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Opinion

BACE1 inhibition as a therapeutic strategy for Alzheimer's disease

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1. The identification of β-secretase as β-site amyloid precursor protein cleaving enzyme (BACE)

Following the discoveries of β-amyloid (Aβ) and the first amyloid precursor protein (APP) mutations that cause familial Alzheimer's disease (AD), it soon became clear that the β- and γ-secretase enzymes were prime therapeutic targets for the development of small-molecule inhibitor drugs for the treatment of AD. Thus, their molecular identities were vigorously pursued. The properties of Aβ generation and secretase activities in cells and tissues led to the development of cell-free and cell-based assays that could be exploited for the identification of the secretases. Subsequently, 5 groups independently reported the molecular cloning of the β-secretase enzyme, which they variously named BACE, Asp2, and memapsin 2.[1–5] It is important to note that all the groups agreed on the same polypeptide sequence even though they used different experimental approaches to identify the β-secretase, lending strong support for the conclusion that the authentic β-secretase had been cloned.

BACE has all the molecular and cellular characteristics that had been previously predicted for the β-secretase *in vitro* and *in vivo*. It is a type I transmembrane aspartic protease of 501 amino acids in length that is closely related to the pepsin family of aspartic proteases. The catalytic domain of BACE harbors 2 aspartic protease signature motifs of the sequence DTGS and DSGT that come together to form the active site of the enzyme. As required for β-secretase, the BACE active site is topologically oriented on the same side of the membrane as the β-secretase cleavage site in APP. Additionally, the activity of BACE has an acidic pH optimum and the catalytic domain resides within the lumen of acidic intracellular compartments, including endosomes and the trans-Golgi network. Moreover, BACE levels are highest in neurons of the central nervous system (CNS); BACE has the correct sequence specificity; and BACE overexpressed in cells cleaves APP and increases Aβ production.

Soon after the discovery of BACE, a homologue, BACE2, was identified that has ~64% amino acid similarity to BACE (henceforth referred to as BACE1). The extensive degree of homology between the 2 enzymes suggested that BACE2 might also function as a β-secretase. However, this possibility seemed unlikely because BACE2 is not expressed to a high level in neurons, in contrast to BACE1. Moreover, BACE2 predominantly cleaves APP within the Aβ domain, so that the generation of Aβ is precluded. These data, together with the finding that BACE1-null mice are devoid of Aβ, suggest that BACE2 is not likely to be a β-secretase in the CNS.

2. Physiological functions of BACE1

2.1. BACE1-/- mice

To justify BACE1 inhibitor drug development efforts, it was necessary to provide *in vivo* validation that BACE1 is the primary β-secretase enzyme in the brain. To do so, gene targeting in embryonic stem cells was used to produce BACE1 knockout (-/-) mice.[6–9] Initial reports showed that BACE1-/- mice were viable and fertile and did not have detectable abnormalities. Their normal morphology and behavior, tissue histology, and blood cell and clinical chemistry characteristics suggested that BACE1 inhibition as a therapeutic approach for AD might lack mechanism-based toxicities. Additionally, APP-overexpressing transgenic mice that also lack the *BACE1* gene are devoid of cerebral Aβ, amyloid deposition, and Aβ-associated memory impairments.[10–14] It is important to note that these data validate BACE1 as the major β-secretase in the CNS and indicate that BACE2 does not compensate for BACE1 loss of function, at least for the production of Aβ. Furthermore, they strongly suggested that BACE1 inhibition should be a safe and effective therapeutic strategy for AD.

Although initial studies of BACE1-/- mice indicated that BACE1 was not required for viability *in vivo*, further investigations were necessary to elucidate the physiological functions of BACE1 and to fully understand the potential for mechanism-based toxicities of therapeutic BACE1 inhibition. For example, BACE1 protein is highly concentrated in presynaptic terminals of CNS neurons, suggesting that BACE1 has a role in

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synaptic function. Moreover, in agreement with high BACE1 expression and presynaptic localization in neurons, deeper analyses of BACE1-/- mice have uncovered numerous subtle neuronal phenotypes, such as axon targeting errors, reduced myelination, memory impairments, reduced muscle spindles, neurochemical abnormalities, alterations in neurogenesis and astrogenesis, increased age-related neurodegeneration, reduced spine density, retinal pathology, endophenotypes of schizophrenia, and seizures. Future investigations may reveal even more BACE1-null phenotypes. Any of these BACE1-null phenotypes in theory could represent mechanism-based side effects of BACE1 inhibitor drugs in humans, thus raising a note of caution that therapeutic inhibition of BACE1 might not be completely free of toxicity.

2.2. Substrates of BACE1

The varied phenotypes of the BACE1-/- mice are likely the result of abrogated β-secretase processing of different substrates of BACE1 in addition to APP. Recent proteomic analyses in cultured primary neurons have identified numerous putative BACE1 substrates that have roles in neuronal functions.[15,16] The majority of substrates of BACE1 are, like APP, type I membrane proteins, and a few, like neuregulin 1 (NRG1), have more complex membrane topologies. Cleavage of most substrates by BACE1 releases an ectodomain fragment that diffuses from the cell in the extracellular milieu. There, it may bind to another molecule on the same (autocrine) or a different (paracrine) cell to affect signal transduction or cell-cell interactions. Perhaps the best-studied example is that of BACE1 processing of type III NRG1, which releases an epidermal growth factor–like domain that binds to the ErbB receptor on the Schwann cell for the stimulation of myelination. Because of the lack of β-secretase processing, BACE1-/- mice have decreased shedding of the NRG1 epidermal growth factor domain, which reduces instructive signals to myelinating cells and leads to hypomyelination.

Another example of β-secretase processing of a neuronal substrate involves the cleavage of the neural cell adhesion molecule close homologue of L1 (CHL1) by BACE1. Like APP, CHL1 is a type I membrane protein, and it has a well-known function in axonal outgrowth and neuronal survival. Cleavage of CHL1 by BACE1 liberates a soluble ectodomain fragment that may bind to neuropilin-1 and semaphorin 3A, 2 molecules that are involved in axonal guidance. Thus, the lack of β-secretase processing of CHL1 might account for the presence of mistargeted axons that have been reported in the olfactory bulb and hippocampus of BACE1-null mice.[15–17]

In addition to cleavage by BACE1, a number of BACE1 substrates undergo ectodomain shedding by proteases in the A disintegrin and metalloproteinase domain (ADAM) family. The extent to which a given substrate is processed by BACE1 versus an ADAM family member varies depending on the substrate. Some substrates are almost exclusively cut by BACE1 (e.g., SEZ6, APLP1), whereas other substrates are primarily cleaved by the ADAMs (e.g., APP, neuroligin-1). One would predict from these results that potential mechanism-based side effects that arise from therapeutic BACE1 inhibition might derive from deficient processing of substrates that predominantly undergo ectodomain shedding by BACE1 rather than the ADAMs. Conversely, potential toxicities of BACE1 inhibition may be less associated with substrates that are primarily cleaved by ADAM proteases over BACE1.

2.3. BACE2-/- mice

The significant amino acid similarity shared by BACE1 and BACE2 suggests that it may be challenging to develop BACE1 inhibitors that do not cross-inhibit BACE2. Therefore, the possibility exists that BACE1 inhibitor drugs might also cause BACE2 mechanism-based side effects in addition to those of BACE1. To investigate this question, BACE2-/- mice were produced by gene targeting. Like BACE1-null mice, the BACE2-/- mice were initially shown to be viable and fertile with no reported phenotype.[9] Moreover, other than enhanced early postnatal lethality, BACE1-/- BACE2-/- double knockout mice did not have a more severe phenotype than the BACE1-/- single knockouts.[9] These data suggest that cross-inhibition of BACE2 with BACE1 inhibitors might not be associated with enhanced toxicity in the adult after postnatal development is completed.

Although BACE2-/- mice initially were reported to be normal, further investigations have revealed BACE2 loss-of-function phenotypes. Pancreatic β-cells express significant levels of BACE2. Interestingly, BACE2-/- mice have increased β-cell mass and insulin levels, and the mice exhibit enhanced glucose regulation.[18] These phenotypes appear to be the result of abrogated BACE2 cleavage of proproliferative type I transmembrane protein Tmem27, a protein involved in the regulation of β-cell mass. Given these results, inhibition of BACE2 may be beneficial for the treatment of type 2 diabetes, although further research into this hypothesis is necessary.

In addition to the pancreatic phenotype, BACE2-/- mice on a C57BL/6 genetic background exhibit hypopigmentation that results in a silvery coat, compared with the dark coat of wild-type C57BL/6 mice. This phenotype is caused by lack of BACE2 processing of the melanocyte protein PMEL, which is expressed in pigment cells of the skin and eye. BACE2 cleavage releases a fragment of PMEL into melanosomes that forms a matrix of amyloid fibrils upon which melanin is deposited.[19] Consequently, abrogated processing of PMEL in BACE2-/- mice leads to abnormal melanosome formation and hypopigmentation. These results suggest the possibility that cross-inhibition of BACE2 by BACE1 inhibitors might cause reduced pigmentation in humans.

3. Small-molecule BACE1 inhibitor drugs and clinical trials for AD

The extensive validation of BACE1 as the primary β-secretase enzyme in the CNS has spurred vigorous efforts to develop small-molecule inhibitors of BACE1 in both academia and industry. The first generation of BACE1 inhibitors consisted of noncleavable peptide-based transition state analogues designed after the amino acid sequence in APP at which β-secretase cleaves. Typically, these large peptidomimetic molecules are very potent BACE1 inhibitors *in vitro*, mainly

because the large open active site of BACE1 has evolved to bind polypeptide substrates with high affinity. Unfortunately, the peptide-based BACE1 inhibitors did not possess favorable *in vivo* pharmacologic properties, such as oral bioavailability, long serum half-life, or blood-brain barrier (BBB) penetration. As a consequence, investigators have turned toward designing true small-molecule BACE1 inhibitor drugs. However, the development of nonpeptidic BACE1 inhibitors large enough to bind with sufficient affinity to the enzymatic active site, yet small enough to exhibit satisfactory pharmacokinetics and suitable brain penetration, has proven to be very challenging. Moreover, BACE1 inhibitors should have sufficient lipophilicity to cross both plasma and endosomal membranes for gaining access to the vesicle lumen where the BACE1 active site is located.

A crucial advance in small-molecule BACE1 inhibitor development came with the first X-ray co-crystal structure of BACE1 with a peptidic BACE1 inhibitor.[20] The BACE1 X-ray structure revealed important inhibitor-enzyme interactions that were exploited in rational drug design efforts. Shortly thereafter, new classes of small-molecule BACE1 inhibitors were developed that exhibited improved pharmacologic characteristics, including small molecular weight, plasma membrane permeability, and better pharmacokinetics. However, most second-generation BACE1 inhibitors were substrates of P-glycoprotein, the adenosine triphosphate-dependent drug efflux pump for xenobiotics in the BBB, and therefore could not reach high concentrations in the brain.

More recently, potent third-generation small-molecule BACE1 inhibitors have been developed that achieve satisfactory brain penetration and robust cerebral Aβ reduction in preclinical animal models. Innovative diverse and complex drug development approaches have been employed to design current BACE1 inhibitors. For example, in fragment-based approaches, small molecules are screened from libraries of compounds that exhibit brain penetration and other favorable druglike properties and are selected for BACE1 binding and enzyme inhibition *in vitro*. Hits of the screen are then co-crystalized with BACE1, and X-ray structures are determined. Small molecules exhibiting weak interactions with the BACE1 active site are bonded together to form larger molecules that strongly bind and inhibit BACE1 yet still retain favorable brain penetration and drug properties. These approaches have yielded several orally bioavailable BACE1 inhibitor drugs that have entered into human clinical trials. Several compounds are in late clinical phases, including those from Merck, AstraZenica, Eisai, Jannsen, Novartis, among others. Expected trial completion dates range from 2017 to 2024. Although scant information on their progress has been published, preliminary trial results for several BACE1 inhibitor drugs have been reported at recent conferences and indicate that these compounds cross the BBB, robustly inhibit Aβ production in cerebrospinal fluid, and appear to be well tolerated. However, we do not yet know whether they will be safe and effective for the treatment or prevention of AD.

Competing interests

The author declares no competing financial interests.

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