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The Normal and Pathologic Roles of the Alzheimer's β-secretase, BACE1

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Abstract

As the most common neurodegenerative disease, therapeutic avenues for the treatment and prevention of Alzheimer's Disease are highly sought after. The aspartic protease BACE1 is the initiator enzyme for the formation of Aβ, a major constituent of amyloid plaques that represent one of the hallmark pathological features of this disorder. Thus, targeting BACE1 for disease-modifying AD therapies represents a rationale approach. The collective knowledge acquired from investigations of BACE1 deletion mutants and characterization of BACE1 substrates has downstream significance not only for the discovery of AD drug therapies but also for predicting side effects of BACE1 inhibition. Here we discuss the identification and validation of BACE1 as the β-secretase implicated in AD, in addition to information regarding BACE1 cell biology, localization, substrates and potential physiological functions derived from BACE1 knockout models.

Keywords

Alzheimer's disease; BACE1; beta-secretase

INTRODUCTION

Substantial evidence points to a role for cerebral aggregation of amyloid beta (Aβ) peptide in Alzheimer's Disease (AD)[1]. Aβ is derived from the sequential action of two aspartic proteases, the β- and γ-secretases, on amyloid precursor protein (APP). β-secretase initiates Aβ formation by cleaving APP to generate the N-terminus of Aβ [2–4˜]. This cleavage produces a secreted ectodomain of APP (APPsβ) and a membrane-tethered C-terminal fragment that is 99 amino acids in length (C99). Subsequently, γ-secretase cleaves within the transmembrane region of C99 to release Aβ that is secreted from the cell. Aβ peptides may vary in length (38–42 amino acids) at the C-terminus due to the imprecise cleavage of the γ-secretase. Since Aβ accumulation is implicated in AD pathogenesis, the identity of the β-secretase was highly sought after due to its ideal status as a drug target for lowering

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cerebral Aβ levels. Herein this review will discuss the identification and validation of the aspartic protease, beta-site APP cleaving enzyme-1 (BACE1), as the β-secretase implicated in AD. Information regarding BACE1 cell biology, localization, substrates and physiological functions derived from deletion mutants will also be discussed.

IN VIVO VALIDATION OF BACE1 AS THE ALZHEIMER'S β-SECRETASE

Over a decade ago, five groups reported two unique aspartic proteases that shared 64% amino acid sequence similarity, and that served as potential β-secretase candidates: BACE1 (also termed memapsin 2 and Asp2) [5–9], and BACE2 (also termed Asp1, memapsin 1, and DRAP) [6, 8, 10–13]. Prior to these reports, β-secretase properties had been well-characterized, a sequence of events that, as it turned out, was instrumental for the identification of the β-secretase. In the discussion below we evaluate the properties of β-secretase that served as a tool to clearly validate BACE1 as the β-secretase essential for Aβ formation.

Although β-secretase activity is widely expressed, the highest proteolytic activity is observed in the brain [14, 15]. Consistent with this expression pattern, BACE1 is present in many tissues, but is predominantly expressed within the brain [6, 7, 11, 16]. BACE2, however, is expressed at moderate to low levels across a variety of cell types, but it is low to undetectable in most brain regions. There are a few exceptions, as there is evidence of BACE2 expression in the mammilary bodies, the ventromedial hypothalamus, and other small brain stem nuclei [11, 16].

The optimal pH for β-secretase activity is within a low pH range [17–19], and as such β-secretase localizes primarily to endosomes and the Golgi apparatus [20–22]. *In vitro* enzyme activity assays revealed BACE1 has an acidic pH optimum [7]. Moreover, BACE1 was shown to reside predominantly within acidic intracellular compartments with its active site in the lumen of the vesicle [5–9, 23].

In cells, APP constructs devoid of the transmembrane domain are not cleaved by β-secretase, which implies that β-secretase specifically targets membrane-bound substrates [2]. Thus, one may deduce that β-secretase is either tightly associated with a membrane protein, or membrane-bound itself. In both cases, BACE1 and BACE2 contain membrane-spanning segments [5–10, 12].

Site-directed mutagenesis analysis of the amino acids surrounding the APP cleavage site demonstrates that β-secretase cleavage is highly sequence-specific [2]. Substitutions at this site and nearby positions decrease β-secretase cleavage of APP. In addition, radio sequencing studies have shown that Aβ isolated from amyloid plaques primarily begins at Asp^{+1} [24], but may also start at Glu^{+11} [25]. The activity of BACE1 on wild-type and mutant APP substrates is consistent with the sequence specificity of β-secretase. BACE1 cleaves APP only at Asp^{+1} and Glu^{+11} [7], and cleaves APP with the Swedish familial AD-causing mutation (APPswe; K670N/M671L) more efficiently than wild-type APP [7, 9, 26]. Conversely, an alanine to threonine substitution two residues from the BACE1 cleavage site (A673T) reduces BACE1-mediated APP cleavage and results in a significant decrease in the risk of AD [27]. Interestingly, the A63T APP substitution is additionally protective against

cognitive decline in elderly without AD [27]. BACE2 does not have the same cleavage specificity for APP as BACE1, cleaving APP not only at Asp^{+1} [28–30], but also at two other positions: Phe^{+19} and Phe^{+20} [28].

When cells are transfected with BACE1 and either wild-type or mutant APP, Aβ levels are increased [9]. Additional credence to BACE1 as the β-secretase comes from experiments using cell lines overexpressing APP. When BACE1 is transfected into wild-type APP-overexpressing cells, Aβ, APPsβ and C99 are elevated over controls [5–9]. Conversely, transfection of BACE1, but not BACE2, antisense oligonucleotides into APP-overexpressing cells decreases Aβ and C99 fragments [7, 8].

The strongest evidence for BACE1 as the β-secretase *in vivo* came from analyses of BACE1-deficient mice ($BACE1^{-/-}$) bred to mice overexpressing APP with the Swedish mutation (Tg2576) to produce a $BACE1^{-/-}$;APP bigenic strain [31–34]. In $BACE1^{-/-}$;APP brain extracts, Aβ and C99 fragments are absent [35, 36]. Moreover, neuronal cultures prepared from $BACE1^{-/-}$ tissue that were infected with APP-expressing adenovirus show no evidence of Aβ or C99 [37]. In addition, age-associated cognitive deficits were prevented in *$BACE1^{-/-}$*; *APP* bigenic mice [31–34, 38–41]. Similarly, lentiviral delivery of BACE1 RNAi attenuated Aβ amyloidosis and rescued memory deficits in APP transgenics [38, 42]. The rescue of memory deficits in *$BACE1^{-/-}$*; *APP* mice suggests that BACE1 inhibition has potential to improve cognitive impairment in humans with AD.

To date, the non-β-secretase-like APP cleavage and low-level cerebral expression of BACE2 argues against a role for BACE2 as the primary β-secretase involved in Aβ generation. Rather, it has been suggested that BACE2 plays a role in Down syndrome pathology [35] since the gene resides on chromosome 21 (Saunders *et al*. 1999) and BACE2 is over-expressed in Down syndrome patients [43, 44]. The physiological and pathological role of BACE2 remains unclear. BACE2 is expressed in glial cells and may contribute to Aβ generation within this cell type although the mechanism requires elucidation [35, 45]. Glial cells play a role in AD amyloidogenesis, and early evidence for a role for BACE2 in glial amyloidogenic processing in Down syndrome patients suggests further investigation.

BACE1 exhibits all of the putative β-secretase characteristics, and most strikingly, absence of BACE1 *in vivo* abolishes Aβ formation and subsequent amyloid pathology. Converging evidence from the molecular, biochemical and animal studies described above substantiates BACE1 as the β-secretase.

BACE1 CELL BIOLOGY

The BACE1 gene encodes for a ~70kDa type 1 transmembrane aspartic protease related to the pepsins and retroviral aspartic proteases [5–9]. The BACE1 luminal domain contains two aspartic protease active site motifs at amino acids 93–96 and 289–292, with each motif containing the highly conserved sequence defining aspartic proteases, D(T/S)G(T/S) [7] (Fig. 1). Both aspartates are required for BACE1 proteolytic activity [5, 46], however since BACE1 forms dimers, one aspartate from each monomer may be supplied for proteolysis. BACE1 is synthesized as a 501 amino acid pro-enzyme with a short prodomain in the endoplasmic reticulum (ER) [5–9, 47]. Within the ER, the luminal domain of BACE1 is

glycosylated on four Asn residues [48] and transiently acetylated on seven Arg residues [49]. Once translocated to the Golgi apparatus, complex carbohydrates are attached and the N-terminal prodomain is removed by furin convertases [5, 46, 50–52], leading to the 70kDa form. Although the pro-enzyme possesses proteolytic activity, this activity increases ~two-fold following removal of the prodomain [53, 54]. After maturation, BACE1 is transported from the trans-Golgi network (TGN) to the cell surface where it is reinternalized into early endosomes [55, 56]. Endosomal sorting of membrane proteins involves the interaction of C-terminal sorting signals (di-leucine-based motifs [(DE)*XXX*L(LI) or D*XX*LL] and tyrosine-based motifs [NP*X*Y or Y*XX*Ø]) with trafficking molecules [57]. BACE1 does not harbor tyrosine-based motifs, but does contain an acidic di-leucine motif (DISLL; residues 496–500) [55, 58] on its cytosolic tail that regulates BACE1 shuttling between the TGN and endosomes [55, 56, 58–60]. This sequence is recognized by Golgi-localized gamma-ear-containing ADP ribosylation factor (ARF)-binding (GGA) proteins (GGA1-3) [61]. BACE1 phosphorylation of Ser-498 facilitates GGA1-3 binding to regulate BACE1 recycling between the cell surface and endosomal compartments [56, 62–64]. Recently, the DISLL sequence was shown to be part of a longer sequence (DDISLL; residues 495–500) that regulates BACE1 endocytosis via interaction with the clathrin-associated heterotetrameric adaptor protein 2 (AP-2) complex [65]. Thus, the DDISLL sequence functions dually to alternatively interact with the GGAs and AP-2. Additionally, a clathrin-independent mechanism of BACE1 endocytosis has also been identified, which occurs via interaction of BACE1 with the small GTPase, ADP ribosylation factor 6 (ARF6) [66]. The low pH of the late Golgi/trans-Golgi network and early endosomal compartments, coupled with the maturation of BACE1, increases BACE1 enzymatic activity [7].

BACE1 is S-palmitoylated on four Cys residues located at the junction of the transmembrane and cytosolic domains [51, 67], and this modification facilitates BACE1 partitioning into lipid rafts. Increased targeting of BACE1 to the lipid raft had been suggested to enhance β-secretase processing of APP [68, 69]. However, another study has reported that non-raft localized palmitoylation-deficient BACE1 is equally active in APP processing and Aβ secretion as raft-associated palmitoylated BACE1 [67]. Although BACE1 can process APP in both raft and non-raft 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 [70].

BACE1 PROMOTER STRUCTURE AND REGULATION

The BACE1 gene promoter has been sequenced and analyzed, including the location of specific regulatory domains as revealed by deletion analysis [71–74]. The BACE1 gene includes an ~30 kilobase (kb) region of chromosome 11q23.2 −11q23.3 and consists of 9 highly conserved coding exons [10, 74]. Canonical “CAAT” and “TATA” boxes are lacking from the BACE1 promoter, though six unique functional domains and three structural domains of increasing sequence complexity are located in near proximity to the ATG start codon [75]. Sequence analysis of the promoter region and 5' untranslated region (5'-UTR) predicts numerous transcription factor-binding sites, including: specificity protein 1 (Sp1; [71–74]), signal transducer and activator of transcription 6 (STAT6;[74]), GATA-1 [73, 74], activator protein-1 (AP1;[74]), activator protein-2 (AP2;[72, 74]), cyclic AMP response

element binding protein (CREB;[74]), nuclear factor-κB (NFκB;[74]), hypoxia-inducible factor-1 (HIF-1) and heat shock factor-1 (HSF-1;[74]), estrogen and glucocorticoid receptors[74],yin yag 1 (YY1;[73]), and myocyte enhancer factor-2 (MEF2;[72]). This indicates that BACE1 expression may be regulated in response to cell signals that influence transcription, and in fact a handful of these and other transcription factors have been validated to affect BACE1 gene expression (Sp1 [71], YY1 [76], STAT1 [77], STAT3 [78], NFκB [79–81] and HIF-1 [82]). It is important to note that the BACE1 promoter is differentially regulated depending on cell type [72, 75] and additionally that its regulation differs from that of BACE2 [83].

Inflammation has been linked to AD brain pathology. AD brains show evidence of an inflammatory response, and long-term non-steroidal anti-inflammatory drug (NSAID) use has been shown to reduce the risk of AD [84]. Accordingly, pro-inflammatory molecules can elevate astrocytic BACE1 expression [77], and BACE1 levels rise at sites of glial activation prior to plaque development [85]. The BACE1 gene promoter also contains a binding site for the transcriptional regulator peroxisome proliferator-activated receptor γ (PPARγ; [86]). Activation of PPARγ represses BACE1 gene promoter activity, whilst proinflammatory cytokines that reduce PPARγ levels lead to increased BACE1 mRNA [86]. Thus, the effects of inflammation and NSAIDs on AD may be mediated by the activation of PPARγ and subsequent repression of BACE1 gene expression. Additional AD risk factors that can elevate BACE1 mRNA levels include traumatic brain injury [87], hypoxia [82, 88, 89] and oxidative stress [90]. Interestingly, Aβ peptide was recently shown to regulate BACE1 gene expression via a specific Aβ-interacting domain (AβID) in the BACE1 promoter [91, 92]. This may represent a feed-forward mechanism that could exacerbate amyloidogenesis and AD pathogenesis.

BACE1 LOCALIZATION

BACE1 is localized to the TGN and endosomal pathway [5–7, 47, 55], co-localizing with APP in endosomes [5, 23]. As mentioned previously, BACE1 also shuttles between the cell surface and early endosomes [55, 56]. Intracellular BACE1 localization is regulated by various adapter proteins. GGA proteins regulate trafficking of BACE1 between the late Golgi and early endosomes by interacting with the BACE1 C-terminal DXXLL motif via a VHL domain [61, 63, 93]. 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 [59, 60, 64]. 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 [60, 94]. Reduced GGA3 levels increase localization of BACE1 to early endosomes and also stabilize BACE1 by preventing its trafficking to lysosomes where it is degraded. Monoubiquiti-nation of BACE1 at lysine 501 promotes lysosomal degradation, which is dependent upon the recognition of the ubiquitinated lysine residue by GGA1 [95]. Thus, GGA3 over expression reduces BACE1 levels and subsequent Aβ formation.

The reticulon (RTN)/Nogo family members have been identified as negative regulators of BACE1 [96, 97]. Over expression of RTN proteins results in prolonged BACE1 retention in the ER with concomitant decrease in BACE1-mediated APP cleavage [98]. Sorting nexin6 (SNX6) is another BACE1-associated protein that influences BACE1 subcellular localization and acts as a negative regulator of BACE1 activity [99]. Inhibition of SNX6 increases Aβ as well as retrograde transport of BACE1 to the trans-Golgi network. Sortilin is the most recently identified modulator of BACE1 trafficking [100]. When overexpressed, sortilin increases BACE1-mediated APP cleavage, while RNAi-mediated knockdown decreases Aβ. Identification and characterization of BACE1 interactors will yield fruitful information regarding novel proteins that control BACE1 trafficking, and hence Aβ production.

Besides BACE1 trafficking, the specific localization of BACE1 in the brain may provide important clues as to the roles of BACE1 in the CNS and the molecular and cellular bases of BACE1 functions. Our previous work suggested that BACE1 is concentrated in presynaptic terminals, especially in mossy fibers of the stratum lucidum in hippocampal CA3 [101]. We recently demonstrated unequivocal BACE1 localization to vesicles within mossy fiber terminals using using immuno-electron microscopy and immunofluorescence confocal microscopy [102]. On occasion, BACE1-positive vesicles were located near synaptic active zones. The specific localization of BACE1 to membranous vesicular structures within presynaptic terminals suggests an important but as yet undetermined function of BACE1 substrate processing at the synapse. Given these data, the presynaptic terminal is likely the principal site of BACE1 localization in the brain.

BACE1 KNOCKOUT MICE

Shortly after the identification of BACE1, several groups undertook efforts to generate *BACE1*$^{-/-}$ mice. Generation of these mice would be valuable for addressing whether BACE1 played a vital role *in vivo*, as well as provide evidence for potential mechanism-based side effects of anti-BACE1 therapeutics. Several knockout strategies were employed: (1) removal of the ATG start codon via deletion of exon 1 [37] (2) insertion of a β-galactosidase gene downstream of the ATG start codon [36], (3) removal of the N-terminal active site motif via deletion of exon 2 [31], (4) removal of the C-terminal half of the protease domain [36], and (5) insertion of a neomycin cassette within exon 1 to introduce a premature stop codon [35]. Although β-secretase activity was effectively abolished in *BACE1*$^{-/-}$brains, initial analyses revealed no effect on gross behavioral and neuro-muscular function [36], tissue morphology, histology, blood or urine chemistry [31, 36]. Subsequent analyses, however, revealed complex neurological deficits that point to additional roles for BACE1 other than APP cleavage. Elucidating BACE1 physiological functions is essential for predicting potential mechanism-based toxicities associated with BACE1 inhibition as a therapeutic approach for AD. To date, the following phenotypes have been reported for *BACE1*$^{-/-}$mice: growth retardation [35], memory deficits [33, 34, 38, 103], hypomyelination [104–106], seizures [39, 107, 108], axon guidance defects [109–111], motor coordination deficits [112], schizophrenia-like behaviors [113], retinal pathology [114], hyperactivity [35, 113], spine density reduction [113] and metabolic abnormalities [35, 115, 116] (Table 1). Moreover, one report notes an increased neonatal lethality among *BACE1*$^{-/-}$pups that is not

attributable to maternal nursing defects [35]. It is unclear whether these pheno-types are ascribed to the lack of BACE1 in adult or during embryonic or postnatal development. The generation of conditional *BACE1*$^{-/-}$mice would be an invaluable tool to address these questions.

BACE1 SUBSTRATES

Previously, the majority of investigative reports focused on BACE1 proteolysis of APP; however, the recent identification of additional BACE1 substrates [117–119] hints at lesser-known physiological functions in which BACE1 may be involved. Identification of these substrates is useful for designing potent and selective BACE1 inhibitors. The existence of additional substrates also suggests that the inhibition of BACE1 for AD may not be free of mechanism-based toxicity. Numerous BACE1 substrates are transmembrane proteins, many of which function in cell signaling, immune or inflammatory responses, and which suggests a role for BACE1 in this capacity. Among these known BACE1 substrates are: Golgi-localized membrane-bound a2,6–sialyltransferase (ST6Gal I) [120–123], interleukin-1 type II receptor (IL1R2) [124], P–selectin glycoprotein ligand–1 (PSGL-1) [125], APP homologs APLP1 and APLP2 [117, 126–128], and low density lipoprotein receptor–related protein (LRP) [117, 129], among others [117–119]. A unbiased screen for novel BACE1 substrates identified 64 type I transmembrane proteins, three glycophosphatidylinositol (GPI)-linked and one type II transmembrane protein [117]. Although the majority of the substrates from this screen have yet to be validated, several were shown to be cleaved by BACE1 in cell culture: ephrin type A receptor (ephrin-A5), Golgi phosphoprotein 4 (GOLIM4), leucine-rich repeats and immunoglobulin-like domains proteins 2 (LRIG-2) and 3 (LRIG-3), insulin-like growth factor 2 receptor (IGF2R) and semaphorin-4C (SEMA4C). A more recent study by Kuhn *et al*. identified an additional 34 novel BACE1 substrates from primary neurons using a method developed by the authors referred to as secretome protein enrichment with click sugars (SPECS) [118]. Of the novel substrates identified, several were validated *in vivo*. These substrates, which suggest BACE1 involvement in synapse formation and neurite outgrowth, include: seizure protein 6 (SEZ6), CHL1, contactin-2, and the cell adhesion protein L1.

A potential role for BACE1 also exists in modulating sodium currents, as evidenced by cleavage of $Na_v\beta_{1-4}$ [130–133] and alteration of sodium currents in BACE1$^{-/-}$mice [35]. Moreover, BACE1 has been implicated in the regulation of myelination and myelin sheath thickness via cleavage of neuregulin–1 [104, 105] and neuregulin-3 [106]. [117–119]. BACE1-dependent processing of the Ig-containing β1 iso-form of neuregulin-1 (IgNrg1β1) is associated with muscle spindle formation and maturation, additionally implicating BACE1 in the control of motor coordination [112]. Lastly, we recently reported a role for BACE1 in axon guidance that is dependent upon BACE1-mediated cleavage of Close Homolog of L1 (CHL1) [110, 118, 119]. BACE1$^{-/-}$ mice exhibit axon guidance defects in the hippocampus and olfactory bulb that phenocopy axon targeting errors observed in CHL1$^{-/-}$mice [110, 134, 135]. Moreover, CHL1 is processed by BACE1 [117–119], and CHL1 and BACE1 co-localize in primary neuron growth cones and in presynaptic terminals in hippocampus and olfactory bulb [110], suggesting that BACE1 cleavage of CHL1 is necessary for proper axon guidance. Additional studies will be necessary to validate putative

BACE1 substrates *in vivo* and thereby provide further insight into the diverse array of biological functions of BACE1.

BACE1 THERAPEUTIC INHIBITION FOR ALZHEIMER'S DISEASE TREATMENT

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 [104, 105] and central optic nerves [104]. *BACE1*$^{-/-}$ mice also display retarded re-myelination of injured sciatic nerves [106]. In addition, recent studies have demonstrated that *BACE1*$^{-/-}$ mice exhibit increased frequency of spontaneous and kainite-induced seizures [107, 108], phenotypes that may be related to deficient cleavage of the BACE1 substrate Na_vb_2. Hypomyelination, increased seizures, motor coordination and axon guidance deficits observed in *BACE1*$^{-/-}$ mice raises concerns that therapeutic BACE1 inhibition may be associated with similar untoward effects in humans.

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 CNS while limiting mechanism-based toxicities. Laird and co-workers showed a significant reduction of Aβ deposition in brains of 12 month-old *APPswe;PS1DE9;BACE1*$^{+/-}$ mice as compared to that of *APPswe;PS1DE9;BACE1*$^{+/+}$ mice; however, no significant differences were observed in brains of 20 month-old *APP-swe;PS1DE9;BACE1*$^{+/-}$ animals [38]. It is unclear why the older mice in this study did not show reduced amyloidosis. In a similar study, McConlogue and colleagues reported significantly reduced Aβ burden in the brains of 13 and 18 month-old *PDAPP;BACE1*$^{+/-}$ mice [40]. Although the two studies had some differences, neither indicated a negative phenotype associated with the *BACE1*$^{+/-}$ mice. Taken together, the data suggest 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 non-hydrolizable transition state analog such as statine [136]. The X-ray structure of BACE1 co-crystallized with peptidomimetic inhibitors [137] greatly facilitated the rational design of BACE1 inhibitors. More recently, later generation non-peptidic compounds with low nM IC50 potencies have been generated (reviewed in [138, 139]).

Although initial drug development efforts with peptidomimetic BACE1 inhibitors were encouraging, BACE1 has since proven to be a challenging medicinal chemistry target. The reasons for this appear to be several. First, BACE1 has a large hydrophobic substrate-binding site designed to fit polypeptides, thus making it difficult to inhibit the enzyme with

small non-peptidic compounds that have desirable drug-like characteristics. Ideally, BACE1 inhibitor drugs should be less than 500 MW, orally bioavailable, metabolically stable, intrinsically potent, and highly selective for BACE1 over BACE2 and other aspartic proteases. Compounds must also be hydrophobic enough to penetrate both plasma and intracellular membranes to gain access to the lumen of the compartment were 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 non-peptidic small-molecule BACE1 inhibitors have been reported that meet these criteria and show efficacy in lowering cerebral Aβ levels in animal models of AD (reviewed in [138, 139]). It is likely that other BACE1 inhibitor drug candidates will soon be entering into human clinical trials. These encouraging results suggest that therapeutic approaches involving BACE inhibition for the treatment or prevention of AD may be a reality in the future. However, given recent data hinting at important physiological roles for BACE1, careful titration of BACE1 drug dosage will be necessary to minimize mechanism-based side effects.

CONCLUSION

BACE1 is the key enzyme initiating Aβ synthesis *in vivo*, making it a prime drug target for AD treatment. The past decade has shown significant progress in our understanding of BACE1 molecular and cellular properties, and increasing progress identifying and characterizing BACE1 substrates other than APP. Recently identified BACE1 substrates hint at potential roles for BACE1 in immunological and inflammatory responses, modulation of sodium currents, regulating nerve myelination, synapse formation, motor coordination, seizure susceptibility and axon guidance. Further investigations are crucial to define the precise role BACE1 may play in these processes, and the extent to which BACE1 inhibition will influence these essential biological functions. Additional phenotypes resulting from BACE1 deficiency may be revealed in studies of $BACE1^{-/-}$ mice under specific challenges. The collective knowledge acquired from investigations of BACE1 deletion mutants and characterization of BACE1 substrates has downstream implications for the discovery of new AD therapeutic targets and predicting side effects of BACE1 inhibition.

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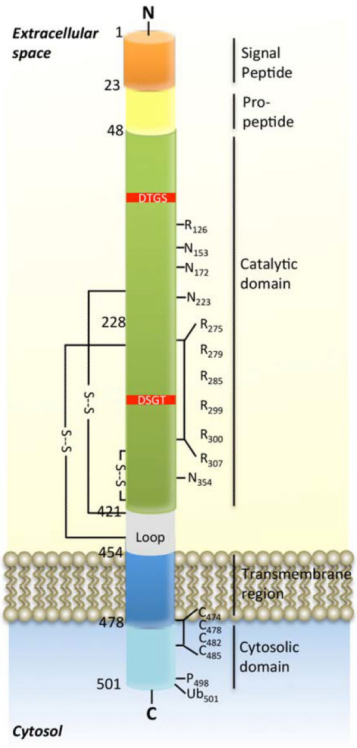

Fig. (1). BACE1 structural organization and post-translational modifications

Colored regions depict BACE1 domains with the corresponding amino acid numbers. The BACE1 catalytic domain contains two aspartic protease active site motifs, DTGS and DSGT, at positions 92–95 and 289–292, respectively (red bars). Acetylation (R), glycosylation (N), S-palmitoylation (C), phosphorylation (P) and ubiquitination (Ub) sites are indicated where known. Three disulfide bonds (S--S) connect amino acids 216–420, 278–443 and 330–380.

Table 1

BACE1 knockout mouse phenotypes.

Phenotype	Substrate	References
Axon guidance defects	CHL1	Rajapaksha *et al.*, 2011 [109] Cao *et al.*, 2012 [111] Hitt *et al.*, 2012 [110]
Hyperactivity	NRG1	Dominguez *et al.*, 2005 [35] Savonenko *et al.*, 2008 [113]
Hypomyelination	NRG1	Willem *et al.*, 2006 [105] Hu *et al.*, 2006, 2008 [104, 106]
Memory deficits	Unknown	Ohno *et al.*, 2004, 2006, 2007 [33,34,103] Laird *et al.*, 2005 [38]
Metabolic abnormalities	Unknown	Dominguez *et al.*, 2005 [35] Meakin *et al.*, 2012 [116] Hoffmeister *et al.*, 2013 [115]
Muscle spindle defects	NRG1	Cheret *et al.*, 2013 [112]
Neurodegeneration with age	Unknown	Hu *et al.*, 2010 [108]
Postnatal lethality, growth retardation	Unknown	Dominguez *et al.*, 2005 [35]
Retinal abnormalities	VEGFR1	Cai *et al.*, 2012 [114]
Schizophrenia endophenotypes	NRG1	Savonenko *et al.*, 2008 [113]
Seizures	$Na_v\beta_2$	Kobayashi *et al.*, 2008 [39] Hu *et al.*, 2010 [108] Hitt *et al.*, 2010 [107]
Spine density reduction	NRG1	Savonenko *et al.*, 2008 [113]