β -Secretase as a Therapeutic Target for Alzheimer's Disease

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Summary: β -Secretase (memapsin 2, BACE1) is an attractive target for the development of inhibitor drugs to treat Alzheimer's disease (AD). Not only does this protease function at the first step in the pathway leading to the production of amyloid- β (A β), its gene deletion produces only mild phenotypes. In addition, β -secretase is an aspartic protease whose mechanism and inhibition are well known. The development of β -secretase inhibitors, actively pursued over the last seven years, has been slow, due to the difficulty in combining the required properties in a single inhibitor molecule. Steady progress in this field, however, has brought about inhibitors that contain many targeted characteristics.

In this review, we describe the strategy of structure-based inhibitor evolution in the development of β -secretase inhibitor drug. The current status of the field offers grounds for some optimism, in that β -secretase inhibitors have been shown to reduce brain A β and to rescue the cognitive decline in transgenic AD mice, and an orally available β -secretase inhibitor drug candidate is in clinical trial. With this knowledge base, it seems reasonable to expect that more drug candidates will be tested in human, and then successful disease-modifying drugs may ultimately emerge from this target. **Key Words:** β -secretase, amyloid precursor protein secretase, inhibitor drug, Alzheimer's disease.

INTRODUCTION

β -Secretase (memapsin 2, BACE1) is the first protease that processes amyloid precursor protein (APP) in the pathway leading to the production of amyloid- β (A β). Because an excess level of A β in the brain is intimately related to the pathogenesis of Alzheimer's disease (AD), β -secretase has long been regarded as a therapeutic target for AD in the development of inhibitor drugs for reduction of A β .¹ The cloning and identification of β -secretase, first reported in 1999,^{2–6} invigorated research on both the protease and its inhibitor drugs. Currently, β -secretase is a major drug target for AD, and the development of its inhibitor drug is being pursued in many laboratories around the world. This review presents a summary of the current understanding of β -secretase and progress in inhibitor drug development against this target. The emphasis is on the review of key developments and strategies. For a more comprehensive documentation of inhibitor development, readers are referred to previous reviews.^{7–12}

β -SECRETASE, THE TARGET

β -Secretase as a therapeutic target for AD offers several advantages. First, it initiates the production of A β and is without a compensatory activity, as demonstrated by the lack of A β in β -secretase gene deletion.^{13–15} The inhibition of this target should achieve A β -reduction and thus represents a disease-modifying therapy, and the inhibition of β -secretase would also eliminate all the harmful downstream steps in the pathogenesis of AD. Second, deletion of the β -secretase gene in mice produced only minor behavioral changes,^{16,17} suggesting the likelihood of patient safety of a β -secretase inhibitor drug aimed at partial inhibition of the protease activity. The absence of a serious phenotype also suggests that the elimination of processing for other β -secretase substrates is not a serious safety concern for the clinical application of β -secretase inhibitors. This has been confirmed in the processing of neuregulin 1 by β -secretase, which is required for the axonal myelination in young mice but not in older mice.^{18,19} Direct infusion of β -secretase inhibitor into the brains of adult mice strongly reduced A β levels but did not change the myelination of the axons.²⁰ Third, in view of the successful precedents in the drug development of HIV protease, also an aspar-

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FIG. 1. A ribbon model of β -secretase catalytic domain derived from its crystal structure.²⁷ The active site is a long cleft located between the N-terminal lobe (blue) and the C-terminal lobe (gold). An eight-residue inhibitor is bound to the active site.

tic protease, the likelihood of developing a clinically useful β -secretase inhibitor seems good.

Several proof-of-concept studies have confirmed β -secretase as a therapeutic target for AD. A β -secretase inhibitor conjugated to a carrier peptide for penetrating the blood–brain barrier (BBB) was shown to reach the brain and reduce $A\beta$ in AD mice.²¹ Immunization of β -secretase that reduced $A\beta$ in the brain also improved the cognitive performance of AD mice.²² Heterozygous β -secretase knock-out transgenic AD mice with 15% reduction of brain $A\beta$ showed a dramatic reduction of amyloid plaques at old age,²³ suggesting that limited $A\beta$ reduction may achieve the therapeutic goal.

β -Secretase and APP are both type 1 transmembrane proteins present at the cell surface. The hydrolysis of APP by β -secretase, however, takes place intracellularly, after the proteins are endocytosed into endosomes or in endoplasmic reticulum and Golgi en route to the cell surface (for review, see LaFerla et al.²⁴). The subcellular compartments where β -secretase functions have acidic

interior, near the optimal pH for the protease activity. A β -secretase inhibitor drug should ideally be able to penetrate subcellular membranes to reach the sites of $A\beta$ production. In addition, because the bulk of $A\beta$ in the body is produced in the brain, a β -secretase inhibitor drug must have the ability to penetrate the BBB.

β -Secretase is a membrane-anchored aspartic protease. Detailed knowledge on the active site and catalytic process had a strong influence on the strategy of β -secretase inhibitor development. Kinetic and specificity studies showed that the protease interacts with approximately 11 substrate residues,^{25,26} with affinity to many hydrophobic residues but somewhat broad in specificity. The crystal structures of β -secretase^{27,28} confirm that the active site is a long cleft for substrate recognition, with two catalytic aspartic residues positioned at the site of bond hydrolysis (FIG. 1). Along the active-site cleft are side-chain pockets that interact with amino acid residues of the substrates to correctly position the substrates for hydrolysis. These structural

FIG. 2. First-generation β -secretase inhibitor OM99-2 (compound 1). The eight-residue inhibitor, $K_i = 1.8$ nmol/L, is derived from amino acid sequence Glu-Val-Asn-Leu-Ala-Ala-Glu-Phe with the peptide bond between Leu and Ala changed to a transition-state isostere hydroxyethylene group (red box).

elements are used in creating interactions of the protease with designed inhibitors.

STRATEGY FOR THE DEVELOPMENT OF DRUG PROPERTIES

We assumed that a successful β -secretase inhibitor drug should be a small, potent, and selective transition-state analog with good membrane penetration and pharmaceutical properties. Small size, of less than 500 Da, usually permits a free passage through the BBB; however, experience from the development of HIV protease inhibitor drugs suggests that a collection of all desired drug properties would likely require a size of 600 to 700 Da for the first-generation β -secretase inhibitor drugs. Fortunately, some compounds within this size range can pass through the BBB, as exemplified by the HIV protease inhibitor indinavir.²⁹ A high potency with K_i value in the nmol/L range would be desirable to minimize the effective dosage and, thus, any potential side effects. For selectivity, the transition-state inhibitors for β -secretase are not expected to significantly inhibit other type of proteases (such as serine protease).

The presence in human of only a limited number of aspartic proteases therefore offers an advantage in the design of inhibitor selectivity. The binding of β -secretase inhibitors to pepsin and gastricsin is not a concern, because these proteases are present only in the gastric juice in the active form, but rapidly denature upon entering the intestine to release bound inhibitors. Renin, an important aspartic protease in the regulation of blood pressure, has an extremely high specificity, so it is not likely to be inhibited by a β -secretase inhibitor. The main aspartic proteases that routinely require selectivity analysis are memapsin 1 (BACE2) for its close specificity to β -secretase³⁰ and cathepsin D for its ubiquitous presence in nearly all the cells. Cathepsin E inhibition is not routinely monitored, because its specificity and inhibition patterns are close to those of cathepsin D.

Structural knowledge of β -secretase suggests that a preferable approach for the development of its inhibitors would be structure-based design cycles, rather than screening of existing chemical libraries. The screen approach would produce many hits, because of the presence in the β -secretase active site of many side-chain binding pockets. The binding intensity, or K_d , of these 'hits' are likely near the K_m values, in the mmol/L to μ mol/L range. To improve the K_d of the hits to nmol/L range, as required for a drug, the approach would need to incorporate the transition-state binding principle of aspartic proteases.³¹ The fact that HIV protease and renin inhibitor drugs in clinical use are all transition-state analogs supports such a contention, and suggests that it would also be true for β -secretase. Using a hit that frequently binds only to a side chain pocket as a starting point for the evolution to a transition-state inhibitor of clinical significance would have required a similar process used in the structure-based design. Thus, for the development of β -secretase inhibitors, the screening approach does not offer particular advantage, as is illustrated by the lack of highly potent β -secretase inhibitors that have been reported as having started from a screened hit.

The first-generation inhibitors OM99-2 (compound 1; FIG. 2)³² and OM00-3²⁶ were designed as transition-state analogs using β -secretase residue preferences in eight subsites and a hydroxyethylene isostere. These inhibitors were in fact very potent, with K_i values of 1.6 nmol/L and 0.3 nmol/L, respectively, and provided an excellent starting point for the evolution of inhibitor structure and properties. Furthermore, the crystal structures of these two inhibitors bound to β -secretase provided detailed information on the interactions at the atomic level. Many of these interactions must be preserved in future inhibitor designs. To gain the desirable drug-like properties in this process, we decided on the following strategic points. First, size reduction was to be accomplished by cutting outside residues. The resulting

FIG. 3. Representative β -secretase inhibitors (compounds 2–7) emerging from structural evolution to gain drug-like properties.

loss of potency was to be regained by exploring better interactions with the central residues. Second, we expected to learn as much as possible from the activity and function relationship of the new crystal structures, with the goal of developing structural motifs for various functions applicable in future designs. Third, knowing that the design of any one of the drug properties cannot be accomplished without affecting the other drug properties, rapid return of data on functional analysis was of paramount importance to decide the direction of evolution.

The main assays included K_i against memapsins 1 and 2 and cathepsin D, and the inhibition of $A\beta$ production in cell line and animal models. We have found transgenic mice Tg2576 to be useful for the monitoring of *in vivo* potency.^{21,33} The streamlined assay battery was designed to speed the structural evolution. Additional property analyses were added to evaluate significant candidates when they emerged. This data feedback plan permitted a rapid structural evolution and a critical evaluation of serious drug candidates.

EVOLUTION OF INHIBITOR STRUCTURES

At an early stage, extensive structural modifications aiming at the size reduction starting at OM99-2 revealed that the removal of the four outside residues P4, P3, P3', and P4' resulted in greatly reduced potency.³⁴ A representative inhibitor is compound 2 (FIG. 3), which, despite well-optimized side chains, has a K_i value almost 1000 times higher than that of OM99-2. The inclusion of P3 Val combined with the optimization of P2 and C-

terminal blocking group brought the K_i back to the nmol/L level, as represented by compound 3 (FIG. 3). Evidence from these studies suggested that, from the structural template derived from OM99-2, high potency could be attained by inhibitors with five subsites, from P3 to P2', resulting in a molecular size of approximately 700 Da.

The inclusion of design of selectivity into the inhibitors was developed along with the structure evolution. Because the inhibitors studied are transition-state analogs, the cross-inhibition is likely limited to other human aspartic proteases. The early inhibitors, such as OM99-2, had little selectivity *versus* memapsin 1 or cathepsin D. When the structural basis of differential inhibition were found in various structural modules, it became possible to design highly selective β -secretase inhibitors.³⁵ As shown in Figure 3, our initial effort led to the design of selective inhibitor 4 with a K_i of 0.3 nmol/L against β -secretase; it displayed 1186-fold selectivity over memapsin 1 and 436-fold selectivity over cathepsin D. Subsequently, we designed inhibitor 5, which has demonstrated a K_i of 0.12 nmol/L against β -secretase but with K_i values for memapsin 1 and cathepsin D greater than 3800-fold and 2500-fold, respectively. The structural basis of selectivity *versus* memapsin 1 resides mainly in P₃-oxazole, which affected a local conformational change better accommodated in β -secretase, compared with memapsin 1.³⁵ The P₂-sulfone group in compound 5 also provides a hydrogen-bonded network in

FIG. 4. Hydroxyethylamine isostere-derived drug-like memapsin 2 inhibitors (compounds 8–11).

β -secretase that cannot be accommodated in cathepsin D, thus differentiating inhibition potency.

We also investigated various means to restrain the conformational freedom of the inhibitors, including the linking of P2 and P4 side chains to form macrocyclic inhibitors.³⁶ A representative inhibitor 6 (FIG. 3) contains a cycloamide urethane macrocyclic ring. A surprisingly good K_i of 14 nmol/L for this inhibitor suggests that the introduction of rings to constrain the backbone freedom may be pursued. Further evolution of the inhibitor structures along this line gave rise to compound 7 (FIG. 3), which contains a substituted isophthalamide ring at P2 and optimized side chains from structure-based design and energy minimization.³⁷ Inhibitor 7 has many improved targeted properties. It is potent (K_i = 1.1 nmol/L), and has moderately good selectivity (K_i values of 31 nmol/L and 41 nmol/L *versus* memapsin 1 and cathepsin D, respectively). At 648 Da, it penetrated the cell membrane well and inhibited A β production in cultured cell with an IC₅₀ of 39 nmol/L.

Administration of inhibitor 7 (8 mg/kg i.p.) in Tg2576 mice effected a 30% reduction of plasma A β ₄₀ at 4 h after a single administration.³⁷ Because the production of A β in young Tg2576 mice is almost exclusively in the brain,³⁸ these observations suggest that at least part of the inhibition of A β production in AD mice is likely to be the result of inhibition of β -secretase in the brain by inhibitor 7.

The knowledge generated from these studies has afforded the design of a new generation of inhibitors that contain many of the targeted drug properties. One such example is inhibitor GRL-8234 (compound 8; FIG. 4). This inhibitor contains a hydroxyethylamine isostere with a P1-phenylmethyl side chain, a functionalized P2-isophthalamide ligand, and a P2'-hydrophobic benzyl derivative. The inhibitor exhibited a memapsin 2 K_i value of 1.8 nmol/L. Most strikingly, it has shown a β -secretase cellular IC₅₀ value of 1 nmol/L. This impressive cellular activity is in marked contrast to inhibitors with hydroxyethylene isosteres.

As it turned out, the choice of ligands and substituents

are all critical to the observed activity of inhibitor 8. The P1-leucine side chain containing inhibitor 9 (K_i $\approx$ 916 nmol/L) was significantly less potent. Inhibitor 10 (K_i = 425 nmol/L) with a P2'-isopropyl group is also less potent than inhibitor 8 with a P2'-methoxybenzyl derivative. Similarly, replacement of P3-phenylmethyl derivative (inhibitor 11) resulted in marked attenuation of activity, with inhibitor 11 showing a β -secretase K_i of 552 nmol/L. The remarkable cellular activity of compound 8 may be attributable to the balance of its lipophilic and basic amine properties. Our X-ray crystallographic analysis of protein–ligand structure of compound 8 shows that both the hydroxyl group and the secondary amine group form a network of tight hydrogen bonding with the active site aspartic acid residues Asp32 and Asp228. The P2-sulfonamide derivative fits well in to the S2-site and makes extensive hydrogen bonding with β -secretase.

Inhibitor 8 has shown good selectivity over other aspartyl proteases (39-fold selective over memapsin 1 and 23-fold selective over cathepsin D). Inhibitor 8 has shown very impressive *in vivo* properties in transgenic mice. Administration of compound 8 (8 mg/kg i.p.) to Tg2576 mice resulted in up to 65% reduction of A β ₄₀ production after 3 h. These impressive properties have provided the basis of further modifications of this series of inhibitors.

GENERAL DEVELOPMENT OF β -SECRETASE INHIBITORS

Kimura et al.³⁹ reported phenylnorstatine-based compounds 12 and 13 (FIG. 5) as potent β -secretase inhibitors (compound 12, IC₅₀ = 4.8 nmol/L; compound 13, IC₅₀ = 1.2 nmol/L), in which tetrazole rings were demonstrated to be an appropriate bioisosteric replacement for the carboxylic acids at both P4 and P1' positions of a previous series of compounds. In an attempt to develop pharmaceutically useful compounds, the same group began investigation of bioisosteres of the acidic tetrazole ring. Introduction of a 5-fluoroorotyl group at the P4

FIG. 5. Phenylnorstatine-based inhibitors (compounds 12–14).

position and L-cyclohexylalanine residue at the P2 position resulted in inhibitor 14, which maintained optimal enzyme inhibitory activity ($IC_{50} = 5.6$ nmol/L) while displaying 84% β -secretase inhibition in cultured cells at a concentration of 100 μ mol/L.⁴⁰

With the aim of obtaining inhibitors with more favorable pharmacokinetic profiles, several research groups concentrated their efforts on the development of compounds based on the hydroxyethylamino isostere, assuming that the presence of a basic nitrogen would impart higher cellular potency, and also taking into consideration the successful results obtained in the field of HIV protease inhibitors. Inhibitors 15 and 16 (FIG. 6), developed by Maillard et al.⁴¹ following this rational approach, displayed impressive potency in both enzymatic (compound 15, $IC_{50} = 5$ nmol/L; compound 16, $IC_{50} = 20$ nmol/L) and cell-based assays (compound 15, $EC_{50} = 3$ nmol/L; compound 16, $EC_{50} = 15$ nmol/L). A similarly high enzymatic potency was observed for inhibitors 17 and 18 (FIG. 6) in which the C-5 position of the isophthalamide ring was functionalized with a polar primary

amide in order to increase affinity for β -secretase and selectivity over cathepsin D.⁴²

A drawback for this class of compounds was the predicted poor metabolic stability due to microsomal *N*-debenzylation and *N*-depropylation.⁴² Further work⁴³ was performed by replacing the isophthalate *N*-terminus by acyclic sulfones. The authors first identified the racemic carbobenzyloxy-derivative compound 19 as a lead compound endowed with good enzymatic activity, but more potent against cathepsin D ($IC_{50} = 67$ nmol/L).⁴³ Subsequently, structure-based design resulted in the synthesis of derivative compound 20, with highly improved enzymatic inhibitory activity ($IC_{50} = 2$ nmol/L) and cellular potency ($IC_{50} = 1$ nmol/L). The X-ray crystal structure of compound 20-bound β -secretase highlighted a close association between the pyridyl nitrogen and the Arg235 in the S2 site. The authors suggest that, because cathepsin D S2 pocket is more lipophilic, and consequently is less tolerant of the introduction of polar groups with respect to β -secretase, the pyridyl

FIG. 6. Hydroxyethylamine-derived diverse inhibitors (compounds 15–21).

FIG. 7. Tertiary carbinamine and reduced amine inhibitors (compounds 22–25).

moiety of compound 20 is also the main determinant of improved selectivity *versus* cathepsin D enzyme ($IC_{50} = 474$ nmol/L).⁴⁴

Inhibitor 21 (GSK188909; FIG. 6) was described as the first orally bioavailable β -secretase inhibitor capable of lowering brain A β in APP transgenic mice,⁴⁵ and the studies leading to the discovery of this orally active hydroxyethylamino isostere-based inhibitor have been reported.^{46–48} GSK188909 inhibited β -secretase activity with an IC_{50} of 5 nmol/L and also showed good selectivity with respect to memapsin 1, renin, and cathepsin D. It caused a decrease in A β_{40} and A β_{42} production in cell-based assays expressing both wild-type and Swedish-variant APP sequences ($IC_{50} = 5$ and 30 nmol/L, respectively). When orally administered to TASTPM mice, along with the P-glycoprotein (P-gp) inhibitor GF120918, GSK188909 (250 mg/kg) caused a 68% and 55% decrease in A β_{40} and A β_{42} , respectively, 9 h after the dose. Notably, although no reduction in A β was evident in brains of mice dosed with GSK188909 alone, subchronic dosing (250 mg/kg twice daily for 5 days) resulted in a small but significant decrease in brain A β_{40} and A β_{42} (18% and 23%, respectively).

A basic nitrogen is also present in a series of tertiary carbinamine-derived inhibitors described by Rajapakse et al.,⁴⁹ in which the primary amine is reported to interact with the catalytic aspartates of β -secretase. Their optimized inhibitor (compound 22; FIG. 7) showed high potency in enzymatic and cellular assays ($IC_{50} = 12$ and 65 nmol/L, respectively) and good selectivity toward both renin and cathepsin D, but only moderate selectivity

toward the high homolog memapsin 1 ($IC_{50} = 620$ nmol/L). Macrocyclization of this series of inhibitors led to lactone compound 23 (FIG. 7), which displayed increased potency *versus* β -secretase ($IC_{50} = 2$ nmol/L). Both series of compounds, however, suffer from poor brain penetration, mostly due to high efflux of P-gp.⁵⁰

A macrocyclization approach as a tool to improve affinity and selectivity by locking a molecule in a bioactive conformation also guided the design of inhibitor 24 (FIG. 7) based on an isophthalamide scaffold coupled to a reduced amide isostere.⁵¹ This compound displayed an enzyme IC_{50} of 4 nmol/L, a cellular IC_{50} of 76 nmol/L, and, most important, an improved membrane permeability and reduced P-gp susceptibility. When administered in mouse at a dose of 100 mg/kg i.v., it produced a 25% decrease in A β_{40} levels in brain extracts. A series of interesting inhibitors based on a 2,6-diamino-isonicotinamide core coupled to a truncated reduced amino isostere as the aspartate binding element^{52,53} is typified by compound 25 (FIG. 7), displaying cellular IC_{50} of 49 nmol/L and *in vivo* activity in transgenic mice expressing human wild-type APP. After intravenous administration of a 50 mg/kg dose of inhibitor 25, a maximal reduction of A β_{40} (34%) at 3 h from dosing was observed, and the concentration of drug in the brain was 1.9 μ mol/L.⁵³

Strategies to discover nonpeptidomimetic small-molecule hits, to be optimized as β -secretase inhibitors, mostly involved screening of large libraries of compounds. For example, a low molecular weight acylguanidine β -secretase inhibitor was discovered through

FIG. 8. Acylguanidine and aminoquinazoline-derived inhibitors (compounds 26–28).

high-throughput screening of the Wyeth corporate compound library.⁵⁴ Optimization of the hit using structure-based design led to the design of compound 26 (FIG. 8), which displayed a β -secretase inhibition IC_{50} of 0.110 μ mol/L. A 0.24-nm (2.4 Å) resolution cocrystal structure of β -secretase with a closely related analog of compound 26 revealed that the acylguanidine moiety forms hydrogen bonds with the catalytic aspartates, whereas the polar functionality of the guanidine N substituent extends into the S1' pocket, forming hydrogen-bonding interactions through bridging water molecules.⁵⁴ Moreover, the crystal structure revealed that the inhibitor stabilizes the enzyme in an open conformation, contrary to most peptidomimetic inhibitors, which bind to β -secretase in a closed-flap form. Preliminary structure–activity relationship (SAR) investigations^{55–57} resulted in moderate improvement of β -secretase inhibitory potency, but poor selectivity for memapsin 1 enzyme and poor permeability, as assessed in a Caco-2 drug transport model⁵⁷ remain major drawbacks of this class of compounds. It has been suggested that the inherently poor cellular permeability of the acylguanidine inhibitors could be due to the guanidinyl functionality, and bioisosteric replacement of this group is currently under evaluation.⁵⁷

Aminoquinazoline compound 27 ($K_i = 0.9 \mu$ mol/L) (FIG. 8) was also discovered by screening a vast library of compounds. The X-ray structure of compound 27 bound to β -secretase revealed that the aminoquinazoline moiety binds to the catalytic aspartates, whereas the lateral chain adopts a hairpin conformation in which the cyclohexyl ring occupies the S1 site stabilizing the enzyme in an open conformation.⁵⁸ Structure-based optimization of compound 27 led to the discovery of compound 28 (FIG. 8), showing striking potency for a small-molecule, nonpeptidomimetic compound ($K_i = 11$ nmol/L). This inhibitor, although showing moderate selectivity for cathepsin D and renin and being a potential substrate for P-gp, as indirectly evaluated by the efflux

ratio calculations in the caco-2 model, lowered A β levels in plasma by 40% to 70% in rats after oral administration (30 mg/kg).

ALTERNATIVE THERAPEUTIC APPROACHES TARGETING β -SECRETASE

In principle, *in vivo* action of β -secretase on APP hydrolysis would require the participation of many other cellular components and would thus provide alternative opportunities for therapeutic intervention; however, few therapeutic approaches outside of β -secretase inhibitors have so far been reported. Chang et al.²² reported a proof-of-concept study on the reduction of A β by immunizing AD mice with the ectodomain of β -secretase. It was shown that antibodies against β -secretase penetrated the BBB, inhibiting A β levels in the brain and improving the cognitive performance of AD mice. The antibodies in this approach serve as inhibitors for β -secretase activity and thus do not require the participation of immune cells for A β reduction. This may indeed lower the risk of autoimmune response as observed in A β immunization. A conceptually related approach is immunization using peptides derived from the β -secretase cleavage site in APP. A study with AD mice using this approach has shown promise.⁵⁹

β -Secretase undergoes extensive intracellular transport in performing its function of APP hydrolysis. Because β -secretase functions optimally in an acidic solution, it has little activity at the cell surface, where the pH is near 7. When both APP and β -secretase are endocytosed into endosomes, the cleavage takes place to generate A β , which is ultimately secreted extracellularly. Regulation of the endocytosis of APP and β -secretase has been shown to be affected by apolipoprotein E (ApoE), mediated by ApoE receptor 2 and adaptor protein X11.⁶⁰ If such a mechanism is operative at a signif-

icant level *in vivo*, compounds that weaken ApoE interaction with ApoE receptor 2 may lead to the reduction of A β .

β -Secretase in the endosomes is transported to the Golgi network, then back to the cell surface.⁶¹ This recycling process requires the participation of a family of adaptor GGA proteins.^{61–63} The observations that the reduced expression of GGAs led to an increase of A β ^{64,65} and that the dileucine motif in the cytosolic domain of β -secretase is essential for its endocytosis^{66,67} suggest that the components involved in the trafficking of β -secretase may participate in the regulation of A β production and may be targets for A β -reduction therapy. The challenge, however, is to discover the means to effectively modulate the activities of these components for achieving A β reduction.

PERSPECTIVES

Despite the attractiveness of β -secretase as a therapeutic target of A β -reduction therapy for AD, the emergence of an effective small-molecule inhibitor drug has been slow. The difficulty in developing such a drug can be traced to several factors, including the lack of success in high-throughput screening, the need to design BBB-penetrating inhibitors with high potency and selectivity, and the relatively unaccommodating nature of the β -secretase active site for substitutions away from the preferred substrate structures. Nonetheless, significant advances have been made in the last few years, which have demonstrated that most of the required drug properties can be designed into a single inhibitor molecule. More encouragingly, β -secretase inhibitors have also been shown to reduce A β in the brain and to rescue the cognitive decline in AD mice.³³ A β -secretase inhibitor drug candidate, CTS-21166, has completed the Phase I clinical trial (see CoMentis announcement at http://www.athenagen.com/index.php?athenagen/press_releases/48). The proof-of-concept studies on the therapeutic models using β -secretase inhibitors lend support to the idea that *in vivo* A β reduction can be achieved with this approach. Based on the prevailing view on the fundamental role of A β in the pathogenesis of AD, there seems to be good reason to expect that β -secretase inhibitor drugs may alter the course of the disease. The progress in the knowledge base for β -secretase inhibitors appears to have reached a stage at which the ultimate emergence of β -secretase inhibitor drugs is probable.

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