

AD, Alzheimer's disease; APP, amyloid precursor protein; APP_{swe}, amyloid precursor protein with the Swedish familial Alzheimer's disease-causing mutation; Aβ, amyloid beta; BACE1, β-site amyloid precursor protein cleaving enzyme 1; GGA, Golgi-localized γ-ear-containing ARF-binding protein; IL, interleukin; RNAi, RNA interference.

Competing interests

The authors declare that they have no competing interests.

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